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17th World Congress of the International Society on Toxinology
&
Venom Week 2012
Honolulu, Hawaii, USA, July 8–13, 2012

Abstract Editors:
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Abstracts Toxins 2012

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This issue of Toxicon contains the abstracts presented at the 17th World Congress of the International Society on Toxinology (IST), held jointly with Venom Week 2012, 4th International Scientific Symposium on All Things Venomous, in Honolulu, Hawaii, USA, July 8–13, 2012.

The World Congress of IST is held every three years, most recently in Recife, Brazil in March 2009. The IST World Congress is the primary international meeting bringing together scientists and physicians from around the world to discuss the most recent advances in the structure and function of natural toxins occurring in venomous animals, plants, or microorganisms, in medical, public health, and policy approaches to prevent or treat envenomations, and in the development of new toxin-derived drugs. The Venom Week Symposiums, previously held in 2005, 2007 and 2009, are held in the USA with an international faculty and focus, and present the most recent advances in terrestrial and marine envenomations of medical and veterinary importance, in venom and antivenom basic science, in the handling and breeding of venomous animals in zoos and aquaria, in venomous animal science, and in regulatory issues. As both meetings were scheduled to be held in the summer of 2012 in the USA, the two organizations decided to hold a joint meeting in Honolulu.

The program of the meeting is characterized by two parallel tracks, basic sciences and clinical sciences, thereby providing venues for presenting advances in these two broad areas of toxinology research as well as translational aspects that bridge these two components. Some sessions on important topics are organized by eminent scientists in their field, whereas other sessions were developed from the more than 300 abstracts submitted to the meeting.

The joint meeting of the 17th World Congress of the IST and Venom Week 2012 is intended to be a forum of scientific exchange among the members of the toxinology communities from around the globe. It is hoped that the unique location of Hawaii enhances the meeting experience. Hawaii is indeed a very special place. The most northern part of Polynesia, it is the most isolated land mass in the world with a sizable population. It is characterized by an unparalleled cultural diversity, where the East meets the West, and where the host culture is Hawaiian. Although part of the United States, it is a fitting place to serve as host for the Asia-Pacific Region of the IST for its World Congress. Oahu, the island on which Honolulu is located, is a Hawaiian name and means “Gathering Place”. Appropriately, it will be the gathering place for the world toxinology community in 2012.

Many thanks go to the Executive Committee of IST, President Dr. Ponnampalam Gopalakrishnakone (Singapore), Secretary/Treasurer Dr. Julian White (Australia), and President-Elect Dr. Alan Harvey (United Kingdom), to the members of both the international Scientific Organizing Committee, the Local Organizing Committee, and to the many sponsors for helping to make the 17th IST World Congress / Venom Week 2012 Meeting a reality and success.

Meeting Co-Chairs
Steven A. Seifert, MD (USA)
Carl-Wilhelm Vogel, MD, PhD (USA)

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Jean-Philippe Chippaux (BENIN)

A. Biotoxins as Bioweapons

1. Detection Technologies for Biological Toxins

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Review: The ad hoc group of the states parties to "the convention on the prohibition of the development, production and stock piling of biological and toxin weapons and on their destruction" has listed the following toxins, bacteriotoxins (Botulinum toxin, clostridium perfringens toxins, staphylococcal enterotoxins, shigatoxins), Phycotoxins (Anatoxins, ciguatoxins, saxitoxins), Mycotoxins (trichothecenes), Phytotoxins (Abrins,Ricins) Zootoxins (Bungarotoxins). In addition, few other toxins which are not in this list such as brevetoxins, conotoxins and other neurotoxins also will be reviewed in addition to the work done by venom and toxin research programme at National University of Singapore.

An overview of the different technologies available for the detection of biological toxins, originating from animals, plants and microbial organism will be described. The methodologies/technologies vary from fieldable ones to highly sensitive one to user friendly ones. Depending on the purpose of detection of toxin vary in different circumstance, quantitatively or qualitatively starting from Ouchterlony diffusion test to classical ELISA kit which has been used for many years for the detection of biological toxins as well as HPLC, LCMS. In the recent past our research group has developed immunosensitive field – Effect Transistors (ISFET) (1), ultrasensitive protein array based on electrochemical enzyme immunoassay (EETA) (2), silicon chip base optical immunoassay (OIA) (3) for the detection of toxins from animal venoms as well as toxin from microbial organisms such as Botulinum toxins, SEB toxin, T-2 toxins as well as plant toxins such as ricin or abrin.

References

Zakaria.E.S, Gopalakrishnakone, P. & Pavel, N. Immunologically sensitive field effect transistors. Encyclopedia of Medical Devices and Instrumentation. Ed: Weber JC, Pubs: John Wiley & Sons, 2006, p. 98-110.
Le, V.D., Fang, X., Zichao Y., Gopalakrishnakone, P. & Gao, Z. An ultrasensitive protein array based on electrochemical enzyme immunoassay. Frontiers in Biosciences 10, 1654-1660, 2008.
Saravanan, G.,Rajasekar, G. & Gopalakrishnakone, P. Fieldable Optical Immunoassay for toxin detection.
Ler, S.G, Lee, F.K. & Gopalakrishnakone, P. Trends in detection of warfare agents: Detection methods for Ricin, SEB and T-2 toxin. Journal of Chromatography A,1133,1-12(2006).

Keywords: biological toxins, detection, lethal
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2. Medical Aspects of Bioterrorism

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Introduction: Bioterrorism is a terrorist action involving the intentional release or dissemination of a biological warfare agent (BWA), which includes some bacteria, viruses, rickettsiae, fungi or toxins. BWA is a naturally occurring or human-modified form that may kill or incapacitate humans, animals or plants as an act of war or terrorism. BWA is a weapon of choice for mass destruction and terrorism, because of there is usually an incubation period, lesser quantities are required than chemical warfare agents, they are easily distributed with devices that are readily obtained, they are usually odorless, colorless, difficult to detect, with no need of specialized equipment for production. BWA may be disseminated as an aerosol, spray, explosive device, and by food or water.

Classification: Based on the risk, BWAs have been prioritized into three categories of A, B and C. Category A includes microorganisms or toxins that easily spread, leading to intoxication with high death rates such as Anthrax, Botulism, Plague, Smallpox, Tularemia and Viral hemorrhagic fevers. Category B has lower toxicity with wider range, including Staphylococcal Entrotoxin type B (SEB), Epsilon toxin of Clostridium perfringens, Ricin, Saxotoxins, Abrin and Trichothecene mycotoxins. The C category includes emerging pathogens that could also be engineered for mass spread such as Hanta viruses, multidrug-resistant tuberculosis, Nipah virus, the tick-borne encephalitis viruses, hemorrhagic fever viruses and yellow fever.

Clinical manifestations of biotoxins in human: Clinical features and severity of intoxication depend on the agent and exposed dose, route of entry, individual variation and environmental factors. Onset of symptoms varies from 2-24 hours in Ricin to 24-96 hours in Botulism. Clinical manifestations also vary from irritation of the eyes, skin and mucus membranes in T_2 toxin to an acute flaccid paralysis of bilateral cranial nerve impairment of descending manner in botulism. Most of the pyrogenic toxins such as SEB produce the same signs and symptoms as toxic shock syndrome including a rapid drop in blood pressure, elevated temperature, and multiple organ failure.

Management: There is no specific antidote or effective treatment for most of the biotoxins. The clinical management is thus more supportive and symptomatic. Fortunately vaccines are now available for most of BWA. Therefore, immunization of personnel at risk of exposure is recommended.

Conclusion: Biotoxins are very wide and bioterrorism is a heath and security threat that may induce national and international problems. Therefore, health professionals and the general population should be aware of the potential for bioterrorism.

Keywords: biotoxin, bioterrorism, biological warfare agent
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3. Capsaicin: A Novel Antidote against Botulinum Neurotoxin A.

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Review: Botulinum neurotoxin A (BoNT/A) is the most potent toxin produced by *Clostridium botulinum*. BoNT/A holotoxin consists of a 100 kDa heavy chain (Hc) and a 50 kDa light chain (Lc) linked by a single disulfide bond. BoNT/A Hc **binding** to its receptors on the neural membrane sets the stage for **endocytosis** into the Motor Nerve Terminals (MNT). Within early endosomes, BoNT/A undergoes pH dependent separation of the Hc and Lc. The Hc **translocates** the Lc into the cytosol where it **cleaves** SNAP-25 and **inhibits** neurotransmission. The ease of production and transportation accounts for the accidental or intentional misuse of BoNT/A. The extensive use of BoNT/A to treat clinical diseases also presents opportunities for such misuse. BoNT/A, as a bioweapon, can be disseminated via aerosol or by contamination of water or food supplies, to cause widespread casualties. The need for prolonged intensive care and ventilatory support for the affected patients will impose severe economic burden on our health care system. Until now, no therapeutic strategies are available to treat BoNT/A intoxication. The antitoxins that are available will be of no use once BoNT/A has entered the MNT. Therefore, developing therapeutic drug strategies to treat botulism is of primary importance. Our research work demonstrates a novel anti-BoNT/A effect of capsaicin, an active ingredient of chili peppers. Capsaicin, a transient receptor potential vanilloid receptor 1 agonist, demonstrated a prophylactic as well as a therapeutic effect against BoNT/A. Preinjection of capsaicin at the mouse hindlimbs protected the uptake and neuroparalytic effects of BoNT/A (Thyagarajan *et al.* 2009). The prophylactic effects of capsaicin were abrogated by capsazepine, a TRPV1 antagonist. Injection of capsaicin, at 12 and 24 hr post BoNT/A, remarkably accelerated the recovery of animals from BoNT/A mediated neuroparalysis. *In vivo*, capsaicin injected mice showed better performance on rotarod, demonstrated higher compound muscle action potential and muscle strength. Also, *in vitro*, capsaicin treatment potentiated the restorative effects of 2,4 diaminopyridine (DAP; a potassium channel blocker). Capsaicin injection enhanced recovery by shortening the duration of neuroparalysis by 50% compared to BoNT/A alone (no capsaicin) injected controls.

Conclusions: Collectively, we demonstrate that capsaicin has the potential to be developed as a novel agent to prevent, and to treat BoNT/A intoxication. This discovery significantly advances our current knowledge of BoNT/A antagonists and forms the basis of novel therapeutic approach from the natural product, chili peppers. Further research to understand the mechanisms of action of capsaicin is in progress.

Keywords: capsaicin, Botulinum neurotoxin A, neuromuscular junction
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4. Biohazards of Botulinum Neurotoxins

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Review: Botulinum neurotoxins (BoNTs) are produced by toxigenic strains of *C. botulinum* in seven serotypes (named A through G). They are responsible for the clinical syndrome of botulism blocking the acetylcholine release from peripheral nerves and thus causing a flaccid paralysis. Respiratory failure secondary to paralysis of the respiratory muscles can lead to death unless appropriate therapy is promptly initiated. Structurally, BoNTs are composed of three domains: a Light Chain (LC), which acts in the cytosol as a metalloprotease and cleaves protein components (SNAREs) of the neuroexocytosis apparatus; a translocation domain (HN) and a receptor binding domain (HC). The high affinity binding, due to a double receptor interaction and the extremely specific enzymatic activity, make botulinum toxin the most lethal substance known, with a LD50 of 1 nanogram of toxin per kilogram body mass. Nevertheless, it also serves as a remarkably effective treatment for hypercholinergic disorders such as blepharospasm, strabismus, hemifacial spasm, certain types of spasticity and other ailments and for cosmetic anti-aging treatments. Due to the severity and potency of this neurotoxin, its importance as a biological weapon is of major concern to public health officials and, in fact, it belongs to "category A" of agents which represent serious risk when misused for military and/or criminal activities. Despite its widespread accessibility and high lethality, however, BoNT has never been used successfully for purposes of warfare or bioterrorrism. Several countries investigated botulinum toxin as a biologic agent since World War II. Botulism's sole link to a presumed successful planned use as a weapon is provided by Paul Fildes, a high-ranking British specialist in bacterial weapons development during World War II, who has alluded to the fact that he contributed to the assassination of Reinhard Heydrick, head of the Gestapo. More recently, in the early 1990s, before their attack with Sarin on the Tokyo subway system, the Japanese cult Aum Shinrikyo, had released an ineffectively produced *C. botulinum* preparation in Japan. During the United Nations' inspections of Iraq's capabilities for biologic warfare in 1991, botulinum toxin was clearly an area in which research had been directed. More botulinum toxin was produced than any other weaponizable agent in Iraq. Botulinum toxin can be absorbed by either gastrointestinal or respiratory epithelium but does not penetrate intact skin. The clinical forms of botulism depend on the mode of contamination but botulism through inhalation can only be the result of a deliberate act using an aerosol.

Keywords: botulinum toxins, botulism, bioterrorism
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5. Bioterrorism and Biological Toxins

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Review: The use of biological agents as a warfare goes back to ancient time, but it is only in modern times that several countries have developed programs of research and development of biological warfare. More recently, initiatives to control the possible use of these weapons have emerged and three lists (A, B and C) of biological agents of different potential danger have been drawn. In this presentation I will discuss one toxin producing bacterium included in list A: Bacillus anthracis and two plant protein toxins included in list B. The mechanisms of action of the two anthrax toxins and their immunosuppressive properties will be illustrated together with the mechanism of action and pathogenetic consequences of the poisoning with ricin or abrin will be illustrated. Finally, possible methods of preventing their action will be considered.

Keywords: anthrax toxin, ricin, abrin, bioterrorism
10.1016/j.toxicon.2012.04.006

6. The Diverse Roles of Botulinum Toxins

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Review: Botulinum neurotoxin is a cause of the naturally occurring disease botulism, a pharmaceutical, and a potential biowarfare/terrorism agent. The bacterium *Clostridium botulinum* is the primary source of this toxin in human disease. *Clostridium botulinum* exists as serotypes A-G, although human disease is almost entirely restricted to types A, B, and E. Up to now there has been no systematic assessment of the differences in disease presentation by different strains. Because botulinum neurotoxins are the most potent substances known, the CDC has listed it as a type A biothreat. The neurotoxin consists of a 150 KDa molecule comprised of a 100 kDA heavy chain (HC) and a 50 kDA light chain (LC). Its toxicity results from its interaction with cholinergic nerve terminals at the neuromuscular junction (NMJ). The HC is responsible for facilitating the pH-dependent endocytotic translocation of the toxin into motor nerve terminals at the NMJ. Once internalized the LC cleaves the SNARE proteins that are responsible for facilitating the release of acetylcholine into the NMJ. The resulting failure of acetylcholine release is responsible for the subsequent loss of skeletal muscle function and the ensuing paralytic syndrome. However, the various strains of botulinum toxin do not all cleave the same SNARE proteins. The degree to which these differences influence the clinical presentation has not been well characterized. There are three potential preventive and therapeutic approaches to botulism syndromes: vaccines, pharmacologic interventions and therapeutic antibodies. The immunologic measures almost certainly are only effective before internalization of the neurotoxin. Vaccines, therefore, are unlikely to be effective therapeutically. Vaccines, however, have been used for many years to protect researchers or military personnel who are to be at risk for encountering botulinum toxin. Such vaccinations are effective in inducing neutralizing antibodies. Therapeutic antibodies directed

against botulinum toxin represent the most promising of the post-exposure therapeutic approaches on the short-term horizon. They must be given before a clinically significant amount of neurotoxin is internalized into the NMJ. In the US there is currently one approved therapeutic antibody preparation. A number of pharmacologic agents are currently being studied, however none have yet shown great promise. Theoretically these might be effective in the post-NMJ internalization phase. Currently we are assessing the diversity in the presentations of botulinum syndromes caused by various strains. It is anticipated that elucidation of these difference might provide information on potential therapeutic approaches to botulism syndromes. Results of these studies will be presented.

Keywords: Botulism, neurotoxin, biothreat
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7. Biological Weapons and Toxins: A Short History and Look at the Future

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Review: This review is a look back at the past and into the future; of the history of toxins used as weapons, and the possible dangers facing us in the future. Before 2001, there was recognition that biological weapons, including toxins, were weapons that required state sponsorship. But that is no longer true. As the asymmetries of weapons and politics have changed, so has the probable use of toxins as weapons. Our understanding and expectation of likely use of toxins in weapons has changed. Even the word “toxin” is commonly used inappropriately to mean toxic chemical rather than a chemical produced by organisms. Prior to 2001, toxins were evaluated on their ability to be manufactured or purified from natural resources. Many infectious disease specialists regard bacterial toxins as the eventual cause of cell damage and cell death. Where are we in the spectrum of toxins as weapons, from a clear use of biological weapons to a purified chemical? Do we need purified chemicals? Are toxins the ultimate dual use agents? Do toxinologists need to examine their publications for possible illicit use?

Keywords: toxin, biological weapon, bioterrorism
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8. Biotoxins and Bioterrorism: Ricin and Saxitoxin

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Introduction: Natural biotoxins have been considered by nation states for use as chemobiological weapons, and several specifically developed for weaponisation. Ricin gained notoriety when it was allegedly used as an agent of assassination, and saxitoxin was the key component in a weapon system designed to target individuals and be unattributable. Terrorist and extremist groups have included biotoxins in their planning, and documents describing the extraction and preparation of ricin have been found in the possession of terrorist cells.

Toxicology: Ricin is a glycoprotein found in the seeds of the castor oil plant (*Ricinus communis*). The native molecule consists of two polypeptide chains, A and B, which are linked by a disulphide bond that is easily reduced. On systemic absorption, the B chain (a lectin) binds to cell surface receptors present on eukaryote cells but not in prokaryotes. There is endocytic uptake of the toxin into the cytosol where the chains dissociate and the A chain binds to the 28s ribosomal subunit, blocking protein synthesis and resulting in the death of the cell. Clinical features develop over several hours and, in one notorious case, death occurred 3 days after injection of a pellet containing ricin. Saxitoxins are a group of compounds produced by a variety of marine dinoflagellates and freshwater cyanophytes (blue-green algae). Ingestion of shellfish that have extracted and concentrated these toxins may result in paralytic shellfish poisoning in humans. This toxic effect results from high affinity binding of saxitoxin to voltage dependent sodium channels in the membranes of excitable cells that then inhibits depolarization. Peripheral nerves appear to be particularly susceptible.

Medical Countermeasures: Symptomatic and supportive treatments are the primary approach to the medical management of patients poisoned with either of these biotoxins. A ricin anti-toxin is under development and assessment, but currently there is no antidote to saxitoxin poisoning.

Conclusion: The biotoxins ricin and saxitoxin are relatively easily weaponised and highly toxic to humans. As such they are bioterrorist threat agents, especially since state programmes have demonstrated their potential use in assassination.

Key words: biotoxins, bioterrorism, ricin, saxitoxin
10.1016/j.toxicon.2012.04.009

B. Drug Discovery & Development

9. Genotoxic Potential of *Micrurus corallinus* Venom on the DNA of Human Lymphocytes

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Background: The articles that report the effects of venoms and their toxins, isolated from Brazilian wildlife, on human DNA are restricted to *Apis mellifera* and *Crotalus d. terrificus*. There is little information on the genotoxic and/or mutagenic potential of venoms and isolated toxins of broad medical and scientific interest, but due to the toxic and

pharmacological effects induced by them, the studies that characterize their action becomes important. Thus, the objective of this study was to investigate the effects of such venom on the DNA of human lymphocytes, in vitro, using both the comet assay and cytokinesis-blocked micronucleus test.

Methods: Treatments were performed on whole blood at different venom concentrations; upon testing completion, nucleoids and binucleated lymphocyte cells have been analyzed by comet assay and micronucleus test, respectively. The cells were maintained for 3 hours in the presence of venom samples for the comet assay, and for 72 hours, during cultivation, in the presence of a cytokinesis-blocking agent (cytochalasin B) for the micronucleus test. The comet assay and micronucleus test were performed by means of fluorescence microscope and optical microscope, respectively.

Results: We were able to observe different levels of damage (<5%, 5-20%, 20-40%, 40-85% and >85%) for the comet assay, except at concentration of 10 μg/mL, which did not induce damage above 85%. It seems that this venom has a dose-dependent genotoxic response, since higher concentrations resulted in increased genotoxic potential. However, all treatments induced damage lower than that observed when doxorubicin (drug control) was used. The presence of micronuclei in binucleated cells indicates the transmission of DNA damage to the first generation of treated cells, thus characterizing a genotoxic potential. Only concentrations of 15 and 30 μg/mL were able to induce the formation of 3.6 and 4.1 micronuclei/1000 binucleated cells, respectively, values higher than those observed in negative control, 1.4, and lower than the positive control, 12.8 (cisplatin 6 μg/mL).

Discussion: The results of the comet assay and micronucleus test complement and confirm the genotoxic potential of the *M. corallinus* venom, with emphasis on concentrations equal to and higher than 15μg/mL. Similar effects were observed for the *C. d. terrificus* venom (Marcussi et al., 2011), also neurotoxic, but at lower concentrations.

Conclusions: Genotoxic studies with animal venoms and toxins are necessary because some venom molecules served as templates for the preparation of pharmaceuticals and countless others are being investigated for this purpose.

Keywords: *Micrurus corallinus*, comet, micronucleus.
10.1016/j.toxicon.2012.04.010

10. Comet Assay and Micronucleus Tests to Assess Damage to the DNA of Human Lymphocytes Induced by the *Bothrops jararaca* Venom

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Background: *Bothrops jararaca* is a species found mainly in Brazil, but can also be found in other South American countries such as Paraguay and northern Argentina. There are no reports about the impact of its venom on the DNA of animal cells and, thus, the objective of this study was to evaluate the genotoxic potential of crude venom on human lymphocytes, in vitro, using the micronucleus test and comet assay technique.

Methods: Whole blood was incubated at different venom concentrations for the purpose of investigating the formation of micronuclei in binucleated cells using optical microscopy, after blocking cytokinesis with cytochalasin B (micronucleus test). The comet assay consisted of analysis of DNA fragmentation by means of electrophoretic run for subsequent observation of comets using a fluorescence microscope.

Results: The venom of *B. jararaca* induced the formation of micronuclei considering untreated blood, but the number of micronuclei formed was less than that observed in the positive control group (cisplatin), even at a venom concentration 5 times higher than that of the control drug. The cytokinesis-block proliferation index (CBPI) was similar for treated and untreated cells, i.e., there was no change in cell growth. Various levels of cell damage could be observed during the comet assay, especially level 2 (20-40%) and level 3 (40-85%). Increased levels of cell damage could be observed in the same assay after doxorubicin has been used as positive control.

Discussion: The results of the comet assay demonstrate DNA strand breakage induced by the *B. jararaca* venom, mostly of local toxic action, as observed by Marcussi et al. (2011) for the *Crotalus d. terrificus* venom, whose action is primarily neurotoxic. The results suggest that different toxins such as peptides, phospholipase A2, proteases, L-amino acid oxidase and hyaluronidase may have genotoxic potential through distinct mechanisms, which are still unknown.

Conclusion: The fact of this venom has been able to induce damage to the DNA of human cells, however, to a lesser extent than that observed for antitumor drugs, emphasizes the need for further studies to characterize in a broadly manner the effects of molecules isolated from venoms, aiming at their future application for the development of new drugs, potentially more specific and less harmful to the human body.

Keywords: *Bothrops jararaca*, comet, micronucleus, human lymphocytes.
10.1016/j.toxicon.2012.04.011

11. Effect of *Zingiber officinale* Plant Extract on the Differential Control of Growth, Apoptotic Activity and Gene Expression in Human Breast Cancer Cells

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Objective: The present study aimed to examine the ability of the methanol extract of the medicinal plant ginger (*Zingiber officinale*) from Saudi Arabia, to inhibit the proliferation and colony formation in breast cancer cell lines, MCF-7 and MDA-MB-231.

Methods: Cell viability, colony formation, dna fragmentation, apoptotic assays, and reverse transcription-pcr and western blot analysis were used in the study.

Results: Ginger treatment resulted in dose-dependent sequences of events marked by apoptosis, as shown by loss of cell viability, chromatin condensation, DNA fragmentation, activation of caspase 3, and cleavage of poly (ADP-ribose) polymerase. At the molecular level, the apoptotic cell death mediated by ginger could be attributed in part to up-regulation of Bax and down-regulation of Bcl-2 proteins. Ginger treatment modulated expression of proteins involved in cell cycle regulation; it decreased expression of cyclin D1, cyclin-dependent kinase-4 (CDK-4), but increased expression of CDK inhibitor, p21. It also inhibited the expression of the two prominent molecular targets of cancer, c-Myc and the human telomerase reverse transcriptase (hTERT).

Conclusion: These findings suggest that the ginger may be a promising candidate for the treatment of breast carcinomas.

Keywords: *Zingiber officinale*, medicinal plants, apoptosis, gene expression, breast cancer, cell line
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12. Structural Interpretation for the Subnanomolar Affinity of Muscarinic Toxin 7 for Human Muscarinic Acetylcholine Receptor 1 by Modeling Protein-Protein Interaction

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Background: Human muscarinic acetylcholine receptor 1 (hM1) is closely related to several diseases, such as Alzheimer's disease, schizophrenia, and peptic ulcer. MT7, a muscarinic toxin isolated from the snake venom of *Dendroaspis angusticeps*, is the only natural selective hM1 allosteric binder with subnanomolar affinity (K_d = 14pM). MT7 is a peptide with 66 residues, possessing a three-finger structure; and hM1 is a GPCR membrane protein, sharing the conserved structure of seven transmembrane regions. Understanding the binding mode of hM1- MT7 will give insights to discover small molecular selective ligand for hM1.

Methods: The structure of hM1 was constructed by homology modeling, and the bilayer membrane was created by the VMD program. The initial interaction coordinate of hM1-MT7 was generated by protein-protein docking in PatchDock program. Explicit membrane molecular dynamics (MD) simulations with Amber program were utilized to produce the dynamic trajectories of hM1-MT7. Binding energy between hM1 and MT7 was calculated with MM/PBSA method and decomposed into every residue to reveal the binding mode of hM1-MT7.

Results and Discussion: The hM1-MT7 binding mode is discovered to consist of five residue interaction clusters, which are separated in three interaction regions. By analyzing the cluster representative structures, the cluster residues form an interaction network, which shows a multiple-point-to-site binding mode. It is revealed in hydrogen binding statistical analysis that Glu170 (hM1) and Arg34 (MT7) are both locked in electrostatic cages with counter charges, respectively. It makes hM1-MT7 hard to dissociate, leading to the high binding affinity of MT7. This is confirmed by the dynamic distances calculation between these residues, and it is consistent with biological mutant experiments.

Conclusions: MT7 is discovered to bind to hM1 in a multiple-point-to-site mode. By forming a core in the interaction network, Glu170 (hM1) and Arg34 (MT7) are responsible for the subnanomolar affinity, which is consistent with the biological experimental data.

Keywords: muscarinic toxin, muscarinic acetylcholine receptor, molecular dynamics simulations, protein-protein docking
10.1016/j.toxicon.2012.04.013

13. Crotamine Pharmacology Revisited: Novel Insights Based on the Inhibition of Kv Channels

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Background: Crotamine, a 5KDa peptide possesses a unique biological versatility. Not only its cell-penetrating activity has become of clinical interest but moreover, its potential selective anti-tumor activity is of great pharmacological importance. Before, several studies have attempted to elucidate the exact molecular target responsible for the crotamine-induced skeletal muscle spasm. The aim of this study was to investigate whether crotamine affects voltage-gated potassium (K_V) channels in an effort to explain its *in vivo* effects.

Methodology/Principal findings: Crotamine was studied on ion channel function using the two-electrode voltage clamp technique on 16 cloned ion channels (12 K_v channels and 4 Na_v channels), expressed in *Xenopus laevis* oocytes. Crotamine selectively inhibits K_v1.1, K_v1.2 and K_v1.3 channels with an IC_{50} of ~300 nM and the key amino acids responsible for this molecular interaction are highlighted. Our results demonstrate for the first time that the symptoms which are observed in the typical crotamine syndrome may result from the inhibition of K_v channels.

Conclusions/significance: The ability of crotamine to inhibit the potassium current through K_v channels unravels it as the first snake peptide with the unique multifunctionality such as cell penetrating, antitumoral activity and K_v channel inhibiting properties. The potent and selective K_v channel inhibiting properties, as demonstrated in this work, can be an advantage for the use of crotamine or its derivatives as anti-tumor drug. This new property of crotamine might explain

some experimental observations and opens new perspectives of pharmacological uses.

Keywords: Crotamine, voltage-gated potassium channel, snake toxin, *Crotalus durissus terrificus*, anti-tumor drug
10.1016/j.toxicon.2012.04.014

14. Evaluation of the Cytotoxic Activity of *Rhinella schneideri* Toad Poison on Tumor Cells and on Healthy Mononuclear Cells

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Introduction: Amphibian poisons, especially from Anura order, contain a variety of biologically active compounds such as biogenic amines, cardiotonic steroids, alkaloids and peptides. Cardiotonic steroids have been shown to induce apoptosis in a wide spectrum of tumor cell. However, the detailed molecular mechanisms of inducing apoptosis are still unclear. The aim of this study was the comparative evaluation of the cytotoxicity of *Rhinella schneideri* poison (Rsp) on tumor cell lines and on healthy mononuclear cells.

Material and Methods: A MTT (3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay was used to detect cell viability using tumor cell lines B16F10 (Murine Melanoma Cells), HL-60 (Murine Acute Promyelocytic Leukemia Cells), HepG2 (Hepatocellular Carcinoma Human Cells), PC-12 (Murine Pheochromocytoma Cells) and PBMC (Human Peripheral Blood Mononuclear Cells). The cells (5 x 104 per well) were plated in 96-well plates and incubated with Rsp (5, 10, 20, 50 and 100 μg/mL) for 24 h. Then, the MTT was added (10 μL) and after 3 h of incubation at 37C°, in 5% of CO2, it was observed the production of formazan crystals by viable cells. The solubilization of crystals was performed by addition of DMSO (100 μL) and the absorbance (DO) at 570 nm was measured. The cell viability was calculated by the equation: cell viability (%) = (DO treated group/DO control group) x 100%.

Results: In the presence of Rsp (5, 10, 20, 50 and 100 μg/mL) the cell viability (%) to HL-60 and B16F10 were 19.1, 19.1, 19.0, 17.5, 20.4, and 40.3, 37.1, 38.2, 32.4, 34.8, respectively. The values obtained to HepG2 and PC-12 were 82.4, 76.7, 79.2, 83.1, 84.2 and 72.8, 85.6, 93.4, 92.7, 76.9, respectively. The cell viability to PBMC was also high (87.4, 77.2, 81.3, 95.6, 99.7).

Discussion: The MTT assays showed that the tumor cell lines HL-60 and B16F10 are more sensitive to Rsp, which induced a marked inhibition of cell proliferation. The cell lines HepG2 and PC-12 showed high cell viability, meaning that Rsp slightly interfered with the replication process of these cells. Rsp showed no cytotoxicity to PBMC cells.

Conclusion: These results show that Rsp has selective cytotoxic activity for the different tumor cells evaluated, indicating the potential application of its components as therapeutic agents in oncology.

Financial support: CAPES, CNPq, FAPESP.

Keywords: *Rhinella schneideri*, cardiotonic steroids, cell viability, anti-cancer activity
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15. New Perspective for Therapy Against Seizures Using Molecules From *Rhinella schneideri* Toad Poison

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Background: The number and diversity of compounds produced by amphibians in their glands is surprisingly high. Parotoid gland secretions from toads are useful source of chemical compounds with potential medical-pharmaceutical applications, among them biogenic amines, cardiotonic steroids, alkaloids and peptides. The aim of this study was to isolate and characterize components from *Rhinella schneideri* (Rs) poison and evaluate its potential use for inhibiting CNS seizures.

Material and Methods: The soluble poison was submitted to chromatography in HPLC system using C2C18 column. Five main fractions were obtained and Rs5 showed neuroprotective action. Male Wistar rats (250 g) were cannulated in the right lateral ventricle, following stereotactic coordinates. Rats were divided into groups (n=6) and the control animals received injections of saline (0.9%; i.c.v.) followed by the convulsants PTZ (80 mg/kg,i.p.) or NMDA (20 μg/μL, i.c.v.). Different concentrations of Rs5 (0.05, 0.1, 0.25, 0.5, 1.0, 1.5 and 2.0 μg/μL; i.c.v.) were analyzed. Additionally, the lower concentration (0.05 μg/μL) was used concomitant with carbamazepine (CBZ) (20 μg/μL – protected 50% of rat against seizures) for evaluation of synergism between the two drugs.

Results: Pretreatment with Rs5 at concentrations of 0.5, 1.0, 1.5 and 2.0 μg/μL protected 40, 60, 84 and 100% of rats against tonic-clonic seizures induced by PTZ, respectively. Additionally, 50, 62, 62, 83 e 100% of rats treated with Rs5 at concentrations of 0.05, 0.1, 0.5, 1.0 and 2.0 μg/μL, respectively, were protected of seizures induced by NMDA. To evaluate the Rs5 toxicity rats were submitted to the rotarod assay after injection of Rs5 (2 μg/μL e 6 μg/μL μg/μL) and ataxia was not observed.

Discussion: Rs5 concentration able to inhibit seizures is lower than the concentration of CBZ which promotes the same effect, showing that it is a molecule with high neuroprotective activity. Additionally, was demonstrated synergism between CBZ (20 μg/μL) and Rs5 (0.05 μg/μL) showing 100% protection against seizures. Furthermore, at the rotaroad assay no animal fell from the apparatus indicating that Rs5 did not cause motor impairment.

Conclusion: Taken together, these results show that Rs5 can be considered a molecule with high potential for development of a new drug for seizures therapy.

Financial support: CNPq, FAPESP.

Keywords: seizures therapy, neuroprotective effect, *Rhinella schneideri*
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16. Marine Pharmacology and the Late 2011 Marine Pharmaceuticals Pipeline

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Review: As the renaissance in the pharmacology of marine natural products continues (Glaser and Mayer, Biochemical Pharmacology 78:440-448, 2009), the status of the clinical marine pharmaceuticals pipeline was assessed in early 2012. There were five FDA-approved marine-derived drugs in the US market, namely cytarabine (Cytosar-U®, Depocyt®), ziconotide (Prialt®), eribulin mesylate (Halaven®), brentuximab vedotin (Adcertis®), and omega-3-acid ethyl esters (Lovaza®), while vidarabine (Vira-A®) was no longer available, and trabectedin (Yondelis®) being EU-registered. As of January 2012 there were 11 marine-derived compounds in the clinical marine pharmaceutical pipeline, which was recently reviewed (Mayer *et al.* Trends in Pharmacological Sciences 31:255-265, 2010). Included in the eleven marine-derived compounds were three new additions, namely monoclonal antibodies conjugated to synthetic dolastatin derivatives that were in either Phase I, Phase II or Phase III clinical trials. Finally, the preclinical marine pharmaceutical pipeline remained an active global enterprise with researchers from several countries reporting novel mechanisms of action for multiple marine chemicals (Mayer *et al.* Comparative Biochemistry and Physiology C 153: 191-222, 2011). Thus the clinical and preclinical pharmaceutical pipelines continued to be very active in early 2012. (http://marinepharmacology.midwestern.edu/).

Keywords: marine-derived pharmaceuticals; pharmacology; drug development
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17. Novel Marine Compounds in Studies of Nicotinic Acetylcholine Receptors

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Background: Nicotinic acetylcholine receptors (nAChRs) belong to superfamily of Cys-loop receptors, containing also glycine, GABA-A, 5HT3 and some others, and are the best characterized. Definite subtypes of nAChRs are shown to be in association with nicotinic addiction and chronic pain, as well as with different diseases (muscle dystrophies, schizophrenia, Alzheimer's and Parkinson's diseases). This is a strong argument for the search of novel potent and highly selective cholinergic ligands to distinct receptor subtypes and for designing new ones on the basis of known compounds. In addition to such well developed sources of bioactive compounds as snake and *Conus* mollusk venoms, at present other marine creatures are paid great attention.

Methods: We characterized a series of low molecular weight compounds extracted from some sea anemones and ascidia for their ability to bind to glycine and different nicotinic receptors as well as to acetylcholine-binding proteins (AChBPs), naturally-occurring spatial homologs of the extracellular ligand-binding domains of all Cys-loop receptors. Among them were alkaloids, polysulphites, sphingolipid- and steroid-like substances.

Results and Discussion: Some of them were found potent competitors of radioiodinated α-bungarotoxin in binding to classical agonist binding sites of nAChRs, which was in a good accordance with results of preliminary computational modeling and docking. In contrast, some other compounds potentiated the radioligand binding to definite targets. Functional activity of all compounds was studied electrophysiologically on glycine and α7 nicotinic receptors expressed in cells revealing several effective blockers. The data obtained mean that sea anemones and ascidia are the additional rich sources of Cys-loop receptors' ligands which could be perspective biomarkers or drugs. The possibility of preparation of potent markers for the α7 nAChR and different AChBPs was demonstrated with the use of peptides designed on the basis of α-conotoxin PnIA from *Conus pennaceus*. Three novel analogs were found to be highly effective and selective ligands and were successfully prepared in a radioactive form with retained potencies and specificities for the α7 nAChR and/or AChBPs.

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Keywords: marine compounds, nicotinic receptors, radioactive ligands
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18. Comparative Toxicity of Binase towards Tumor and Normal Cells

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Review: Due to their biological activity ribonucleases are able to become the basis for the development of novel drugs in malignant neoplasms therapy. The work characterizes

cytotoxic activity of *Bacillus intermedius* ribonuclease towards solid tumor cells: pulmonary adenocarcinoma (A549), human fibrosarcoma (HT1080) and murine glioma C6 (ATCC). The enzyme possesses high cytotoxic effect on A549 and C6 cells and does not at the same time inhibit proliferation of HT1080 cells. This fact could be explained by the different expression levels of *ras-oncogenes* by the tested cell lines. Ribonuclease did not show cytotoxicity towards human umbilical vein endothelial cells (HUVEC) in concentration range of 0.1-300 μg / ml. The data obtained indicate that detection of certain oncogenes in tumor cell as markers of their susceptibility to binase cytotoxic action is very promising.

Keywords: binase, cytotoxicity, ras-oncogenes, A549, C6, HT1080, HUVEC cell lines
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19. Purification and Determination of Antibacterial Constituent, L-Amino Acid Oxidase from *Calloselasma rhodostoma* and *Ophiophagus Hannah*

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Background: The emergence and re-emergence of diseases in addition to appearance of superbugs such as *vancomycin- and methicillin-resistant Staphylococcus aureus* has exposed the urgent and dire need for alternative antibiotics. Recent research has shown evidence of snake venoms, namely *Ophiophagus hannah* (King cobra) and *Calloselasma rhodostoma* (Malayan pit viper) exhibiting strong potential antibacterial activity against *Staphylococcus aureus*.

Methodology: Antibacterial activity was assessed against 22 bacterial strains including resistant *Acinetobacter baumanii*, *Enterococcus faecalis, Klebsiella pneumoniae,* methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus aureus* strains using a hole-plate diffusion method, whereby the inhibition zone of the venoms was compared to conventional antibiotics. Following that, using a combination of successive purifications and the antibacterial assay (bioassay guided purification), the characterization of the active constituent was achieved through gel filtration, ion exchange and affinity-binding chromatography. The homogeneity and identity of the enzyme was determined by gel electrophoresis and mass spectrometry.

Results: The active antibacterial constituent for both venoms was determined to be a homodimeric enzyme, L-Amino Acid Oxidase (LAAO). SDS-PAGE analysis revealed a single band of the purified enzyme with a molecular weight of approximately 65kDa and 66kDa from *Ophiophagus hannah* (OH) and *Calloselasma rhodostoma* (CR), respectively. Both OH-LAAO and CR-LAAO showed positive and effective inhibition of several Gram positive bacteria including methicillin-resistant *Staphylococcus aureus* with a minimum inhibitory concentration (MIC) of less than 10μg/mL, whereas both enzymes were less effective in inhibiting most Gram negative bacterium, which required MIC values above 20μg/mL. The MIC result consistently suggests that the antibacterial activity of OH-LAAO is more effective than CR-LAAO. MIC values for CR-LAAO have not been previously reported. As a comparison, suitable antibiotics were also tested and observed.

Discussion: Past studies have stated that specific binding is necessary and potentially vital as the bacterial growth inhibition is achieved by high concentrations of H_2O_2 when in close contact to bacterial surfaces. The MIC results propose that CR- and OH-LAAOs probably attach more strongly to Gram-positive bacterial surface than to Gram negative bacterial surface and are therefore more effective in inhibiting Gram positive bacterial growth inhibition.

Conclusion: OH and CR-LAAO have effective antibacterial activity, especially against Gram positive bacteria. This finding encourages further work to elucidate their potential in the development of a novel antibiotic.

Keywords: *Ophiophagus hannah* (OH), *Calloselasma rhodostoma* (CR), L-amino acid oxidase (LAAO), methicillin-resistant *Staphylococcus aureus* (MRSA), antibacterial
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20. CFTR as a New Target for Crotoxin: Potential Application for Cystic Fibrosis

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Background: Crotoxin is the major heterodimeric toxin from the South American rattlesnake (*Crotalus durissus terrificus*) possessing PLA2 activity. Crotoxin principally exhibits pre-synaptic neurotoxicity through interaction with protein receptors, and displays beneficial properties such as bactericidal, antitumoral and antiviral activities. It has been shown that crotoxin also potentiates L-type calcium currents and may offer potential clinical application in the control of cardiac function. The aim of this study was to investigate the interaction of crotoxin with the Cystic Fibrosis Transmembrane conductance Regulator (CFTR), a cAMP activated Cl^- channel whose dysfunction is responsible for cystic fibrosis, and to characterize the potential physiological relevance of this interaction.

Methods: We used X-ray crystallography to solve the three-dimensional structure of crotoxin. For functional studies, two series of experiments were performed: (i) surface plasmon resonance (SPR) for kinetic interaction studies and (ii) voltage-current (I/V) experiments in *Xenopus oocytes* expressing CFTR. The latter allowed us to determine cAMP-activated Cl^- - CFTR currents measured in the presence and absence of crotoxin.

Results: The crystal structure of crotoxin solved at 1.35 Å resolution revealed key residues involved in the stability and toxicity of this potent heterodimeric beta-neurotoxin (Faure et al., 2011, J.Mol. Biol. 88, 69-76). Using SPR we

demonstrated a direct specific binding of crotoxin and its PLA2 subunit to the nucleotide-binding domain (NBD1) of CFTR. Using I/V measurements we detected an increase in cAMP-activated Cl^- - CFTR current when crotoxin or its CB subunit were injected into *Xenopus oocytes*. Since the deletion of Phe508 in NBD1 is the most frequent mutation leading to cystic fibrosis, we also studied the binding of crotoxin to the F508delCFTR mutant. We found that crotoxin and its CB subunit effectively increase the channel current, showing a potentiating or correcting effect on F508delCFTR.

Conclusions: This new crystallographic data and the unexpected beneficial effect of crotoxin on mutated CFTR provide an original perspective to investigate the pharmacotherapy of cystic fibrosis.

Keywords: crotoxin, crystal structure, cystic fibrosis
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21. Anti-endotoxin Effects and Pharmacology of the Immune Selective Anti-Inflammatory Derivatives (ImSAIDs)

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Background: Endotoxin is a frequent cause of morbidity in human medicine, often resulting in systemic immune activation and sepsis. Recently, scientists have characterized the peptide, phe-glu-Gly, derived from salivary proteins. feG exhibits potent and rapid anti-inflammatory and anti-endotoxin properties, making it a potential drug development target for toxins that initiate the inflammatory response.

Methods: Analysis and summary of peer-reviewed literature related to the immuno-pharmacology of the peptide phe-glu-Gly (feG).

Results: feG attenuates the cardiovascular and inflammatory effects of endotoxin and anaphylaxis by reducing hypotension, leukocyte chemotaxis, and vascular leak. feG affects activated inflammatory cells, especially neutrophils, by regulating integrins and inhibiting intracellular production of reactive oxygen species. feG is active at low doses (100 μg/kg) and has a long (9-12 hour) biological half-life. Furthermore, feG has exhibited no toxicity in studies to date.

Discussion: The mechanism by which feG exerts its effects is distinctly different from steroids or non-steroidal anti-inflammatories (NSAIDs). This new mechanism appears to hold promise for therapeutic intervention of endotoxemia and perhaps other toxins which affect vascular integrity and immune activation.

Conclusions: As a biological therapeutic, feG holds promise in toxicity or envenomations where and over-exuberant inflammatory responses may result. Further work is required to understand its applications in venom related events such as tissue destruction, immune cell activation, vascular leak, and anaphylaxis.

Keywords: endotoxin, ImSAIDs, antitoxin
10.1016/j.toxicon.2012.04.022

22. Engineering of an HB-EGF Inhibitor from the Diphtheria Toxin Receptor–Binding Domain for the Treatment of HB-EGF-Related Diseases

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Background: HB-EGF is a growth factor ligand of the EGF receptors (EGFR) ErbB1 and ErbB4. It is involved in rapidly progressive glomerulonephritis, ovary and pulmonary cancer, vasospasm connected with cerebral contusion, and perhaps diabetic retinopathy and restenosis. Inhibition of the HB-EGF - EGFR activation pathway may have strong therapeutic potency. However, treatment with chemical or antibody EGFR inhibitors lack specificity and lead to serious adverse effects. The membrane precursor form of HB-EGF, pro-HB-EGF, is the natural receptor for diphtheria toxin. We designed a powerful inhibitor of HB-EGF, both in its soluble and membrane-bound precursor forms by engineering of the diphtheria toxin receptor-binding domain (DTR).

Methods: We combined structure modeling, site directed mutagenesis and directed protein evolution to improve considerably the therapeutic potential of DTR. Three codon-biased libraries each covering 4 or 5 residues, mostly hydrophobic and potentially responsible for the insolubility of the protein produced in *E. coli* were screened for the generation of soluble mutants. Site-directed mutagenesis of the binding site of DTR was performed to enhance affinity. CD4 T-cell epitopes were identified by experimental and *in silico* approaches and mutated to decrease immunogenicity of DTR. Antigenicity of the evolved DTRs was evaluated by ELISA using sera from healthy diphtheria toxin-vaccinated donors. Biological activity of evolved DTRs was measured by their capacity to inhibit diphtheria toxin toxicity and HB-EGF dependent cell proliferation.

Results: DTR1 carrying mutations Y380K/Q387E/L390T/A395T was the most soluble mutant. Surprisingly, it is 60 fold more affine for HB-EGF than native diphtheria toxin ($K_d \sim$ 49 pM). DTR3 carrying the mutations F389Y and G510A in the binding site was 5 fold more affine than DTR1 ($K_d \sim$ 9.5 pM). DTR9 was derived from DTR3 by the introduction of the mutations N399K, V452T, V483Q, H492E, S494K, T517E and E497D (or T436H) destroying 12 most dominant CD4 T cell. DTR9 was 4 fold more affine than DTR3 ($K_d \sim$ 2.2 pM). ELISA analysis of the sera of 20 healthy donors showed that most circulating anti-diphtheria toxin antibodies target the catalytic domain of the toxin and that the antigenicity of DTR9 is strongly diminished as compared to the whole toxin.

Conclusion: DTR9 is a potent inhibitor of the soluble and membrane precursor forms of HB-EGF. Tenth of mg of protein are purified from one L of bacterial culture without optimization. Solubility is greatly enhanced, affinity is 1400

fold higher than that of the parental toxin and immunogenicity and antigenicity are greatly decreased.

Keywords: Diphtheria toxin, HB-EGR, therapeutic protein
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23. Transformation of the Naturally Occurring Frog Skin Peptide, Alyteserin-2a into a Potent Anti-cancer Agent

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Background: Cell-penetrating peptides are present in skin secretions of certain frogs and constitute a component of the animal's system of host defense that protects against predators and pathogenic microorganisms. These compounds have potential for development into potent anti-cancer agents especially for use against tumors that cell have developed resistance to commonly used drugs. Alyteserin-2a (ILGKLLSTAAGLLSNL.NH2) is a cationic, amphipathic, α-helical peptide, first isolated from skin secretions of the midwife toad *Alytes obstetricans*. The peptide displays moderate cytotoxic potency against A549 human non-small cell lung adenocarcinoma cells (LC_{50} = 80 μM) and human erythrocytes (LC50 = 140 μM).

Methods: Structure-activity relationships were investigated by synthesizing analogs of alyteserin-2a in which the hydrophilic amino acids in the peptide were replaced by one or more L-lysine or D-lysine residue. The anti-tumor activities of the peptides were determined in A549 cells by measurement of ATP concentrations using a CellTiter-Glo luminescent cell viability assay during a 24 h incubation. Hemolytic activities of the peptides were determined by measurement of the release of hemoglobin from human erythrocytes.

Results: The substitutions Ser7 → L-Lys, Gly11 → L-Lys, Ser14 → L-Lys and Gly11 → D-Lys in alyteserin-2a produced analogs with increased (2 - 4 fold) cytotoxic potency against A549 cells but the hemolytic activity of the peptides also increased by a corresponding amount. The substitution Asn15 → L-Lys produced the most potent analog against tumor cells increasing the activity against A549 cells by approximately 6-fold (LC_{50} = 13 μM). Potency was also increased 5-fold against HepG2 human hepatocarcinoma cells (LC_{50} = 19 μM), 4-fold against MDA-MB-231 breast adenocarcinoma cells (LC50 = 14 μM), and 4-fold against HT-29 human colorectal adenocarcinoma cells (LC_{50} = 30 μM). The hemolytic activity of [N15K]alyteserin-2a (LC50 = 50 μM) was 2.8-fold greater than the native peptide. [N15K]alyteserin-2a is very soluble in physiological media and relatively easy to synthesize.

Conclusions: Increasing cationicity while maintaining amphipathicity increases the anti-tumor activity of alyteserin-2a. Although too toxic itself for systemic use, [N15K]alyteserin-2a is identified as a lead compound for the development of further analogs with improved cytotoxicity against tumor cells, but decreased activity against non-neoplastic cells.

Financial support: This work was supported by the Terry Fox Fund for Cancer Research.

Keywords: anti-cancer, frog, peptide, cytotoxin
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24. Recombinant Hybrid Proteins from Cobra Venom Factor and Human C3: Promising Agents for Therapeutic Intervention in Complement-Mediated Diseases

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Background: Cobra Venom Factor (CVF) is the anti-complementary protein in cobra venom. Whereas it does not exert direct toxicity, it exhaustively activates complement which is believed to help expedite the toxic venom components to reach their targets through an increased blood flow and vascular permeability at the site of envenomation as a consequence of the release of the complement-derived anaphylatoxins C3a and C5a. CVF activates complement by forming a C3- and C5-cleaving enzyme, the C3/5 convertase, with prey complement factor B, analogously to C3b, the activated form of complement component C3. CVF and C3 (from all vertebrate species, including humans) have been shown to share extensive structural homology as evidenced by DNA sequence, amino acid sequence, immunological cross-reactivity, and crystallographic domain structure. In contrast to C3b, which forms a rather short-lived convertase consistent with its biological function of localized complement activation on a target cell surface, the convertase formed with CVF is very stable and resistant to the complement regulatory proteins of the host.

Methods: We have created hybrid proteins of human C3 with CVF by exchanging functionally important regions, or selected amino acid residues, in human C3 with the corresponding regions or amino acid residues from CVF.

Results: The resulting hybrid proteins are human C3 derivatives with the CVF-specific function of forming a stable convertase in human serum, causing complement inhibition by consumption. As adverse complement activation is an important component of the pathogenetic process of many diseases, these novel hybrid proteins, referred to as humanized CVF, represent a novel class of complement-inhibiting agents. In several preclinical models of disease, complement depletion with humanized CVF is a potent therapeutic approach, including myocardial infarction reperfusion injury, age-related macular degeneration, myasthenia gravis, and others.

Conclusions: In addition to being a novel therapeutic agent, hybrid proteins of CVF and human C3 (or C3 from other species, including cobra) also represent important experimental tools to identify functionally important regions in CVF.

Keywords: cobra venom factor, complement, venom-derived drug development
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25. Viability of Fibrin Sealant from Snake Venom as Scaffold to Rat Marrow-Derived Mesenchymal Stem Cells

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Background: The aim of this study was to evaluate the *in vitro* viability of biomaterial Fibrin Sealant (FS) derived from snake venom as a scaffold for Rat marrow-derived mesenchymal stem cells (MSCs). The FS is characterized as a biological adhesive material, and is produced and was supplied by the Center for the Study of Venoms and Venomous Animals, CEVAP, Brazil.

Methods: Stem cells were collected from the bone marrow of femurs and tibias of mice 30 days old. MSCs were characterized using flow cytometry with CD 44 and CD 90 MSC positive markers and MSC and CD34 (mononuclear stem cells) negative marker. To confirm the characterization of the MSCs, cultivations of the first pass were used to differentiate MSCs into specific cell lineage (osteogenic, chondrogenic and adipogenic). To evaluate the in vitro growth and cell viability with the biomaterial, inverted light optical microscopy, fluorescence microscopy and scanning electron microscopy and transmission were used. We also observed the spontaneous differentiation ability of SF in contact with MSCs to osteogenic, chondrogenic and adipogenic lineage.

Results: Different microscopy showed that the SF used was able to accomplish the capture and maintenance of MSCs. On further increased magnification, it was possible to observe the cell interaction with adjacent cells and cellular extensions into the interior and surface of the biomaterial. After eight days of preparation the confluence of the cell culture was around 90% and the MSCs adhered to the surfaces of the sealant. Future studies will be performed to implement the fibrin sealant as a scaffold enriched *in vivo* experimental models to investigate the time required for mechanical support for the MSC to remain at the target site and to achieve the expected differentiation.

Conclusions: The collection, cultivation and characterization of rat MSCs were possible. The SF was effective as a biological scaffold and interacted with the MSCs keeping them viable alternatives providing clinical and surgical therapies more efficient for regenerative processes.

Financial support: CAPES, FAPESP 09/06280-0.

Keywords: fibrin sealant, scaffold, snake venom, mesenchymal stem cells
10.1016/j.toxicon.2012.04.026

26. nAChR Antagonist as Chemotherapeutic Agents of Certain Lung Cancer Types Expressing Cholinergic System. A Case of Snake Three-Fingered Toxins and Alkylpyridinium Polymers from Marine Sponge

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Background: The most common form of lung cancer is non-small cell lung cancer (NSCLC). NSCLC have been shown to express molecules that are part of the cholinergic system, including α7 nicotinic acetylcholine receptors (nAChR). Nicotine and acetylcholine act as agonists of α7 nAChRs provoking cell proliferation. It has been reported that exposure to nicotine protects cancer cells expressing α7 nAChRs against the apoptosis induced by anticancer drugs. Antagonists of α7 nAChRs can attenuate the proliferative effects of agonists and could thus be considered as potential anticancer agents. One option is well studied snake three-fingered neurotoxins i.e. α-bungarotoxin or α-cobrotoxin. Recently one of the synthetic alkylpyridinium polymers (APs) closely related to natural APs from marine sponge, which are cytotoxic for certain types of cancer cells, was shown to be a strong antagonist of α-7 nAChR.

Methods: We have studied the survival of A549 adenocarcinoma cells upon treatment by α-bungarotoxin, α-cobrotoxin and APS8 one of the APs synthetic analogues. In addition the APS8 were also used to study signaling pathways, apoptosis and proliferation of A549 NSCLC cells and normal cells. Evidence of apoptosis activation by APS8 in NSCLC was quantitatively analyzed by annexin V-FITC/propidium iodide uptake analysis with fluorescent cytometry. Cell morphology of APS8 induced apoptosis was investigated with a combination of the fluorescent DNA-binding dyes acridine orange and ethidium bromide. Expression of proteins upon treatment of cells by APS8 with or without nicotine was studied using Bio-Ray protein chips. We investigated mechanism underlying the apoptotic activity of APS8.

Results: We have shown that α-bungarotoxin and α-cobrotoxin are about 50 fold less cytotoxic for A549 cells as compared to APS8 analogue. APS8 has significant selective cytotoxicity towards NSCLC cells, while has no influence on normal lung fibroblast cells MRC5. Upon treatment with APS, a large proportion of cancer cells undergo apoptosis, while normal cells are not affected. APS8 also attenuates the antiapoptotic effects of nicotine. Treatment of NSCLC with APS8 upregulates proapoptotic proteins from Bcl-2 family, while anti-apoptotic proteins are down-regulated. APS8 markedly increased the expression levels of death receptors. Our results imply that both intrinsic and extrinsic pathway of apoptosis are activated by APS8.

Conclusion: Apoptosis of cancer cells induced with nAChR antagonist APS8 may be a new and promising method in lung cancer treatment.

Keywords: nAChR antagonists, cancer cells, apoptosis
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27. Development of a Virtual System to Support Clinical Toxinology Research

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Review: This research aimed to develop a Virtual Support System for Clinical Research for managing a clinical trial in toxinology. It will be deployed in the Clinical Research Unit (UPECLIN), Botucatu Medical School – UNESP. This unit participates in the National Clinical Research Network (RNPC), along with 31 other centers in Brazil. The assembly of the proposal was needed because the current significance of clinical research, the need to harmonize actions to teaching all centers involved, and finally embed socially the research subjects and health professionals away from centers of excellence. Thus, this initiative will support a multicenter, controlled, randomized phase II clinical trial, which will examine 260 patients with venous ulcers treated weekly for 90 days, to test the topical use of fibrin sealant derived from snake venom. This system was designed to house courseware to support research, conceptual information and data from the project, in addition to ethical concepts, bioethical and telecare, for the research subjects. It also has teaching modules for on-line training of health professionals. This pathology is a common occurrence and when complicated by infection or chronic, is a serious public health problem. Therefore, the development of the virtual system to support toxinology clinical studies was necessary to assist in the translation from experimental research to clinical trials, collaborating with diseases of the same importance as well as shares of RNPC.

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Keywords: virtual system, clinical trials, fibrin sealant, toxinology
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28. Sea Anemone Peptides Modulate TRPV1 Activity and Produce Analgesia Without Hyperthermic Effect

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Background: The transient receptor potential vanilloid 1 receptor (TRPV1) is a nonselective cationic ion channel. It can be activated by a variety of stimuli: noxious heat (>43°C), protons, osmotic pressure, various lipid messengers and exogenic ligands such as capsaicin, the pungent ingredient of chili peppers. TRPV1 takes part in development and progress of chronic pain and inflammation. Therefore, there is a considerable interest in identification and development of novel agonists and antagonists of TRPV1 for the treatment of these pathological states. At present there are a lot of small organic molecules that interact with the capsaicin binding site and in allosteric manner block TRPV1 activation. Clinical trails of such molecules revealed that TRPV1 involved in body temperature control and most of TRPV1 antagonists cause hyperthermia.

Methods: Electrophysiology, microplate reader measurement of intracellular Ca^{2+}, single cell Ca^{2+} imaging, mice models of pain, core body temperature measurement.

Results: Earlier, analgesic polypeptides named APHC1, APHC2 and APHC3 were isolated from extract of sea anemone *Heteractis crispa*. We characterized these polypeptides as potent TRPV1 modulators and compared their pharmacology in various *in vitro* and *in vivo* models. All polypeptides partially blocked capsaicin-induced response of receptors on CHO or HEK293 cells expressing TRPV1, but none of them influenced on thermal activation. It is very likely that sea anemone peptides APHC1-3 displayed differential activity on different modes of TRPV1 activation. APHC1-3 showed significant antinociceptive and analgesic activity *in vivo* models in reasonable doses (0.01-0.1 mg/kg) and did not cause hyperthermia. Intravenous administrations of polypeptides APHC1-3 prolonged hot-plate latency, blocked capsaicin-, acetic acid- and formalin-induced behavior and inhibited CFA-induced hyperalgesia.

Discussion: New polypeptide antagonists of TRPV1 cause significant analgesia and no hyperthermia effect in experimental animals. Therefore, they could be a base for molecular design of TRPV1 antagonists with desirable properties.

Conclusions: Polypeptides APHC1-3 belong to new class of TRPV1 modulators that produce significant analgesic effect without hyperthermic action.

Keywords: TRPV1, *Hetractis crispa*, analgesic
10.1016/j.toxicon.2012.04.029

29. Russelobin, a Non-toxic Thrombin-like Serine Protease from the Venom of Russell's Viper (*Daboia russelli russelli*): Possible Applications in Cardiovascular Drug Development

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Background: Russell's viper venom is rich in proteolytic enzymes which affect the haemostatic system of an envenomated victim. However, relatively few proteases have been characterized from this venom. Here we report the purification and characterization of a thrombin-like serine protease from the venom of *Daboia russelli russelli* (Pakistan).

Methods: A Russell's viper thrombin-like enzyme (RVTLE) was purified from crude venom by size exclusion chromatography followed by reversed-phase HPLC and characterized for biochemical and pharmacological properties, fibrinogen clotting activity and *in vivo* toxicity.

Results: RVTLE, named Russelobin, is a glycosylated, monomeric protein having a molecular mass of 51.3 kDa. The deglycosylated enzyme (after removal of N-linked sugars) showed a protein band of 22.2 kDa in SDS-PAGE. *De novo*

sequencing of Russelobin via LC/MS/MS analysis of tryptic digested peptides identified 5 unique peptides. An NCBI-BLAST search of these peptides against a snake venom protein database revealed significant similarities to snake venom thrombin-like enzymes and serine proteases. Russelobin hydrolyzed chromogenic substrates in the following order: plasma kallikrein>thrombin > factor Xa>trypsin >plasmin. Russelobin showed BAEE-esterase activity but was devoid of TAME-esterase activity. The enzyme was significantly inhibited by serine protease inhibitors (AEBSF, benzamidine HCl) and α_2-macroglobulin. Russelobin showed optimum activity at 45 °C, pH 9.0 and was very stable against five cycles of freeze-thawing. Russelobin was able to clot human fibrinogen by cleaving the Aα chain of fibrinogen. The Bβ chain was degraded to a much slower rate and the γ chain of fibrinogen remained intact. HPLC analysis of fibrinopeptides release pattern showed that Russelobin preferentially released FPA, and the release of FPB was observed only after prolonged incubation of enzyme with fibrinogen. However, human thrombin released both the FPA and FPB to an equal extent. Plasmin-mediated degradation of fibrin formed by the action of Russelobin was found to be significantly higher than degradation of the fibrin clot formed by human thrombin. Russelobin did not show cytotoxicity against COLO-205 and MCF-7 cells. The i.p injection of Russelobin at dose of 1 mg/kg body weight of mice was found to be non-toxic.

Discussion: Russelobin is only superficially similar to human thrombin and it results in plasma defibrinogenation. FPB is minimally cleaved, producing an easily (physically) disrupted clot which is rapidly degraded *in vitro* by plasmin.

Conclusion: The application of Russelobin in cardiovascular drug development is indicated due to its low toxicity and its potential utility as a defibrinogenating agent. We thank Kentucky Reptile Zoo for providing RVV.

Keywords: thrombin-like snake venom protease; Russell's viper; fibrinogen clotting protease; serine protease
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30. Antimicrobial Peptides from Arachnid Venoms and their Biological Activity in the Presence of Commercial Antibiotics

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Background: Since the development of penicillin in the 1940s, the synthesis and use of different antibiotics have had an important impact in human health. The emergence of resistant strains has made bacterial infections gradually difficult to treat with available antibiotics. Particularly, bacteria have developed different mechanisms for acquiring or modifying their genes, an accelerate mechanism of adaptation for survival of both, pathogenic and non-pathogenic organisms. The discoveries of some antimicrobial peptides (AMPs) in spider and scorpion venoms as well as in other living organisms have driven the idea of using them as new antibiotic tools. In this work we characterize chemically and prove the antimicrobial effects of two antimicrobial peptides isolated from the spider *Lachesana sp.* and the scorpion *Centruroides suffusus suffusus* (*C.s. suffusus*) venoms. Additionally we present a new strategy of synergistic approaches to improve their antibiotic capacity.

Methods: *Lachesana sp.* and *C.s. suffusus* soluble venoms were fractionated by HPLC. AMPs from these two venoms were screened using the zone inhibition test on agar cultures of *Staphylococcus aureus*. The arachnid AMPs were further purified and its primary structure sequenced by Edman degradation and mass spectrometry. The AMPs were evaluated with and without the presence of the commercial antibiotics chloramphenicol, ampicillin, novobiocin, streptomycin and kanamycin using a dilution susceptibility test in growth medium.

Results: Two AMPs were obtained, one from the venom of *Lachesana sp.* and other from *C.s. suffusus*, and named La47 and Css54 respectively. La47 (26 mer) is identical to the AMP latarcin 3a from *Lachesana tarabaevi*, and Css54 (25 mer) has a 41 % identity to the AMP Pin2 from *Pandinus imperator*. La47 and Css54 showed the largest synergic effect by inhibiting the growth of *Staphylococcus aureus or Escherichia coli* in the presence of the commercial antibiotics streptomycin and kanamycin.

Discussion: It is well accepted that the AMPs mode of antibiotic action is through pore formation of the bacterial membranes, which leads to membrane depolarization, and then to diffusion of extracellular material into the bacterial cytoplasm. In this respect, streptomycin and kanamycin could find the way to the interior of the bacterial cell and inhibit the bacterial machinery of protein transcription, which would guide to bacterial death.

Conclusions: These data show a motivating outlook for potential clinical treatments of bacterial infections using mixtures of both AMPs and commercial antibiotics.

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Keywords: venom, scorpion, antimicrobial
10.1016/j.toxicon.2012.04.031

31. Spider-venom Peptides that Target the Human NaV1.7 channel: Potential Analgesics for the Treatment of Chronic Pain

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Background: Chronic pain is a major worldwide health issue, with patients often receiving inadequate treatment.

The voltage-gated sodium (Na_V) channel $Na_V1.7$ has recently been identified as a promising target for treatment of chronic pain. Gain-of-function mutations in the *SCN9A* gene encoding the pore-forming α-subunit of $Na_V1.7$ cause painful neuropathies whereas, loss-of-function mutations result in a congenital indifference to all forms of pain[1]. Thus, blockers of $Na_v1.7$ are likely to be useful analgesics for the treatment of persistent pain. However, it will be critical to ensure that such blockers do not inhibit other Na_V subtypes with essential physiological functions, such as $Na_V1.5$ that is restricted to the heart and is critical for the rising phase of the cardiac action potential. Many venomous animals have evolved toxins that modulate the activity of Na_V channels. Spider venoms in particular are rich in Na_V channel modulators, with one third of all known ion channel toxins from spider venoms acting Na_V channels.[2]

Methods: Based on their primary structure and cysteine scaffold, 12 distinct families of spider toxins that modulate the activity of Na_V channels (NaSpTxs) were identified[3]. Several of these families show activity at $Na_V1.7$; one family in particular is interesting from a pharmacologically perspective as some members display subtype selectivity. In order to determine the molecular epitopes that govern the interaction of members of this NaSpTx family with $Na_V1.7$ and other Na_V subtypes, we have developed a recombinant expression system for several family members in order to produce isotopically labeled peptides for NMR structural studies and point mutants for functional analyses.

Results: We have discovered that one member of this NaSpTx family is the most potent blocker of $Na_V1.7$ discovered to date, and that variations in activity within this family are correlated with subtle changes in the structure and dynamics of the peptide pharmacophore.

Discussion: Examining the potency and selectivity of naturally occurring sequence variants within this peptide family, as well as point mutants thereof, will enable us to precisely determine the molecular epitopes that mediate their interaction with $Na_V1.7$ as well as key off-target Na_V subtypes.

Conclusion: Structure-function relationship studies of this NaSpTx family will allow us to rationally design blockers of $Na_V1.7$ with improved potency, selectivity, and analgesic potential.

References

Cox, J.J. *et al.*(2006) *Nature* **444**, 894–898.
www.arachnoserver.org.
Klint, J.K. *et al.* (2012) Toxicon. Submitted.

Keywords: spider-venom peptides, NaV1.7, therapeutics, analgesics, structure-function relationships, pharmacophore, drug development
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32. Development of High Throughput Calcium Channel Assays to Accelerate the Discovery of Novel Toxins Targeting Human Cav2.2 Channels

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Background: Voltage-gated calcium channels (Ca_v) are multidomain membrane proteins that play essential roles in the control of neurotransmitter release and nociceptive transmission. Different Ca_v auxiliary subunits have been shown to influence the pharmacological and physiological properties of Ca_v, although the precise role of each subunit is not completely understood. $Ca_v2.2$, a validated analgesic target, is potently and selectively inhibited by small peptidic toxins (ω-conotoxins) expressed in cone snail venoms. Some of these peptides have become useful pharmacological tools, while others have shown potential as therapeutic leads due to their specificity for $Ca_v2.2$. Ziconotide, a synthetic version of ω-conotoxin MVIIA, inhibits $Ca_v2.2$ with high potency, and it is currently in the market for treatment of severe intractable pain. Unfortunately, the poor selectivity of ziconotide and other available drugs still represent a challenge for the effective treatment of chronic pain, which affects billions of people worldwide. Thus, a better understanding of the mechanisms involved in pain pathways and the identification of novel and more selective drug candidates is urgently needed.

Aims: The aims of this study were to provide insights into the contribution of Ca_v specific subtypes, and auxiliary subunits, to the pharmacology of Ca_v antagonists, and thus provide a molecular basis for the involvement of $Ca_v2.2$ in pain pathways; and to develop high throughput assays to discover novel selective inhibitors for $Ca_v2.2$ from cone snail and spider venoms.

Key Results and Discussion: We identified the Ca_v subtypes and auxiliary subunits endogenously expressed in the human neuroblastoma SH−SY5Y cell line, and used Ca_v specific blockers to pharmacologically characterize Ca_v channels in these cells. These results allowed us to develop high throughput radioligand binding and fluorescent calcium imaging assays to accelerate the discovery and characterization of inhibitors of human $Ca_v2.2$. ω-Conotoxins, such as CVID, MVIIA and GVIA displaced the highly selective radiolabeled peptide ^{125}I-GVIA from SH−SY5Y cell membranes with high affinity ($pIC_{50} \sim 11$). Surprising discrepancies in toxin inhibition were found between the cell membranes and the whole cells in the binding assays, as well as in the calcium imaging assays, suggesting an influence of auxiliary subunits on ω-conotoxin affinity.

Keywords: Cav2.2, calcium channels, SH-SY5Y cells, spider and cone snail venom toxins
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33. Mass Spectrometry as a Tool to Search Specific Ligands for G-Protein-Coupled Receptors

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Background: G-protein-coupled receptors (GPCRs) constitute the largest family of transmembrane proteins, their importance arises from their role as signal transmitters and regulators of cellular response. They control almost all physiological processes in humans and consequently they

emerge as the molecular target of around 40% of the commercial available drugs. However, only about 60 of 356 GPCRs subtypes are targeted by those marketed drugs. The 220 remaining GPCRs are still unexploited, mostly due to the lack of specific ligands. Since the number of bioactive compounds found in animal venoms can reach up to 40 millions, the use of this natural library as a source for new ligands and pharmacological tools discoveries comes to light. This work aimed at the development and the improvement of a new mass spectrometry based technique able to identify new ligands of vasopressin receptors type 2 (V_2R), a member of GPCRs superfamily.

Methods: Vasopressin receptors type 2, overexpressed in a commercial cell line, were incubated with vasopressin plus a simple mix of known peptides (e.g. bradykinin, angiotensin and P14R peptide). After the incubation, two fractions were obtained: one containing the peptides that bound to the receptors ('bound fraction') and the second the peptides that not bound ('free fraction'). Both fractions were analyzed with a MALDI-TOF/TOF mass spectrometer to identify the peptides that bound to the V_2R and to verify that only the vasopressin was detected in the bound fraction. The second step of this work was linked to the incubation of V_2R with crude (or fractions of) animal venoms not only to ensure the workability of the established protocol when applied for a complex mixture of unknown compounds, but also to discover new specific ligands.

Results: The MS analyses showed the presence of the 3 cited peptides in the free fraction, while in the bound fraction we just observed a peak correspondent to vasopressin. These results demonstrate the feasibility of the protocol in fishing for specific ligands for V_2R.

Conclusion: The present work developed and improved a new methodology to screen for specific ligands for V_2 receptors in either a simple mixture or a complex mixture, in consequence, this may assist in the discovery of new ligands. Moreover this methodology may be used to find new ligands for different subtypes of receptors or even characterize orphan receptors, since the protocol can be adapted for other GPCRs subtypes.

Keywords: MS, vasopressin receptors, venom
10.1016/j.toxicon.2012.04.034

34. Miniaturization of μ-Conotoxins as Peptidomimetic Strategy to Develop Selective Sodium Channel Blockers

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Review: In recent decades cone snail toxins ("conotoxins") have become of great interest in the search for novel subtype-selective modulators of voltage-gated ion channels, including voltage-gated sodium channels (Na_vs). Malfunctioning of Na_vs is the underlying cause of numerous diseases and the availability of subtype-selective modulators of these channels is therefore of utmost importance.

Methods: We studied novel, synthetic bioactive peptides that specifically act on Na_vs. They were designed starting from two known, naturally occurring conotoxins BuIIIC (from *Conus bullatus*) and KIIIA (from *Conus kinoshitai*). Both toxins have been shown to potently block VGSC isoforms. Based on their structure and on recent insights in the development of "cono- and peptidomimetics", a series of minimized peptides were created, consisting out of 12 to 16 amino acids, with two disulfide bridges. After proper folding, mass and structure verification, their blocking activities on different Na_v isoforms (Na_v 1.2-1.8) were investigated by two electrode voltage clamp.

Results: The most promising compound shows the following IC_{50} v alues: 77.8 nM ± 5.9 for $Na_v1.2$, 53.4 nM ± 2.0 for $Na_v1.4$, 2373.1 nM ± 94.1 for $Na_v1.5$, 115.7 nM ± 27.4 for $Na_v1.6$, respectively. Its structure was determined by NMR spectroscopy, showing a scaffold that contains no α-helix but in which some of the key residues seem to be superimposable with key residues of KIIIA.

Discussion: Recently, block of Na_vs (in particular $Na_v1.6$ and $Na_v1.2$) was suggested as a novel therapeutic strategy in multiple sclerosis. Therefore, we tested our miniaturized compound in an animal model of multiple sclerosis, experimental autoimmune encephalomyelitis. Surprisingly, our results could not underscore this strategy since mice to which the compound was administered, showed a worsening of the disease. This was for example represented by higher mean clinical scores and a lower amount of phosphorylated axons, demonstrated by a series of immunohistological experiments.

Conclusions: In summary, our results show that it is possible to design very small but potent and selective peptides based on known pharmacophores of μ-conotoxins. On the other hand, our *in vivo* studies with a specific blocker of Na_vs, suggest a complex and ambiguous role of Na_vs in an animal model of multiple sclerosis.

Keywords: conotoxin, peptidomimetics, sodium channel
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35. Development of Guanidinium-Toxin-Based Sodium Channel Blockers as PET Imaging Agents and Therapeutics for Diagnosing and Treating Pain

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Review: Existing treatments for moderate-to-severe pain, including opioids, are not always effective and suffer from a wide range of side effects, including potential for addiction. Guanidinium toxins (GTxs) are potent, naturally-occurring small molecules that inhibit voltage-gated sodium channels (Nav) by binding to the channel pore and blocking Na ion transit. Because existing Nav blockers (lidocaine, bupivacaine, etc.) already serve as clinically important anesthetics and analgesics, GTxs also have

potential as in that regard. In fact, GTxs have been tested successfully in clinical trials for several indications including pain, chronic headache, and anal fissures. GTxs inhibit six of nine mammalian isoforms of Navs at low nanomolar concentrations. Significantly, the cardiac isoform, Nav1.5, is resistant; greatly reducing the risk of cardiotoxicity compared with existing Nav inhibitors. We have demonstrated that modification of GTxs through *de novo* chemical synthesis allows the introduction of improved pharmacologic properties. For example, novel GTx analogues show local anesthetic and analgesic activity in rat nociception assays for as long as nine days. No toxicity was observed at doses up to 15 times the minimally effective dose. We continue to evaluate the efficacy and PK-ADMET properties of our GTx lead compounds to guide development of a long-lasting GTx-based therapeutic that offers significant improvements over current treatments for moderate-to-severe pain. GTx analogues also have the potential to serve as diagnostic pain imaging agents. Accurate identification of chronic pain generators is a significant clinical challenge currently. Nav expression is enhanced in neuronal and inflamed tissues that exacerbate nociceptive activity. This feature can be exploited to image and localize these pro-nociceptive tissues. Thus far, we have successfully imaged significant increases in Nav density in an injured nerve using a novel radiolabeled GTx analogue, 18F-saxitoxin ([18F]STx), and PET-MRI in a neuropathic pain model (spared nerve injury; SNI). SNI rats received [18F]STx (i.v.) under anesthesia, and dynamic scans of right and left thighs were obtained for 30 minutes with small animal PET. Significantly increased [18F]STx labeling can be seen in the SNI nerve compared to control side 10-20 minutes after injection. Blocking with 'cold' [19F]STx eliminates such a difference between the right and left nerves, confirming the specificity of the tracer. The results indicate that [18F]STx shows potential as a specific radiotracer for visualizing Nav channels in the setting of neuropathic pain and for providing a clinical tool for objective, image-guided therapies to treat chronic pain.

Keywords: analgesics, pain, imaging, guanidinium toxin, saxitoxin, sodium channels
10.1016/j.toxicon.2012.04.036

36. Random Peptide Library Based on a Spider Neurotoxin, and Utilization of the Library in *in vitro* Evolution Directed to GPCR Ligands

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Background: *In vitro* evolution is one of the effective approaches in protein engineering to obtain peptides specifically recognizing target molecule(s). We previously succeeded in generating IL-6 receptor (IL-6R) agonists and antagonists by *in vitro* evolution from a random peptide library with a three-finger neurotoxin scaffold (Naimuddin *et al*, 2011). Peptides were initially selected by binding to soluble portion of IL-6R, and then their physiological activities were confirmed by cell-based assay. Soluble proteins could be mostly the target. In contrast, to apply *in-vitro* evolution techniques to membrane proteins, optimization for protein solubilization/reconstitution is critical and is a major bottleneck in the process. To overcome the problems, we have developed a new technique named PERISS (intra periplasm secretion and selection) method, and successfully applied to select peptides targeting muscarinic receptor m2 subtype.

Methods: (1) Construction of random peptide library; the peptide GTx1-15 (34 aa) was used as a template. Peptide domains located on the surface and the domains proposed to be involved in target recognition were estimated. The corresponding regions in the GTx1-15 cDNA were replaced by random nucleotides $(NNS)_n$. (2) PERISS method; the peptides and the target m2 receptor were expressed in the periplasm and in the inner membrane of *E. coli*, respectively. Interaction between the peptide and the target, and the following selection were achieved in the periplasmic space. The peptides interacting with the target m2 were collected as binding complexes [peptide-target-*E. coli*]. The selected peptides were recombinantly expressed in *E. coli*, and purified. Binding activity of the peptide to m2 receptor was measured by [^{3}H]-NMS replacement assay.

Results: The peptide library was constructed based on a neurotoxin GTx1-15, which we originally isolated from the spider venom and characterized as a T-type calcium channel modulator (inhibitor cysteine knot; Ono *et al*, 2011). Structure elements for ICK scaffold were maintained in the library, while potential diversity is 10^{15} in calculation. We performed the PERISS method to screen from the library for peptides selectively recognize m2 receptor. After six rounds of the PERISS, selected peptide sequences showed convergence. One of the peptides showed moderate affinity (K_i^{app} ~300 nM) and subtype selectivity to m2 receptor.

References

Naimuddin, M., *et al.*, *Molecular Brain* (2011), 4, Article 2: Ono, S., Kimura, T., Kubo, T., *Toxicon* (2011) 58, 265-76: Naimuddin, M., Kubo, T., *Anal Biochem* (2011) 419, 33-39: Kimura, T., Ono, S., Kubo, T., *Intl J Peptides* (2012) 2012, Article 731293.

Keywords: in vitro evolution, scaffold, peptide, neurotoxin, GPCR, three finger, ICK
10.1016/j.toxicon.2012.04.037

37. A Sea Anemone Toxin to Treat Autoimmune Diseases: ShK and its Analogs

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Background: CCR7$^-$ effector memory T (T_{EM}) lymphocytes are involved in chronic inflammatory diseases such as

multiple sclerosis, type 1 diabetes mellitus and rheumatoid arthritis. These cells up-regulate the Kv1.3 potassium channels upon activation and these channels play a major role in T_{EM} cell activation and function. Blockers of Kv1.3 and other potassium channels are found in many venoms, including that of sea anemones. In 1995, Castañeda and colleagues extracted a potent peptidic K^+ channel blocker from the Caribbean sea anemone *Stichodactyla helianthus* and named it ShK, for *Stichodactyla helianthus* K^+ channel toxin.

Methods: We have used ShK as an *in vitro* and *in vivo* proof of concept for targeting Kv1.3 channels for the treatment of autoimmune disease. We have also used this peptide as a template for the development of highly selective Kv1.3 channel blockers.

Results: Rationale modification of the peptide ShK has yielded several potent and highly selective Kv1.3 channel blockers, such as ShK-186. ShK-186 preferentially targets human and rat T_{EM} cells *in vitro* and *in vivo*. It prevents and treats rat models of chronic inflammatory disease (delayed type hypersensitivity, experimental autoimmune encephalomyelitis – a model of multiple sclerosis, and pristane-induced arthritis – an animal model of rheumatoid arthritis) without preventing clearance of acute infections or displaying overt toxicity.

Discussion: ShK-186 is currently undergoing preclinical trials for the therapy of autoimmune diseases. Dr. Shawn Iadonato will further discuss pharmacokinetic and pharmacodynamic perspectives in another session.

Conclusions: ShK and its analogs represent a new class of immunomodulatory compounds for the treatment of chronic inflammatory diseases.

Keywords: autoimmune disease, potassium channel, sea anemone toxin
10.1016/j.toxicon.2012.04.038

38. Development of the Sea Anemone Toxin ShK-186 for the Treatment of Autoimmune Diseases: PK and ADME Perspectives

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Background: ShK-186, a specific peptide inhibitor of the Kv1.3 channel, is being developed for the treatment of autoimmune diseases with a specific focus on multiple sclerosis. The Kv1.3 channel is critical to the proinflammatory properties of effector memory T cells, and blockade of the channel diminishes the activation and expansion of this cell population *in vitro* and *in vivo*. The drug is effective in reducing the clinical signs and histopathological correlates of disease in animal models of MS, rheumatoid arthritis, psoriasis, skin hypersensitivity, and autoimmune glomerulonephritis (see presentation by Dr. Christine Beeton).

Methods: We have recently completed a series of IND-enabling nonclinical studies in rat and cynomolgus monkey that will be used to support first-in-human clinical trials of ShK-186. A key component of these studies was a thorough characterization of the absorption, distribution, metabolism, excretion and pharmacokinetic properties of the drug in rodent and primate species. Bioanalytical methods were developed (HPLC-MS/MS, ELISA) to measure ShK-186 levels in plasma from rat and cynomolgus monkey. In addition, an ^{111}In-labeled DOTA conjugate of ShK was developed that retained full channel blocking potency and that enabled detailed PET-scanning studies of peptide biodistribution *in vivo*.

Results: ShK-186 shares several important properties with other charged venom peptides including biphasic slow absorption from the injection site and wide distribution to peripheral tissues. These properties result in persistent whole blood concentrations of the peptide above the K_d for Kv1.3 channel blockade for 5 days in rat and greater than one week in squirrel monkey. Exploratory studies in the rat delayed-type hypersensitivity and chronic-relapsing experimental autoimmune encephalomyelitis models demonstrate that dose administration every 3 – 5 days provides maximum therapeutic benefit. These data support approximately once-weekly dose administration in human clinical trials.

Discussion: While >30 peptides are FDA-approved products, the market potential of these molecules has been tempered by an expectation of frequent parenteral administration. Charged venom peptides may alternatively provide durable pharmacological effects consistent with better patient tolerability and compliance and improved commercial success.

Conclusions: We have completed the IND-enabling nonclinical program for ShK-186. The nonclinical pharmacological properties of the peptide support once-weekly administration in human clinical trials.

Keywords: sea anemone, autoimmune, ShK, multiple sclerosis, EAE, encephalomyelitis hypersensitivity, pharmacokinetic, ADME, metabolism, venom, peptide, drug
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39. Discovery and Development of χ-Conopeptides for the Treatment of Pain

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Background: A new class of small peptides (χηι-conopeptides MrIA and MrIB) were discovered in the venom of the moluscivorous cone snail *Conus marmoreus* that noncompetitively inhibited the norepinephrine transporter (NET).

Method: Based on its novel mode of action and specificity, MrIA was used as a lead molecule by Xenome Ltd.

Early studies in pain models revealed MrIA potently reversed neuropathic pain (allodynia) that developed in rats following loose ligation of the sciatic nerve.

Results: Extensive analoguing identified Xen2174 as a product candidate for the treatment of moderate to severe pain based on its improved stability, wide therapeutic index, and long-lasting antinociception in rodent models of neuropathic and post-surgical pain. Toxicology data and a Phase I intravenous study supported the investigation of bolus intrathecal (IT) doses of $\leq$ 40 mg in humans. A single bolus IT injection in a cohort of mixed oncology patients (N=37) with chronic pain demonstrated that Xen2174 was safe and tolerated at doses ranging from 0.025 to 30 mg. Clinical data over 4 days post-treatment suggested a possible association between dose and reduction in patient pain, especially in patients with a baseline VAS pain score $\geq$ 40 out of 100.

Conclusions: The favourable clinical safety profile of single IT doses of Xen2174, and an association with pain reduction in this open-label study, support the continued clinical investigation of Xen2174 for the relief of moderate to severe pain. Recent SAR and mutational studies confirm that χ-MrIA inhibits NET by binding to the mouth of the transporter in the "occluded" state.

Keywords: conotoxin, pain, norepinephrine transporter
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40. The Anti-cancer Activity of the Venom from Spider Macrothele raveni *in vitro* and *in vivo*

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Background: The spider *Macrothele raveni* is distributed in the hilly areas of Guangxi Province, China. It has been found that the venom from the spider *M. raveni* contains a mixture of compounds with different types of biological activity.

Methods: U251 glioblastoma cells were treated with the spider venoms of different concentrations and time. The anti-cancer activity of the venom from *M. raveni in vitro* was analyzed by some functional assays including lactate dehydrogenase (LDH) release assay, flow cytometry and western blotting. BALB/c nude mice were used in experiment of tumors inhibition *in vivo*.

Results: The LDH release assay showed that the venom at concentrations of 10, 20, 40 μg/mL inhibited U251 cell proliferation and it affected cell viability in dose- and time-dependent manners using [^{3}H]-methyl thymidine incorporation assay. U251 cells treated with the venom were accumulated on G_2/M and G_0/G_1 phase of cell cycle detected by flow cytometry. Western blotting analysis indicated one of the pharmacological mechanisms of spider venoms was to activate the expression of *p*21 gene. In addition, *In vivo* examination of the inhibition of the size of tumors of nude mice using the spider venom (1.6, 1.8, 2.0 μg/g mice) revealed that tumors size was significantly decreased from controls by 21 days of treatment and at all points of analysis for 7 week ($P<0.01$).

Discussion: Our data suggest that inhibition of the spider venom from *M. raveni* may be a potential drug for human glioblastoma carcinoma. To date there have been no studies on examining the activity of anti-tumor of the venom from the spider *M. raveni*.

Conclusion: The venom from the spider *M. raveni* inhibits the activation and differentiation of glioblastoma cell and induces apoptosis.

Keywords: macrothele raveni, spider venom, U251 cells, anti-cancer activity
10.1016/j.toxicon.2012.04.041

41. Anti-Inflammatory Effect of Honey Bee Venom on Wistar Rats Induced Poly Cystic Ovarian Syndrome by Estradiol Valerate

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Background & Aims: Polycystic Ovarian Syndrome (PCOS) is a low grade inflammatory disease characterized by hyper androgenemia, hirsutism, endothelial dysfunction, pathological angiogenesis and chronic anovulation. C-reactive protein (CRP) is a protein found in the blood released by adipocytes, which plays a key role in adipocytes, which plays a key role in PCOS. Cyclooxygenase-2 is a key enzyme which converts arachidonic acid into prostaglandins and is triggered by inflammatory stimuli, such as cytokines. Its expression increases in PCOS. Honey bee venom (HBV) contains a variety of biologically active components having various pharmaceutical properties. This study was designed to detect the possibility of HBV application as an anti-inflammatory therapeutic agent. In the present study, the anti inflammatory role of honey bee venom on expression of COX-2 and level of CRP, indexes of inflammatory, in Wistar rats with polycystic ovarian syndrome was investigate.

Methods: 1mg/100grB.W Estradiol Valerate (EV) was subcutaneously injected to induce PCOS in mature female rats (170−200 gram). After 60 days, HBV was administered for consecutive 10 days and its results in PCOS treatments were investigated. Rats were sacrificed and ovaries were sectioned to determine histomorphometrical and immunohistochemical evaluations in ovary and liver. Testosterone and estradiol detected by Chemo Luminesance Immuno Assay. In order to detect CRP, ELISA kit was used and determines the absorbance by reading at 450 nm in three groups of EV-induced PCOS, HBV-treatment and normal intact animals.

Results: Thickness of theca layer, number and diameter of cysts and atretic follicles and levels of CRP significantly

decrease in HBV group comparing with PCOS group. Moreover, corpus luteum, as a sign of ovulation, was observed in HBV-treated ovaries which were obviously absent in PCOS group. The immunohistochemical analyses for cyclooxygenase-2 expressions showed a decrease expression of cyclooxygenase-2 enzyme in envelop layers of follicles in group 3.

Conclusions: Our results suggest that beneficial effect of HBV may be mediated by the inhibitory effect of HBV on CRP and COX-2 levels.

Keywords: polycystic ovarian syndrome, honey bee venom, Cyclooxygenase-2, C Reaction Protein, Wistar rat.
10.1016/j.toxicon.2012.04.042

42. Effect of Conotoxins on GABAC Receptor

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Background: Due to the lethal effects of toxins produced by *Conus* snails on human beings, the interest for their study has increased, not only for their great diversity, but for the quantity of compounds that form the neurotoxic cocktail as well. These toxins, commonly known as conotoxins or conopeptides, modulate ionic channels and receptors of the CEntral Nervous System. We know that alpha- and psi-conotoxins modulate subtypes of nicotinic acetylcholine receptors (nAChR) and sigma-conotoxins modulate the activity of serotonin receptor (5-HT_3). Both of these receptors belong to the Superfamily of cys-loop receptors, which also includes glycine (GlyR) and gamma-amino butiric acid subtype A and C ($GABA_AR$ and $GABA_CR$) receptors.

Methods: This project consisted on an electrophysiological evaluation of the effects of venom fractions on the $GABA_CR$. The venom was isolated from *Conus spurius*. The purification of the crude venom was performed by HPLC using a C_{18} analytical reverse-phase column Vydac C18 (4.6 mm x 250 mm, particle size 5 μm, 300 Ångstrom pore size). The electrophysiological characterization was performed on the homomeric ρ_1 $GABA_C$ subtype ($GABA_{C\ \rho1}$) receptor expressed in *Xenopus laevis* oocytes. Each oocyte was clamped at -60mV with two-electrode system and was constantly perfused with an electrophysiological solution. GABA gated-currents were elicited with 1 μM gama-amino butyric acid (GABA) pulses. The neurotoxic fractions were applied to each oocyte in a 5-min static bath.

Results: Only one fraction out of 25 had effect on GABA-elicited currents with a 58% of inhibition.

Discussion: This is the first evidence of activity on GABA receptors by conotoxins.

Conclusions: This work helps to elucidate the functional mechanism of this receptor subtype.

Keywords: conotoxins two electrode technique, GABAC ρ1 receptors.
10.1016/j.toxicon.2012.04.043

43. Melittin Peptide Kills *Trypanosoma cruzi* Epimastigotes and Trypomastigotes Forms by Different Cell Death Phenotypes

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Background: The *Apis mellifera* venom contains several components with a wide variety of pharmaceutical properties, such as melittin, which comprises 40-50% of the venom's dry weight. Previous studies demonstrated its leishmanicidal, anti-microbial and anti-tumor activities. Chagas' disease, caused by *Trypanosoma cruzi*, is estimated to affect 16-18 million people in Central and South America, and the patients' treatment is based on drugs such as Benznidazole, which exhibits toxic effects and rarely beneficial in the disease chronic phase. Therefore, the development of new chemotherapeutic agents from natural sources, is a lining research to be exploited. This study displays the melittin activity against *T. cruzi* (CLBrener clone) epimastigotes and trypomastigotes, and over the host cells.

Methods: Four-day-old culture epimastigotes were cultivated for 4 days in LIT medium containing 1.34, 2.68 and 5.36 mg/ ml of melittin. Tissue culture trypomastigotes were incubated in RPMI medium containing 0.1, 0.2 and 0.4 mg/ ml melittin for 1 day. Melittin effects on epimastigotes growth and trypomastigotes lysis was evaluated by daily counting with a Neubauer chamber. The peptide effects over parasites morphology were analyzed by electron microscopy. The parasites viability was evaluated by flow cytometry using propidium iodide (PI) staining. To test the melittin cytotoxicity to the host cells, peritoneal macrophages were treated or not with 1 and 5 mg/ ml for 48h and examined by MTS assay.

Results: The treatment resulted in a reduction of parasites number, in a dosis-dependent manner. The IC_{50} for epimastigotes inhibition growth is 2.44 ± 0.39 mg/ ml and LD_{50} for trypomastigotes lysis is 0.14 ± 0.05 mg/ ml. Epimastigotes presented between 70 to 99% PI-positive staining, and trypomastigotes 62 to 69%. The ultrastructure led us to believe the occurrence of different programmed cell death pathways, where epimastigotes were killed by autophagy (e.g. structures suggestive of autophagosomes) and trypomastigotes by apoptosis (e.g. abnormal nuclear chromatin condensation and kDNA disorganization). The formazan precipitate did not occurred in the macrophages treated with 5 mg/ ml, with a significant absorbance reduction compared to untreated cells.

Discussion: Our data demonstrate that melittin was effective against the epimastigote and trypomastigote forms of *T. cruzi*, which displayed different death phenotypes, at concentrations non-toxic to host cells.

Conclusions: These findings confirmed the great potential of natural products, such as melittin peptide, as a source to screen e develop new drugs for the treatment of neglected diseases, such as Chagas' disease.

Keywords: Melittin, Apis mellifera venom, programed cell death, Chagas disease, *Trypanosoma cruzi*.
10.1016/j.toxicon.2012.04.044

44. Leishmanicidal Effects of a Phospholipase A2 Isolated from *Crotalus viridis viridis* Snake Venom

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Background: Treatment of Leishmaniasis, caused by *Leishmania* sp., a disease which afflicts millions of people worldwide, is based on drugs as amphotericin B, pentavalent antimonial and pentamidine, which exhibit toxic effects and limited efficacy. Therefore the search for new drugs is a lining research to be exploited. Several toxins have been used as therapeutic agents and pharmacological tools for drug development. Snake venom phospholipases, exhibited different *in vitro* effects such as modulate cell proliferation, bactericidal activity and *Plasmodium falciparum* inhibition growth. *Crotalus viridis viridis* (Cvv) phospholipase A2 (PLA2) is a 14 kDa, non-neurotoxic, basic single-chain myotoxin, previously described. This work is based in the Cvv venom PLA2, and its effects over *L. amazonensis* promastigotes.

Methods: The crude venom extract was loaded onto to a reverse phase analytical (C8) column using a high performance liquid chromatographer. A linear gradient of water/acetonitrile with 0.1% trifluoroacetic acid was used. The peak contained the isolated PLA2 (confirmed by SDS-PAGE and mass spectrometry) was collected, lyophilized, resuspended in distilled water and its protein content measured. *L. amazonensis* promastigotes were incubated in Schneider medium, with 0.3125 to 10 mcg/ ml PLA2, at 24oC, and the effect on the cells proliferation was evaluated by counting with a Neubauer chamber, up to 72 h. The parameter used to estimate proliferation inhibition was the IC_{50}, which corresponds to the drug concentration that inhibited 50% cell growth. Parasites viability was assessed by flow cytometry using propidium iodide (PI) labeling, and the morphological alterations were examined by light microscopy through Giemsa staining.

Results: The treatment caused a significant inhibition (32% to 82%) in the parasites growth, dosis- and time-dependent, soon the first 24 hours. The data obtained allowed us to estimate the IC_{50}/ 24 h of 2.50 ± 1.42 mcg/ ml, decreasing to 0.77 ± 0.5 mcg/ ml after the final 72 h. The morphological alterations presented by treated parasites were rounded and unusual cell body shapes, with loss of membrane integrity, corroborated by the 96.6% of PI-positive labeling.

Discussion: We presented the employee of Cvv PLA2 against *L. amazonensis* promastigotes, which inhibited the parasites *in vitro* growth. Further studies by electron microscopy are underway in order to detect the intracellular targets and the PLA2 effects over amastigotes forms.

Conclusions: This work presented that Cvv PLA2 can be object of further research for a new leishmanicidal agent, as we have looked forward to achieving.

Financial Support: CAPES, CNPq and FAPERJ.

Keywords: PLA2, *Crotalus viridis viridis, Leishmania sp.*, Leishmaniasis
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45. Carbon-13 and Nitrogen-15 Turnover in Serum of Bubaline Donors of Biological Material for Medical Use

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Background: Toxicology is a fascinating area for potential bioprospect molecules in the development of new drugs. The synergism between the animal toxins and biological materials can be used to formulate a new compound. In this regard, the fibrin sealant produced by CEVAP (Centro de Estudos de Venenos e Animais Peçonhentos) is part of this strategy. The knowledge of the assimilation of carbon and nitrogen isotopes in different tissues or fractions shows the turnover, which suggests the incorporation of diet as a function of feeding time. The traceability involves biological samples that reflect the diet, allowing detection of the inclusion of animal meal in the final product. This method may prevent the transmission of diseases such as Creutzfeldt-Jakob disease, characteristic of human prion, which is also transmitted by the action on the medical use of drugs or implants. The aim was to evaluate the $\delta^{13}C$ and $\delta^{15}N$ turnover in serum of bubaline for the certification of "green buffalo" as a donor of biological material in the development of products for medical use.

Methods: Three young buffaloes (Murrah) were evaluated, during 117 days, under two different composition of isotopic feeding: 1) Diet with vegetable protein source, constituted by C_3 e C_4 photosynthetic plants species (0-21 days); 2) Diet with animal protein source, constituted by bovine meat and bone meal (22-117 days). Isotopic ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) were measured in a Mass Spectrometer Delta V Advantage Isotope Ratio MS. To quantitatively measure the speed of isotopes replacement, after determining the time interval, we used the exponential function of time (*turnover*), expressed by equation 1: $\delta\,‰\,{}^{13}C/{}^{15}N\,(t) = \delta\,‰\,{}^{13}C/{}^{15}N\,(f) + [\delta\,‰\,{}^{13}C/{}^{15}N\,(i) - \delta\,‰\,{}^{13}C/{}^{15}N\,(f)]\,e^{-kt}$. The half life of Carbon-13 and Nitrogen-15, provided 50% of each diet ($t = T$), was calculated by the equation 2: $T = \ln 2/k$.

Results: Isotopic values of carbon-13 were initially expressed by $\delta^{13}C = -12{,}93 \pm 0{,}20‰$, reaching values of $\delta^{13}C = -11{,}83 \pm 0{,}16‰$ to 117 days. For nitrogen-15, the values ranged from $\delta^{15}N = 7{,}43 \pm 0{,}45‰$ to $\delta^{15}N = 8{,}68 \pm 0{,}24‰$. The half-life values were $T = 11.7$ days for carbon-13 and $T = 14.6$ days for nitrogen-15.

Conclusion: The evaluation time of 95 days is sufficient to incorporate the isotopic signal of diet supplemented with animal protein, fundamental for the certification of "green buffalo" such a donor of biological material.

Keywords: animal toxins, stable isotopes, fibrin sealant
10.1016/j.toxicon.2012.04.046

46. Biological Features of an Enantiomeric Antimicrobial Peptide from Scorpion Venom

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Background: Pin2 is an antimicrobial peptide (AMP) originally isolated and purified from the venom of the scorpion *Pandinus imperator*. It has been proved to be highly active on disrupting bacterial and eukaryotic cell membranes. Pin2 could be an original drug to be used as pioneering peptide antibiotic for topical infections; however, it is easily cleaved in the presence of proteolytic enzymes. Therefore, to explore innovative derivatives of Pin2 an enantiomeric form was chemically synthesized. In this work we investigated some biological characteristics of D-Pin2 in the presence of a clinical isolated pathogenic strain of *Pseudomonas aeruginosa*.

Methods: *P. aeruginosa* was clinically isolated from an infected wound. It was microbiologically verified by morphological, biochemical and molecular assays. *P. aeruginosa* samples were grown on King B liquid media for 2 days to allow production of proteases. The supernatants were separated by centrifugation at 4°C and filtered. The protein was precipitated using acetone. The proteolytic degradation of D-Pin2 in the presence of trypsin, human serum and protein extracts from *P.aeruginosa* was assayed for 3, 12, and 24h at 37°C. Native L-Pin2 was used as control. The content of D- or L- Pin2 was followed by HPLC.

Results: D-Pin2 was found to be less hemolytic and more efficient against Gram – and + strains. However, the minimal inhibitory concentration in the presence of *P. aeruginosa* was 50% higher than that of native L-Pin2, but D-Pin2 was resistant to proteolytic activity of trypsin and enzymes from human serum. It is known that *P. aeruginosa* secretes several proteases including protease IV and alkaline protease, among others. Therefore, when D-Pin2 samples were incubated with precipitated extracts of *P. aeruginosa*, it was found a decrease of 20, 50 and 100 % of D-Pin2 at 3, 12 and 24h, respectively, indicating that *P. aeruginosa* may contain enantio-selective proteases.

Discussion: It is assumed a possible degradation of D-Pin2 by the activity of D-aminoacid dehydrogenase or racemase reported in *P.aeruginosa*. D-aminoacid dehydrogenase from *P.aeruginosa* is highly active on D-alanine and some D-amino acids. *P.aeruginosa* extracts with D-aminoacid activity are not reported. Could there be the presence of a protease degrading D-aminoacids?

Conclusion: D-Pin2 has less hemolytic activity and it is more resistant to protease degradation; therefore, it could be a drug candidate for topical infections but will be less effective towards *P. aeruginosa*.

Acknowledgements

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Keywords: Pin-2, degradation, *P. aeruginosa*
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47. Rational Development of Novel Leads from Animal Secretion Based on Coagulation and Cell Targets

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Review: Animal venoms and secretions are sources of novel pharmacologically active molecules of potential therapeutic value. We have screened venoms aiming to discover, identify and isolate peptide molecules active in the mammalian haemostatic system. This research initiative yielded a portfolio of promising drug candidates comprising Lopap from bristles of the *Lonomia obliqua* caterpillar and Amblyomin-X from saliva of the *Amblyomma cajennense* tick. These novel recombinant proteins and synthetic peptides turned out to be multifunctional molecules, which are presently under different phases of development processes. Amblyomin-X is a potential candidate to treat cancer and metastasis. Lopap is a prothrombin activator which belongs to the lipocalin family and displays serine protease-like activity with procoagulant effect. It also induces cytokine secretion and antiapoptotic pathways in human cultured endothelial cells. A Lopap-derived peptide is capable of inducing collagen synthesis in fibroblast culture and in the animal dermis. In this context is crucial to elucidate the multifunctional properties of those molecules and provide a further data integration allowing the identification of potential targets as well as possible mechanisms of action, and then the prediction of novel leads (new chemical entities, NCEs) as drug candidates optimizing their therapeutic effects considering the system biology. *In vitro* and *in vivo* models reinforce therapeutic applications. The *in silico* methods (docking, molecular dynamics simulations, protein homology modeling, quantitative structure-activity and/or structure-property relationships, chemometric analysis) will generate molecular and mathematical models to establish the mechanisms of action hypothesis and support the rational design of novel leads with improved pharmacokinetic and pharmacodynamic profiles. Lopap and Amblyomin-X have patents licensed to pharmaceutical industries and are subjects of efforts to reach firm proofs of concept. The non-clinical phase studies are currently underway for Amblyomin-X.

Financial support: FAPESP, INCTTOX-CNPq.

Keywords: lonomia, amblyomma, coagulation
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48. Use of Connectivity Maps and Platform Technologies for Drug Lead/Biosimilar Discovery in Venoms

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Background: Venoms have long been mined as resources for new drugs or drug leads. Traditionally, drug discovery has

taken the approach of screening the venom libraries based on specific, pre-identified targets. Once a hit is detected, the venom is subjected to fractionation to isolate the compound(s) of interest. This approach has proven moderately successful, particularly with high throughput screens with good targets; however, this approach essentially is predetermined to find what you are looking for and miss novel activities not associated with the target. Novel genomic approaches to drug discovery have recently been demonstrated to be useful for identifying drugs for repurposing for applications directed at new diseases. One such approach is the use of gene expression profiles of known bioactive compounds and drugs informatically associated with the gene expression profiles of certain disease states. This has been described as Connectivity Maps (1). In our study, we have extended the principle of Connectivity Maps as a novel approach for discovering bioactivities in venoms in a non-biased manner.

Methods: As a proof of concept we carried out gene expression profiling of both Gila monster (*Heloderma suspectum*) venom, and a venom component Byetta (exenatide), a drug currently on the market to treat diabetes, followed by informatic analysis using Connectivity Map databases. Similarly, we analyzed peptidomes from several snake venoms.

Results: Using Connectivity Mapping on Gila monster venom we were able to identify activity in the venom connected with several diabetes drugs. Likewise, Byetta also mapped to diabetes drugs thus suggesting that use of this approach would have identified the anti-diabetes activity in the venom. Interestingly, the venom also mapped to several hypotensive drugs, which is not surprising given the hypotensive action of envenomation on victims. Connectivity mapping of the peptidomes of several viperid venoms indicated interesting potential activities in those libraries as well.

Discussion: The use of platform technologies such as gene expression profiling coupled with informatic analyses using large databases has proven of recent value in repurposing of drugs for different diseases. Similar approaches as demonstrated in these studies suggest an alternative, complementary approach for screening venoms for novel activities with the goal of identifying drug leads and/or biosimilars for further development.

Conclusions: Use of Connectivity Mapping on Gila monster venom validated this approach as a proof of concept that novel bioactivities in animal venoms may be discovered in a non-biased manner. (1) Lamb et al., *Science*, 2006.

Keywords: connectivity maps, platform technologies, drug discovery, biosimilars
10.1016/j.toxicon.2012.04.049

C. Evolution of Toxins & Venom Glands

49. The Origin and Evolution of Metalloproteinases in the Venom of Snakes

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Background: Snake venom metalloproteinases (SVMPs) are a pathologically-important, often major, toxin component of snake venoms, particularly in the venoms of viperid snakes. The SVMPs are members of the large multi-locus adamalysin gene family alongside ADAM (a disintegrin and metalloproteinase) and ADAMTS (ADAM with thrombospondin motifs) proteins. Here we discuss the evolution of SVMPs from: (i) their single ancestral recruitment into the venom of advanced snakes, to (ii) the diverse structural and functional isoforms observed in venom today.

Methods: Phylogenetic analyses were used to reconstruct the evolutionary history of the adamlysins, with a focus on the SVMPs. Subsequently, the mode and tempo of SVMP evolution was analysed using ancestral sequence reconstructions, positive selection tests and macromolecular structure modeling.

Results and Discussion: The ancestral recruitment of SVMPs into venom resulted from the duplication of an ADAM28-like gene. Consequently, basal SVMPs are most closely related to reptilian ADAM28 and mammalian ADAM28, ADAM7 and ADAM decysin-1 proteins. Notably, ancestral SVMPs exhibit complete conservation of cysteine residues with their non-venom ADAM homologs, demonstrating that the gain and loss of cysteine residues thought to be important for facilitating structural changes/post-translational modifications are the direct result of mutations following the recruitment of SVMPs into venom. Following this recruitment event, novel SVMP domain scaffolds have been generated in viperid snakes (P-II and P-I classes) through the duplication of SVMP genes coupled with the action of positive selection. P-III SVMPs first evolved into the P-II structure through the single evolutionary loss of the cysteine-rich domain, whilst multiple independent losses of the P-II disintegrin domain have resulted in convergent evolution of P-I SVMPs. In both instances of domain loss, adaptive evolution is a major driving force – positive selection was found to predominately act on amino acid residues predicted to be surface-exposed on the molecular surface of new SVMP scaffolds.

Conclusions: These results highlight how changes to the genetic structure of venom toxins can catalyze the accelerated evolution of novel proteins and facilitate major structural and functional alterations. The generation of different molecular scaffolds (P-I, P-II and P-III SVMPs) encoded by the same multi-locus gene family appears to facilitate protein neofunctionalization, whilst also presenting an evolutionary advantage through the retention of multiple genes capable of encoding functionally distinct proteins.

Keywords: snake venom metalloproteinases, molecular evolution, toxin, Serpentes, phylogenetics, positive selection
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50. Tetrodotoxin in North-American Newts

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Background: The red spotted newts, *Notophthalmus viridescens*, from the eastern part of North-America and newts of the genus *Taricha*, which are distributed along the west-coast of the continent, are known to be poisonous due to the presence of high concentrations of tetrodotoxin (TTX) in their skin, but also in all organs.

Methods: TTX and its analogues, 6-*epi*TTX and 11-oxoTTX were analyzed in methanolic extracts of newt specimens and of their organs by using post-column LC-fluorescent detection. TTX was also localized in tissue sections using a monoclonal antibody-based immunoenzymatic technique.

Results: TTX and its analogues were detected in varying concentrations in *Notophthalmus viridescens* newts in adults as well as in the terrestrial efts. When kept in captivity, toxin levels decreased over the years, offsprings totally lacked TTX. In a newt population from Pennsylvania more than 50 percent were found to be infected with intestinal parasites (nematodes, trematodes, cestodes) which were positively stained for TTX by immunohistochemical technique. Moreover, insect predators such as mantids (*Tenodera* spp.) were observed occasionally feeding on the newts. In the Californian newt, *Taricha torosa*, only TTX was identified in the skin and internal organs. The toxin was also present in the eggs and larvae, which was histochemically confirmed. However, none of the toxińs analogues could be detected.

Discussion: Variability of the toxin levels in the newts is a common phenomenon ranging from zero to extremely high concentrations. The biogenetic origin of TTX is still a matter of discussion. An exogenous source of TTX via the food chain or its synthesis by symbiotic bacteria like in marine animals may explain its high variability in certain populations. The role of TTX in protecting the newts from predation or parasitism is questionable, because parasites and some predators (insects, snakes) may sustain or adapt to the toxicity.

Conclusion: The occurrence of a toxin like TTX in amphibians (newts, frogs, toads) offers many opportunities to study evolutionary, ecological and biosynthetic aspects of a natural compound.

Keywords: tetrodotoxin, newts, toxin evolution
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51. Evolution of a Neurotoxin from a Defensin

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Review: Evolution of toxic proteins (toxins) represents key functional innovations convergently occurring in phylogenetically diverse animal lineages. However, the evolutionary scenario of such innovations remains largely unknown. Previous studies have shown high structural conservation between scorpion neurotoxins affecting voltage-gated potassium channels (VGPCs) (abbreviated as α-KTxs) and antibacterial insect defensins. Here we highlight an evolutionary role for a small deletion event taking place in an insect defensin which results in a neurotoxic α-KTx. In comparison with insect defensins, α-KTxs lack an amino-terminal loop (n-loop) but possess two conserved residues (LysCys$_4$XaaAsn, underlined here) involved in a direct interaction with the pore region of VGPCs. We found that five insect defensins from Hemiptera and Hymenoptera also contain the two functional residues at equivalent positions to α-KTxs and thus represent missing evolutionary links between these two classes of peptide families. Deletion of the n-loop of one such peptide navidefensin2-2 removes steric hindrance of peptide-channel interactions and results in a VGPC-targeted neurotoxin (herein termed navitoxin) that selectively blocks three different mammalian VGPC isoforms with nanomolar to micromolar affinities. Mutations of the two crucial residues diminished or significantly decreased the affinity of navitoxin on these channels, demonstrating that this defensin-derived neurotoxin binds to VGPCs in the same manner with α-KTxs. Taken together, these results indicate that evolution of toxicity can be achieved by one small deletion event. Our finding might also be important in considering toxicity of antibacterial defensins as drugs.

Keywords: defensin, voltage-gated potassium channel, neurotoxin
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52. Divergence in the Biological Activity and Composition of Venom from Mackay (QLD) and Barossa/Adelaide (SA) Populations of Australian *Pseudonaja textilis* (Serpentes: Elapidae): An Important Role of Procoagulants in Rodent Prey Incapacitation

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Background and Aims: The eastern Brown snake *Pseudonaja textilis* has evolved extremely toxic venom to subdue prey, which consists mainly of mice, rats, lizards and frogs. A study on museum specimens found that individuals from Queensland grow larger in body size than those in South Australia, and that the proportion of endothermic prey consumed increases with snake body size (Shine, 1989). A more potent venom would confer advantage in preventing escape of a large rodent prey or an injury to the snake. It was thus interesting to compare the biological activities of the Queensland *P. textilis* venoms to those of South Australia in rats to see if the venom from Queensland would affect the physiological functions of a rat more severely and more rapidly than the venom from South Australia.

Methods: Sprague-Dawley rats were used as the prey model. The phrenic nerve-diaphragm preparation was used to assess neurotoxicity, and a rat plasma clotting time assay to examine coagulopathic activity of the venoms (obtained from Venom Supplies, SA). Proteomic profiling of venom composition was carried out by size-exclusion HPLC.

Results: Mackay *P. textilis* (QLD) venom was found to be more coagulopathic than venom from specimens from the Barossa and Adelaide region (SA), with extremely fast onset of clotting. Surprisingly, venom from Mackay specimens was found to be significantly less neurotoxic than the Barossa/Adelaide venom. When compared to Australian taipan *Oxyuranus scutellatus* and *O. microlepidotus* venoms, both *P. textilis* venoms were significantly more coagulopathic. Venom composition was found to differ greatly between the Mackay and Barossa/Adelaide populations, with Mackay venom having larger peaks in the high molecular weight region. In contrast, in the Barossa/Adelaide venom, lower molecular weight toxins were more abundant.

Discussion: Coagulopathic activity is strongly associated with rodent prey incapacitation. A higher coagulopathic potency of the Queensland *P. textilis* venom has likely evolved to incapacitate its large rat prey rapidly. *Oxyuranus* species have less coagulopathic venoms than *P. textilis*, but they are known to deliver higher quantities of venom. Prothrombin activator of *P. textilis* is a high molecular weight protein and a large peak in this region of the Mackay profile is likely associated with its extremely strong procoagulant activity. Larger peaks in the neurotoxin molecular weight regions of the Barossa/Adelaide sample may contribute to its more potent neurotoxic activity.

Reference:
Shine, R. (1989). Constraints, allometry and adaptation: Food Habits and Reproductive Biology of Australian Brownsnakes (*Pseudonaja*: Elapidae). Herpetologica 45:195-207.

Keywords: *Pseudonaja textilis*, venom, biological activity, divergence, coagulopathy, prey incapacitation
10.1016/j.toxicon.2012.04.053

53. Evolutionary Expansion of Venom Genes in the King Cobra Genome

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Background: Snake venom is a complex mixture of proteins and peptides evolved to immobilize prey and deter predators. The rapid evolution of venom toxins is part of a continuous predator-prey 'arms race' that represents a classic model for studying molecular evolution. Snake toxins are thought to evolve from normal physiological proteins through gene duplication and recruitment to the venom gland. However, in the absence of genomic resources, these hypotheses have remained mainly speculative.

Methods: Using Illumina sequencing technology we have produced a draft genome of an adult male Indonesian king cobra (*Ophiophagus hannah*). We have deep-sequenced a king cobra venom gland, accessory venom gland and pooled body organ transcriptomes to help establishing intron-exon boundaries and identify pseudogenes. The genomic sequence data were first assembled de novo into contigs, which were subsequently oriented and merged in scaffolds. Haploid genome size was estimated using flow cytometry to be around 1.36-1.59 Gbp. In total, we generated 41.2 Gbp (approximately 28x genome coverage) of sequence data. Our assembled draft has an N50 contig size of 3,982 bp, and an N50 scaffold size of 226 Kbp. The contigs sum to 1.45 Gbp, and the scaffolds (which contain gaps) to 1.66 Gbp.

Results: Comparative genomics revealed evidence of tandem duplication of toxin genes encoding physiological L-amino acid oxidase, cysteine-rich secretory proteins and metalloproteinases, followed by recruitment through selective expression in the venom gland. By contrast, nerve growth factor toxins appear to have evolved by duplication and dual recruitment, while hyaluronidase and phospholipase B evolved by recruitment of existing physiological genes without further duplication, similar to acetylcholinesterase. We also identify 21 different three-finger toxin (3FTX) genes in the genome, suggesting a massive expansion of this family. We find a significant variation in the expression levels of these different 3FTX genes in the venom.

Conclusions: These data show that venom genes originate and evolve through multiple distinct mechanisms. These sequences provide a valuable resource for studying rapid evolution of gene sequences and the evolution of recruitment of genes to different tissues, and could help unravel the molecular basis of the evolution of new gene function.

Keywords: genome, king cobra, venom
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54. Individual Venom Profiling of *Crotalus durissus terrificus* Specimens from a Geographically Limited Region: Crotamine Assessment and Captivity Evaluation on the Biological Activities

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Background: *Crotalus durissus terrificus* (*Cdt*) venom major components comprise crotoxin, crotamine, gyroxin and convulxin. Crotamine exerts a myotoxic action, among others, but its expression varies even amid snakes from the same region. Biochemical, enzymatic and pharmacological variations of venoms may be associated with the geography, climate, gender, age, and diet, as well as captivity time and venom extraction intervals. The present study aimed to characterize the *Cdt* venom from the Botucatu region, (SP, Brazil), by assessing its biochemical, pharmacological and enzymatic properties.

Methods: Venoms from newly captured snakes and already-captured animals were characterized comparatively to verify the sexual, environmental (length of captivity) and ontogenetic variations that could influence the venom composition. Protein concentration, SDS-PAGE and RP-HPLC were performed and the coagulant, toxic (LD_{50}) and crotamine activities were assayed. Individual SDS-PAGE analyses (315 samples) were performed and the biological activities of the venom of 60 adults (captive and newly captured males and females) and 18 newborns were compared with the Brazilian Reference Venom.

Results: Crotamine was found in 39.7% (125/315) of the samples, as determined by SDS-PAGE and RP-HPLC. Protein concentration differed significantly between adults (75%) and newborns (60%). RP-HPLC and SDS-PAGE analyses showed highly variable protein concentration and copious crotoxin isoforms; however, the LD_{50} values decreased during the captivity time. *Cdt* venom biological activities were similar among adult groups, but diminished during the captivity period.

Conclusions: The current findings demonstrate that venoms vary significantly in terms activity and protein concentration, despite originating from the same specie and region.

Financial support: Support: CAPES, FAPESP 09/06280-0.

Keywords: snake venom; environmental variation; sexual variation; ontogenetic variation; *Crotalus durissus* venom.
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55. Intraspecific Variation of Biological Activities in Venoms from Wild and Captive *Bothrops jararaca*

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Background: The venom of *Bothrops jararaca* is composed of complex mixture of molecules, mainly lectins, metalloproteinases, serinoproteinases, desintegrins, phospholipases and peptides. This composition may vary according to the snake's age, sex and region of origin.

Methods: The present work evaluated individual variation of *Bothrops jararaca* venom in the Botucatu region, Sao Paulo state, Brazil, through enzymatic, biochemical and pharmacological characterization, utilizing *in vitro* tests and biological assays. The activities were compared with those of Brazilian Reference Venom (BRV). Protein concentration varied between adult and juvenile groups.

Results: The electrophoretic profiles were similar, with molecular masses ranging between 25 and 50 kDa, but with intraspecific variations. RP-HPLC revealed protein concentration variations. Coagulant activity did not differ among adult groups, but there was a large variation between juvenile and BRV, which coagulated more intensely. Venoms from adults presented greater hemorrhagic activity; especially males recently arrived from the wild. On the other hand, the juvenile kept in captivity and adult males presented more elevated values. Edematogenic activity verified an increase in edema in all groups. At the mean lethal dose (LD_{50}), toxicity varied significantly between groups, being the venom from captive females three times more toxic than that from juvenile.

Discussion/Conclusions: The results of this study illustrate the intra- and interspecific complexity present in snake venoms, possibly represented in this case by ontogenetic, sexual and environmental variability in *Bothrops jararaca* venom. The data presented herein suggest a possible venom specialization in light of the factors studied. Furthermore, it is proposed that Brazilian public health authorities track down deeply the constitution of the pooled venom employed in the immunization of serum-producing animals since. Given the large territorial extension of Brazil, this variability would require regional monitoring and evaluation of the efficacy of bothropic antivenom on treatment of snakebite and the permanent sequela observed.

Financial support: CAPES, FAPESP 09/06280-0.

Keywords: snake venom; environment variation; sexual variation; ontogenetic variation; *Bothrops* venom.
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56. The Evolutionary Origins of Monotreme Crural Glands

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Background: Monotremes (echidna and platypus) are egg-laying mammals. One of their most unique characteristics is that males have crural glands that are seasonally active. Male platypuses produce venom in significant quantities during the breeding season, presumably to aid in competition against conspecifics. Platypus envenomation

of humans causes excruciating pain and prolonged swelling that is unresponsive to conventional painkillers. The fact that male echidnas also produce secretions during the breeding season was only discovered recently. Given that echidnas are not able to erect their spurs, the composition and purpose of these secretions remains to be determined.

Methods: We used Illumina sequencing to identify highly expressed genes in the crural gland of both species.

Results: Peptides identified which are likely to be responsible for the unique symptoms of platypus envenomation included serine proteases, metalloproteinases, antimicrobials, cytokines and protease inhibitors. The echidna crural gland transcriptome appeared markedly different to the platypus with no correlation between the top 50 most highly expressed genes between the datasets. Gene ontology terms associated with the top 100 most highly expressed genes in echidna, but not platypus, showed functional terms associated with steroidal and fatty acid production.

Conclusions: The non-aggressive behavior of the animal during the breeding season, the structure of the spur, along with the histology of the echidna venom gland suggests that echidna venom has a vastly different purpose to platypus venom. Our early results support that echidna crural gland proteins may function in chemical communication consistent with a role in reproduction and that over evolutionary time the echidna crural gland must have lost its venomous capabilities.

Keywords: platypus, echidna, evolution
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57. Structural Characteristics and Evolution of A Novel Venom Phospholipase A2 Gene from *Protobothrops flavoviridis*

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Review: *Protobothrops flavoviridis* (Habu, *Viperidae*, *Crotalinae*) inhabit the southwestern island of Japan. A variety of phopholipase A_2 (PLA_2) isozymes constitute major toxic components of *P. flavoviridis* venom. A novel PLA_2 gene, named *PfPLA 6*, was found in 6,328-bp NIS-1(5')-a segment preceding 25-kbp NIS-1(3') segment, which harbors five PLA_2 isozyme genes, in *P. flavoviridis* genome, and sequenced. Its open reading frame (ORF) of *PfPLA 6* encodes basic PLA_2 with SLVQ sequence in N-terminus, which is specific for [Lys^{49}]PLA_2 subgroup, and Asp at position 49, which is specific for [Asp^{49}]PLA_2 subgroups. Thus, *PfPLA 6* seemingly encodes a hybrid of [Lys^{49}]PLA_2 and [Asp^{49}]PLA_2. However, *PfPLA 6* is a pseudogene because the protein encoded by its ORF and its corresponding cDNA have not been isolated. Comparison of the nucleotide sequences of 71 *Viperidae* (*Viperinae* and *Crotalinae*) venom PLA_2 isozyme genes including *PfPLA 6*, particularly focusing on characteristic deletion of 12-bp segment called S1EX 1 or 55-bp segment called S2EX 1 in exon 1 and interposition of 219-bp segment called SINT 2, being in accord with short interspersed nuclear element (SINE), in intron 2, enabled us to propose a novel classification of Viperidae PLA_2 genes into three types, A (with S2EX 1 but without SINT 2), B (without S1EX 1but with SINT 2), and C (without S2EX 1 but with SINT 2). It is thought that A-type PLA_2 genes including *PfPLA 6* without SINT 2, which are all pseudogenes, are evolutionarily ancestral to B-type and C-type PLA_2 genes with SINT 2. This classification also clarified that Viperidae PLA_2 genes are B type and Crotalinae PLA_2 genes are C type. Since eleven *P. flavoviridis* PLA_2 isozyme genes including four inactive genes hitherto characterized have both S2EX 1 and SINT 2, they are all categorized as C type. As *PfPLA 6* is a pseudogene, an active prototype of *PfPLA 6* could be assumed to be the ancestral PLA_2 gene. Phylogenetic analysis of *P. flavoviridis* PLA_2 isozymes also suggested that *PfPLA 6* is ancestral to other PLA_2 genes. Putative evolutionary processes from this A-type prototype PLA_2 gene to descendent PLA_2 genes were discussed. This work will be published in "*Bioscience, Biotechnology, and Biochemistry*" this year.

Keywords: *Protobothrops flavoviridis*, phospholipase A2 gene, classification, SINE, ancestral gene.
10.1016/j.toxicon.2012.04.058

58. Functional Redundancy in Venoms is an Evolutionary By-Product

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Background: Functional redundancy in proteins is a rare phenomenon, with gene duplicates typically evolving on divergent molecular evolutionary trajectories. Venoms represent a rare case of functional redundancy, making it of a great interest to the evolutionary biologist. A long-standing theory has been that toxin diversity is a result of directional (positive) selection, with the reciprocal mutation-reaction process between prey and hunter being the major evolutionary selection pressure. This 'immediacy' hypothesis does not take into account historical processes that affect venom evolution. We used the scorpion cysteine stabilized αβ (CSαβ) toxins as a model for an investigation of the evolutionary forces that shape venom complexity.

Methods: All scorpion toxins belonging to the CSαβ scaffold were extracted from UNIPROT, aligned and analyzed for their phylogenetic association through Bayesian inference. This analysis was further used to assess position specific and pairwise analysis of selection within the toxin families.

Results and discussion: It was revealed that the functional redundancy of CSαβ toxins is the result of genetic

drift. Additionally, we showed a correlation between functional diversity of this scaffold in the venom and the paleogeographic distribution of the scorpions. These findings present a paradigm shift to a model that requires strong directional selection pressure to induce structural and functional recruitments, but allow the accumulation of paralogs under neutral selection.

Conclusion: We suggest that functional redundancy of venoms is for a long-term evolutionary benefit rather than a consequence of the immediate–potency arms-race.

Keywords: venom evolution, genetic drift, functional redundancy
10.1016/j.toxicon.2012.04.059

59. Understanding the Chemical Diversity of Spider Venoms Using a Combined Genomic, Transcriptomic and Proteomic Approach

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Background: Spiders and other venomous animals depend on the production of complex venom for defense, prey capture, and competitor deterrence. The major components of most spider venoms are disulfide-rich peptides of mass 3–8 kDa. Recent mass spectrometric analyses indicate that the venoms of some Australian funnel-web spiders contain >500 different peptides. However, very little is known about the genetic mechanisms used by these mygalomorph spiders to generate such a complex chemical cocktail.

Methods: In order to address this problem, we used a combined proteomic, transcriptomic, and genomic approach to determine the complete range of peptides expressed in the venom of these spiders, including the underlying architecture of the toxin-encoding genes, the nature of the mRNA transcripts, and the three-dimensional structure of the mature toxins.

Results: Proteomic analysis of the venom of an Australian funnel-web spider (*Hadronyche infensa*) using both state-of-the-art OrbiTrap and LC-MALDI 4800 TOF-TOF mass spectrometers yielded >2000 peptide masses. Two cDNA libraries were constructed for analysis of the venom-gland transcriptome: one from a single individual sequenced with Sanger sequencing and a pooled library sequenced using 454 technology. A two-step bioinformatic pipeline (ArachnoBase and ToxSeek) was developed to analyse the >270,000 high-quality reads that were obtained. These analyses revealed a total of 32 superfamilies of venom peptides and proteins, including: (i) 25 disulfide-rich toxin families; (ii) 3 families of enzymes; (iii) 1 family of cysteine-rich secretory proteins (CRISPs); and (iv) 3 families of secreted proteins. The number of structural classes is currently under investigation, with selected toxins being expressed in *E. coli* with the aim of solving their 3D structure using NMR. Genomic analyses have revealed that the disulfide-rich toxins are produced from intronless genes.

Conclusion: This work provides the most comprehensive overview to date of the toxin landscape of a prototypic mygalomorph spider venom. Neither of the transcriptomic studies provided evidence for multi-toxin transcripts or for alternative splicing as a mechanism for generating toxin diversity. The results are consistent with the one-gene:one-toxin paradigm proposed previously for venomous cone snails. The holistic approach described here should enhance our understanding of how spiders and other venomous animals evolved such complex venoms.

Keywords: spider venom, venom proteome, venom evolution, proteomics, transcriptomics, genomics, bioinformatics
10.1016/j.toxicon.2012.04.060

60. Glycan Structures and Intrageneric Variations of Acidic Phospholipases A2 from Tropidolaemus Venom

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Background: Pitvipers belonging to the *Tropidolaemus* genus are endemic to southeastern Asia, and it may consist of four species. *Tropidolaemus* venoms are unique in that they contain special neurotoxins known as waglerins and only a very little amount of hemorrhagins. However, the other venom components are not well understood.

Results: Phospholipases A_2 (PLA_2s) were purified and characterized using the venoms obtained from two different regions, Sumantra and Sulawesi. An active PLA_2 could be isolated from both the samples with an approximate yield of 4∼7% (w/w). Mass analyses and *N*-terminal sequencings revealed that the PLA_2s in both the samples were different although their waglerins were identical. The samples probably derived from two species, namely *T. wagleri* and *T. subannulatus*. This is consistent with the recent taxonomic and geographic study of this genus. The N-terminal sequences of both the PLA_2s were homologous to the acidic PLA_2s of the other pitviper venoms which contain a conserved Glu6 residue. Interestingly, both PLA_2s appeared to be glycosylated at Asn14. Hydrolysis by PNGase F reduced their apparent masses from 16 to 14 kDa. The released glycans were analyzed by MALDI-TOF and were found to be complex type oligosaccharides without sialylation. The acidic PLA_2 from the Sumantra sample induced the aggregation of mouse platelets, while the PLA_2 from Sulawesi inhibited the aggregation of mouse platelets induced by ADP and collagen. Furthermore, enzymatic removal of glycans from the PLA_2s did not significantly alter their effects on lipid hydrolysis and platelet aggregation.

Conclusion: *Tropidolaemus* venoms demonstrate interesting novelty and biodiversity. This is the first report of glycosylated snake venom PLA_2s whose glycan structures have been solved. The presence of glycans on these enzymes

warrants further analyses which may provide useful insights into the functional regulation of these molecules.

Keywords: *Tropedolaemus* venom, antiplatelet phospholipases, mass profile of N-glycans
10.1016/j.toxicon.2012.04.061

61. A Phylogenetic Framework for the Study of Convergence and Divergence in Scorpion Venoms

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Background: Mining on scorpion venoms using high throughput techniques has revealed a large prevalence of non canonical components in scorpion venoms, which in fact outnumber the typical toxin class. The evolutionary histories of these scorpion venom components show puzzling patterns, where both extensive divergence and recurrent recruitment are thought to have played important roles.

Methods: Non-redundant datasets were built for five protein families found in scorpion venoms from at least three different taxonomic families. Non-venom orthologs were identified by database searching and tested as proxies for rooting venom proteins phylogenetic trees. Maximum Likelihood and Bayesian trees for each protein family were reconstructed and compared to a draft species tree based on public mitochondrial sequences.

Results: High confidence rooted trees were obtained for three venom protein families. In the remaining two families, non-venom orthologs were found nested within venom clades, thus suggesting independent recruitment events.

Discussion: Mapping of venom proteins trees onto the species tree reveals a tangled pattern of continuous recruitment and lineage-specific diversification. Reconstructing the evolutionary history of venom proteins remains a difficult task due to multiple confounding factors. The use of a robust species phylogeny would certainly aid to resolve the discrepancies found in the different gene trees and help in the distinction between convergent recruitment and divergence.

Conclusions: Inasmuch genomes can be regarded as the historical records of previous selection processes, the molecules making up the venoms should reflect the history of the interspecies interactions that the producing lineages have faced. It follows that reconstruction of their evolutionary histories should 1) clarify how venoms have attained their remarkable complexity and 2) shed light over the multi-organismic processes that have shaped the evolution of venomous organisms in the context of their ecosystemic networks.

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Keywords: convergence, divergence, evolution, scorpion, toxin, venom
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62. Tentacles of Venom: Molecular Evolution of Coleoid Venoms

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Background: New insights into the evolution of venom systems and the importance of the associated toxins cannot be advanced without recognition of the true biochemical, ecological, morphological and pharmacological diversity of venom systems. A major limitation of the use of venom proteins has been the very narrow taxonomical range studied. Entire groups of venomous animals remain virtually completely unstudied. One such group are the coleoids (cuttlefish, octopuses, and squid), which have only been recently revealed by us to share a common venomous ancestor.

Methods: Eleven cuttlefish, octopuses, and squid species were field collected from tropical through to polar waters, thus providing wide taxonomical and ecological coverage. Venom molecular evolution was analysed using a combined proteomics/transcriptomics approach including mass spectrometry, 2-gels, and cDNA libraries. The comparison of proteomic and transcriptomic data allowed for rapid identification of peptide/protein types present in the venoms and subsequent determination of full-length transcript sequences.

Results: The combined approach revealed not only protein/peptide types convergently recruited into the chemical arsenals of other venomous animals but also discovered novel molecular scaffolds unique to coleoid venoms. Our bioactivity studies also revealed unique temperature specific adaptations of enzymes found in the Antarctic species.

Discussion: Coleoid venoms were revealed to be as complex as other venoms that have traditionally been the recipient of the bulk of research efforts. The presence of multiple peptide/protein types convergently present other animal venoms reveals new information as to what characteristics make a peptide/protein type amenable for recruitment into chemical arsenals.

Conclusion: Coleoid venoms have significant potential not only for understanding fundamental aspects of venom evolution but also as an untapped source of novel toxins for use in drug design and discovery.

Keywords: coleoid, venom, venomics, proteomics, transcriptomics, toxin evolution
10.1016/j.toxicon.2012.04.063

63. Discovery of the Nicotinic Receptor Toxin Anabaseine in a *Polystyliferan Nemertine*

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Background: Nemertines, a phylum of predominantly marine worms, prey on other animals and defend themselves with toxins. The anoplan nemertines lack a proboscis stylet for puncturing their prey and secrete neurotoxic and cytotoxic peptides. The hoplonemertines are enoplan (armed) worms that have a proboscis armed with one more stylets. Hoplonemertines are systematically divided into the relatively diverse order Monostylifera that is relatively common in certain benthic zones and the less readily collected order Polystylifera that are bathypelagic as well as benthic in distribution. Monostyliferan nemertines are known to produce alkaloidal toxins (including anabaseine) that affect nicotinic acetylcholine receptors and pyridine chemoreceptors (Bacq, 1936; Kem, 1971; Kem and Soti, 2001; Kem et al., 2006). Anabaseine has been a lead compound in the design of alpha7 nicotinic receptor agonists to treat disorders of cognition such as Alzheimer's disease and schizophrenia (Kem et al., 2004; Freedman et al., 2008).

Methods: We recently obtained two live specimens of the sublittoral benthic polystyliferan *Paradrepanophoros crassus* (PC) from the northwest coast of Spain. Bacq (1936) found this species to contain a substance(s) acting like nicotine on autonomic ganglia. We used Ehrlich's reagent (Kem et al., 1971) for anabaseine detection and HPLC and mass spectrometric methods for isolation and identification of anabaseine and related alkaloids. Ethanolic preservative was used to extract the toxins.

Results: We have demonstrated that Pc contains contains high concentrations of anabaseine in its body proper and proboscis but lacks 2,3'-bipyridyl, nemertelline and other alkaloids found in the benthic monostyliferan *Amphiporus angulatus*.

Conclusions: Our data indicate that the biosynthetic machinery for producing anabaseine probably was acquired by a common ancestral hoplonemertine before the evolutionary divergence of these two hoplonemertine orders.

Keywords: anabaseine, nicotinic, nemertine
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64. Centipede Venoms: Old and Unusual

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Background: At 420 million years old centipedes represent one of the oldest extant arthropod venom systems. However, despite the ancientness of these venoms, almost nothing is known about their components or molecular evolution.

Methods: Five centipede species were selected to provide both wide taxonomic coverage and a phylogenetic timeline to date evolutionary events. Emphasising the Scolopendridae due to their large size, availability, and clinical importance, the following species were selected to represent over 400 million years of evolution and to allow for comparisons at the order, subfamily, genus, and species level: *Thereuopoda* sp., *Ethmostigmus rubripes, Cormocephalus westwoodii, Scolopendra alternans*, and *Scolopendra morsitans*. Milked venoms were analysed using a combined proteomics/transcriptomics approach including mass spectrometry, 2-gels, and cDNA libraries. The comparison of proteomic and transcriptomic data allowed for rapid sequence determination and, crucially, identification of posttranslational modifications.

Results: We provide the first comprehensive insight into the chilopod "venome", revealing novel scaffolds unique to centipede venoms as well as scaffold types convergently recruited into other venoms. We also present multifunctional transcripts and transcripts with tandemly repeated sequences, which are unusual to invertebrate venoms.

Discussion: Centipede venoms appear to differ substantially from the venoms of other arthropods in the abundance of high molecular weight components. The ancient evolutionary history of centipedes is also apparent from the differences at the highest taxonomic level, which diverged about 400 mya.

Conclusion: The presence of a wide range of novel proteins and peptides in centipede venoms highlights these animals as a rich source of novel bioactive molecules. Understanding the evolutionary processes behind these ancient venom systems may not only aid in directing bioprospecting efforts, but it will also broaden our understanding of which traits make proteins and peptides amenable to neofunctionalisation.

Keywords: centipede, venom, venomics, proteomics, transcriptomics, toxin evolution
10.1016/j.toxicon.2012.04.065

65. The Phylogenetic Scale of Venom Variation in Haplogyne Spiders

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Background: Haplogynes are a higher-level clade of araneomorph spiders that includes many taxa of interest with respect to their venom composition. Some notable haplogynes include pholcids ("daddy long legs" or cellar spiders), plectreurids, and families in the scytodoid superfamily such as spitting spiders (Scytodidae) and the sicariid family that includes brown recluse (*Loxosceles*) and six-eyed sand spiders (*Sicarius*).

Methods: With a goal of analyzing the phylogenetic scale of venom variation in spiders in general, we are comparing venom gland transcriptomes and proteomes from representatives of this group selected based on their phylogenetic position. Our data include some comparisons among relatively closely related taxa (common ancestor within the last 30 million years) and among other more

distant relatives (common ancestor roughly 200 million years old). Our center of focus is comparisons among lineages of sicariids, that been evolving for over 100 million years in the context of being generalist, ground-dwelling predators of arthropods. We compare sets of sicariid toxins with toxins from scytodidae, pholcidae and non-haplogyne archaeid spiders.

Results: We have discovered venom peptide toxin families that appear to be distinct for sicariidae, and others that are expressed across haplogynes. While some toxins are phylogenetically widespread, there are striking differences among lineages in relative abundance of these toxins. We will discuss patterns of positive selection within some of the most common and widespead toxin lineages. Together these data help illuminate the evolutionary dynamics of venom functional complexes in spiders.

Keywords: diversity, evolution, phylogeny
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66. β-defensin-like genes in Brazilian Poisonous Snakes

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Background: β-Defensins are antimicrobial peptides (AMP) found in vertebrates. They constitute an important and conserved component of innate immunity with antimicrobial activities. These molecules are also reported in the venoms of sea anemones, snakes and platypus. In *Crotalus durissus terrificus*, it is known two peptides with β-defensin scaffold: crotamine, a small basic myotoxin of rattlesnake venom, and crotasin, a gene expressed abundantly in several rattlesnake tissues, but scarcely in the venom gland. In this study we prospected β-defensin-like genes in snakes of Viperidae family by PCR.

Methods: Genomic DNA from different species of *Bothrops, Bothropoides*, *Rhinocerophis* and *Lachesis* snakes were used as template. The primers were designed based on the signal peptide (SP) and 3'UTR, conserved sequences from crotamine and crotasin genes. The amplifyed sequences were aligned with CodonCode Aligner and the phylogeny analyzed by maximum parsimony using TNT1.1.

Results: From nine Viperidae snakes we obtained ten different sequences. The genes presented 3 exons and 2 introns that code the SP and the mature β-defensin. The size of first intron vary greatly (0.4 - 1.7kb) whereas the second was conserved (~153 bp). The mature peptides (MP) have the six cysteines (conserved in the β-defensin family), basic amino acids residues clustered in the carboxi region, net charge from +2 to +12, a N-terminal glutamine, a conserved glycine at ninth position and a C-terminal lysine.

Discussion: The gene organization is similar to crotamine, crotasin and other genes found in lizard and teleost fish. The SP and introns sequences are more conserved than MP sequences. The K_a/K_s ratio suggested an accelerated evolution on exon 2. Phylogenetic analyses using introns or exons did not recover the phylogenetic relationships obtained with mitochondrial DNA for Viperidae species, probably due to differential selection pressure of these two types of data. Between these nuclear sequences, the intron phylogeny has a better resolution, recovering individuals of the same species as sister groups.

Conclusions: The gene structure of β-defensin in snakes is different from that found in mammals and birds, but is similar to that found in another reptile, the green lizard *Anolis carolinensis*. As expected to this family of genes, it was observed an accelerated evolution on exon 2, what increase the peptide variability and enable the animal to protect against diverse pathogens.

Financial Support: FAPESP and INCTTox.

Keywords: rattlesnakes, β-defensins, gene structure, toxin evolution
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67. Comparative Analysis of Transcriptomes of *Phoneutria pertyi* and *P. nigriventer* Venom Glands

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Background: Species of the genus *Phoneutria* known as "aranha-armadeira" or armed spiders are responsible for a large number of spider bites in Brazil. Until recently, all studies on the venom of the genus have been restricted to the species *P. nigriventer*. However, some recent proteomic studies revealed that other species venoms also contains a wide variety of proteins and peptides, including neurotoxins which act on the ion channels and chemical receptors of the neuro-muscular systems of insects and mammals. Thus, these venoms have emerged as invaluable tools for research, drug discovery and drug development with application in medicine and agriculture.

Methods: In order to find novel venom components with biological activity and to provide a database for comparative study with the previously described *P. nigriventer* venom gland transcriptome, we constructed a plasmidial cDNA library from the species *Phoneutria pertyi* mRNA venom gland to generate Expressed Sequence Tags (ESTs) data.

Results: After editing, 710 good quality reads were clustered and 295 unique sequences were obtained (106 contigs and 189 singlets). Of these, 197 (67%) had a high degree of homology to spiders toxins deposited in the Uniprot database, most are *P.nigriventer* toxins isoforms. We observed that *P. pertyi* venom gland transcriptome were more abundant in insecticidal toxin sequences than *P. nigriventer* one. We also found new sequences for

putative toxins in *P. pertyi* transcriptome, indicating that they can be novel toxins.

Conclusions: These results show that although these spider venoms contain a similar range of toxins isoforms, the expression levels of each type of toxins are different, and also they contain toxins with unique sequences, what can suggest adaptation to different environments.

Keywords: *Phoneutria* venom gland, transcriptome, neurotoxin
10.1016/j.toxicon.2012.04.068

68. Substrate range and specificity of the SicTox phospholipase D toxins

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Background: Certain phospholipase D (PLD) toxins from the venoms of the *Sicariidae* spider family can induce necrotic lesions in mammalian tissue. The gene family ('SicTox') containing these PLD toxins is split into two well resolved clades: alpha and beta. The alpha-clade members generally exhibit enzymatic activity towards the substrate sphingomyelin (SM); in contrast, the beta-clade toxins exhibit diminished PLD activity towards SM. We are studying the catalytic activities of SicTox family PLD enzymes to gain insights into the evolution of substrate specificity and eventually correlate them with the biological function of these proteins as toxins.

Methods: We have cloned and can heterologously express a representative member from both the alpha- and the beta- clades. The beta-clade toxin is from the *Sicariidae* species *Sicarius terrosus*. The alpha-clade member is from *Loxosceles arizonica*, whose venom is known to be significantly harmful to humans. We are utilizing ^{31}P-NMR spectroscopy and spectrofluorometric assays to screen for phospholipase activity against a broad range of substrates.

Results: The alpha-clade enzyme is multispecific and exhibits PLD activity towards sphingomyelin and lysophosphatidylcholine. In contrast, the beta-clade toxin has diminished PLD activity for either of the choline-containing substrates. This is the first time a SicTox gene product has been biochemically characterized from a *Sicarius* species.

Conclusion: We can heterologous express and purify active SicTox enzymes. ^{31}P-NMR spectroscopy and enzyme coupled spectrofluorometric assays allows us to screen a large variety of phospholipid compounds for phospholipase activity to compare their catalytic efficiencies. The results to date indicate a difference in the substrate profiles of the proteins from the alpha- and beta- clade PLD toxins.

Keywords: SicTox, phospholipase D, *Sicariidae*
10.1016/j.toxicon.2012.04.069

D. Hemostasis

69. Recurrent, Persistent, or Late, New-Onset Hematologic Abnormalities in Crotaline Snakebite

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Background: Hematologic effects from rattlesnake envenomation exhibit a phenomenon of recurrent, persistent or late, new onset (late) abnormalities in some Fab antivenom-treated patients 4 or more days post-envenomation. Indicators that reliably identify or exclude those patients at risk of late hematologic effects have not been developed.

Methods: This was a retrospective, observational case series of rattlesnake bite records at two US poison centers. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for D-dimer, fibrinogen, platelets, platelet count trend, INR and PTT associated with late hematologic abnormalities, were determined.

Results: Three hundred seventy six cases were reviewed. Sixty cases met inclusion criteria. Overall, 17 of 60 patients (28%) had a hematologic abnormality as a result of envenomation. Eleven of 60 patients (65% of those with a hematologic abnormality; 18% overall) developed late hematologic abnormalities 4 or more days post-envenomation. Four patients had late, new onset hypofibrinogenemia and/or thrombocytopenia. All were associated with early D-dimer elevation and/or platelet rise in response to FabAV treatment, respectively. Normal hematologic parameters in the first 48 h post-envenomation and the lack of a greater than 20% rise in platelets within 4 h post-antivenom administration had a 100% NPV for late hematologic effects.

Conclusions: Patients with early onset hypofibrinogenemia, a positive D-dimer, thrombocytopenia, or a 20% increase in platelet count within 4 h post-treatment had significant likelihood of late hematologic effects. Patients in whom fibrinogen, D-dimer, INR, PTT, and platelet counts remained normal throughout the first 48 h post-envenomation, and who did not exhibit a $>$ 20% increase in platelet count within 4 h post-antivenom administration, did not develop late hematologic effects. This work, if validated, may help to identify those patients at particular risk of hematologic recurrence; those with risk of new, late onset hematologic effects; and those in whom late hematologic effects are unlikely to occur.

Table 1
Sensitivitey, Specificity, Positive and Negative Predictive Values compared between the clinical trials, the published data of Ruha, et al, and this study.

Current Study (n = 60)					Clinical Trials^ (n = 42)				Ruha et al.* (n = 66)			
Pre-48 hour Parameter	**Sens**[ł]	**Spec**[ł]	**PPV**[ł]	**NPV**[ł]	**Sens**	**Spec**	**PPV**	**NPV**	**Sens**	**Spec**	**PPV**	**NPV**
Fibrinogen	60%	89%	43%	94%	67%	59%	76%	76%	67%	78%	43%	94%
D-dimer	100%	56%	21%	100%	87%	69%	65%	89%				
Fibrinogen wnl[ł] & D-dimer wnl	-	100%	-	100%								
Fibrinogen wnl & D-dimer increased	100%	-	14%	-								
Platelet Count	75%	81%	43%	95%	78%	83%	58%	93%	75%	85%	43%	96%
Platelet Trend	100%	76%	43%	100%								
Platelets wnl & Trend < 20% increase	-	100%	-	100%								
Platelets wnl & Trend > 20% increase	100%	-	25%	-								
INR[ł]	100%	63%	14%	100%	71%	68%	33%	91%				
PTT[ł]	50%	85%	20%	96%	40%	85%	29%	90%				
ANY ABNORMAL	**72%**	**-**	**28%**	**-**	**100%**	**-**	**76%**	**-**	**100%**		**43%**	
ALL WNL[§]	**-**	**100%**	**-**	**100%**		**100%**		**63%**	**-**	**100%**	**-**	**88%**

Abbreviations: ŧSensitivity (Sens), specificity (Spec), Positive Predictive Value (PPV) and Negative Predictive Value (NPV), Within normal limits (wnl), International normalized ratio (INR), Partial thromboplastin time (PTT)
^In the clinical trials, FSPs, not D-dimers, were measured and the data presented is for FSPs.[1]
*In Ruha's study, fibrinogen was combined (and/or) with PT/INR and fibrinogen data is for the combined analysis.[5]
§ The clinical trials did not include abnormal FSPs in their definition of hematologic abnormality and did not include platelet trend. The study by Ruha, et al did not include D-dimer or platelet trend.

Table 2
Cases with recurrent, persistent or late, new onset thrombocytopenia.

Case #	Lowest early platelets	Platelets w/in 4 h	>20% platelet increase	Lowest 4+ days platelets	Late Thrombocytopenia
8	25	146	Y	85	R
10	25	336	Y	61	R
5	59	237	Y	36	R
3	95	230	Y	102	R
4	47	ND	ND	138	R
23	112	ND	ND	56	P
2	154	243	Y	47	L
6	196	247	Y	91	L

Units: Platelets = x1000/uL.
Abbreviations: R=Recurrent; P=Persistent; L=Late, new onset; Y=Yes; ND=Not Done.

Table 3
Cases with recurrent, persistent or late, new onset (late) hypofibrinogenemia.

Case #	Lowest early Fibrinogen	D-dimer	Lowest 4+ days Fibrinogen	Late hypofibrinogenemia
3	<50	Positive	<50	R
9	133	Positive	54	R
4	83	ND	<50	P
7	252	Positive	134	L
5	272	Positive	121	L

Units: Fibrinogen = mg/dL.
Abbreviations: R=Recurrent; P=Persistent; L=Late, new onset; ND=Not done.

Table 4
Cases with recurrent, persistent or late, new onset increased INR or PTT

Case #	Highest early PT/INR	Lowest Fibrinogen	D-dimer	Highest 4+ days PT/INR	Any Late Abnormality
3	1.9 (INR)	< 50	Positive	8.8 (INR)	R
3	24 (PTT)	< 50	Positive	120 (PTT)	L
4	1.5 (INR)	83	ND	1.4 (INR)	P
5	1.3 (INR)	121	ND	1.4 (INR)	R
11	41 (PTT)	167	Positive	43 (PTT)	R

Units: Fibrinogen = mg/dL; INR = samples/normal; PTT = seconds
Abbreviations: International normalized ratio (INR); Partial thromboplastin time (PTT); R=Recurrent; P=Persistent; L=Late, new onset; ND=Not done.

Previously published: *Clinical Toxicology* (2011); 49: 324–329.

Keywords: *Crotalinae*, hematologic effects, recurrence
10.1016/j.toxicon.2012.04.070

70. Purification and Characterization of New Platelet Aggregation Inhibitor with Dissociative Effect on ADP-Induced Platelet Aggregation, from Protobothrops Venom in Japan

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Background: Snake venom disintegrins contain the RGD or KGD sequence in a homologous position, and inhibit the interaction between fibrinogen and the integrin αIIbβ3. The class P-III snake venom metalloproteinases (SVMPs) contain disintegrin-like, and cysteine-rich domains, and also inhibit platelet aggregation. Recent work has shown interaction between platelet integrins and disintegrin or cysteine-rich domains of P-III SVMP such as jararhajin [1] from *Bothrops jararaca* venom and atrolysin A [2] from *Crotalus atrox* venom. In this study, we report the purification and characterization of new inhibitors, with a dissociative reaction of ADP-induced platelet aggregation, from *Protobothrops* venom.

Methods: Proteins were purified by gel-filtration and ion-exchange chromatography. Platelet aggregation tests followed the turbidimetric method of Born [3]. The binding assay of fibrinogen and αIIbβ3 was performed by ELISA.

Results and Discussion: We purified three inhibitors, named as SV-PAD-1 (from *P. elegans* venom; [4]), PT-PAD (from *P. tokarensis* venom), PF-PAD (from *P. flavoviridis* venom). Three proteins showed a single protein band and these molecular weights were about 110 kDa on SDS-PAGE under reducing conditions. We suggest that these proteins are P-III SVMP because of these proteins showed enzymatic activity and those activities were completely inhibited by

EDTA. SV-PAD-1, PT-PAD, and PF-PAD strongly inhibited ADP-induced platelet aggregation. IC_{50} values of SV-PAD-1, PT-PAD, and PF-PAD were about 20-53 nM, and that of elegantin (from *P. elegans* venom; [5]) a disintegrin, was 300 nM. Furthermore, three inhibitors promptly dissociated platelet aggregation in rabbit platelet-rich plasma stimulated with 2 μM ADP. 50% dissociative concentrations of SV-PAD-1 and PT-PAD on ADP-induced platelet aggregation were 16 nM and 12 nM, however elegantin did not dissociate on platelet aggregation even at 1 μM. In the binding assay between fibrinogen and αIIbβ3, SV-PAD-1 and PT-PAD hardly inhibited fibrinogen binding to αIIbβ3, whereas only PF-PAD inhibited binding between αIIbβ3 and fibrinogen.

Conclusions: SV-PAD-1, PT-PAD, and PF-PAD are unique proteins from snake venoms that promptly dissociated ADP-induced platelet aggregation. We suggest that the mechanism(s) of action of these three inhibitors is different from that of elegantin and these inhibitors will be useful for the investigation of the mechanism of action of platelet dissociation.

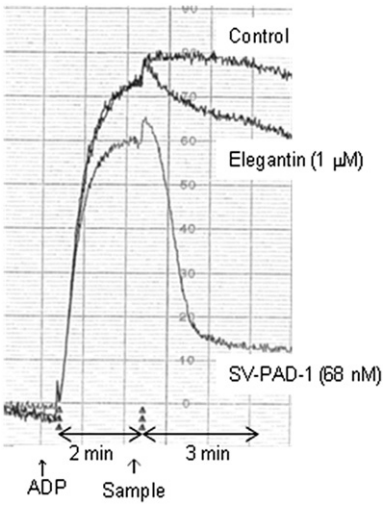

Fig. 1. Dissociation of SV-PAD-1 and elegantin on ADP-induced platelet aggregation. The experimental procedures and conditions were described in Methods.

References

Lambert L. J. et al., (2008) J. Biol. Chem. 283, 16665-16672.
Jia L. G. et al., (2000) Arc. Biochem. Biophy. 373, 281-286.
Born G. V., (1962) Nature 194, 927-929.
Oyama E. et al., (2009) Toxicon 53, 706-712.
Scaloni A. et al., (1996) Biochem. J. 319, 775-782.

Keywords: protobothrops venom, platelet aggregation, dissociation, purification
10.1016/j.toxicon.2012.04.071

71. Antiplatelet Activity and Mass Spectrometric Study of Venoms from Two Iranian Vipers, *Echis carinatus* and *Cerastes persicus fieldi*

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Background: Platelet aggregation inhibitory effect and anticoagulant properties of fractions separated from the venoms of *Cerastes persicus fieldi* and *Echis carinatus* were investigated.

Methods: The partial fractionation was performed on a Sephadex G-100 column.

Results: Two fractions separated from *Cerastes persicus fieldi* showed antiplatelet aggregation activity on ADP (200 μM)-induced platelet aggregation (ca 80% inhibition). The molecular mass of the most potent platelet aggregation inhibitor from Iranian *Cerastes persicus fieldi venom* was determined to be 4462 Da by LC-ESI-MS. Mass spectrometry of the whole venom of Iranian *Cerastes persicus fieldi* resulted in two major components with molecular masses of 564 and 1158 Da. Attempts to measure the antiplatelet aggregation activity of crude *Echis carinatus* venom and its fractions were not successful due to the protein coagulation of the plasma samples after addition of the venom. LC-MS study of the whole venom from Iranian *Echis carinatus* permitted us to detect a component with molecular mass of 13 kDa. Anticoagulant activities of the venoms were also evaluated. Total venom of *Echis carinatus* showed anticoagulant activity in PT test, while its fractions showed procoagulant activity.

Keywords: anticoagulant, antiplatelet aggregation, snake venom, *Echis carinatus*, *Cerastes persicus* fieldi, gel filtration, LC-ESI-MS
10.1016/j.toxicon.2012.04.072

72. A Novel Family of Factor Xa Inhibitors from the Salivary Gland of Sandfly Vector of Leishmaniasis, *Lutzomyia longipalpis.*

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Background: Salivary glands from blood sucking arthropods display a notable repertoire of anti-hemostatics, including vasodilators, platelet and coagulation inhibitors. Several distinct families of anticoagulants targeting FXa have been described, including Kunitz inhibitors, serpins, antistasin-like inhibitors, among others.

Results: We discovered that the main anticoagulant of the sandflies, L. longipalpis, and P. papatasi, is part of a novel family of FXa inhibitors, herein named Lufaxin, which does not display sequence similarity to any anticoagulant discovered so far, or protein deposited in the database. Lufaxin is a 34 kDa protein with 8 disulphide bridges, and was found to be a slow, tight, reversible and

specific inhibitor of FXa. Lufaxin blocks FXa catalytic activity and prothrombinase assembly, but does not inhibit thrombin, trypsin, FIXa, FXIIIa, tryptase, plasmin, FX or DEGR-FXa (catalytic site inhibited FXa). SPR revealed that Lufaxin interacts with FXa with high affinity (kD ~ 5 nM), while analytical experiments demonstrated that the interaction is stoichiometric. Notably, Lufaxin also prevents PAR2 activation in glioblastoma cell lines activated with FXa, and prevents paw edema formation triggered by FXa injection. Finally, Lufaxin potently inhibits thrombus formation in vivo (triggered by chloric ferric), promotes bleeding, and prolongs PT and aPTT ex vivo.

Discussion: Lufaxin is a novel family of FXa inhibitors, which displays both antiinflammatory and antithrombotic activity.

Conclusions: Its unique sequence represents a novel tool to understand the structure of FXa, or is a prototype to develop novel anticoagulants targeting FXa.

Keywords: Factor Xa, anticoagulants, blood sucking
10.1016/j.toxicon.2012.04.073

73. Novel Anticoagulants from Snake Venom

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Review: Anticoagulants prevent the formation of unwanted blood clots that can lead to heart attack or stroke and that result in a large number of deaths in developed countries. Currently available drugs have some drawbacks, including their non-specific actions. Therefore, novel anticoagulants that target specific steps in the coagulation pathway are being sought. Recently, we have isolated and characterized several anticoagulants from various snake venoms. These anticoagulants belong to the three-finger toxin family and exert their effects on the extrinsic pathway of the blood coagulation system. Despite similarity in overall three-dimensional structure, three-finger toxins represent an interesting family of anticoagulants as they are able to recognize various distinct targets in the blood coagulation system. Some of these toxins target the extrinsic tenase complex, whereas others target the prothrombinase complex. Further, they all exhibit their anticoagulant functions through distinct mechanisms. Here, we will describe the functional characterization of a few of these novel anticoagulants. We will also discuss the mechanisms of their anticoagulant functions. Understanding the structure-function relationships of these anticoagulant toxins will help in designing potential leads for future anticoagulation therapies and hence are of great interest.

Keywords: snake venom, three-finger toxins, anticoagulants
10.1016/j.toxicon.2012.04.074

74. Plant Latex Proteases: An Insight into their Procoagulant Activity

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Background: Latex, a milky fluid characteristic of certain angiospermic plant families, has been widely used from time immemorial as a hemostatic agent. There has been a lack of sufficient scientific and biochemical evidence on the mechanism of hemostasis and the present study was to characterize the procoagulant nature of latex proteases.

Methods: Latex from plants belonging to *Apocyanaceae*, *Asclepiadaceae* and *Euphorbiaceae* families were processed to get the protein-rich fraction, which was used as the protease source. The proteolytic assay was carried out using casein as substrate and the nature of proteases was substantiated using specific protease inhibitors. The effect of latex proteases on blood coagulation was determined using citrated plasma from healthy volunteers and from Hemophilia patients. Thrombin-like and Plasmin activity of the crude and purified protease was determined using purified fibrinogen and washed plasma clot respectively by spectrophotometric and electrophoretic methods. Pergularain e I, a "thrombin-like" cysteine protease from *Pergularia extensa* latex was purified to homogeneity using cation exchange chromatography. The homogeneity and molecular mass were determined by RP-HPLC and MALDI-TOF analyses. The cleavage site of Pergularain e I was identified by MALDI-TOF analysis using purified fibrinogen.

>Results: The protein-rich fractions of different latexes showed moderate to high proteolytic activity and hydrolyzed casein in a dose-dependant manner. The protease activity of Apocyanaceae and Euphorbiaceae was inhibited by PMSF and Benzamidine, whereas, IAA inhibited Asclepiadaceae latex proteases. Protease fraction from all the latexes exhibited procoagulant activity and decreased the plasma recalcification time in a dose-dependant manner. Asclepiadaceae family cysteine proteases possessed "thrombin-like" activity, enabling the clotting of plasma without supplementation of Ca^{2+}. Cysteine proteases also decreased clotting time in plasma from hemophilia patients. Pergualrain e I, a 23.356 K.Da basic glycoprotein contained about 20% carbohydrate. It cleaved fibrinogen between Arg and Gly, similar to thrombin.

Discussion: Proteases are abundant in plant latex and are responsible for the pharmacological effects of the latex. Any given plant family contains only one type of protease. The latex cysteine proteases exhibit procoagulant activity by Thrombin-like activity, whereas the mechanism of serine proteases is not clear. The clot inducing and dissolving activity of latex proteases is vital for their involvement in wound healing process. Pergularain e I acts similar to thrombin to release Fibrinopeptides A and B from fibrinogen.

Conclusion: The detailed study on characterization and molecular mechanisms of latex proteases will provide biochemical basis for the extensive use of latex as a hemostatic agent and will be of potential therapeutic use in thrombotic disorders.

Keywords: latex proteases, thrombin-like, hemophilia, fibrinopeptides
10.1016/j.toxicon.2012.04.075

75. The P-I Metalloproteinase from *Cerastes cerastes* Snake Venom Inhibits Human Platelet Aggregation

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Background: Snake venoms contain various metalloproteases that are highly toxic, resulting in a severe bleeding by interfering with the blood coagulation and by degrading the basement membrane or extracellular matrix (ECM) components. It has been suggested that hemorrhagic metalloproteases interact in a specific way with platelet surface proteins resulting in an alteration of platelet function. However, the exact mechanism of venom-induced hemorrhage is not yet fully understood.

Methods: The P-I class SVMP, CcH1, was purified from *Cerastes cerastes* venom by a combination of gel filtration, ion exchange, affinity and RP- HPLC chromatography. This SVMP CcH1 is a 25 kDa weakly hemorrhagic metalloproteinase causing a variety of local tissue damage. In this study, we report the functional characteristics of CcH1 on platelet aggregation and we evaluated the effect of CcH1 on extracellular matrix components (laminin and type IV collagen).

Results: CcH1 was able to preferentially hydrolyze the α-chain of fibrinogen and fibrin. Proteolytic activity of the enzyme was completely inhibited by metal chelating agents but not by other typical protease inhibitors. This enzyme principally degrades the laminin and type IV collagen dose-dependent manner. It is interesting; however, that CcH1 strongly suppresses ADP-induced human platelet aggregation in a dose-dependent manner.

Discussion: The hemorrhagic mechanism of CcH1 could be summarized as follows. i), CcH1 is able to hydrolyze fibrinogen key molecule in blood coagulation and platelet aggregation. ii), this molecule has proteolytic activity on extracellular matrix proteins which are critical components in structure-function of the vascular basement. It is possible to hypothesize that the metalloprotease domain of CcH1 could contribute to the hemorrhagic activity of the enzyme. Such hypothesis can be strongly supported by the reports that the class P-III metalloproteases usually exhibit more hemorrhagic activity than the class P-I metalloprotease containing only a metalloprotease domain.

Conclusions: the P-I metalloproteinase CcH1 presents a fibrinolytic activity and hydrolyze also laminin and collagen IV. This weakly hemorrhagic proteinase inhibits also ADP-induced human platelet aggregation. This study contributes to the understanding of the functional mechanisms of metalloproteinases. Furthermore, the weak hemorrhagic of CcH1, could be used as a therapeutic tool in the treatment and prevention of thrombotic diseases.

Keywords: snake venom, hemorrhagic metalloproteinase, platelet aggregation
10.1016/j.toxicon.2012.04.076

76. An Antiplatelet Peptide, Lahirin, from Indian Monocled Cobra Venom

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Background: A low Molecular weight (6.5 kDa) fibrinogenolytic peptide has been purified from the Indian monocled cobra (*Naja kaouthia*) venom and was named Lahirin. It is a α-fibrinogenase which cleaves the Aα chain of fibrinogen in a dose and time dependent manner. It also dissolved fibrin clots developed *in vitro*. Lahirin was found to inhibit platelet aggregation process in whole human blood.

Methodology: 1) Purification of Lahirin was achieved by repeated cation exchange chromatography. 2) Platelet aggregation studies of Lahirin was performed using chronolog whole blood aggregometer.

Results: Lahirin was found to inhibit ADP dependent platelet aggregation process in whole human blood in a dose dependent manner. However, Lahirin did not inhibit either collagen or ristocetin induced platelet aggregation. Thrombin induced platelet aggregation process was drastically slowed down by Lahirin treatment.

Discussions: Lahirin inhibits platelet plug formation by either binding with P2Y1/ P2y12 receptors or by digesting fibrinogen molecules binding platelets with each other to form platelet plugs. However, the rate of fibrinogen digestion by Lahirin is too slow to explain its fast platelet aggregation inhibition activity. As it is specifically inhibiting the G protein coupled receptor mediated platelet aggregation process i.e., ADP, Thrombin and Arachidonic acid, Lahirin might be a protease which either directly act on membrane receptors or interferes with the intracellular signaling molecules of the GPCR signaling pathway.

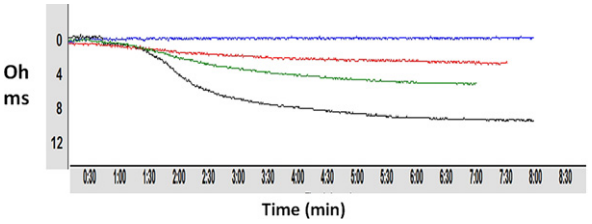

Fig. 1. ADP induced Platelet Aggregation process. **Blue** Negative control, **Black** ADP induced aggregation. **Green** ADP induced aggregation of 20 μg Lahirin treated blood. **Red** treated with higher concentration of Lahirin (40 μg).

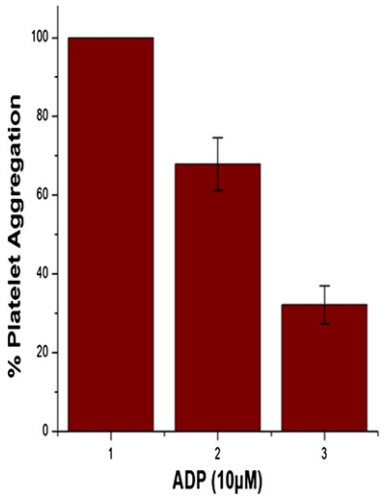

Fig. 2. 1) Control Human whole blood 2) 20 μg Lahirin treated blood and 3) 40 μg Lahirin treated blood (n=10).

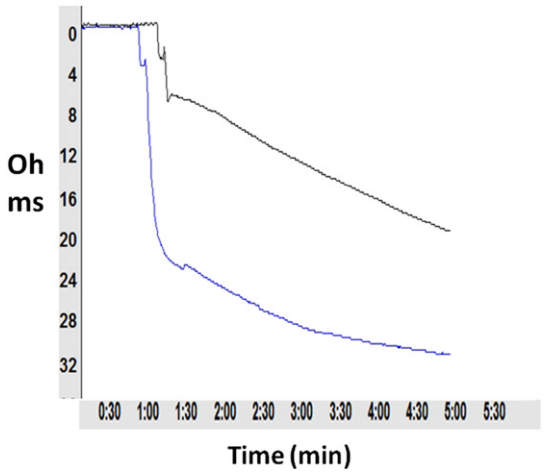

Fig. 3. Thrombin induced platelet aggregation process. Blue represents Control Human whole blood platelet aggregation after thrombin treatment. Black represents thrombin induced aggregation of Lahirin (40 μg) treated blood.

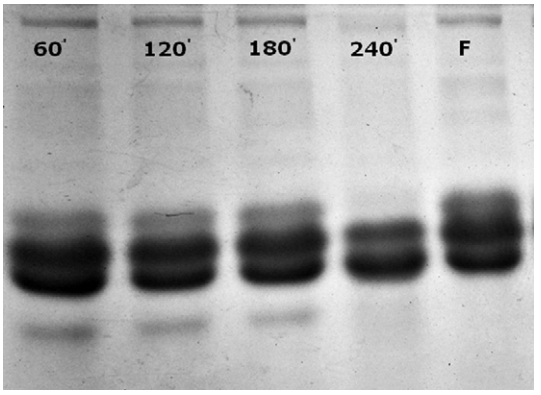

Fig. 4. Time dependent fibrinogenolytic activity of the Lahirin by 10% SDS-PAGE.

Keywords: fibrinogenolytic, antiplatelet, peptide
10.1016/j.toxicon.2012.04.077

77. Anticoagulant Activity of Moon Jellyfish (*Aurelia aurita*) Tentacle Extract

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Background: Biochemical and pharmacological profile of jellyfish venom is not well studied in jellyfishes found along the western coast of India. The venom is expected to contain novel pharmacologically active molecules of therapeutic importance. In this study, *Aurelia aurita* tentacle extract was investigated for its anticoagulant activity *in vitro*.

Methods: Fibrinogenolytic Assay, Fibrinolytic Assay and Platelet Aggregation Studies using Whole Blood Aggregometer.

Results: The Jellyfish Tentacle Extract (JFTE) showed very strong fibrinogenolytic activity by cleaving Aα and Bβ chains of fibrinogen molecule. JFTE completely liquefied fibrin clots. JFTE appears to contain several anticoagulant proteins or peptides. Some of which appear to be metalloproteases and some serine proteases. JFTE also inhibited ADP and collagen dependent platelet aggregation, in a dose dependent manner.

Discussion: Purification and characterization of the protein(s) responsible for anticoagulation is under progress.

Conclusion: JFTE affects the haemostatic system at three different levels: Platelet aggregation, Fibrinogen digestion and Fibrin clot digestion.

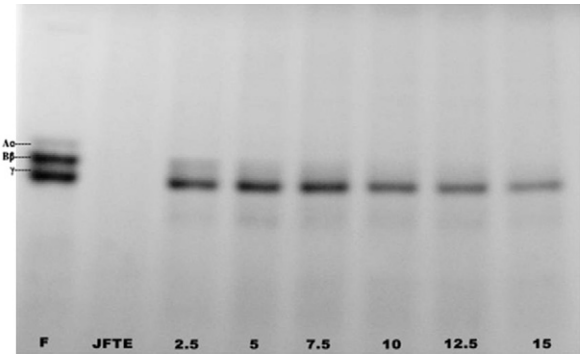

Fig. 1. Dose dependent fibrinogenolytic activity of JFTE. Fibrinogen (2 mg/mL) was incubated independently with different concentrations of JFTE at 37°C for 180 min. (F) Fibrinogen alone. (JFTE) JFTE alone. Numbers at the bottom of each lane indicates dose of JFTE in μg.

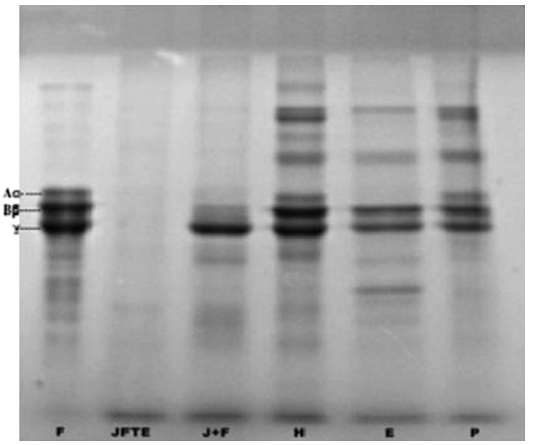

Fig. 2. Inhibition of fibrinogenolytic activity of JFTE by 12% SDS-PAGE. (F) Fibrinogen (2 mg/mL) alonge. JFTE (H) Fibrinogen incubated with 2.5 μg JFTE pre-treated with heat at 100°c for 1 min. (E) Fibrinogen incubated with 2.5 μg JFTE pretreated with 2 mM EDTA. (P) Fibrinogen incubated with 2.5 μg JFTE pre-treated with 1 mM PMSF.

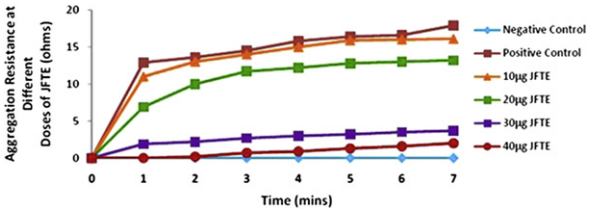

Fig. 3. Time depended inhibition of ADP-induced platelet aggregation by JFTE. Positive Control: Blood+ADP and Negative Control: Blood+0.85% Saline.

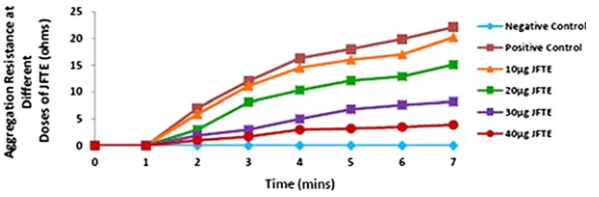

Fig. 4. Time dependent inhibition of collagen-induced platelet aggregation by JFTE. Positive Control: Blood+Collagen and Negative Control: Blood+0.85% Saline.

Keywords: Moon Jellyfish, anticoagulant activity, haemostasis
10.1016/j.toxicon.2012.04.078

78. Evaluation of the Efficacy of Treatment Using Bothropic or Bothropic/Crotalic Antivenin in *Bothrops jararacussu* (VIPERIDAE) Experimental Envenomation

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Background: Snakebites are a public health problem in Brazil. The most effective treatment is antivenin therapy and the effectiveness of Bothrops antivenin for the neutralization of Bothrops jararacussu venom (VBju) has been discussed by many groups. The aim of this study was to compare the efficacy of different antivenin treatments in the experimental envenomation induced by VBju.

Methods: Groups of mice (n=5) were injected i.v. with 2 x Minimum Defibrinogenating Dose (0,50mg/kg) of VBju, or saline (control), and treated with botropic (BA), crotalic (CA) and botropic/crotalic (BA/CA) antivenin, or saline, 1 hour (h) after venom inoculation i.v. Blood samples were collected in 3, 6, and 12 hs after treatment, by the orbital plexus. Plasma fibrinogen levels (PF) were determined and the thrombelastography (TE) was carried out on whole blood samples with the ROTEM® coagulation analyzer. The NATEM® test was used to assess native whole blood clot formation. Test parameters as clotting time (CT), clotting formation time (CFT), alfa-angle and maximum clotting Firmness (MCF) were acquired for 1 h. Statistical analysis *were* accessed by *one-way ANOVA and Tukey test*.

Results and Discussion: Regarding PF at 3 h, the animals envenomed and treated with different antivenin showed a huge variation in the groups. At 12 h, there were no statistical difference in the PF levels of groups treated with saline and that treated with antivenins, indicating that fibrinogen was spontaneously normalized, independently of the treatment. On the other hand, at 6 h, the animals treated with BA/CA showed significantly higher PF levels (1.89± 0.18g/L), when compared with those treated with BA (1.39±0.7g/L), CA (0.93±0.11g/L) or saline (1.17±0.08g/L). PF of all control groups were within normal range (2.48-3.49 g/L). Results of TE are in agreement with the PF obtained from different groups. Thus, at 3 h, most samples of animals injected with VBju remained unclottable, while at 12 h, the parameters were significantly restored. Moreover, at 6 h, the TE graphic profiles had demonstrated that animals treated with BA/CA had a good recovery, showing lower CFT (176.75±28.45 sec.), when compared with those of BA (350.66±83.38 sec.), CA (unclottable) and saline (434.25±159.01 sec.).

Conclusion: BA/CA treatment is better than BA in the B. jararacussu experimental envenoming in mice.

Financial support: INCTTOX, CNPq and CAPES.

Keywords: *Bothrops jararacussu* envenomation, treatment, coagulopathy
10.1016/j.toxicon.2012.04.079

79. Hemostatic Disturbances Evoked by Young and Adult *Bothrops jararaca* Snake Venoms: Analysis of the Envenoming Process and the Recovery after Specific Antivenin Treatment

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Background: Ontogenetic variations in the pro-coagulant enzymes of the young B. jararaca venom (YBjv) makes it more coagulant than that from adult snakes (ABjv).

Moreover, YBjv is not so efficiently neutralized by specific antivenin in vitro as ABjv. Herein we compared hemostatic disturbances induced by ABjv and YBjv in rats and assessed their recovery after treatment with Bothrops antivenin by thromboelastography (TE).

Methods: Male Wistar rats were inoculated with ABjv, YBjv (1.6 mg/kg, s.c), or saline, and treated with Bothrops antivenin (BA) or saline (100 μL i.v.) 1 hour (h) after venom inoculation. Blood samples of not treated rats were collected 3, 6 and 24 h after injection to evaluate platelet count (PLT) and plasma fibrinogen (PF). Thromboelastography (TE) was carried out on citrated (recalcified) and native (without anticoagulant) blood samples, collected 6 and 24 h after injection and treatment with BA or saline. Parameters as clotting time (CT), clot formation time (CFT) and alpha angle were acquired for 1 h.

Results: At 3 h, PLT count of ABjv and YBjv rats dropped, respectively, 13 and 3 times and PF levels fell abruptly (34.0 ± 2.0 and 42.0 ±8.0 mg/dL) in regard to controls (PLT 1058 ±48.0 x 10^9/L; PF 252 ±8.0 mg/dL,). At 6 h the PF levels still pretty low. Even so, it could be noticed a slightly recovery trend, more evident by the PLT count, which increased significantly (ABjv 217.0 ±89.0 and YBjv 306.0 ±51.0 x 10^9/L). At 24 h, the PLT count were much higher (ABjv 624.0 ±44.0 and YBjv 721.0 ±37.0 x 10^9/L), and the PF consumption level was partially reverted (ABjv 94.0 ±8.0 and YBjv 57.0 ±11.0 mg/dL). By TE analysis, at 6 h, it was noticed that the CT, CFT, alpha angle remain greatly altered on non-treated rats, especially in YBjv. At 24 h, these parameters were not totally normalized, but it was noticed a recovery trend, which is consistent with the PLT and PF data for this time.

Discussion: With the BA treatment, all the parameters were significantly restored especially at 24 h after the venoms inoculation. Thus, despite differences in enzymatic variations and *in vitro* neutralization potential among ABjv and YBjv, the treatment with BA was equally efficient in reversing the hemostatic disturbances caused by both venoms.

Financial support: FAPESP, CNPq and CAPES.

Keywords: ontogenetic variation, *Bothrops jararaca* snake, antivenin treatment, thromboelastography
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80. Purification of a Prothrombin Activator from *Bothrops moojeni* Snake Venom

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Background: Bothrops snake envenomation is associated with several local and systemic effects, in which coagulation disorders are responsible for one of the most severe manifestations. The involvement of venom proteases capable of activate blood coagulation factors and initiate the coagulation cascade, leads to blood in coagulability on patients. The aim of the present work was to isolate a prothrombin activator from *Bothrops moojeni* snake venom, and assess its ability for thrombin generation and coagulation activity.

Methods: The protease was purified in five chromatographic steps, in order, of a size exclusion chromatography on Sephacryl S-200 column followed by affinity chromatography in Blue-Sepharose, hydrophobic interaction in Phenyl-Sepharose, Mono Q ion exchange chromatography and bioaffinity chromatography using Lentil-Lectin Sepharose. The non-bonded fraction of the former procedure was analyzed in SDS-PAGE and represents the isolated protease. In order to evaluate the potential of the toxin on prothrombin activation, amidolytic and proteolytic assay were performed. The amidolytic assay was carried out incubating human prothrombin and colorimetric substrate S-2238 with several concentrations of the activator. Substrate cleavage by the thrombin generated was monitored by measuring the formation of p-nitroaniline. Assay was also performed after preincubation of the protease with 15mM of EDTA or benzamidine for 30 minutes at 25oC. For proteolytic assay, human prothrombin was incubated with the protease in a molar ratio of 15:1 at 37oC for different time periods. The analysis of prothrombin fragments were performed in SDS-PAGE under reduction condition. The involvement of *B. moojeni* activator on coagulation of normal and deficient of factor X human plasma was assessed by recalcification time using turbidimetric assay.

Results: The SDS-PAGE analysis of the purified protease shows a protein with ~90kDa under non-reduction conditions and ~70, 18 and 15 kDa domains in the presence of β-Mercaptoethanol. Concerning the protease prothrombin activation capacity, the amidolytic activity indicates that the toxin is able to generate active thrombin in a dose-dependent manner, being inhibited by EDTA but not benzamidine. Analysis of the proteolytic reaction showed complete consumption of prothrombin by the protease over the time interval, generating thrombin as major fragment. In addition, *B. moojeni* prothrombin activator was able to induce normal and factor X deficient human plasma coagulation.

Discussion and Conclusion: The present work describes the isolation of a possible PIIId class metalloprotease from the venom of *Bothrops moojeni* capable of cleaving prothrombin molecule, generating thrombin as major product, and inducing human plasma coagulation through coagulation factor II activation.

Keywords: snake venom, *Bothrops moojeni,* metaloprotease, prothrombin activator
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81. BaPLA2-IV, a New Type Thrombin Inhibitor, Non Cytotoxic Phospholipase A2 From *Bothrops atrox* Venom

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Background: Snake venoms are composed of biological active molecules that modulates several biological events such as blood coagulation by acting on physiological

components. Thrombin is a key enzyme that participates on blood clotting and cellular carcinogenesis. The present work intents to investigate the involvement of BaPLA$_2$-IV, an acidic phospholipase A$_2$ isolated from *Bothrops atrox* snake venom, on blood coagulation and cellular proliferation induced by thrombin.

Methods: The PLA$_2$ was isolated from *B. atrox* venom by three chromatographic procedure using size exclusion, ion exchange and reverse phase chromatography. The anticoagulant activity was performed by incubating thrombin with several concentrations of the PLA$_2$ and added of bovine fibrinogen as an initiator of the reaction. The recalcification time assay was assessed incubating different concentrations of PLA$_2$ in human plasma and monitored turbidimetrically at 650nm. The thrombin clotting time (TCT) was evaluated by incubing human plasma with different concentrations of BaPLA$_2$-IV with subsequently addition of thrombin, and the time for clot formation was measured visually. Prothrombin time (PT) and activated partial thromboplastin time (APPT) was performed by assay kits, in which several concentrations of PLA$_2$ was tested. To determine the effect of BaPLA$_2$-IV on platelet aggregation, the enzyme was pre-incubated with rich platelet plasma (PRP) and added of ADP (5μM) and collagen (5μg/mL), using aggregometer. In order to evaluate BaPLA$_2$-IV involvement on cell proliferation, several concentrations of the toxin was incubated with thrombin, and B16F10 cell line treated with the mixture. The proliferative activity was measured by MTT method.

Results and Discussion: BaPLA$_2$–IV, in low concentrations, prolonging the fibrin clotting time. The same activity was not observed with high concentrations PLA$_2$, in which reduced coagulation time. A similar effect was observed in recalcification time assay, showing that BaPLA$_2$-IV acts as an anticoagulant agent in low concentrations. The TCT assay showed that the toxin at 5μg/20μL was capable of reduce, in ∼57% clot formation induced by thrombin, prolonging the TCT time. Taking in account PT and APPT assay, at the same concentrations of experiment above, BaPLA$_2$-IV had no apparent effect on both assays. The PLA$_2$ was able to inhibit platelet aggregation induced by ADP and collagen in 82% and 80%, respectively. BaPLA$_2$-IV in concentrations from 0.01 to 1μg/mL was capable to completely inhibit cell proliferation induced by thrombin.

Conclusion: BaPLA$_2$-IV exerts an interesting response modulating thrombin activity in different biological responses, as blood clotting and cell proliferation.

Keywords: snake venom, *Bothrops atrox*, phospholipase A2, thrombin inhibitor.
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82. Anticoagulant Activity of Crotalic Venoms on Whole Blood Samples from Chickens and Rats

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Background: The venom of the rattlesnake *Crotalus durissus* has neurotoxic and myotoxic activities and also can cause coagulation disturbance. It is constituted mainly by crotoxin, convulxin, gyroxin and crotamine. This coagulation disturbance is mainly due to gyroxin, a serine protease with thrombin-like activity. Venom activities can vary according to toxin composition or substrate. A Western blotting analysis showed that crotalic venoms can present three different band patterns of gyroxin: two bands of 32.5 and 35.5 kDa, one strong or one weak band of 32.5 kDa.

Objective: To analyze if this polymorphism could result in differences in coagulant activity, we tested the effect of the three patterns of crotalic venom on plasma and citrated whole blood samples from different animal species.

Methods: Three pools of venoms: VP1 (two gyroxin bands), VP2 and VP3 (one strong and one weak band of 32.5 kDa respectively) were tested by determining the Minimum Coagulant Dose (MCD) on citrated bovine plasma, as well as by thromboelastometry (TEM) after recalcification of citrated whole blood samples from chickens and rats.

Results: MCDs on bovine plasma were 92.6, 80.7 and 164.7 μg/mL to VP1, VP2 and VP3 venoms, respectively. However, preincubation of all venoms at 300 ng/mL significantly prolonged the clotting time of recalcified chicken and rat whole blood samples ($p < 0.05$ using t-test). The observed prolongation of clotting time was about 3-4 times in chicken whole blood, whereas in rats was around 1.5 times.

Discussion and Conclusions: The MCDs of crotalic venoms on bovine plasma observed here are in agreement with reports published earlier. In addition, the variation of MCDs suggests an influence of gyroxin polymorphism on coagulant activity of these venoms, since, VP3 that presented the minor amount of gyroxin showed the highest MCD. Surprisingly, after preincubation of citrated whole blood samples from chickens and rats with calcium and VP1, VP2 or VP3, all venoms presented anticoagulant activities. As these venoms did not inhibit totally the blood coagulation, it indicated that there was no depletion of coagulant factors as fibrinogen or other components as platelets. The anticoagulant activities were dose-dependent but the mechanism of action is unknown and it was not explored here.

Financial Support: FAPESP, INCTTOX, PIBIC-CNPq.

Table 1
Clotting time obtained from the thromboelastometric profile after recalcification of citrated whole blood samples from chickens preincubated with crotalic venom.

ng/mL	VP1	VP2	VP3
PBS (Control)	(5)	(6)	(6)
	100% ± 20 %	100% ± 20%	100% ± 20%
3	(2)	(1)	(1)
	110.7 ± 27.2	111.2	99.1
30	(5)	(6)	(6)
	162.6 ± 55.7*	154.3 ± 60.6	132.5 ± 22.4*
	p = 0.045	p = 0.063	p = 0.025
300	(5)	(5)	(6)
	409.2 ± 208.2*	313.0 ± 66.7*	467.2 ± 239.6*
	p = 0.011	p = 0.000	p = 0.004

330 μL of citrated whole blood samples were preincubated with 20 μL of crotalic venom for 5 min at 37^{0}C and recalcified with 20 μL of $CaCl_2$ at 0.2M. Values are expressed as mean and standard deviation in %, considering the clotting time of samples treated with PBS (control) as 100% ± 20%; *, $p < 0.05$ = statistically significant in relation to control (Student`s *t* test); (), number of experiments.
Abbreviations: ng/mL, nanograms per milliliter; VP1, 2 and 3, pools of crotalic venoms; PBS, phosphate buffered saline.

Table 2
Clotting time obtained from the thromboelastometric profile after recalcification of citrated whole blood samples from rats preincubated with crotalic venom.

ng/mL	VP1	VP2	VP3
PBS (Control)	(6)	(6)	(6)
	100% ± 20 %	100% ± 20%	100% ± 20%
3	(6)	(6)	(5)
	116.1 ± 37.9	100.4 ± 16,7	113,4 ± 18,4
	p = 0.381	p = 0.974	p = 0.282
30	(6)	(5)	(5)
	128.9 ± 33.3	111,3 ± 21,5	126,5 ± 23,2
	p = 0.098	p = 0.388	p = 0.072
300	(6)	(5)	(5)
	154.8 ± 39,4*	131.9 ± 13.9*	127,7 ± 14,3*
	p = 0,013	p = 0.009	p = 0.029

330 μL of citrated whole blood samples were preincubated with 20 μL of crotalic venom for 5 min at 37^{0}C and recalcified with 20 μL of $CaCl_2$ at 0,2M. Values are expressed as mean and standard deviation in %, considering the clotting time of samples treated with PBS (control) as 100% ± 20%; *, p < 0,05 = statistically significant in relation to control (Student`s *t* test); (), number of experiments.
Abbreviations: ng/mL, nanograms per milliliter; VP1, 2 and 3, pools of crotalic venoms; PBS, phosphate buffered saline.

Keywords: gyroxin, thromboelastometry, minimum coagulant dose
10.1016/j.toxicon.2012.04.083

E. History of Toxinology

83. Clodomiro Picado, Róger Bolaños, and the Beginnings of Toxinological Research in Central America

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Review: Snakebite envenomings constitute an important public health issue in Central America. The scientific study of snakes, snake venoms, envenomings and their treatment in this region started with the pioneer work of Clodomiro Picado, an outstanding Costa Rican scientist who worked for three decades at the Clinical Laboratory of San Juan de Dios Hospital, in San José. Picado performed meticulous descriptions of the Costa Rican snake fauna and characterized the toxicity of venoms. In addition, he developed a collaboration with Vital Brazil, of Instituto Butantan, Brazil, which allowed the import of Brazilian antivenoms to Costa Rica. Moreover, he promoted a law which enforced land owners to have antivenoms for the treatment of workers bitten by snakes. In 1931, he published *Serpientes Venenosas de Costa Rica* (Venomous Snakes of Costa Rica), which summarized two decades of research. Thirty-five years later, a successful interinstitutional project, known as *Programa de Sueros Antiofídicos* (Antivenom Program), involving the Costa Rican Ministry of Health, the University of Costa Rica, and the Embassy of the United States of America in Costa Rica, with the leadership of Róger Bolaños, succeeded in producing, for the first time in Central America, antivenoms for the treatment of viperid and elapid snakebite envenomings. As a consequence, the Instituto Clodomiro Picado was founded in 1970, with Bolaños appointed as its first Director. Over the years, this institute, which belongs to the University of Costa Rica, has evolved as an active research, teaching, extension and production center which greatly contributes to a better understanding of Central American snakes and their venoms, to improving the treatment of envenomings, to graduate and undergraduate teaching, and to the prevention of snakebites in the region.

Keywords: snakebites, Costa Rica, Clodomiro Picado, Róger Bolaños, antivenoms
10.1016/j.toxicon.2012.04.084

84. The Poisoner's Garden: Plants Used to Curse and Kill

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Review: Since the early days of human civilization, poisonings have been a primary mode of murder, and although some victims were accidentally poisoned, those that are more easily remembered are the intentional murders of our wicked past. Although Dr. Hawley Crippen may have murdered for love, Dr. George Lamson murdered for money and Dr. Thomas Cream murdered for fun, they each used different toxins to do the deed and were successful until the police of the Scotland Yard caught up to them. Unrelated to these murderers, some victims of plant poisons include Socrates, Georgi Markov, Abraham Lincoln's mother, Nancy Hanks Lincoln, and the armies of Xenophon as reported by Pliny in 401 BCE. In the 1900's, Henri Girard Landru attempted to murder by mushroom to obtain insurance money from his friends and lovers, while Charles Cullen RN murdered patients with the use of many different pharmaceuticals, including digoxin, over the course of fifteen years before finally being caught. Although plant poisons now appear to be an accessory of the past, the long tradition of intentional poisonings have rekindled the intrigue and horrors of those rueful rendezvous for would-be-assassins of our modern era. Digitalis was most recently used by Lisa Leigh Allen to murder her cop husband in the US, and Lakhvir "Curry Killer" Singh, a spurned lover in the UK, murdered her ex-lover with aconite. Although rarely used today, plant poisons continue to be some of the most tragic and least implicated tools for a murderer or murderess at large.

Fig. 1. Thomas Cream, MD.

Fig. 2. Hawley Crippen, MD.

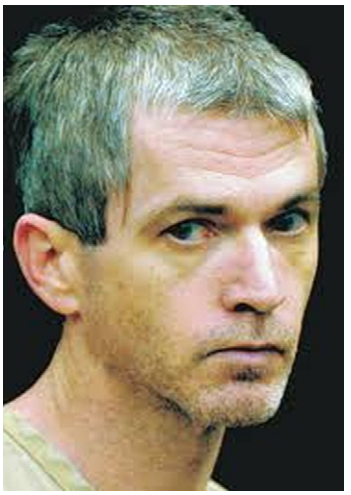

Fig. 3. Charles Cullen, RN.

Fig. 4. Nancy Hanks Lincoln.

Keywords: plants; toxins; poisoners; history
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85. TOXIN REVIEWS: The First 30 Years

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Review: During the last 30 years several toxin journals have come and gone, leaving TOXICON and TOXIN REVIEWS as the two enduring scientific journals in the toxin research field. Organization of TOXIN REVIEWS began in 1980 at the invitation of Maurits Dekker, a legendary figure in scientific publishing, co-founder of Interscience Publishers and father of Marcel Dekker. The journal was organized as one of three journals in the JOURNAL OF TOXICOLOGY series with CLINICAL TOXICOLOGY and CUTANEOUS AND OCULAR TOXICOLOGY. The series was intended to build on the strength of CLINICAL TOXICOLOGY. Publication by Marcel Dekker, Inc., out of offices on two floors of a building in Manhattan, New York City, began in 1982 at two issues/year. In 1989 (Volume 8) Anthony T. Tu, Colorado State University, was added as Co-Editor. In 1991 (Volume 10) the journal increased to three issues/year, and in 1994 (Volume 14) it increased to four issues/year. In 2002 (Volume 21) Dr. C. Yu, National Tsing Hua University, Hsinchu, Taiwan, was added as Associate Editor. Marcel Dekker, Inc. continued as the publisher of record through 2004 (Volume 23). After Marcel Dekker retired, his sons ran the company for a few years, then sold it in 2003 to Taylor & Francis, a Division of Taylor & Francis Group, an Informa company headquartered in London, UK. Taylor & Francis became the publisher of record in 2005 (Volume 24) from offices in Philadelphia, USA. The name of the journal was changed from JOURNAL OF TOXICOLOGY - TOXIN REVIEWS to just TOXIN REVIEWS, because their software did not have enough spaces for the whole name. Name changes are problematic because a journal's location in libraries (alphabetical order) changes and the journal gets split up in the stacks. In 2007 (Volume 26), the production offices were moved back to New York City and the name of the publisher of record was changed to Informa healthcare. In 2008 (Volume 27) the cover was redesigned. In 2009 (Volume 28) the Associate Editor position was replaced with Dr. Hamed Abbas, US Dept. of Agriculture and production offices were moved to London, UK. The publisher redesigned the cover again and changed the format to quarto so the journal would fit into their Pharmaceutical Sciences series. In 2010 some production office activities were moved to Sweden. In 2011 Drs. Shier and Tu stepped down, and Dr. R. Manjunatha Kini, National University of Singapore, was appointed Editor-in-Chief.

Keywords: TOXIN REVIEWS, journal, review
10.1016/j.toxicon.2012.04.086

86. From Medicine to Toxinology and Back to Medicine, and the Eternal Role of Snakes in Therapy

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Review: The author's work over three decades as an experimental pathologist involved multiple aspects of the complement system, from basic science to its role in disease, to an experimental concept of directing complement activation therapeutically, and to devise a novel concept of treating diseases with complement pathogenesis. Much of this work was based on the structural and functional homology of Cobra Venom Factor (CVF) to human complement component C3, resulting in a humanized CVF protein as a novel therapeutic agent. At the end, as it turns out, nothing is really new: as multiple scientific meetings on complement were held in Greece, the author had an opportunity to visit the ancient temple in Epidaurus, honoring Asclepius, the demi-god of medicine and healing (Fig. 1). In this healing facility – called the Asclepion, the ancient Greek equivalent of a hospital and spa – patients were treated using snakes, a procedure immortalized in the caduceus, the symbol of the medical profession to this very day (Fig. 2).

Fig. 1. Statue of Esclepius, in the museum at Epidaurus, Greece.

Fig. 2. Caduceus, symbol of the medical profession.

Keywords: Asclepius, Caduceus, snakes in therapy
10.1016/j.toxicon.2012.04.087

87. Antivenom Production by Instituto Butantan: 110 Years of Experience

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Instituto Butantan was founded in 1901 to combat plague. Doctor Vital Brazil was its first director and also a pioneer researcher on the toxicology of snake venoms. Currently, the Institute stands out in the public health sector for its scientific achievements and the production of vaccines and antivenom sera, being the largest biopharmacetical producer in Latin America. Its main outputs are vaccines, anti-venoms, anti-toxins, blood products and other biopharmaceuticals. Being a strategic research center, Butantan has partnerships with several public and private institutions for the development of new vaccines. The 170 researchers from Instituto Butantan published 325 scientific articles in international magazines with significant impact factors in 2010 alone. Among these studies, it can be highlighted those that screen new bioactive components of poisonous animals. Several patents have been filed in this area, indicating the innovative potential of the institute. Instituto Butantan also stands as science and technology repository for teaching with its three science museums specializing in biodiversity, microbiology and science history. This is one of the most visited public parks by foreign tourists in Sao Paulo. The importance and magnitude of Instituto Butantan as a center for public health is the result of an intense interaction between research,

production of much needed biopharmaceuticals and cultural activities, which are critical to maintaining its excellence. The Institute is prime example of the accomplishments of Latin American Science put the service of public health. It has endured the test of time, survived two dictatorships and came unscratched 111 years after its foundation as the most important Immunology Center in Brazil.

Keywords: antivenom, Instituto Butantan, immunology, vaccines, blood products, venomous animals
10.1016/j.toxicon.2012.04.088

88. Saul Wiener: From Kristallnacht to Toxinology and Fragile X.

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Background: The rise of Nazi Germany led to a tide of Jewish refugees many of who subsequently contributed to scientific advances in the Allied countries. For example, Ernest Chain fled Berlin in 1933 and undertook pioneering studies of Australian snake venoms at Oxford before moving onto penicillin. Similarly, Wilhelm Feldberg, dismissed from the Physiological Institute in Berlin in 1933, spent 1936-38 in Australia, examining the tissue responses to venoms, work that ultimately resulted in the identification of the leukotrienes. The subject of this presentation, the late Saul Wiener, made major contributions to toxinology and human genetics. This presentation reviews the life of this founding member of the International Society of Toxinology, who died in Melbourne on September 15, 2010.

Methods: This biography uses Wieners primary publications, secondary papers about his work, the literature on Jewish refugee doctors and scientists in Australia, as well as interviews with the subject, his wife and his colleagues.

Results: Wiener was born in Germany (July 25, 1923) to parents of Polish origin but migrated to Melbourne after Kristallnacht in 1938 and completed Medicine at the University of Melbourne in 1947. Soon thereafter he enrolled as a PhD student and studied Rheumatic Fever in the Department of Microbiology. His 1953 degree, made him equal second, as an Australian medical graduate achieving an Australian PhD. Thereafter, whilst employed as a research officer in the Commonwealth Serum Laboratories (1952-58), he developed the Redback spider antivenom and the world's first marine antivenom, against Stonefish. He also researched the funnel web spider, pioneered the study of *Chironex fleckeri* box jellyfish venom and first demonstrated the toxicity of fresh cone snail venom. In the late 1950s he also explored active human immunisation with snake venom. His 1960 MD thesis was probably the first higher degree in toxinology in Australia and included perhaps the first Australian toxinology publication in the journal *Nature*. After leaving CSL in 1958, Saul's interest in immunology led to a year as a Fulbright Scholar at Columbia University, where he developed new skills in chromosome analysis. Returning to Melbourne he commenced as a staff specialist (Allergist) at the Royal Melbourne Hospital. His research moved into cytogenetics, including some of the earliest work on Familial X-linked mental retardation ('Fragile X').

Conclusion: Like many Jewish German refugees, Saul Wiener contributed much to medical science, to his new home in Australia and thence to the world of broader world of toxinology.

Keywords: history, toxinology, antivenoms, venom research, Saul Weiner
10.1016/j.toxicon.2012.04.089

89. Cobra Venom Factor, an Intriguing Venom Component: Over 100 Years of Research, and Counting

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Review: Cobra Venom Factor (CVF) is the unusual venom component found in the venom of *Naja sp.* and some other Elapid genera, with most of our knowledge derived from the Asian cobras *Naja naja* and *Naja kaouthia*. CVF, strictly speaking, is not a toxin. CVF forms a bimolecular enzyme with prey complement component Factor B, called the C3/C5 convertase. The CVF-containing enzyme is stable and exhibits resistance to the complement regulatory proteins, allowing it to continuously activate complement components C3 and C5, leading to the release of the C3a and C5a anaphylatoxins, which are believed to facilitate the entry of the toxic venom components into the bloodstream through an increased vascular permeability. The anticomplementary activity of cobra venom was first described in 1903. In 1913, the anaphylatoxin-generating activity of cobra venom was described, at a time when the complement origin of the anaphylatoxins was unknown. After a relative dormancy of 50 years, CVF was purified as the responsible venom component for both activities in the late 1960ies. Subsequently, the molecular interaction of CVF with the complement system became understood, with CVF simultaneous playing an important role in unraveling the biochemistry of the alternative complement pathway. Subsequent milestones include the biochemical characterization of CVF (and its convertase (1982)), identification of its structural similarity to C3 (1984), molecular cloning (1994), recombinant expression (2004), and solution of its crystal structure, alone and in complex with Factor B (2009). The observation that CVF can be safely administered to laboratory animals, causing temporary complement depletion (1970), made CVF a widely used tool over the past 40 years to investigate the biological functions of complement in innate and adaptive immunity as well as its role in the pathogenesis of many diseases. Transgenic mice expressing CVF and a permanently low level of complement have been generated for the same purpose (2002). CVF was also coupled to monoclonal antibodies for targeted complement activation, such as killing of tumor cells (1982). More recently, human C3 derivatives have been created by replacing short stretches of sequence with homologous CVF sequence, forming stable convertases and depleting complement. These human C3 derivatives, termed humanized CVF, represent a novel class of experimental therapeutics that have been shown to be effective in multiple preclinical models of disease with

complement pathogenesis including paroxysmal nocturnal hemoglobinuria (PNH) (2008), myocardial ischemia reperfusion injury (2009), age-related macular degeneration (AMD) (2010), and myasthenia gravis (2011), with no adverse effects observed, including in primates (2010).

Keywords: cobra venom factor, complement
10.1016/j.toxicon.2012.04.090

F. Insects

90. Proteomic Analysis of the Molecular Targets of a Peptide from Wasp Venom Through Developing of an Analytical Platform

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Background: The organisms' answers to changes in their environment or even in their development generate intracellular signals which are translated, amplified and converted to physiological/ pharmacological answers toward the signals transduction system from plasma membrane. G-proteins are responsible for detection of specific and temporal characteristics of the majority of signal transduction mechanisms. Currently, more than 50% of medicines utilized in the world, act directly or indirectly, by activating or blocking the G-protein coupled receptors (GPCRs). Therefore, the subject of this study was the development of an affinity chromatography platform by using the mastoparan peptide Protopolybia-MP III, previously reported as specific ligand of GPCRs from rat mast cells.

Methods: The peptide Protopolybia MP-III (INWLKLGKAVIDAL-NH_2) was synthesized by using F-moc strategy and then, coupled to the chromatographic resin Sepharose 4B, in order to set up an affinity chromatography protocol; the column (12 x 2 cm, 15 mL) was built in TRIS-HCl buffer pH 8.0. Rat peritoneal mast cells were collected and lysed with NaCl 1M; the membrane extract was reconstituted into proteoliposomes of 300 nm of diameter. The elution of proteoliposomes suspension was carried-out at a flow rate of 0.5 mL/min and monitored at 280 nm; fractions of 1 mL were collected. The liganded proteins (under proteoliposome form) were removed under salt gradient from 0 to 1 M NaCl in the same equilibration buffer. The proteins eluted in each proteoliposome fraction were extracted with a solution of 0.02% (w/v) SDS and submitted to 1-D SDS-PAGE, stained with 0.025% (w/v) Coomassie Brilliant Blue and submitted to proteomic analysis. Protein profile showed five protein bands with molecular weights of 18 to 66 kDa, which were excised from gel, processed and sequenced by ESI-IT-MS/MS.

Results and Discussion: This experimental approach, associated to SDS-PAGE, *in gel* trypsin digestion and proteomic analysis, permitted the identification of five endosomal proteins: Rho GTPases Cdc 42 and the exocyst complex component 7- as components of the Ca^{+2} independent FcεRI-mediated exocytosis pathway; synaptosomal-associated protein 29 and the GTP-binding protein Rab-3D as components of the Ca^{+2} dependent FcεRI-mediated exocytosis pathway, which are related to cell signal transduction.

Conclusion: This novel analytical strategy permitted the identification of some GPCRs, promoting a better understanding of the mast cell activation by peptides from wasp venom.

Keywords: mastoparan; wasp venom; exocytosis; proteoliposomes; proteomics; mass spectrometry; affinity chromatography
10.1016/j.toxicon.2012.04.091

91. Proteomic analyses of the Venom from the Giant Ant *Dinoponera quadriceps*: A Comparative Study and Characterization of the Major Components of the Venom Derived from 4 Different Areas of Brazil

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Background: The order Hymenoptera, which embraces wasps, bees and ants, constitutes the largest group of venomous animals. Within the 120,000 already described species, the group of ants represents around 15,000 species. The Ponerinae subfamily is symbolized by the world's biggest ants (3-4 cm) and is found in subtropical zones of South America. In this group of ants, the genus *Paraponera* e *Dinoponera* reveals particularly remarkable venom describing medical interest. Their sting may produce acute pain, cold sweating, nausea, a vomiting episode, malaise and tachycardia. Moreover, peptides isolated from the genus *Paraponera* have been described as ion channel modifiers and antimicrobial toxins. Despite those reports, the information about the biological properties and composition of their venom is still very limited.

Objectives: To study the venom of the giant neotropical *Dinoponera quadriceps* ant collected in 4 geographically different regions of Brazil. By using combinatorial mass spectrometric approaches, we aim to: (i) characterizing the venom composition of these ants and (ii) establishing a comparative analysis of the venom from 4 geographically different regions in Brazil.

Methods: The ants were captured in the surroundings of Contendas, Manoel Vitorino, Caetite and Feira de Santana (Bahia, Brazil). Venoms were extracted by mechanical stimulation and then dried. An aliquot of each was analyzed by MALDI-TOF/TOF (Ultraflex II, Bruker Daltonics, Bremen, Germany) and ESI-Q-TOF (Synapt-G1, Waters, Manchester, UK) in direct infusion or with a liquid nanochromatographic separation (nanoACQUITY, Waters, Manchester, UK) step before the MS analysis. In

order to determine the cysteine content and the presence of disulphide bridges, an aliquot of the venom was reduced (dithiothreitol) and alkylated (iodoacetamide) before being analyzed.

Results: The combinatorial mass spectrometry analyses demonstrate that ant venom is a copious source of new natural compounds. Several peptides were identified and selected for "de novo sequencing". Regarding to the comparative study of the 4 regions, we observed slight differences in the expression of some peptides and even the absence of others. The analysis of the reduced and alkylated venom carried out until now revealed that the venom, as expected, did not hold peptides with cystines.

Conclusion: This study has reported for the first time the mass fingerprint of *Dinoponera quadriceps* venom derived from 4 areas of Brazil. Further characterization of the compounds is being made for a better understanding of its biological properties.

Keywords: proteomics, ant venom, MS
10.1016/j.toxicon.2012.04.092

92. Centipede Envenomation: 104 Cases from Bangkok, Thailand

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Introduction: This study describes epidemiology, clinical manifestations, and various treatments for envenomation by centipedes in patients presenting to an urban tropical emergency department in Bangkok, Thailand.

Methods: We retrospectively analyzed cases of centipede stings admitted to BMAMC/ Vajira Hospital, Bangkok, between 2004 and 2009; data were collected on demographics, local and systemic effects, treatments, complications, and disposition.

Results: There were 104 cases included in this study. 52% of patients were female. Mean age was 28 years (range, 1 month to 76 years). Most of the patients (77%) had no underlying diseases. Median time to ED presentation was 40 minutes (range, 15 minutes to 48 hours). Most injuries (85.9%) occurred between 6 pm and 6 am. Injury incidence was highest in April and May, and again in October through December. Envenomation sites were recorded in 96% (100/104) of patients; nine patients were stung more than once. Feet (29.4%) and hands (22.9%) were the most commonly injured sites. Local effects were common: 96% of patients reported localized pain, and 78% had localized swelling. Systemic effects were found in 12 cases (11%); symptoms and signs included nausea, vomiting, rashes, fever, systemic swelling, palpitations, abdominal pain, wheezing, chest tightness, flushing, and pruritus (see table). Anaphylaxis was diagnosed in 3 cases.

Patients were treated primarily for pain control, with 98.1% receiving analgesic drugs, and 33.7% treated with local anesthesia. Prophylactic antibiotics, tetanus immunization, antihistamines, and steroids were prescribed in 73%, 28%, 24.%, and 10% of cases, respectively. Return ED visits were recorded for 5 patients (4.8%), for worsening local effects, gastroenteritis, or vertigo. Only one case developed cellulitis and received intravenous antibiotics. There were no deaths.

Conclusions: Nearly all patients had local effects, while systemic effects were rarely observed and resolved with minor supportive care. The occurrence of hypersensitivity reactions in 3 patients suggests that, in a minority of patients, centipede venom may, like hymenoptera venom, be immunogenic. More than half of patients presented in the evening to an urban hospital within 1 hour of envenomation, confirming the synanthropism and nocturnal behavior of centipede species in urban Thailand.

Table: Systemic effects of centipede envenomations.

Systemic effects	Number (%)
Nausea	8 (7.7)
Vomiting	6 (5.8)
Rash	4 (3.8)
Fever	2 (1.9)
Systemic swelling	2 (1.9)
Palpitations	2 (1.9)
Abdominal pain	1 (1)
Wheezing	1 (1)
Chest tightness	1 (1)
Flushing	1 (1)
Itchiness without rash	1 (1)

Keywords: centipede envenomation; Thailand; venomous arthropods
10.1016/j.toxicon.2012.04.093

93. Mechanisms Implicated In Cell Proliferation and Cell Survival Induced By Recombinant Losac, A Cell Adhesion Molecule From *Lonomia oblique*

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Background: Losac is the first members of the Ig-like immunoglobulin superfamily exhibiting proteolytic function. It was purified from the bristle extract of the caterpillar of *Lonomia obliqua* (Lepidoptera). Previously, we have shown that purified Losac is capable to induce cell proliferation, cell survival and the liberation of hemostatic mediators in endothelial cells. However little is known about the mechanism and structural features implicated in these activities. As an initial step towards the functional characterization of Losac, we found the clone with the transcript coding for Losac through the screening of a bristle's cDNA library.

Methods: Recombinant Losac (rLosac) was expressed in *E. coli* BL21(DE3). Several cell cultures and methodologies were applied aiming to unravel molecular mechanisms modulated by rLosac. Cell viability and mitochondrial

metabolic functions were assessed using MTT assay. Cell cycle was analyzed by flow cytometry. Nitric oxide (NO) liberation was determined in cell supernatants according to the Griess reaction. The content of tissue plasminogen activator (t-PA) in the supernatant was measured with an ELISA kit. Cell signaling studies were analyzed by Western blotting.

Results: In HUVECs (human umbilical vein endothelial cells), rLosac elicits the same activities observed for the native Losac purified from bristles: cell proliferation, cell survival and liberation of NO and t-PA. Moreover, in a quiescent state, rLosac-induced cell proliferation was inhibited by staurosporine (a PKC inhibitor) but no by L-NAME (an inhibitor of eNOS) or LY294002 (a PI3K inhibitor). Western blotting analysis of quiescent cells showed the presence of phospho-p44/42 MAPKs in the basal state and an increase in their levels by rLosac treatment. In DRG (dorsal root ganglia) neurons, no morphological effects (in number and appearance) were observed after treatment with rLosac (10-200 nM). Instead, rLosac was able to significantly increase the mitochondrial function. This activity was inhibited by LY294002 and PD98059, both inhibitors of the PI3K and MAPK signaling pathways respectively. In fibroblast (FN-1), rLosac protected cells from serum-deprived apoptosis.

Discussion: All these results indicate that rLosac trigger cell viability through the activation of survival pathways in the early hours of cell contact with Losac and subsequent gene expression, DNA synthesis and proliferation.

Conclusion: A potential biotechnological application of rLosac is minimizing cell death of animal cell culture (production purposes) and consequently increasing the productivity.

Financial Support: FAPESP, CNPq, CAT-CEPID.

Keywords: Lonomia, cell adhesion molecule, cell proliferation, cell survival
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94. Bee Venom and Pain

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Review: In this presentation, I will summarize a 15-year history of the studies on the nociceptive effects of bee venom and its main active polypeptide melittin in mammals. In this series of studies, behavioral assays, electrophysiological recordings, pharmacological and neurochemical approaches were used. It has been revealed that melittin, as a unique algogen, on one hand, acts on phospholipase A2 (PLA_2) –lipoxygenases (LOXs) / cyclooxygenases (COXs) pathways, resulting in activation and sensitization of transient receptor potential vanilloid receptor 1 (TRPV1) by LOXs/COXs-mediated products in primary nociceptive sensory neurons. On the other hand, melittin can activate TRP canonical (TRPC) channels probably through diacylglycerol (DAG), potent $TRPC_{3/6/7}$ channels activator, produced by G-protein coupled receptors (GPCRs)-mediated phosphorylation of PLC that might involve ATP P_2Y receptors in the periphery. Activation of TRPV1 and TRPCs leads to tonic firing of primary nociceptive sensory neurons that results in long-term plastic changes (central sensitization) in the spinal dorsal horn where plays very important roles in driving persistent spontaneous nociceptive behaviors and pain hypersensitivity induced by subcutaneous injection of bee venom and melittin (mimicking bee sting injury).

Keywords: bee venom, melittin, pain
10.1016/j.toxicon.2012.04.095

95. Biological and Immunochemical Characterization of *Premolis semirufa* Caterpillar-Bristles Toxic Components

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Background: Pararama, the popular name of the larval form of the moth *Premolis semirufa* inhabits rubber plantations in the Amazon region and the accidental contact of the skin with the caterpillar's bristles or cocoons results in immediate and intense heat, pain, edema, and itching. In many cases a chronic inflammatory reaction with immobilization of the joints occurs. Despite being a serious problem in occupational medicine and a social problem affecting the Brazilian Amazon region, since the rubber tappers can no longer return to their activities, which are the source of their livelihood, studies on the pathogenesis of Pararama are scarce. Thus, the aim of the present study was to analyze the biological and immunochemical characteristics of *Premolis semirufa* caterpillar's bristles crude extract.

Results: Analysis of the bristles extract in *in vitro* assays revealed the presence of proteolytic and hyaluronidase activities but no phospholipase A_2 activity. *In vivo* assays, using mice, showed that the extract was not lethal, but caused significant edema and induced intense infiltration of inflammatory cells to the envenomation site. Furthermore, the bristles components stimulated an intense and specific antibody response but autoantibodies such as anti-DNA or anti-collagen type II were not detected.

Conclusions: Together, these data show the existence, in the *Premolis semirufa*'s bristles extract, of a mixture of different enzymes that may be acting together in the generation and development of clinical disease manifestations. Moreover, this study demonstrates the production of high antibody titers in mice inoculated with the extract, which may also contribute to the genesis of the inflammatory reactions observed in the envenomation. The absence of autoantibodies indicate that the molecular mechanisms causing disease after multiple contact with the *Premolis semirufa*'s bristles differ from that observed in chronic synovitis, such as the rheumatoid arthritis. The bristles toxic action, high antibody response with the formation of immune complexes and complement

activation may also play a role in the establishment of the disease. These aspects will be further investigated in future studies.

Keywords: *Premolis semirufa*, Pararama, caterpillar, inflammation
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96. Chemical and Biological Characterization of a Novel Neuropeptide in the Venom of Solitary Digger Wasp

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Background: Solitary wasps are known to inject their venoms into insects or spiders, paralyzing the prey in order to feed their larvae. Therefore, the solitary wasp venoms should contain a variety of neurotoxins acting on nervous systems. In fact, polyamine toxins, peptide neurotoxins and a protein paralyzing toxin have so far been found in several solitary wasp venoms. Besides the neurotoxins, we have found that cytolytic peptides are also present in the solitary wasp venoms. In our continuing survey of biologically active substances in solitary wasp venoms, we found FMRFamide-related peptides in the venoms of the solitary digger wasps *Sphex argentatus argentatus* and *Isodontia harmandi*. Reported herein are the isolation, sequencing and biological evaluation of these neuropeptides.

Methods: The venom extracts with 50%MeCN/H_2O/0.1% TFA were purified by reverse-phase HPLC, and the isolated peptides were chemically characterized by mass spectroscopy**.**

Results: Analysis of MS/MS spectra by MALDI-TOF/TOF revealed the sequence of a novel peptide as EDVDHVFLRF-NH_2, which is highly homologous to leucomyosupressin (pQDVDHVFLRF-NH_2) and SchistoFLRFamide (PDVDHVFLRF-NH_2), the FMRFamide-related neuropeptides from cockroach and locust, respectively. Indeed, this new peptide inhibited contraction of the locust oviduct at 50 nM with the same extent as SchistoFLRFamide. A non-amidated peptide (EDVDHVFLRF) was also isolated, but this showed no activity in the locust oviduct contraction.

Conclusions: This is the first example of FMRF-related neuropeptide to be found in solitary wasp venom.

Keywords: solitary wasp venom, neuropeptide, FMRFamide-related peptide
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97. Insulin-Binding Protein-Like Venom Protein of the Solitary Hunting Wasp, *Eumenes pomiformis* (Hymenoptera: Eumenidae)

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Background: Insulin/insulin-like peptide-binding protein (IBP) and its transcript are abundantly found in *Eumenes pomiformis* venom and the venom gland/sac-specific EST library, respectively. *E. pomiformis* IBP (EpIBP) is most similar to insect IBP-like proteins that are known to inhibit insect growth and insulin signaling. To investigate the toxicity of EpIBP, *in vivo* expressed EpIBP was injected into lepidopteran larvae.

Methods: EpIBP was expressed by *Escherichia coli*. EpIBP in the inclusion body was unfolded and then refolded. The third instars of *Spodoptera exigua* were fixed in an incised silicone tubing, and the expressed EpIBP was injected into the abdominal cavity (0.5 μg/0.5 μl/larva) using a nano-injector and a glass capillary.

Results and Discussion: *S. exigua* larvae that were injected with the *in vivo* expressed EpIBP showed a 20% lower pupation rate than the control larvae at the fifth day after the injection, although their body weight was not significantly different to the control when the larvae were provided artificial diet after the injection. EpIBP extended the larval stage without inducing paralysis of *S. exigua* larvae. To investigate the effects of EpIBP on caterpillar under a starvation condition, survivorship and body weight of the EpIBP-injected larvae that were not provided artificial diet after the injection were observed until all the tested larvae died. The survivorship of the EpIBP-injected larvae was 24% and 36% higher than the control larvae at the fourth and fifth day after the injection, respectively. The body weight of the injected larvae also showed a statistically significant difference under the starvation condition compared with the control. At the fourth day after the injection, the body weight of the control larvae reduced to 59%, which is approximately 10% lower than that of the EpIBP-injected larvae. It has been reported that IBP of *Drosophila melanogaster* (Imp-L2) and its homolog Sf-IBP from *S. frugiperda* repress the insulin/insulin-like peptide signaling pathway. These results suggest that EpIBP might inhibit the metabolism of the caterpillars, which is likely related with the insulin-like peptide signaling pathway, suppress the loss of body weight and eventually extend the larval stage.

Conclusion: This finding suggests that the large amount of IBP in *E. pomiformis* venom likely functions to suppress the larval growth of prey and to allow the prey to endure starvation under paralysis conditions.

Keywords: venom, insulin-binding protein, growth regulation
10.1016/j.toxicon.2012.04.098

G. Marine

98. Transcriptomic and Peptidomic Characterisation of the *Conus marmoreus* Venom: Insights on Conopeptide Diversity and Venomic Processing

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Background: Next generation sequencing technologies are revolutionising venom peptide discovery with an unprecedented speed and coverage. As of today, 3

transcriptomes of cone snail venom ducts have been published, and surprisingly only a limited number of genes encoding these bioactive peptides (< 100 per species) were recovered for each species. Therefore, there is a striking disparity between the number of transcriptomic sequences and the number of peptides (~ 200 to > 1,000) reported per venom from MS studies. In the present study, we have integrated for the first time next generation sequencing with proteomic technologies to comprehensively define the conopeptide composition of the venom from a single species of Conidae (*Conus marmoreus)*.

Methods: A total of 92 conopeptide precursor sequences from 16 gene families (Superfamilies) were extracted from transcriptomic data using sequence similarity search tools and phylogenetic analyses.

Results: Using the highly sensitive 5600 TF MS instrument, we identified > 3000 unique peptides from one single LC-ESI-MS/MS run. All conopeptides derived from transcriptomic sequences could be matched to mass spectrometry data at the MS level within 100 ppm accuracy, from which 69 (75%) had series of partial or complete MS/MS coverage confirming these matches.

Conclusions: This study reveals that conopeptide diversity arises from a more limited set of genes, mainly through alternative cleavage sites, residue-specific post-translational modifications, and selective N- and C-terminal truncations. This integrated strategy is expected to accelerate the discovery of drug leads from cone snail venoms.

Keywords: conopeptide, transcriptomic, proteomic
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99. The Venom of *Conus geographus* Revisited

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Background: *Conus geographus* deservedly has a reputation as the most dangerous of all cone snails, with reported human fatality rate as high as 65 % . While crude venom gland extracts have been used for the past 50 years to determine animal LD_{50} and isolate some potent paralytic toxins, the venom actually injected by *C. geographus* into its victim has never been studied. We hypothesised that the injected venom may prove different from the crude venom extracted from the venom duct, as recently demonstrated for other cone snail species (1-3).

Methods: To test this hypothesis we developed for the first time a milking procedure that allows venom collection from this species. Venom samples were then analysed using LC-MS methods.

Results: There was a linear correlation between the volume and dry weight of venom injected and the size of the shell. We also report on the molecular composition and intraspecific variations of the injected venom, and demonstrate significant differences between injected and dissected venoms from the same individual. Finally, extrapolation of our data to an historic fatal case of *C. geographus* envenomation allowed the determination of the human lethal dose, which may help in the management of future victims.

Conclusions: The injected venom of *C. geographus* proved to be a highly complex mixture of mainly small molecular weight compounds. Significant intraspecific variations were noted, as well as major differences between milked and dissected venoms. Since it is directly relevant to human envenomations, further research on the injected venom is needed to uncover the associated lethal biological effects.

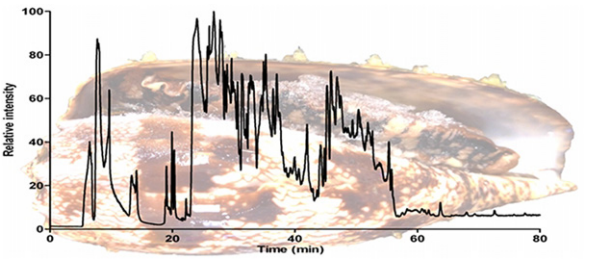

Fig. 1. Relative intensity (%) v. Time (min).

References

Biass, D., Dutertre, S., Gerbault, A., Menou, J. L., Offord, R., Favreau, P., and Stocklin, R. (2009) Comparative proteomic study of the venom of the piscivorous cone snail Conus consors. *J Proteomics* **72**, 210-218.
Dutertre, S., Biass, D., Stocklin, R., and Favreau, P. (2009) Dramatic intraspecimen variations within the injected venom of Conus consors: an unsuspected contribution to venom diversity. *Toxicon* **55**, 1453-1462.
Jakubowski, J. A., Kelley, W. P., Sweedler, J. V., Gilly, W. F., and Schulz, J. R. (2005) Intraspecific variation of venom injected by fish-hunting Conus snails. *J Exp Biol* **208**, 2873-2883.

Keywords: *Conus geographus*, proteomics, injected venom
10.1016/j.toxicon.2012.04.100

100. The Nematocyst Venom of Hydra: What is it Composed of and How Did it Evolve?

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Background: The genomes of cnidarians encode many putative toxins. However, to date, it is not clear which of these toxins are injected through the nematocysts and which are found in other tissues. Here, we characterize the nematocyst venom of the model cnidarian *Hydra magnipapillata* and trace the genomic underpinnings of the venoms evolution.

Methods: We identified soluble components from isolated nematocysts of *H. magnipapillata* using shotgun tandem mass spectrometry. Bioinformatic analyses were performed to compare the identified venom components

with the arsenal encoded in the *Hydra* genome, and the biological activity of selected proteins was verified by recombinant expression.

Results and Discussion: We identified 162 proteins in the soluble fraction of *Hydra* nematocysts, of which 25 encode putative toxins. These include potential neurotoxins affecting K^+ channels, phospholipase A2s (PLA2s) and several groups of hemolysins, but no orthologs of the well-studied anemone peptide neurotoxins affecting Na^+ channels. Venom proteins seem to have evolved through several distinct mechanisms: the actinoporin gene family diversified strongly in Hydra, with at least two of six members maintaining hemolytic activity despite significant changes in sequence compared to similar toxins from sea anemones (e.g. equinatoxin 2). In contrast, type III PLA2s represent an ancient gene family of which several members were recruited to the venom, with the venom isoforms from distantly related organisms revealing signs of convergent evolution likely representing similar selective pressure. Finally, several venom proteins contain an ShK domain similar to K^+ channel toxins, as well as other domains associated with venom in multiple organisms such as CRISP, C-type lectin and Zn-metalloprotease. In all cases, the ShK domains are encoded by a separate exon, suggesting a mechanism whereby exon shuffling provides a "venom recruitment shortcut": secreted proteins into which these exons are integrated will potentially bind K^+ channels found on excitable tissue – a major target for neurotoxins. Our results paint a dynamic picture of the evolution of cnidarian venom and highlight a potential mechanism for generating multi-functional venom proteins from the available pool of molecular "building blocks".

Keywords: Hydra, nematocysts, mass spectrometry, exon shuffling, gene duplication, convergent evolution
10.1016/j.toxicon.2012.04.101

101. Manipulation of Zoanthids in Home Marine Aquarium: Unexpected Cause of Intoxication Involving a Whole Family

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Background: A new danger has recently been identified in marine aquaria as highly toxic Palythoa zoanthids have entered the home marine aquaria trade. The Dutch Poisons Information Center was recently involved in a palytoxin poisoning of all members of one family.

Case report: Two adults and 2 teenagers were presented at the emergency department with nausea, headache, fits of dry cough, shortness of breath, chest pain, tachycardia and fever. Approximately three hours earlier, the father attempted to destroy a zoanthid colony (about 10 polyps) using boiling water. Immediately a foul odour was noticed and within one hour, all members of the family experienced signs of intoxication. All victims were hospitalised. On examination, a decreased PO_2 saturation was noticed and elevated leukocyte count was found. Repeated chest radiogram showed no infiltrates. Treatment consisted of O_2 administration, nebulised salbutamol and acetaminophen. During the following days, symptoms gradually subsided and all patients were discharged 2 days later.

Results: Analyses of the Dutch Poisons Information Center database revealed 7 previous cases related to presumed palytoxin exposure after handling/elimination zoanthids in home marine aquaria. The first consultation was in 2006. In five cases symptoms developed after dermal/parenteral contact and in 2 cases after inhalation of aerosols. Symptoms varied from dizziness, nausea, headache, shivering, cold-warm sensations, malaise, paresthesia, numbness especially of the hands/arms, to mild to moderate dyspnoe and myalgia. Contact dermatitis was seen after dermal contact.

Discussion: At present, intoxications by palytoxin are identified on the basis of symptoms. Identification of palytoxin in serum/urine of the patient or in zoanthids is very difficult. The major concern is finding a lab capable of analysing the samples.

Conclusions: As highly toxic Palythoa zoanthids have entered the home marine aquaria trade in Europe including the Netherlands, Poisons Information Centers need to be aware of the circumstances under which palytoxin can induce intoxications. Efforts should be made to be able to identify the source of intoxication, using human and zoanthids samples.

Keywords: zoanthid, intoxication, aquaria
10.1016/j.toxicon.2012.04.102

102. Convergent Evolution of Sodium ion Selectivity in Metazoan Neuronal Signaling

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Review: The ion selectivity of metazoan voltage-gated Na^+-channels is critical for neuronal signaling and has long been attributed to a ring of four conserved amino acids (DEKA) that constitute the ion selectivity filter at the channel pore. The sea anemone *Nematostella vectensis* is a member of the early-branching venomous metazoan phylum Cnidaria and has five genes encoding Na^+-channel homologs. Expression and characterization of these channels in *Xenopus* oocytes revealed that four of them preferably conduct calcium ions, whereas one channel is Na^+-selective despite a non-consensual DKEA selectivity filter. Mutagenesis and physiological assays indicated that pore elements additional to the selectivity filter determine in this channel the preference for Na^+ ions. Phylogenetic analysis shows that Na^+-channel homologs have already been present in unicellular organisms and are found in most animals, but vertebrates. Na^+-selective channels bearing the DEKA selectivity filter appeared first in the

urbilaterian and exists in most 'higher' animals (e.g., mollusks, insects, chordates). The Na^+-selective channel of *Nematostella* is clustered within a channel group unique to Cnidaria, which diverged >500 million years ago from Ca^{2+}-conducting Na^+-channel homologs. The identification of cnidarian Na^+-selective ion channels distinct from the channels of 'higher' animals (Bilateria) indicates that selectivity for Na^+ in neuronal signaling emerged independently in these two animal lineages, and might have answered needs of higher complexity and faster, more accurate neuronal signaling that would be separated from intracellular signaling mediated by Ca^{2+} ions. These findings change the common view about the evolution and molecular requirements for Na^+ ion selectivity.

Keywords: Na+-channels, ion selectivity, evolution
10.1016/j.toxicon.2012.04.103

103. Emergent Marine Toxins in Europe: New Challenges for Scientists and Regulatory Authorities

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Review: The most common HAB (Harmful Algae Bloom) toxins are regulated and monitored in European countries, which has decreased the risk of shellfish consumption regarding PSP (Paralytic Shellfish Poisoning), ASP (Amnesic Shellfish Poisoning) and DSP (Diarrhoeic Shellfish Poisoning). The monitoring of these toxins in bivalves is established since many decades ago, but new toxin vectors are being found, including gastropods and equinoderms, that are edible but not regulated. Other emergent toxins such as cyclic imines (spirolides, gymnodimines, pinnatoxins and pteriatoxins) are being studied but their toxicity and prevalence is not known and there are no regulatory limits in Europe or anywhere in the world. There are also increasing reports on other emergent marine toxins of tropical origin such as tetrodotoxin (TTX), ciguatera (CTX) and ovatoxin and palitoxin analogues (PTX) in European waters. Most of these are very toxic by oral route attaining high levels in vertebrates or invertebrates that enter human food chain. The existence of migratory routes for marine species via Suez Canal and the global warming effects may be some of the causes accelerating these processes. Recently, the production of the neurotoxic amino acid β-N-methylamino-L-alanine (BMAA) by marine and estuarine cyanobacteria isolated from Portuguese coastal environments has called our attention due to the potential implication on human neurodegenerative diseases. There is evidence that BMAA may also be taken up by food chains in the Baltic Sea, with the highest levels in the brain and muscle of bottom dwelling fishes. We detected BMAA in several strains of cyanobacteria isolated from estuarine environments in levels up to 63 μg/g dry weight. BMAA should be further investigated namely in what concerns the main vectors and toxin levels to perform an integrative risk analysis. In this work, we focus on the emergent marine toxins detected in European waters giving special attention to those that have serious implications in human health. The most recent chemical and biological analytical methods available enable us to detect lower amounts of toxins in more complicated matrices. Chronic toxicity data is needed in order to allow the establishment of Tolerable daily intake (TDI) for some of these “new” toxins. The seasonal and geographical occurrence of these toxins should be cleared so as to allow us to implement accurate risk analysis and help regulatory authorities to establish new guideline values.

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Keywords: emergent toxins, marine environment, Europe
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104. New Actinoporin from the Northern Pacific Sea Anemone *Urticina crassicornis*

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Background: Actinoporins are well known 20 Kda basic cytolytic proteins that had been isolated from about 30 species of sea anemones. We report on isolation, primary sequence and initial characterization of novel actinoporin family member from *Urticina crassicornis*.

Methods: living sea anemones were milked and their exudate concentrated. Afterwards, proteins in the exudate were separated by the means of gel and ion-exchange chromatography. Fractions were analyzed by SDS-PAGE and had their hemolytic activity determined via a turbidimetric method. The fraction containing pure cytolysin named UcT 1 was subjected to N-terminal sequencing. RACE-ready cDNA was prepared from the isolated mRNA using the GeneRacer Kit (Invitrogen), after which 5’ and 3’ rapid amplifications of cDNA ends (RACE) were performed. Primer and the degenerate primer corresponding to the amino acid sequence obtained by Edman degradation of the wild-type UcT were used. The constructs were amplified by PCR. Products of the PCR reaction were separated by Mini agarose electrophoresis and the band of interest was extracted from the gel and cloned with the GeneJET PCR Cloning Kit (Fermentas), using *E. coli* DH5α chemically competent cells. Positive clones were determined by restriction analysis and sequenced. Primers used for 5’RACE were the gene specific reverse primer TGCACGGCTGACACGAATTTGC and the GeneRacer 5’ Primer, both in 1 μM final concentrations. PCR was performed with the same reaction mix as for the 3’RACE reaction. All obtained sequences were processed, analyzed and aligned with the Vector NTI software package (Invitrogen). The isoelectric

point for the obtained protein was determined and its insertion into lipid monolayers was studied.

Results: UcT I shows a typical actinoporin sequence largely identical to EQT II the most studied family member. UcT I is a strongly basic protein (I.p. 9) with potent hemolytic activity. Experiments with lipid monolayers revealed that UcT 1 requires a mixture of SM:DOPC in order to insert itself into the membrane.

Conclusion: New cytolysin from *U. crassicornis* share common functional characteristics to proteins belonging to actinoporin family and share a large degree of identity and homology to EqT II.

Keywords: sea anemone, toxin, cytolysin, actinoporin
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105. Discovery, Characterization, and Functional Implications of Conotoxins from Cone Snails Species of the Americas

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Background: Cone snails are among the most prolific and versatile peptide engineers known. Their venom is a complex concoction composed of modified peptides (conopeptides) that elicit a wide range of neurophysiological responses. The biochemical strategy developed by cone snails to target the multiplicity of neuronal receptors has provided us with a natural library of toxins with great potential therapeutic uses, including the first FDA-approved drug of marine origin, Prialt™. The expression of conopeptides is species-specific, with significant intraspecies and intraspecimen variations. Accordingly, more than 2,000 conopeptides/species can be expressed yielding an enormous library of bioactive compounds. We have concentrated our research efforts in exploring the venom of the 200+ Conus species of that inhabit the waters of the Americas (Western Atlantic and Eastern Pacific regions, respectively).

Methods: Here we described the discovery of conopeptides by using either biochemical-based approaches or efficacious bioassay-guided methods. Specifically, we have used SE and RP-HPLC to isolate nanomolar quantities of novel conopeptides, such as bru1a, bru3a, bru9a and RegIIA. Their sequences were determined by Edman degradation. Testing of these conotoxins included two-electrode voltage clamp recording on nAChRs subtypes expressed in *Xenopus laevis* oocytes. Complementary to this approach, we use *in vivo* electrophysiological measurements to evaluate the effect of conopeptides fractions on the functional outputs of a well-characterized neuronal system in *Drosophila melanogaster* known as the giant fiber circuit (GFC).

Results: These new a-conotoxins have unique selectivity profiles; i.e., RegIIA is a potent inhibitor of a3b4 nAChRs, without blocking the a4b2 subtype. This feature makes RegIIA a suitable probe to evaluate the machinery involved in nicotine addiction. While structurally related to other a-conotoxins, RegIIA has an exquisite balance of shape, charges, and polarity exposed on its structure that enable inhibition of a3b4 nAChRs. A novel a4/3 conotoxin, bru1b, was discovered using GFC approach, and it was further characterized using voltage clamp measurements on a panel of nAChRs subtypes expressed in *Xenopus* oocytes. Additionally, we will describe a new set of mini-M conotoxins of the m1-m3 subclasses. These conotoxins exhibit a remarkable level of structural diversity in their posttranslational modifications, size of intercystine loops and overall 3D structure. One of these mini-M conotoxins, bru3a, is active in the GFC indicating a potential target for these conotoxins.

Conclusions: These novel conopeptides add further molecular diversity to the *Conus* biochemical strategy for predation and are of particular interest as shown to have novel functional implications within known conotoxin superfamilies.

Keywords: conotoxins, nAChRs, oocytes, neuronal circuits, ion channels
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106. Molecular Diversity of Box Jellyfish Toxins

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Review: Box jellyfish (cubozoans) are renowned for their ability to immobilise and kill prey and inflict painful and debilitating stings to humans by injecting potent venoms from their nematocysts. *Chironex fleckeri* is the largest species of box jellyfish and its venom produces extremely rapid and potentially life-threatening effects. Advances in box jellyfish toxinology using bioactivity-guided purification methods, tandem mass spectrometry and molecular cloning techniques have revealed that *C. fleckeri* venom contains a diverse array of proteins that is dominated by a family of abundant high molecular weight venom proteins that are cytolytic, cytotoxic and cause profound cardiovascular collapse in experimental animals. Related toxins with similar biological activities are present in other jellyfish species and comparative analysis of available toxin sequences infers that this expanding family of potent cnidarian toxins forms at least two distinct protein clades. Sequence divergence among family members coupled with experimental evidence suggests there are significant structural variations between

clades that may alter their function and target specificity. In this context, an overview of this unique family of protein toxins is presented, including a brief history of their discovery and recent progress in their purification and molecular characterisation primarily from a bioinformatic perspective.

Keywords: bioinformatics, *Cnidaria*, *Cubozoa*, cytolysin, cytotoxin, nematocyst, venom, toxin
10.1016/j.toxicon.2012.04.107

107. The Chemical Landscape of Cnidarians as Viewed Through the Lens of Pore-Forming Proteins

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Background: Cnidarians such as hydra, sea anemones, corals and jellyfish are simple, mostly sessile animals that depend on bioactive chemicals for survival. Cnidarians utilize sophisticated stinging cells (nematocytes) to inject paralyzing venom into their prey, predators or competitors. In addition to the nematocyte venom, we show here that cnidarians produce cytolytic, "toxin-like" pore-forming proteins (PFPs) in other tissues, and suggest functional roles for these proteins.

Results and Discussion: Equinatoxins (Eqts), well-studied lethal PFPs from the sea anemone *Actinia equina*, are detected by immunohistochemistry in nematocytes and are thus probably used for prey capture. However, Eqts are also secreted into the mucous layer surrounding the anemone from gland-like cells. Eqt-2 can kill fish upon application to the water in which they swim, suggesting it may serve for defense against predators. Surprisingly, while Eqt-2 does not kill bacteria it strongly inhibits their attachment to substrates, suggesting this protein may inhibit the adhesion of pathogenic bacteria or other fouling organisms to the anemone. A second example of non-nematocystic PFPs are Hydralysins (Hlns), neurotoxic PFPs from the green hydra *Chlorohydra viridissima*: in-situ hybridization and immunohistochemistry reveal that Hlns are secreted into the gut cavity upon feeding, where they may promote osmotic disintegration of the prey during feeding. Other potential PFPs may be involved in developmental signaling and immunity in hydra.

Conclusions: We propose a model whereby, in cnidarians, bioactive compounds such as PFPs are secreted both as localized point sources (nematocyte discharges) and across extensive body surfaces, likely combining to create complex "chemical landscapes". We speculate that these cnidarian-derived chemical landscapes may affect the surrounding community on scales from microns to, in the case of coral reefs, hundreds of kilometers.

Keywords: Cnidaria, hydra, nematocyst, pore-forming protein, chemical landscape, diffusion, boundarylayer, antimicrobial
10.1016/j.toxicon.2012.04.108

108. Cardiovascular and Hemolytic Effects of Sp-CTx a Cytolysin Isolated from the Scorpionfish, *Scorpaena plumieri*

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Background: In a previous study, a potent hemolytic/cardiotoxin (Sp-CTx) was isolated from scorpion fish *Scorpaena plumieri* venom. In the present work we aimed to optimize the Sp-CTx purification method and to investigate the mechanisms responsible for its pharmacological effects.

Methods: Sp-CTx was purified to homogeneity from fish fin spines venom through a combination of ammonium sulfate precipitation and two chromatographic steps: hydrophobic interaction and anion exchange. Active fractions were identified by hemolytic assay. Osmotic protection assays using polyethylene glycol polymers of various sizes (30mM) were performed to test whether Sp-CTx induces hemolysis by membrane pore formation. Cardiovascular studies of Sp-CTx were evaluated on male Wistar rats both *in vivo*, and *in vitro*. The effects of Sp-CTx on L-type Ca^{2+} current ($I_{Ca,L}$) in adult rat ventricular cells were investigated using the whole cell patch clamp method.

Results: The yield of the purification procedure was 13% of the hemolytic activity from the whole venom, with a purification factor of 24 fold. Sp-CTx induced-hemolysis decreased with increasing size of osmotic protectants. *In bolus* injection of Sp-CTx in rats (70μg/Kg) produced a biphasic response which consisted of an initial systolic and diastolic pressure increase followed by a sustained decrease of both parameters. In isolated papillary muscle, Sp-CTx (80nM) produced an increase in isometric force. At 8nM concentration, this toxin increased by about 30% the $I_{Ca,L}$ in rat ventricular cells.

Discussion: In the present work, a new purification procedure of Sp-CTx was established. This method requires a smaller number of chromatographic steps and the activity recovery was improved substantially (13 fold). Hemolytic effect was prevented by the presence of osmotic protectants. At 40 nM concentration, 100% cell lysis was observed after 6 min, molecules larger than 3 nm in diameter inhibited 100% cell lysis. As we observed a biphasic response in pressure levels when Sp-CTx was injected in rats we could argue that Sp-CTx targets first calcium channels and after some time non-selective pore formation takes place leading to a decrease in both systolic and diastolic pressure levels. On the other hand, the increase in contraction force would be the result of $I_{Ca,L}$ increase.

Conclusion: The results strongly suggest that Sp-CTx may be a pore-forming protein. These data are in agreement with the significant hemolytic activity observed. Also,

the increase in $I_{Ca,L}$ paralled by the pore formation may provide the basis for Sp-CTx cardiotoxic effects.

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Keywords: *Scorpaena plumieri*, cardiotoxin, cytolysin
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109. A Comparison of the Structural Characteristics of the Nematocysts of the "Fire Corals" *Millepora alcicornis* and *M. complanata*, and their Hemolytic and Vasoconstrictor Effects

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Review: *Millepora* species are colonial polyp cnidarians of the class Hydrozoa that produce calcium carbonate skeletons. These species are commonly known as "fire corals" because a brief contact with them causes intense burning pain, erythema, wheals, and sometimes ulceronecrotic lesions. These local lesions are due to the presence of stinging organelles named nematocysts, which inject an unidentified mixture of toxins through the skin. Up to now, 13 species of the genus *Millepora* are recognized around the world. However, it is not clear whether these species produce different type of toxins each from other and if the same type of toxins are present in the same type of nematocyst. Since, in the present study we made a comparison between the types of nematocysts present in two *Millepora* species that inhabit in shallow-water reefs of the Mexican Caribbean: *M. alcicornis* and *M. complanata*. In this study, we also the differences in profile proteins of their extracts and the vasoconstrictor and hemolytic activities. First, the type of nematocysts of both species was examined using light microscopy, as well as scanning and transmission electron microscopy. Only two types of nematocysts were observed in both species. The most abundant type was identified as macrobasic mastigophore and the other one belonged to the stenotele type. Second, both species induce the two types of activities, but the vasoconstrictor effect induced by the extract of *M. alcicornis* ($E_{max} = 90.1 \pm 5.6$ % of the contraction induced by 1 μM phenylephrine) was slightly more efficient than that induced by the extract of *M. complanata* ($E_{max} = 64.6 \pm 2.7$ %), although there is not a significant difference in the potency. In the other hand, analysis of the hemolytic activities showed that *M. alcicornis* extract ($HU_{50} = 0.05 \pm 0.005$ μg protein/ml) was significantly 10-fold more potent than the extract of *M. complanata* ($HU_{50} = 0.5 \pm 0.02$ μg protein/ml). In conclusion, the results of the present study shown that although *M. alcicornis* and *M. complanta* presents the same types of nematocysts, there are some differences in their protein profiles and in the potency of their extracts to induce hemolysis. These observations suggest that the same type of nematocyst can produce different types of toxins and in variable quantities.

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Keywords: *Millepora complanta*, *Millepora alcicornis* and Nematicysts.
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110. Voltage Sensor Trapping in Voltage-Gated K-Channels by the Marine Neurotoxin Gambierol

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Background: We previously showed that the polyether ladder toxin gambierol (a ciguatera toxin) acts on a novel binding site at the lipid exposed face of the pore domain of Kv channels.

Methods: Voltage clamp recordings of ionic currents and gating currents were obtained from HEK293 cells expressing Kv3.1 channel subunits (WT and insensitive mutants). Concatemers with WT and mutant subunits were used to constrain subunit stoichiometry. Computational Markov models with appropriate gating and drug binding schemes were implemented to investigate the gating modification.

Results: Gambierol inhibited ionic current through Kv3.1 channels with an IC_{50} of 1-2 nM, with a time constant of ~ 60 sec when the channels were held in the closed state at -80 mV. Short (250 ms) steps to +140 mV failed to overcome this inhibition, but 29% of ionic current was recovered after prolonged (5-10 sec) depolarizations to +140 mV, suggesting a lower affinity of the open state. This was further tested by keeping the cells depolarized at +60 mV while washing in the toxin; under these conditions no noticeable block developed. The gating currents of Kv3.1 channels displayed "OFF" currents with a slowed current decay after steps that open the channel gate, indicating that the concerted opening step has been passed (similar to Shaker channels). After application of Gambierol, this slowing quickly disappeared and subsequently, all gating charge movement was eliminated. A tetrameric concatemer with only 1 high-affinity binding site still displayed high toxin sensitivity, but displayed faster unblock at strong depolarizations (>120mV).

Discussion: Given the tetrameric structure of Kv channels, there are 4 binding sites for gambierol on the lipid exposed crevice between S5 and S6. The results indicate that binding of gambierol prevents to the voltage sensors to move to the activated position, by stabilizing a deep closed state, such that binding at a single binding site is sufficient to preclude channel opening (which requires all 4 voltage sensors to reach the activated state). Computational modeling confirmed this mechanism of ultra-strong stabilization of the closed state.

Conclusions: The polyether toxin gambierol acts as a profound gating modifier at a lipid exposed binding site

and with a mechanism that is distinct from the gating modification of hanatoxin-related toxins.

Keywords: Kv channels, gating modification, gambierol, ciguatera
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111. State of the Art of Palytoxin and Analogs Analytical Methods for Seafood Monitoring

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Review: Palytoxin group (PLTXs) are produced by marine organisms, like zoanthids (*Palythoa)* and benthic dinoflagellates (*Ostreopsis)*. Besides originally found in tropical regions, reports on the presence of PLTXs in subtropical and temperate marine waters have grown considerably in the past decade. This is related to the increase of *Ostreopsis* spp. blooms in such environments, which pose considerable risk to the human health. In fact, contamination of seafood with *Ostreopsis spp*. already caused human poisoning incidents after the ingestion of clupeoid fish, some with fatal outcomes [1,2]. Therefore there is an urgent need to establish regulations on PLTX-group toxins in seafood intended for human consumption. Due to lack of exposure assessment and analytical information about these toxins, authorities are not able to establish a reliable guideline limit. European Food Safety Authority (EFSA) gives an estimated value for palytoxin and ostreocin-D of 30 μg/Kg shellfish meat [1]. Chemically these natural products are large and complex molecules, having both hydrophilic and lipophilic behaviors consisting of 64 chiral centers, 40-42 hydroxyl and 2 amide groups. These characteristics confer their heat resistance, water solubility and a large number of possible analogs. The analytical approach to identify and quantify PLTXs, such as HPCE-UV, HPLC-UV and FLD or LC-MS, it's mainly chosen depending on the type of matrix and on the limit of detection required. The most sensitive method for *Ostreopsis* cultures, having a limit of detection (LOD) of 0.75 ng on column [3,4], implies the derivatization of the molecule and until know was not validated for seafood intended for human consumption. In order to better monitor this emergent phenomenon there is a need to develop techniques and to produce certificated standards and reference materials to achieve proper method validation, following International Conference on Harmonization (ICH) and Food and Drug Administration (FDA) guidelines. This work overviews the actually available methods to analyze PLTXs - highlighting their strengths, limitations and adequacy for seafood monitoring, while it points out possible analytical requirements and future approaches.

References

EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009. The EFSA Journal 1306, 1–23. Available from: www.efsa.europa.eu (December 2010).
Ramos, V., Vasconcelos, V., 2010. Mar. Drugs 8, 2021–2037.
Rióbo, P., Paz, B., V., Franco J. M., 2006. Anal. Chim. Acta 566, 217–223.
Rióbo, P., Franco J. M., 2011. Toxicon. 57, 368–375.

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Keywords: palytoxin, analytical methods, seafood monitoring
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112. Cathepsin B/X is Secreted by *Echinometra lucunter* Sea Urchin Spines, a Structure Rich in Granular Cells and Toxins

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Background: *Echinometra lucunter* is a common Brazilian sea urchin; however, few data is available on its spines' structures or toxins. In insects and mollusks the presence of cathepsins has been described. These enzymes would aid in the eggs or larva's growth through protein degradation, and remodeling the calcified part, as Cathepsin K does in mammals bones. Here, we report a cathepsin B/X like activity from the sea urchin spines.

Methods: Spines aqueous extract was obtained in 100 mM NH_4COOH (pH 7.4) at 4°C and was assayed for enzymatic activity, with synthetic substrates, in the presence or absence of inhibitors and DTT. For properly classification of the peptidase in the correspondent clan and family, the reference FRET-substrate cleavage pattern was determined, pH-dependency activity was evaluated and Western-blot analyses were performed. Moreover, spines were analyzed by light and scanning electron microscopy (SEM).

Results: The spine extract was able to cleave both Z-R-MCA and Abz-GIVRAK(Dnp)-OH following pre-incubation with DTT, and was inhibited by E-64. Furthermore, the double-peaked pH curve (5 and 7) and the cleavage site proportion (4:6, R↓A:A↓K), indicate the presence of both mono and dicarboxypeptidase activities. Finally, it was positive for the (human) anti-cathepsin B antibody in the Western-blot. According to spines histological analyses, the presence of granulous cells, positively stained with bromophenol blue, along the entire spine, but concentrated at the tip, were observed. These cells are located within the calcified matrix, which is longitudinally disposed and radiates outwards, as observed by SEM.

Discussion: The cleavage pattern of FRET substrate, the inhibition by E-64 and DTT-activation clearly indicates the presence of a cysteine peptidase in the spine extract. The two optimum pH values are typical for some cathepsins, as well as the carboxy mono and dipeptidyl-peptidase activity, characteristic of cathepsin X and B, respectively.

Moreover, the Western blot analysis confirmed the presence of cathepsin B, among other proteins revealed in the silver-stained SDS-PAGE. Proteins are normally abundant in the granular cells, just like those observed in the bromophenol blue staining that were more abundant at the tip of the spine, a region that can be easily broken due to contact, which would need frequent remodeling.

Conclusion: *E. lucunter* spines extracts showed a proteolytic activity that, based on the selected experiments, could be classified as Cathepsin B and/or X, according to their cleavage site. This enzyme could also participate in the remodeling process and growth of the spine.

Fig. 1. Sea Urchin.

Keywords: sea urchin, *Echinometra lucunter*, spines, cathepsin, granular cells
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113. Effects of Successive Subculture and Various Culture Conditions on Growth and PSP Productivity of the Toxic Dinoflagellate *Alexandrium catenella*

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Background: Production of paralytic shellfish poison (PSP) in toxic dinoflagellates considerably differs among the same species, depending on the strain, habitat, and season, and even between wild cells and cultured cells. We investigated the effects of successive subculture and various environmental factors on growth and toxin production by the PSP-producing dinoflagellate *Alexandrium catenella* (*Ac*), which frequently cause PSP contamination in bivalves in Kyushu, Japan.

Methods: [Exp 1] Wild cells (Wc) of *Ac* were collected from Tamanoura Bay, Fukue Island, Kyushu, in April 2009, and *Ac* cells isolated from the same seawater were successively subcultured in SWM-III medium at 20°C with the light intensity of 60 μmol/m^2/s (12L/12D), and primary, secondary, and tertiary subculture cells (Sc-1, Sc-2, and Sc-3, respectively) were obtained. Toxin profiles of each cell group were analyzed by high performance liquid chromatography with fluorometric detection (HPLC-FLD). [Exp 2] *Ac* cells were cultured in SWM-III medium and trace metal-free SWM-III medium under the same conditions as in Exp 1 for 16 days. Cell numbers were counted periodically during the culture, and toxin profiles at the end of culture were analyzed by HPLC-FLD. [Exp 3] *Ac* cells were cultured under a red LED (RL; 660 nm), blue LED (BL; 470 nm), and white fluorescent light (WFL) for 15 days, and the growth and toxin profiles were evaluated.

Results and Conclusions: Toxin amounts produced by Wc, Sc-1, Sc-2, and Sc-3 were 646, 50, 12, and 15 fmol/cell, respectively. Gonyautoxin (GTX)2,3, GTX6 and saxitoxins (STXs) disappeared during successive subculture, simplifying the toxin profile; C toxin (C)1-4, GTX1,4, and GTX5. No obvious difference in *Ac* growth was detected when trace metals were excluded, but toxin levels were more than double those in the control culture. C1,2 and GTX1,4 levels increased remarkably. Growth of *Ac* was slow under RL, and maximum cell density was 1/3 and 1/4 that under BL and WFL, respectively. *Ac*, however, produced a greater amount of toxin under RL, 3.7 to 9.3 times higher than under BL and WFL. Under RL, the ratio of C1,2 was higher (about 60%), whereas ratios of GTX1,4 and C3,4 were lower. These results indicate that successive subculture leads to a decrease in *Ac* production of PSP, and that production is affected by trace metals and irradiation wavelength during culture.

Keywords: paralytic shellfish poison (PSP), saxitoxin (STX), gonyautoxin (GTX), dinoflagellate, *Alexandrium catenella*
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114. Effect of Byproducts from *Artemia salina* Culture Medium on PSP Toxicity and Toxin Composition of *Alexandrium catenella*

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Background: Presently, it is not clearly known what causes the quantitative and compositional change of paralytic shellfish poisoning (PSP) toxin in the PSP-producing phytoplankton. Most of the investigations carried out so far used various physical and chemical parameters such as temperature, salinity, pH, dissolved nutrient ratio etc. for deciphering their possible influence on the PSP toxicity change. However, it's possible to assume that inhibition of competing co-occurring plankton species could influence the toxin production dynamics in marine phytoplankton under natural condition. In order to understand the influence of other plankton species on the PSP toxicity and composition, we compared PSP produced by *Alexandrium catenella*

by adding the rearing medium of Artemia (*Artemia salina*) to the culture medium vis-à-vis the control medium in which filtered natural seawater was added to culture medium.

Materials and Methods: After rearing for 10 days, the Artemia-rearing medium was filtered through 0.22 μm filters and added to the in sterile enriched seawater medium (ESM) in three culture flasks. In three separate culture flasks, filtered natural seawater was added to the ESM to use as controls. *A. catenella* was cultured in both set of medium for 6 days under light level of 49,000 lux, and 14h light/10h dark cycle at the temperature of 21°C. After completion of the culture experiment, *A. catenella* were collected from each sample for counting cell numbers under a microscope and PSP was extracted from each sample to measure the toxicity level and the toxin composition using high performance liquid chromatography (HPLC). The cell counts and toxin measurements were carried out prior to beginning of the experiments in all the samples.

Results and Discussion: Significant difference ($p<0.05$) was observed in toxicity/per cell values between the sample with the Artemia-rearing medium (88.38±23.30 fmol/cell) and the control medium (38.45±20.44 88.38±23.30 fmol/cell). Concerning the PSP composition, there was significantly higher ($p<0.05$) concentration of PX2 and GTX4 in the sample with Artemia-rearing medium compared to that of the controls. Similar trend was also observed with PX2 and GTX4 toxicity in the sample with the Artemia-rearing medium compared to that of the controls. The results were confirmed by undertaking experiments twice and similar results were obtained both the times.

Conclusions: Our results suggest that the metabolic substances released in the byproducts from biological activity of other plankton could potentially influence toxicity and composition of toxins in *A. catenella*. Ecological significance of such phenomena needs to be further investigated.

Keywords: PSP, *Alexandrium catenella*, toxicity, toxin composition
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115. Two Proteins Homologous to Pufferfish Saxitoxin- and Tetrodotoxin-Binding Protein (PSTBP) Found in the Plasma of Non-Toxic Cultured Specimens of the Pufferfish (*Takifugu rubripes*)

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Background: Marine pufferfish generally possess the potent neurotoxin tetrodotoxin (TTX). Although previous TTX administration experiments using non-toxic cultured specimens of *Takifugu rubripes* have provided essential information on the transfer/accumulation profiles of TTX in the pufferfish body, the molecular mechanism of TTX transportation/accumulation remains unclear. It was recently reported that *T. rubripes* plasma contains ~120-kDa protein that immunocrossreacts with pufferfish saxitoxin- and tetrodotoxin-binding protein (PSTBP) isolated from *Takifugu pardalis*. In the present study, we explored PSTBP homologous genes in *T. rubripes*, a suitable model species for post-genomic research, and investigated the expression of PSTBP homologs in non-toxic cultured specimens.

Methods: Using the *T. pardalis* PSTBP as the query sequence, a BLAST search was conducted against a draft genome sequence of *T. rubripes*. 3'-RACE was performed to obtain complete coding sequences. Multiple sequence alignment was performed using CLUSTAL W/X, and phylogenetic analysis, using the neighbor-joining method with the PAUP program. Expression of mRNAs in various tissues (skin, muscle, liver, testis, and ovary) was examined by RT-PCR. A 120-kDa protein was separated by SDS-PAGE after ammonium sulfate fractionation of the plasma, and identified by N-terminal protein sequencing (Edman chemistry) and MALDI-QIT-TOF mass spectrometry.

Results: Three putative genes (tentatively designated *Tr1-3*) were obtained by the BLAST search/3'-RACE. *Tr1* and *Tr2* genes appeared to be novel, encoding a single lipocalin domain, which is closely related to the C-terminal domain of *T. pardalis* PSTBP. The *Tr3* gene could be a functional counterpart of PSTBP, possessing two lipocalin domains. The phylogenetic tree indicated that lipocalin domains of Tr proteins and *T. pardalis* PSTBP form three distinct clades. *Tr1* and *Tr3* gene transcripts were detected exclusively in the liver. The 120-kDa protein from the plasma was identified as the mixture of Tr1 and Tr3.

Discussion: Tr1 protein may form an SDS-resistant dimer, as Tr1 (single-domain) was co-identified with Tr3 (two-domain) in the same protein band (120 kDa). The possibility that Tr1 is the product of an unidentified gene that encodes tandem-repeated Tr1-like domains, however, cannot be ruled out. Constitutive expression of Tr1 (or Tr1-like) and Tr3 in non-toxic pufferfish would be beneficial for quick self-protection from free toxins upon toxin-intake, and also for efficient toxin transportation/accumulation. *Tr2* gene expression is currently under investigation.

Keywords: pufferfish, *Takifugu rubripes*, tetrodotoxin, toxin-binding protein, PSTBP, homologous gene, MALDI mass spectrometry
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116. Systemic Toxicity of the "Fire Coral" *Millepora complanata*: Isolation of a Non-Protein Vasoconstrictor Fraction with Lethal Activity in Mice

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Background: *M. complanata* is a cnidarian (class Hydrozoa) widely distributed in the coral reefs of the Mexican Caribbean. This hydrocoral is popularly known as "fire coral", since contact with it causes severe pain, skin eruptions and blisters. Previous *in vitro* experiments,

carried out by our research group, showed that the aqueous extract of this hydrocoral contained proteins that induced several pharmacological and toxic effects, which include hemolysis and stimulation of intestinal and arterial smooth muscle contractility.

Methods: In the present study, the systemic toxicity of *M. complanata* aqueous extract was assessed in mice. Additionally, a bioactivity directed chromatographic fractionation of the extract was performed and the bioactive fractions were further analyzed in order to isolate the compounds responsible for the pharmacological and toxicological activities induced by the extract.

Results: The results obtained in the present study indicated that intravenous administration of the extract induced violent convulsions and death in mice within 1 min (LD_{50} = 4.62 μg protein/g of body weight). Doses less than the LD_{50} (1.33, 2.67 y 4.00 μg protein/g) produced kidney and lung histopathological damage attributed to the presence of cytolysins. Histopathological changes were completely eliminated after incubation of the extract in denaturing conditions. Unexpectedly, the denatured extract conserved its lethal effect. These findings suggested that that the *M. complanata* extract contained hemolysins that might be responsible for the histopathological changes observed in kidney and lung. Additionally, this extract contained other unidentified thermostable toxins, likely secondary metabolites, which have lethal effects in mice. Chromatographic analysis of the extract led to the isolation of a 61 kDa vasoconstrictor protein. Additionally, several non-peptidic vasoconstrictor compounds were detected. Particularly interesting, fraction MC1-IIA, obtained by a three-step process (ion exchange, gel filtration and reverse phase chromatography), induced vasoconstriction, delayed hemolysis, and lethal effects in mice. These effects resembled those produced by the crude extract. A subsequent chromatographic analysis of MC1-IIA showed that this fraction contained at least four secondary metabolites, which were named as IIA-2-1, IIA-2-2, IIA-3-1, and IIA-3-2. Analysis of mass spectrometric and NMR spectroscopic data indicated that these metabolites were poly-hydroxylated compounds.

Conclusions: The present study constitutes the first report of the presence of a non-peptidic lethal toxin in an organism of the class Hydrozoa, and evidenced the great structural diversity of the toxins produced by *Millepora* species.

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Keywords: fire coral, histopathological damage, lethal effect.
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117. Identification and Characterization of α-Conotoxins in *Conus purpurascens*

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Background: Cone snails use venom that contain proteins and highly modified peptides (conopeptides) to rapidly immobilize their prey and defend against predators. Conopeptides act directly upon specific ion channels and receptors in both neuronal and neuromuscular tissues. The most ubiquitous family of conopeptides across the *Conus* genus is the α-conotoxins. a-conotoxins antagonize nicotinic acetylcholine receptors (nAChRs). These receptors are associated with several disease states, such as Alzheimer's disease, Parkinson's disease, and nicotine addiction. *Conus purpurascens* is the only fish-hunting species of the Eastern Pacific. The venom of this species is known to contain varying components between individuals, which highly increase the number of conopeptides present in *C. purpurascens.* Although two α-conotoxins have already been fully characterized from its venom, there are numerous unidentified α-conotoxins that have yet to be discovered.

Methods: The venom of thirty-seven captive *C. purpurascens* individuals was extracted using a "milking" procedure and then separated into individual components using size exclusion chromatography (SEC) and reverse-phase high performance liquid chromatography (RP-HPLC). The molecular weights of the isolated conopeptides were determined using matrix assisted laser desorption ionization mass spectrometry (MALDI-MS). The conopeptides were plated on two matrices for MALDI-MS analysis, alpha-cyano-4-hydroxycinnamic acid (α-CHCA) and the partially reducing matrix, 1,5-diaminonaphthalene (1,5-DAN).

Results: The recently discovered α-conotoxin, α-PIC, and other conopeptides of novel molecular weights (1422, 1560, and 1810 Da) were collected.

Discussion: By comparing the molecular weights of conopeptides on each matrix, the number of disulfide bonds can be determined, and therefore, the conopeptide can be classified according to its conotoxin framework. The novel conopeptides will be tested *in vivo* for bioactivity in the *Drosophila melanogaster* giant fibre circuit (GFC). The *D. melanogaster* GFC contains nAChRs that are homologous to those in vertebrates.

Conclusion: Since there is interspecies variation between the venom of *C. purpurascens* individuals, additional novel conopeptides with important biological activity can de discovered. Testing novel conopeptides in the *Drosophila* GFC system is one of the many ways that we can begin to characterize them by their targets. Characterizing α-conotoxins is of great importance as they can be used as pharmaceutical probes for disease pathways and neurodegeneration.

Keywords: *Conus purpurascens*, α-conotoxins, nicotinic acetylcholine receptors
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118. Primary Structures of Proteinaceous Toxins from Three Species of Scorpaeniform Fish (Lionfish, *Pterois lunulata*, Scorpionfish, *Inimicus japonicus* and Waspfish, *Hypodytes rubripinnis*)

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Background: The order Scorpaeniformes includes a number of venomous fish, such as stonefish, lionfish and scorpionfish. These venomous fish possess pungent spines with venom glands or venom sacs in dorsal, pelvic and anal fins. When stung by the spines, local symptoms such as intense pain, swelling and redness are immediately induced in victims; even deaths may occur in sever cases. So far, stonefish proteinaceous toxins, stonustoxin of *Synanceia horrida* and neoverrucotoxin (neoVTX) of *Synanceia verrucosa*, have been well characterized and elucidated for their primary structures. Recently, we determined the primary structures of toxins from two species of *Pterois* lionfish, *P. antennata* and *P. volitans*, based on a cDNA cloning strategy using primers designed from the reported amino acid sequences of the stonefish toxins. It is assumed that proteinaceous toxins of other venomous scorpaeniform fish can be determined for their primary structures by the same strategy.

Methods: Three species of scorpaeniform fish (lionfish *Pterois lunulata* of the family Scorpaenidae, scorpionfish *Inimicus japonicus* of the family Synanceiidae and waspfish *Hypodytes rubripinnis* of the family Tetrarogidae) were used as samples. The crude toxin extracted from dorsal spines of each live specimen was assayed for hemolytic activity against rabbit erythrocytes and lethal activity against mice and analyzed for proteins cross-reacting with neoVTX by immunoblotting and inhibition immunoblotting using the anti-neoVTX antiserum previously raised in a guinea pig. Primary structures of proteinaceous toxins were determined by cDNA cloning using primers designed from the highly conserved amino acid sequences of the stonefish and lionfish toxins.

Results and Discussion: All the crude toxins from the three species of scorpaeniform fish showed both hemolytic and lethal activities with considerably different potencies. Analysis by immunoblotting and inhibition immunoblotting revealed that the scorpaeniform fish contain about a 75 kDa protein (corresponding to the toxin subunit) cross-reacting with neoVTX. Cloning experiments established the amino acid sequences of the scorpaeniform fish toxins (α and β subunits composed of 698-703 amino acid residues). The established sequences share high identities (more than 80%) with one another and also with those of the known stonefish and lionfish toxins. A B30.2/SPRY domain was also recognized in the C-terminal region of the scorpaeniform fish toxins, as in the case of the known stonefish and lionfish toxins. These results suggest a universal distribution of biologically and structurally similar proteinaceous toxins in venomous scorpaeniform fish.

Keywords: primary structure, proteinaceous toxin, scorpaeniform fish
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119. Characterization of Local Inflammatory Response Induced by Scorpion Fish, *Scorpaena plumieri* Venom in a Mouse Model of Tissue Injury

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Background: The *Scorpaena plumieri* fish venom induces a severe pain and edema, observed both clinically and experimentally. In order to understand more about the envenomation syndrome, the present study characterized experimentally the local acute inflammatory response induced by *S. plumieri* venom (SpV) in a mouse model of tissue injury.

Methods: Venom local activities (15 μg) were assayed using mice hind paw method. The local edema was quantified by measuring the thickness of injected paws; histopathological analysis (leukocyte recruitment and morphological changes) were assessed in injected footpads; cytokines (TNFα and IL-6) and chemokine (MCP-1) levels were measured in paw supernatants homogenates by flow cytometry using Cytometric Bead Array – Mouse Inflammation Kit. The mechanism involved in the SpV edematogenic activity was investigated by previous treatment of mice (i.p.) with anti-inflammatory drugs: i) cyclooxygenase non-selective inhibitor, diclofenac sodium (1 mg/Kg); ii) histamine H_1 receptor antagonist, promethazine (1 mg/Kg); iii) serine-proteases inhibitor, aprotinin (8 mg/Kg) and iv) bradykinin B_2 receptor antagonist, HOE-140 (100 mol/Kg).

Results: SpV induced an intense and sustained dose-dependent local edema. In addition, an expressive increase of the number of leukocytes was observed (neutrophil cells were predominant), reaching maximal intensity after 6 h of venom injection. SpV also was able to induce a significant release of pro-inflammatory mediators. Maximal levels of TNF-α (38 ρg/ml), IL-6 (1600 ρg/ml) and MCP-1(2470 ρg/ml) were recorded after 2 h of venom injection. SpV induced edema was significantly reduced by previous administration of aprotinin or HOE-140. However, the pre-treatment with diclofenac sodium and promethazine had less effect on this response.

Discussion: In the present study, we demonstrated that the local inflammatory response induced by SpV is characterized by fast release of some pivotal proinflammatory cytokines and chemokine, which was accompanied by leukocyte recruitment. The onset of the acute inflammatory response (leukocyte accumulation) was broadly consistent with release of TNF detected in footpad homogenates 0.5 and 2 h after venom administration. The significant reduction of edematogenic response by HOE-140 and aprotinin evidences that one of the main pathways involved in SpV-induced edema is the kallikrein-kinin system (KKS).

Conclusion: Our results demonstrate that SpV evokes a complex inflammatory reaction and provide clear evidence of the involvement of the KKS in this response.

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Keywords: *Scorpaena plumieri* venom, edema, inflamatory mediators, kallikrein-kinin system
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120. The Atypical Activity Profile of bru1b, an α-Conotoxin from the Venom of *Conus brunneus*

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Background: Cone snails are venomous marine predators whose venom is a complex mixture of modified peptides (conopeptides). Conopeptides have direct specificity towards voltage- and ligand-gated ion channels and G-protein coupled receptors. More specifically, α-conotoxins target nicotinic acetylcholine receptors (nAChR) and are of great interest as probes for different nAChR subtypes involved in a broad range of neurological function.

Methods: We used *in vivo* electrophysiological measurements of the functional outputs of a well-characterized neuronal circuit in *Drosophila melanogaster* known as the giant fiber system, a circuit of four neurons innervating to two muscles, the TTM and DLM, which are responsible for the fly's escape response. Additionally, bru1b was tested using voltage clamp of *Xenopus* oocytes expressing mammalian nAChRs and in binding assays using *Aplysia californica* acetylcholine binding protein (AChBP). The binding modes of bru1b with the Da7 nAChR were modelled using Modeller9.10v and the crystal structures of the *Aplysia* AChBP-conotoxin known to date.

Results: A new α-conotoxin has been identified that does not have the same activity profile as other α-conotoxins. This conotoxin was originally discovered via bioassay-guided fractionation using the recently developed *Drosophila melanogaster* Giant Fiber circuit assay[1]. After biochemical characterization, it was determined that this conotoxin was an α-4/3 conotoxin that specifically blocked the DLMn neuronal pathway of the giant fiber system at 33 pmol/mg. It was tested against most subtypes of mammalian nAChRs, and found to be inactive at the micromolar range. It was also tested against the *Aplysia californica* AChBP and was found not to bind to it.

Discussion: The atypical activity profile of bru1b was quite unexpected. This conotoxin is an insect-specific Da7-nAChR inhibitor. To date, no *D. melanogaster* nAChR subunits have been expressed in any other expression systems.

Conclusion: The model of bru1b's binding mode with the Da7 nAChR based on the crystal structure of the *Aplysia californica* AChBP provides an explanation of bru1b's interesting activity profile and shows yet another very selective tactic of cone snail venom used to capture and immobilize their prey.

Keywords: conotoxin, drosophila, nicotinic acetylcholine receptor
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121. Venom Composition Changes during Prey Capture in *Conus textile*

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Background: We investigated venom composition variation during prey capture in the mollusc-hunting *Conus textile*. While studies on injected venom have become more common in fish-hunting cone snails, these approaches have not been applied to the analysis of venoms from mollusc-hunters. Observations of the mollusc-hunter *C. textile* during feeding reveal that prey are often injected multiple times in succession. Species that inject prey multiple times during a single feeding event may have compositional changes in their injected venom profiles. Our work investigates the occurrence of venom composition changes after each injection and how these changes are related to the biomechanics of prey-capture.

Methods: *C. textile* injected venom was collected from multiple shots in succession. Injected venom samples from multiple individuals were analyzed by mass spectrometry and reverse-phase high performance liquid chromatography.

Results: We have found novel peptides not previously described from studies on dissected venom. Initial results support intraspecific venom variation as well as complex venom profiles that show compositional differences in subsequent venom injections during a single feeding event.

Discussion: Venom profiles from a mollusc-hunter reveal venom profiles to be more complex than profiles obtained by fish-hunting cone snails. The results obtained by MADLI-ToF MS are mirrored by quantitative differences in venom composition shown by reverse-phase high performance liquid chromatography.

Conclusions: It is not yet clear why mollusc-hunters inject prey multiple times prior to engulfment of prey and how this relates to the observed changes in venom composition. Analysis of injected venom from multiple *C. textile* individuals provides some insights toward understanding this phenomenon. Future studies on the physiological effects and molecular targets of the venom peptides will help reveal the role of the various venom peptides injected during first versus later injections.

Keywords: cone snail, venom, prey capture
10.1016/j.toxicon.2012.04.122

122. Acute Toxicity and Brine Shrimp Cytotoxicity Induced by the Venom of the Fire Coral *M. alcicornis* Collected in the Mexican Caribbean

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Background: *Millepora alcicornis* is a hydrocoral with a characteristic branching-growth pattern common in coral reefs of the Mexican Caribbean. Like other members of this genus, *M. alcicornis* is capable of inducing skin eruptions and blisters with severe pain after contact. At present, very little is known about the systemic toxicity induced by this hydrocoral. Therefore in this study we investigated the systemic toxic effects and the histopathological changes produced by the aqueous extract of *M. alcicornis* in mice. We also assessed the toxic effects of this extract towards the nauplii of the brine shrimp *Artemia salina* and the damages caused by the extract on this crustacean.

Methods: The acute toxic effects were analyzed in mice after intravenously administration of the extract and the histopathological changes were observed by light microscopy. We perform cytotoxicity assay in *Artemia salina* and the damages caused by the extract on the brine shrimp were observed by Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM).

Results: We found that the extract is lethal to mice ($LD_{50} = 17$ μg protein/g) and caused kidney, liver, and lung damage. In addition, this extract is toxic to *A. salina* ($LD_{50} =$ 70.71 μg protein/ml) and provoked several histological alterations in *A. salina* tissues. *M. alcicornis* aqueous extract completely lost its toxicological activity after incubation in a boiling water bath for 20 min.

Conclusions: These results indicate that the aqueous extract of *M. alcicornis* contains heat labile cytolysins that induce lethal effects in mice and in brine shrimps. It is very likely that these toxins are also responsible for the damages produced on mice and crustaceans tissues.

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H. Microbial Toxins

123. Effects of Cyanobacterial Bloom on Fish: Proteomics and Histological Investigation on the Medaka *Oryzias Latipes*

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Background: Cyanobacterial toxic blooms often occur in freshwater lakes and constitute a potential health risk to human population, as well as to fish community or other aquatic organisms. Microcystin-LR (MC-LR, the most commonly detected cyanotoxin in the freshwater environment) is a potent hepatotoxin, deregulating kinase pathway via phosphatases 1 and 2A inhibition. Although toxicological effects have been clearly related to the in vitro exposure of fish to purified microcystins, cyanotoxins are produced by the cyanobacteria together with numerous other potentially toxic molecules, and their global and specific implications on the health of fish are still not clearly established and remain puzzling to assess.

Methods: Medaka fish (*Oryzias latipes)* was chosen as an *in-vitro* model for studying the effects of a cyanobacterial bloom on liver protein contents using a gel-free quantitative approach, iTRAQ, in addition to anatomic-pathological investigations. Fish were gavaged with cyanobacterial extracts (*Planktothrix agardhii)* from a natural bloom (Lake of La Grande Paroisse, France) containing 2.5 μg eq. MC-LR per 5 μl. Two hours after exposure, fish were sacrificed and organs were collected for analysis.

Results: Using proteomic approach, 304 proteins could be identified in treated fish livers, 147 presenting high confidence identification, among which 15 revealed statistically significant variations in comparison with controls (gavaged with water only). Overall, these protein regulations clearly testify to the occurrence of oxidative stress and lipid regulation effects in the liver of medaka fish. Differently to pure mycrocystin-LR gavage experiments, a strong induction of Vitellogenin1 protein was observed for the first time with a cyanobacterial extract. This result was confirmed by ELISA quantification of vitellogenin liver content.

Conclusions: These results suggest the occurrence of an endocrine disruptor effect of estrogen-type of the Planktothrix bloom extract, whether this reproductive effect can be attributed to microcystins need further investigation.

Keywords: medaka, cyanobacteria, proteomics
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124. Biogeography of Microcystins

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Review: Microcystins are toxins produced by cyanobacteria that can be lethal to humans as well as wild animals and livestock. Microcystins are cyclic heptapeptides and are found in all the main continents, being firstly recorded in the species *Microcystis aeruginosa*. Biogeography of toxins is a may help determine any biogeographic pattern that can explain the distribution of a certain toxin. In virtue of the lack of knowledge under this topic we conducted a study to assess if there is any biogeographical trend for this cyanotoxin on a worldwide scale. Three genes involved in the biosynthesis of microcystins (*mcyA*, *mcyD*, and *mcyG*) were retrieved

from the worldwide databases and were geographically related using phylogenetic tools. Results for all three genetic markers indicate the occurrence of a southern hemisphere cluster (South Africa and Australia), Asia had a distinct population from other continental groups and these appear to have no stringency. These data bring to light the importance of evaluating the biogeography of natural occurring toxins since their distribution can be clearly influenced by geography. Events such as speciation and dispersal are phenomena that can explain their present occurrence.

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Keywords: phylogeny, biogeography, *M. aeruginosa.*
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125. Cadherin Binding Is Not a Limiting Step for *Bacillus thuringiensis subs. israelensis* Cry4Ba Toxicity to *Aedes aegypti* Larvae

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Background: *Bacillus thuringiensis ssp. israelensis* (Bti) produces insecticidal proteins known as Cry toxins (Cry4Aa, Cry4Ba and Cry11Aa). These toxins are highly specific to their target insect, and are innocuous to vertebrates and plants. Cry toxins are pore-forming toxins that interact with specific receptor located on the host cell surface. Cry proteins are ingested by susceptible larvae dissolve in the alkaline environment of the gut, thereby releasing soluble protoxins, these are cleaved at specific sites by midgut proteases yielding active protease-resistant fragments. These fragments bind to specific protein receptors on midgut epithelial cells (cadherin receptor and GPI-anchor receptor), leading to membrane insertion and pore formation producing death of the insect. This mechanism is accompanied by toxin oligomerization before membrane insertion. Previous work showed that Cry11Aa binds to cadherin receptor fragment (CR7-11) with high affinity. Binding to cadherin has been proposed to facilitate Cry toxin oligomer formation.

Methods: In the present work we analyzed binding of Cry4Ba and Cry11Aa to cadherin receptor (CR7-11). We determined the affinity by ELISA binding assay and by surface plasmon resonance (SPR).

Results: Cry4Ba binds to CR7-11 in a saturable way with nine fold lower binding affinity (K_d of 134.9 nM by ELISA and K_d of 154 nM by SPR) than that of Cry11Aa (K_d of 14.8 nM by ELISA and K_d of 17 nM by SPR). In the oligomer formation assays, only in presence CR7-11, fragment induced oligomer formation in Cry11Aa in contrast to Cry4Ba that oligomers were formed even when activated in the absence of CR7-11. Pore formation of Cry4Ba oligomers was determined in black lipid bilayers showing the formation of ion channels. We analyzed the toxicity to *Aedes aegypti* of both toxins in the presence of CR7-11. Cry11Aa toxicity was enhanced 2.7 fold in the presence of ten fold excess of CR7-11. In contrast, there was no change in Cry4Ba toxicity. Finally, silencing the cadherin gene by dsRNA showed that silenced larvae were more tolerant to Cry11Aa in contrast to Cry4Ba, that showed similar toxic levels to control larvae.

Conclusions: Although cadherin binding has been shown to be an important binding step of different Cry toxin, the data presented here shows that it is not a limiting step in the toxicity of Cry4Ba to *A. aegypti.*

Keywords: Cry toxins, receptor binding, *Bacillus thuringiensis*.
10.1016/j.toxicon.2012.04.126

126. Magnetic Resonance Imaging and Spectroscopy Study of Microcystin LR Hepatotoxicity

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Background: Acute lethal intoxication with microcystin LR (MC-LR) results in hepatocyte damage and intra-hepatic hemorrhage. Hepatotoxic effects of microcystins have been studied in detail, and inhibition of protein phosphatases seems to be responsible for the majority of the observed effects. However, very little is known about the metabolic changes in hepatocytes of an experimental animal exposed to those toxins. Apoptosis of hepatocytes and blood stasis may decrease the level of ATP and creatine phosphate in hepatocytes of experimental animals. The aim of the present study was to measure the changes in liver structure and relative concentrations of energy rich metabolites in the liver of experimental animals noninvasively using magnetic resonance imaging (MRI) and P31magnetic resonance spectroscopy (MRS).

Methods: Adult male mice strain C57BL/6J (The Jackson Laboratory, USA) age 11-15 weeks, weighting from 19-27 g were used for P31 magnetic resonance spectroscopy, and Sprague-Dawley rats weighting 190 to 240 g were used for MR imaging. Before the experiment the animals were sedated and anesthetized by xylazine (Chanasine, Chanelle Pharmaceuticals, Ireland) and ketamine (Bioketan, Vetoquinol Biowet, Poland). Measurements of liver morphology changes by means of MRI and measurements of phosphorous compounds by P^{31} MRS were performed on a small bore 2,35 T Bruker tomograph before and after the animals had received a LD_{50} of MC-LR intraperitoneally.

Results: Exposure of experimental animals to microcystins caused a dose and time - dependent increase of inorganic phosphate with corresponding decrease of creatine phosphate and ATP peaks. In animals that have survived the treatment the changes were only minor or even absent. MR imaging showed an increased signal intensity from the liver of animals exposed to MC-LR. An increase in the liver size was most evident in animals that died due to MC-LR intoxication. Post mortem investigation showed typical pathohistological changes.

Discussion: Previous data have shown that MRI can detect changes in liver morphology due to higher proton content in the affected portions of the liver. Our data show strong correlation between pathohistological findings and MRI, and P31 MRS showed a decrease in the concentration of energy-rich such as ATP and creatine phosphate with concomitant increase of inorganic phosphate.

Conclusions: This study confirms that MC-LR increases the dephosphorylation of energy rich substances such as ATP and creatine phosphate and impairs the adequate generation of ATP. This is in agreement with the previous findings that microcystins cause mitochondrial swelling and induce apoptosis, which are energy-demanding processes.

Keywords: microcystin, liver, magnetic resonance, imaging, spectroscopy, P31
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127. Urease of Helicobacter pylori: Roles in Inflammation and Platelet Activation

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Background: Ureases (EC 3.5.1.5), nickel-dependent enzymes that hydrolyze urea into ammonia and CO_2, are synthesized by plants, fungi and bacteria. The urease produced by *Helicobacter pylori* (HPU), an etiological agent of gastric ulcers and cancer, is considered a virulence factor since its ureolytic activity enables the bacterium to survive in the acidic medium of the stomach. Gastric colonization by *H. pylori* is usually accompanied by an intense infiltration of polymorphonuclear leukocytes, macrophages and lymphocytes. The degree of mucosal damage correlates with an intense neutrophil infiltration. It is well known that platelets participate in the inflammatory response by modulating the activity of other inflammatory cells and ischemic lesions due to vascular insufficiency may lead to ulcers within the gastric mucosa. Previous data of our group showed that HPU: 1) induces platelet aggregation independent of its ureolytic activity, requiring ADP secretion through the lipoxygenase pathway; 2) induces paw edema in a dose- and time-dependent manner, with an intense neutrophil infiltration.

Methods: In this work, we used a recombinant urease produced in *Escherichia coli* to evaluate biological effects independent of its enzyme activity.

Results: In platelets, our results indicate that HPU interacts with the membrane glycoprotein VI, a well known receptor for collagen, which triggers a signaling cascade requiring metabolites of the platelet 12-lipoxygenase pathway. In human neutrophils, 100 nM rHPU induced extracellular production of ROS, and inhibited their apoptosis (40.5% compared to control), altering the levels of Bcl-X_L and Bad proteins. HPU-induced neutrophil chemotaxis was 88% of that observed by fMLP, a strong chemoattractant used as positive control. These effects of rHPU persisted in the absence of enzyme activity. rHPU-induced paw edema, neutrophil chemotaxis and apoptosis inhibition reverted in the presence of the lipoxygenase inhibitors esculetin or AA861. Neutrophils exposed to rHPU had increased content of lipoxygenase(s) and no alteration of cyclooxygenase(s) level(s).

Conclusion: Our data indicate that HPU, besides allowing the bacterial survival in the stomach, could play an important role in the pathogenesis of the gastrointestinal inflammatory disease caused by *H. pylori*.

Financial support: CAPES, CNPq, FAPERJ and FAPERGS.

Keywords: urease, *Helicobacter pylori*, inflammation
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128. Human iPS Neuronal Platform for Botulinum Neurotoxins

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Background: Human induced pluripotent stem (hiPS) cells hold great promise for providing various differentiated cell models for *in vivo* toxigenicity testing. For *Clostridium botulinum* neurotoxin (BoNT) detection and mechanistic studies several cell models currently exist, but none examine toxin function with species-specific relevance while exhibiting high sensitivity. The most sensitive cell models to date are mouse or rat primary cells and neurons derived from mouse embryonic stem cells, both of which require significant technical expertise for cell preparation.

Methods: This study describes for the first time the use of human iPS derived neurons for BoNT detection. The neurons are already differentiated and cryopreserved and consist of a 98% pure pan-neuronal population of GABAergic, dopaminergic, and glutamatergic neurons (Cellular Dynamics Inc, Madison, WI). Western blot and qPCR data show that the cells express all the necessary receptors and substrates for BoNT intoxication by all BoNT serotypes.

Results: BoNT/A intoxication studies demonstrate that the iPS-derived neurons detect biologically active BoNT/A reproducibly, quantitatively, and with high sensitivity ($EC_{50} \sim 0.3$ Units). Specificity was confirmed by antibody

protection studies, and quantitative detection of BoNT serotypes B, C, and E as well as BoNT/A complex are demonstrated. Direct comparison to BoNT detection using primary rat spinal cord cells showed equal or increased sensitivity and a steeper dose-response curve, reaching 100% SNARE protein target cleavage with significantly less BoNT.

Discussion: These data suggest that neurons derived from human iPS cells provide an ideal and highly sensitive platform for BoNT potency determination, neutralizing antibody detection, as well as for mechanistic studies of other neurotoxins of diverse composition.

Keywords: neurotoxins, pluripotent stem cells, botulinum neurotoxin
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129. Retrograde Trafficking at the Presynaptic Nerve Terminal Using Bacterial Toxins

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Background: A comprehensive understanding of the regulation of presynaptic sorting and retrograde transport of membrane vesicles is vital for understanding neuronal development, plasticity and survival. The appropriate sorting of receptors and ligands at the nerve terminal can occur at the cell surface or following endocytosis, through incorporation into specific membrane domains, mechanisms that are also used by toxins.

Methods: To analyse presynaptic membrane sorting we have used two probes based on bacterial toxins, which target the local recycling and retrograde pathways with a high degree of specificity. The retrograde pathway was targeted using the atoxic B-subunit of cholera toxin (CTB), and the local pathway using the atoxic heavy chain of botulinum A (BoNT/A-Hc). Endocytosis and membrane sorting was analysed in primary hippocampal neurons by both fluorescence and live cell microscopy, in addition to electron microscopy.

Results: Both BoNT/A-Hc and CTB enter neurons predominantly at the presynaptic nerve terminal. Entry of BoNT/A-Hc is activity-dependent. Entry of CTB is restricted to a subset of nerve terminals in resting neurons, but following membrane depolarisation CTB enters all nerve terminals. CTB and BoNT/A-Hc are segregated at the nerve terminal, both at the level of the cell surface and in intracellular vesicles. CTB enters retrograde carriers that are contain the neurotrophin receptor, TrkB, and undergoes retrograde transport to the Golgi complex in the cell body. BoNT/A-Hc predominantly enters synaptic vesicles and early endosomes. BoNT/A-Hc endocytosis is dependent on dynamin, a GTPase involved in both clathrin-mediated and clathrin-independent endocytosis. Inhibiting the activity of dynamin prevents BoNT/A-Hc endocytosis and slows the development of botulism in mice (Harper et al. 2011 JBC 286:35966).

Discussion: Understanding the molecular mechanisms that underpin the sorting and directed transport of internalized proteins at the presynaptic nerve terminal is important not only for understanding intoxication, but also for understanding sorting and targeting of vital survival factors. By using atoxic fragments of toxins that hijack neuronal pathways we are beginning to dissect out the molecular machinery that underlies these processes. We have found that the sorting of proteins destined for different destinations in the cell begins to occur very early in their trafficking, potentially as early as incorporation into specific domains at the plasma membrane. Furthermore, we have demonstrated that specifically inhibiting molecular components of the endocytic machinery is a viable approach to identifying potential targets for therapeutic intervention.

Conclusion: Early regulation of endocytic carriers underlies the fidelity of neuronal presynaptic membrane sorting.

Keywords: trafficking, bacterial toxins, neurons
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130. Water Bacterial Activities Involved in Immune Depression of Haemodialysis Patients

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Background: The present work surveyed haemodialysis (HD) units, in order to assess the bacteriological and toxinological qualities of dialysis water and dialysate; and determine to experimentally to how extent the bacterial activities are involved in immune depression for the patients.

Methods: Quantitative methods were used to enumerate the total count of viable heterotrophic bacteria; total coliforms; *Pseudomonas* spp.; *Aeromonas* spp. (cfu/ml); and endotoxin concentration (EU/ml) in dialysis water and dialysate of HD centers.

Results: The most commonly isolated Gram-negative bacteria from treated water were *Aeromonas* spp., 22.2. Endotoxin concentrations ranged between 3 to 113 (EU/ml) for water and dialysate respectively. The haematological analysis had non significant except haemoglobin level had a significant values ($P = 0.02$). The clinical data showed comparable difference than control patients. Missing, out of work, bypassed and even erroneously arranged treatment facilities are some of the reasons responsible for the detected high levels of bacterial and endotoxin in final product water.

Conclusions: The presence and growth of bacteria in water and dialysate should be controlled and the endotoxin testing must be a part of the regular quality control regime, to minimize the risk of adverse reactions in HD patients.

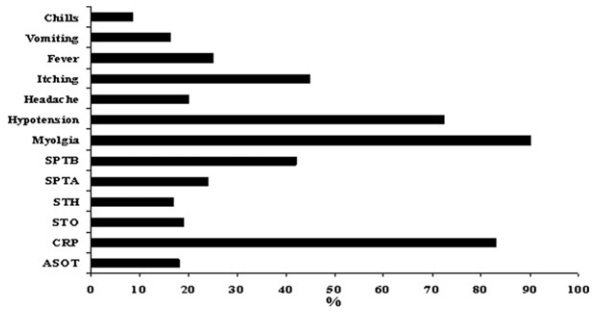

Fig. 1. Percentage of some bacterial toxins, specific antibodies, and pyrogenic reactions in haemodialysis patients.

Table (1): Mean values of total heterotrophic bacteria, total coliforms, *Pseudomonas* spp. and *Aeromonas* (cfu/100ml), endotoxins (EU/ml) in water and dialysate of haemodialysis centers.

	Tap Water	Treated Water	Dialysate
Total heterotrophic bacteria	197.8	234.2	101
Total coliforms	71.5	21. 5	2
Pseudomonas spp.	37.3	9.2	14.4
Aeromonas spp.	56.2	22.2	9.7
Endotoxins	3	17	13

Keywords: bacteriological, toxinological, immune depression, haemodialysis patients
10.1016/j.toxicon.2012.04.131

131. Unexpected Hazard Due to Fuminaisin Toxin Contaminating Herbal Teas in Saudi Arabia

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Background: Fumonisins are phytotoxic mycotoxins that are synthesized by various species of the genus *Fusarium* and are hazardous for human and animal health. The purpose of this study was to investigate fumonisin B1 (FB1) in herbal tea consumed especially by Saudi population.

Methods: FB1 was detected using immunoabsorpant column chromatography with fluorescence detection. 40 commercially available samples were collected and analyzed for FB1**.**

Results: The detectable amount for FB1 was ranged from 0- 266 ppm. All the herbal tea samples were evaluated for the fungal contamination and the presence of mycotoxigenic fungi. Results indicated that predominant mycoflora was distributed in 13 genera and 20 species. From these, the genera *Aspergillus*, *Penicillium* and *Fusarium* that considered extremely important from the mycotoxicological standpoint were the most abundant fungi**.**

Conclusions: The presence of toxigenic moulds represents a potential risk of other mycotoxin contamination and considering the worldwide increased use of herbal products as alternative medicines, it is necessary setting standards for toxigenic moulds in crude herbal tea in order to reduce the risks for consumers' health.

Keywords: fumonisins, herbal tea, toxigenic moulds, mycotoxins
10.1016/j.toxicon.2012.04.132

132. Determination of Histamine and Histamine-forming Bacteria in Striped Marlin Fillets (*Tetrapturus audax*) Implicated in a Food-borne Poisoning

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Background: An incident of food borne poisoning causing illness in 67 victims due to ingestion of fried fish fillets occurred in June, 2011, in Kaohsiung city, southern Taiwan**.**

Methods: Epidemiological, bacterial and chemical investigations were performed**.**

Results: Of the five suspected fish fillets, two samples contained 62.0-mg/100 g (fried sample) and 89.6-mg/100 g (raw sample) of histamine, which is greater than

Fig. 1. DNS sequences of 348-bp region of cytochrome b gene from suspected fish sample and accession No. HQ630754.1 of *Tetrapturus audax* (striped marlin).

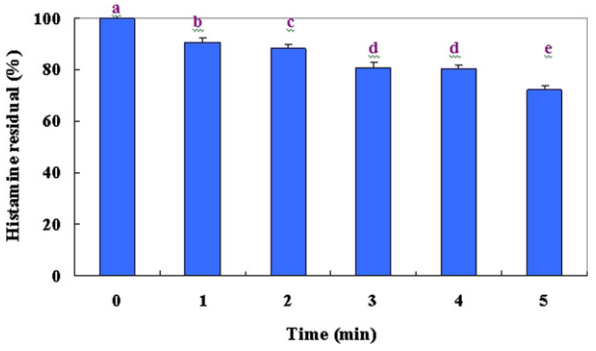

Fig. 2. Histamine residual percentage of marlin fillet implicated in food poisoning at 89.6 mg/100 g histamine by frying for 0, 1, 2, 3, 4, 5 min. Bars represent mean +/− SD of three replications. Bars with the different letters are significantly different ($p<0.05$).

50 mg/100 g of the potential hazard level in most illness cases. Given the allergy-like symptoms of the victims and the high histamine content in the suspected fish samples, this food-borne poisoning was strongly suspected to be caused by histamine intoxication. Five histamine-producing bacterial strains capable of producing 59 to 562 ppm of histamine in trypticase soy broth (TSB) supplemented with 1.0 % L-histidine (TSBH) were identified as *Enterobacter aerogenes* (two strains), *Raoultella ornithinolytica* (two strains) and *Morganella morganii* (one strain). The fish species of suspected samples were identified as striped marlin (*Tetrapturus audax*) by using PCR direct sequence analysis. In addition, the degradation loss of histamine in suspected raw fillets was 28% after 170 °C frying for 5 min.

Conclusions: An outbreak of Scombroid food poisoning was confirmed by clinical symptomatology, epidemiologic investigation, and chemical and bacteriological analyses.

Keywords: histamine; striped marlin; histamine-forming bacteria; scombroid poisoning
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Table 1
Values of the pH, water content, salt content, aerobic plate count (APC), total volatile basic nitrogen (TVBN), total coliform (TC), and *E. coli* in the marlin fillets implicated in food poisoning.

Sample type and no.	pH	Water content (%)	Salt content (%)	TVBN (mg/100g)	APC (log CFU/g)	TC (MPN/g)	*E. coli* (MPN/g)
Fried fillets							
F-1	6.04	62.2	0.81	21.1	2.70	<3	<3
F-2	6.09	65.6	0.88	17.2	5.74	10	<3
F-3	6.19	59.3	0.88	17.2	3.04	<3	<3
Raw fillets							
R-1	6.24	85.5	0.66	21.8	8.02	340	<3
R-2	5.98	87.2	0.61	13.9	7.79	130	<3

Table 2
The levels of biogenic amines in the marlin fillets implicated in food poisoning.

Sample type and no.	Levels of biogenic amine (mg/100 g)								
	Put [a]	Cad	Try	Phe	Spd	Spm	His	Tyr	Agm
Fried fillets									
F-1	2.7	0.2	ND [b]	ND	2.1	ND	17.8	1.2	ND
F-2	0.4	0.5	ND	ND	ND	ND	62.0	3.0	ND
F-3	1.1	0.2	ND	ND	0.4	ND	3.8	1.2	ND
Raw fillets									
R-1	2.7	0.5	ND	ND	1.1	ND	89.6	1.2	ND
R-2	3.0	2.7	ND	ND	0.5	ND	0.8	0.5	ND

[a] Put, putrescine; Cad, cadaverine; Try, tryptamine; Phe, 2-phenylethylamine; Spd, spermidine; Spm, spermine; His, histamine; Tyr, tyramine; and Agm, agmatine.
[b] ND, not detected (amine level less than 0.05 mg/100g).

Table 3
Identification of histamine-forming bacteria isolated from the uncooked marlin fillets implicated in food poisoning by 16S rDNA, based on the output results from NCBI database analysis, and their production of histamine and other biogenic amines (ppm) in culture broth.

Strain	Organism identified	Percentage identity (%)	Source	GenBank accession number	His*	Put	Try	Spd
R-1-1	*Raoultella ornithinolytica*	100	R-1	HQ242731.1	562	2.6	0.2	0.1
R-1-2	*Raoultella ornithinolytica*	100	R-1	HQ242730.1	129	4.6	2.4	0.3
R-1-3	*Enterobacter aerogenes*	100	R-1	JF430156.1	287	2.5	2.0	1.1
R-1-4	*Enterobacter aerogenes*	100	R-1	AB244467.1	246	24.8	ND[b]	0.3
R-2-1	*Morganella morganii*	100	R-2	JF947361.1	59	5.0	1.3	1.7

[a] His, histamine; Put, putrescine; Try, tryptamine; Spd, spermidine.
[b] ND: Not detected (amine level less than 0.05 ppm).

133. Root Toxicity of the Mycotoxin Botryodiplodin in Soybean Seedlings

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Background: The fungus *Macrophomina phaseolina* causes disease in more than 500 plant species. In soybean it causes charcoal rot disease, which is the major cause of soybean yield losses in Mississippi and a significant problem around the world, particularly under dry conditions. *M. phaseolina* infects plants from the reservoir of inoculum in soil by entering plants through the roots. Once inside, it extends mycelium through the vascular system to other parts of the plant. As previously reported, *M. phaseolina* isolated from infected plants and soil in southeastern USA produces the mycotoxin (-)-botryodiplodin, but not phaseolinone. (-)-Botryodiplodin is believed to be a virulence factor that *M. phaseolina* uses to penetrate roots during the initial infection process. The fungus secretes mycotoxin, which kills nearby plant tissue, allowing the fungus to easily enter the plant through the necrotic tissue produced by the toxin.

Methods: The effects of (±)-botryodiplodin on soybean root were investigated in soybean seedlings germinated in soil and transplanted at the cotyledon stage (VC, 4-7 cm growth) to hydroponic growth in individual glass tubes in 10% Villagarcia medium.

Results and Discussion: During 4 days growth at room temperature in continuous light, submergence induced abundant lateral root growth. The seedlings were transplanted to fresh medium containing a range of concentrations (0-80 μg/ml) of (±)-botryodiplodin prepared by chemical synthesis, and cultured an additional 4 days. Lateral root growth was inhibited at ≥4 μg/ml (±)-botryodiplodin. (±)-Botryodiplodin at 80 μg/ml caused severely stunted roots that were stained pink, the color of the pigment produced when botryodiplodin reacts with protein. Inhibition of root growth was quantified by excising roots, drying lateral and tap roots separately and weighing. (±)-Botryodiplodin inhibited tap root growth at IC_{50} = 23.5 μg/ml and lateral root growth at IC_{50} = 4.2 μg/ml. There were no apparent toxic effects to aerial parts of the plants, except secondary to root loss.

Conclusions: The results were consistent with botryodiplodin selectively killing actively growing meristematic tissue at root tips. Production of necrotic tissue at root tips would be expected to provide the fungus with ready access to the plant vascular system. Supported in part by the Mississippi Soybean Promotion Board.

Keywords: mycotoxin, botryodiplodin, soybean
10.1016/j.toxicon.2012.04.134

134. Cytotoxic and Proteolytic Molecules of the Human Parasite *Dientamoeba fragilis*, Identified by RNA seq, Provide Support for its Pathogenic Capacity

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Background: *Dientamoeba fragilis* is a single-celled gastrointestinal parasite of humans with a worldwide distribution. Since its discovery *Dientamoeba* has been largely neglected in clinical circles due to doubts surrounding its pathogenicity. Knowledge regarding the molecular biology of *Dientamoeba* is scarce and only 34 DNA and cDNA sequences (collectively) are available for *Dientamoeba* in Genbank (at the time of writing). Despite numerous reports of clinically apparent dientamoebiasis, no research has been conducted to identify the mechanisms of pathogenicity employed by this organism. It is well established that various pathogenic helminths (worms) and other single-celled human parasites excrete/secrete a range of cytotoxic and proteolytic molecules which induce host tissue destruction and host cell lysis. These molecules represent important factors associated with pathogenicity. It was postulated that *Dientamoeba* also excretes similar cytotoxic and proteolytic molecules and that these molecules are involved in the pathogenisis of clinically apparent dientamoebiasis. This hypothesis was explored using high throughput sequencing technologies to determine whether *Dientamoeba* expresses similar cytotoxic factors *in vitro*.

Methods: *Dientamoeba* parasites were isolated into xenic culture systems from the stools of patients presenting at St Vincent's Hospital, Sydney (Australia), with gastrointestinal complaints. Total RNA was extracted from cultures using standard Tri-reagents. Enrichment of eukaryotic mRNA's from total RNA extracts was achieved using oligo-(dT) chromatography. These mRNA's were sequenced using Roche GS FLX sequencing technology. Sequence reads were assembled using Newbler (version 2.6). The identity of resulting contigs/isotigs was predicted using the Blast2GO program. Homologs to specific cytotoxic/cytolytic molecules were also identified using a standalone version of the NCBI tblastn algorithm.

Results: Four cultures of *Dientamoeba* were obtained and two were selected for sequencing. Assembly of sequencing reads yielded approximately 6000 contigs/isotigs (depending on the assembly settings/paramaters). Homology searches using Blast2GO and tblastn identified a repertoire of *Dientamoeba* molecules which are potentially involved in pathogenic processes. These molecules bear strong homology to factors produced by other pathogenic parasites, which are involved in host tissue destruction and host cell lysis.

Conclusions: These preliminary results indicate that *Dientamoeba* produces molecules which are potentially involved in pathogenic processes, though further characterisation of these molecules is required. This will involve purification of these proteins followed by *in vitro* cytotoxicity assays involving mammalian cell lines to determine their true cytotoxic/cytolytic activity. This is the first report describing potentially cytotoxic and proteolytic molecules

in *Dientamoeba*. Importantly, this data provides support for the pathogenic capacity of *Dientamoeba fragilis*.

Keywords: Dientamoeba fragilis, cytotoxin, cytotoxic, cytolytic, proteolytic, gastrointestinal, parasite
10.1016/j.toxicon.2012.04.135

135. Evaluation of Cardiotoxic Effects of Microcystin–LR in Mouse Isolated Hearts

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Background: Microcystin-LR (MC-LR) is related to envenoming of animals and humans following blooms of cyanobacteria and the release of large quantities of the toxin in lakes and rivers used as water supplies. There are no previous studies showing the cardiac effects of MC-LR under oxidative stress conditions, such as ischemia and reperfusion (I/R). The main goal of this study was to analyze the effect of MC-LR on isolated mouse heart cardiac function**.**

Methods: We assessed the effects of MC-LR in mouse isolated hearts perfused with an appropriated nutritional solution by using the modified Langendorff preparation. We analyzed the tension developed, the electrocardiographic records (EKG), the damaged area and the Creatine Kinase (CK) activity in the perfusate. The preparation was perfused under control conditions and after 15 minutes of stabilization, we added different concentrations MC-LR (0.1-0.3 μg/mL) to the bathing media after 10 min of I/R. At the end of the experiments, hearts were gently sliced and exposed to 1% triphenyl tetrazolium chloride (TTC) to assess damaged areas (Am Heart J, 593: 101, 1981). Electrical and contractile properties were analyzed by the WINDAQ program**.**

Results: The heart exposure at MC-LR 0.1μg/mL did not show any changes. At concentrations 0.3μg/mL MC-LR decreased more than 60% on the cardiac tension and on the QRS waves, after 70 minutes compared to control hearts exposed to nutritional solution (n=4). The analysis with TTC test did not show image evidences of damage, although the CK released in the perfusate increased more 100%. The I/R protocols did not change the control records or the TTC image or CK analysis. However, the hearts exposed to 0.1μg /mL after I/R, showed a decrease of 50% of the cardiac tension without changes on the EKG wave sizes or CK released**.**

Discussion: Our experiments showed that MC-LR alone has cardiotoxic effect above 0.3 μg/mL and its cardiotoxic effects is increased by I/R conditions which stress the cardiac tissue**.**

Conclusions: Our results have shown for the first time the direct damage and effects on cardiac function by MC-LR and this effect is increased by oxidative stress conditions.

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Keywords: Cyanobacteria, Microcystin LR., mouse isolated heart, ischemia and reperfusion
10.1016/j.toxicon.2012.04.136

I. Miscellaneous

136. The Allosteric Binding Site for ρ-TIA on the Extracellular Surface of the α1B- Adrenoceptor

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Background: The G protein-coupled receptor (GPCR) superfamily comprise over 1000 membrane receptors that functionally couple extracellular stimuli to intracellular effectors to control many vital processes. Despite the potential of extracellular surface (ECS) residues in Class A GPCRs to interact with subtype specific allosteric modulators, an ECS pharmacophore has not been identified**.**

Methods: Using the turkey beta$_1$-adrenergic receptor structure, we built a new model of the alpha$_{1B}$-adrenoceptor (α_{1B}-AR) to help identify the allosteric modulatory site of ρηο-conopeptide TIA, an inverse agonist at this receptor**.**

Results: Combining mutational, radioligand binding and IP-one signaling studies together with molecular docking simulations using a refined NMR structure of ρ-TIA, we identified eight residues on the extracellular surface (ECS) of the α_{1B}-AR that influenced ρηο-TIA binding. Double mutant cycle analysis and docking confirmed that ρηο-TIA binding was dominated by a salt bridge and cation-π and T-stacking-π interactions**.**

Conclusions: These interactions reveal that ligands binding to extracellular loop 2 are sufficient to allosterically inhibit agonist signaling at a GPCR. The ligand-accessible ECS residues identified provide the first view of an ECS pharmacophore of a GPCR and the first set of structural constraints for the design of novel allosteric antagonists acting at the ECS of adrenergic receptors.

Keywords: conotoxin, adrenergic receptors, toxin binding determinants
10.1016/j.toxicon.2012.04.137

137. Neuropathies of Spinal Cord Development of Rat Pups Maternally Fed on Fried Potato Chips

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Aims/Objectives: Recently, elevated levels of acrylamide in variety of foodstuffs including fried potatoes chips were reported. The study aimed to illustrate the neuropathies of and demyelination of spinal cord of pups maternally fed on diet containing fried potatoes chips.

Study design: Experimental study.

Methodology: Eighty fertile virgin females and fertile males of albino Wistar rats (*Rattus norvegius*). Females were made pregnant after mating and zero date of gestation was determined. They were arranged into three groups; control, acrylamide-treatment (15mg./kg B.wt) and diet containing 50%fried potatoes chips. Pregnant were treated with acrylamide or fed on fried potatoes chips every other day untill 3 week post-partum. The cervical cord was separated at 2 & 3 week-old rat. Fresh samples were subjected for SDS-PAGE analysis; meanwhile other specimens were processed for light and transmission electron microscopy (TEM).

Results: The present findings revealed altered protein expression in both experimental groups. Light and electron microscopic observations exhibited neuropathological alterations especially of ependymal canal and neuronal cells. Demyelination of axons was observed.

Conclusion: Maternal supplementation of fried potatoes chips during gestation and suckling period led to consume large amount of acrylamide generated during cocking as well as its metabolite glycidamide. Both components find their way across transplacental during gestation and breast milk during lactation period interfering with spinal cord differentiation and cause neurotoxicity and demyelination.

Keywords: neuropathies, offspring, fried potatoes chips
10.1016/j.toxicon.2012.04.138

138. Nickel hepatotoxicity in rats and trials for protection using antioxidants

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Objectives: This work has been carried out to investigate the ameliorating effect of curcumin (80 mg/kg bt.w.P.O.) and/or zinc (227 mg/L in D.W) against $NiCl_2$ (1200 ppm in D.W.) - induced hepatotoxicity.

Methods: One hundred and twenty female albino rats were divided into two groups (A & B), of 60 rats each and each group further subdivided into 6 sub-groups of 10 rats each. Group A was administrated the antioxidants concomitantly with nickel for 8 weeks; group B was administered the antioxidants two weeks prior to nickel exposure and 8 weeks concomitantly with nickel. Sub-group A1 & B1 (Control), A2 & B2 (Ni), A3 & B3 (Ni + corn oil), A4 & B4 (Ni + curcumin), A5 & B5 (Ni + Zn) and A6 & B6 (Ni + curcumin +Zn).

Results: Administration of $NiCl_2$ alone to rats induced significant elevation in serum ALT, AST, serum and hepatic renal MDA level, and CAT activity. Also, $NiCl_2$ induced a significant decrease in serum and hepatic GSH levels and SOD activity. In addition, histopathological changes in liver (vacuolization of hepatocytes and increase in number of kupffer cells) were also recorded. Curcumin and or/zinc administration improved the altered biochemical parameters and histological structures, especially in the pre-treated animals. The greatest improvement was seen with pre-treatment using both curcumin and Zn together.

Conclusions: Curcumin and Zn improved the biochemical parameters and histologic features in nickel-induced hepatotoxicity in rats. Pre-treatment with curcumin and Zn combintion provided the greatest protection.

Keywords: nickel, hepatotoxicity, curcumin, zinc, antioxidants
10.1016/j.toxicon.2012.04.139

J. Pharmacology

139. Comparison of Angiotensin Converting Enzyme Inhibitors and Angiotensin II Type 1 Receptor Blockade for the Prevention of Premalignant Changes in the Liver

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Methods: We investigate and compare the possible antitumor activity of clinically used angiotensin converting enzyme(ACE) inhibitors; captopril, perindopril and angiotensin II type 1 receptor (AT1R) blocker, losartan against hepatocarcinogenesis initiated by diethylnitrosoamines (DENA) and promoted by carbon tetrachloride (CCl4). Diethylnitrosamine (DENA) (200 mg/kg i.p.) initiated and carbon tetrachloride (CCl4) (2 ml/kg i.p.) promoted hepatocarcinogenesis in male Wistar rats after 8 weeks.

Results: Hepatocarcinogenesis was manifested biochemically by elevation of serum hepatic tumor markers tested;α-feto protein (AFP) and carcinoembryonic antigen (CEA). In addition, hepatic carcinogenesis was further confirmed by a significant increase in hepatic tissue growth factors; vascular endothelial growth factor (VEGF) and basic fibroblast growth

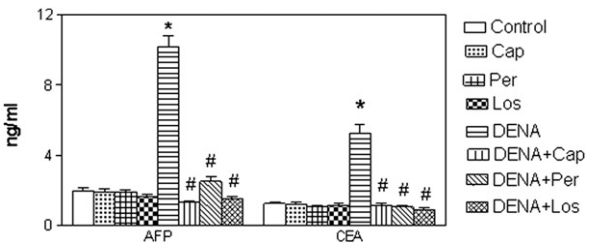

Fig. 1. Effects of oral administration of ACE inhibitors and AT1R blocker for 15 successive days before induction of hepatocarcinogenesis and throughout the experimental period on hepatic tumor markers AFP and CEA. Both tumor markers were measured by ELISA kits. The results were expressed as ng/mL. Each column represents the mean of 10 rats with a vertical bar showing the SEM. *Significant difference from control group ($p<0.05$). #Significant difference from DENA group ($p<0.05$).

factor (FGF). Moreover a marked increase in matrix metalloproteinase-2 and hydroxyproline content were also observed. Hepatocarcinogenesis was further confirmed by a significant decrease in hepatic endostatin and metallothonein level. Long-term administration of the selected drugs for 2 weeks before and throughout the experimental period produced a significant protection against hepatic carcinogenesis. The present results claimed that different doses of the selected drugs succeeded in normalization of serum tumor markers. Furthermore, the drugs reduced the elevated level in the hepatic growth factors, matrix metalloproteinase-2 and hydroxyproline induced by the hepatocarcinogen. Moreover, the amelioration was also accompanied by augmentation of hepatic content of metallothionein and endostatin. Histopathological examination of liver tissues of rats treated with DENA–CCl4 correlated with the biochemical observations.

Conclusions: These findings suggest a similar protective effect of ACE inhibitors; captopril; perindopril and AT1R blocker, losartan against premalignant stages of liver cancer in the DENA initiated and CCl4 promoted hepatocarcinogenesis model in rats. Therefore, RAS especially angiotensin II (Ang II) and AT1R interaction plays a pivotal role hepatocarcinogenesis development.

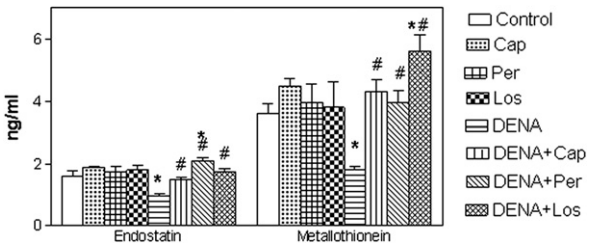

Fig. 2. Effects of ACE inhibitors and AT1R blocker pretreatment before carcinogen intoxication on hepatic metallothionein and endostatin. Both were measure by ELISA kits. The results were expressed as ng/mL. Each column represents the mean of 10 rats with a vertical bar showing the SEM. *Significant difference from control group ($p<0.05$). #Significant difference from DENA group ($p<0.05$).

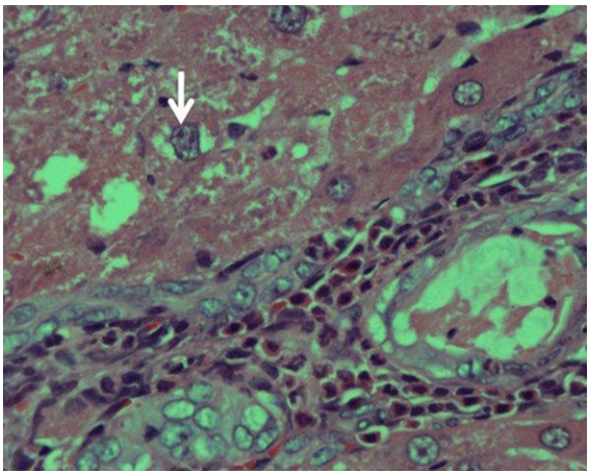

Fig. 3. Liver from rat treated with DENA and CCl4 showing necrotic hepatocytes with occasional dysplastic nuclei (arrow head). The adjacent portal tracts were infiltrated by numerous inflammatory cells including many eosinophils. H&E X 400.

Table 1
Effects of ACE inhibitors and AT1R blocker administration on DENA-induced changes in rat hepatic growth factors; VEGF, TGF-β1 and FGF.

Groups	VEGF pg/ml	TGF-β1 pg/ml	FGF pg/ml
Control	144.43 ± 3.06	132 ± 26	1005+56
Captopril	142.8 ± 802	87 ± 13.5	865 ± 81
Perindopril	133.3 ± 6.22	150 ± 20	1024 ± 41
Losartan	122 ± 4.4	124.4 ± 17.7	1035 ± 54
DENA	210 ± 5.08*	158.5 ± 44.9	1444 ± 52*
DENA+ Captopril	129.2 ± 5.04#	123.7 ± 28.5	1149 ± 57
DENA+ Perindopril	144.7 ± 7.8#	169.8 ± 18.7	1156 ± 83#
DENA+Losartan	128.13 ± 3.9#	74 ± 10.4	1025 ± 116#

All data represent mean values ± SEM (n=10). ACE inhibitors and AT1R blocker were given in drinking water for 15 consecutive days before DENA administration and continue during the experimental period.
* Significant difference from control group.
# Significant difference from DENA group. $P < 0.05$.

Table 2
Effects of ACE inhibitors and AT1R blocker administration on DENA-induced changes in rat hepatic MMP-2, TIMP-1 and hydroxyproline.

Groups	MMP-2 ng/ml	TIMP-1 pg/ml	Hydroxy-proline ng/ml
Control	3.47 ± 0.23	945 ± 22	509 ± 18
Captopril	3 ± 0.24	716 ± 79	534 ± 40
Perindopril	2.4 ± 0.47	852 ± 118	547 ± 56
Losartan	3.3 ± 0.44	753 ± 50	500 ± 40
DENA	14 ± 0.966*	1419 ± 157	893 ± 73*
DENA+ Captopril	4.66 ± 0.57#	1021 ± 55	468 ± 24#
DENA+ Perindopril	5.3 ± 0.57#	1856 ± 147*	497 ± 24#
DENA+Losartan	5.17 ± 0.94#	1312 ± 157	621 ± 32#

All data represent mean values ± SEM (n=10).
ACE inhibitors and AT1R blocker were given in drinking water for 15 consecutive days before DENA administration and continue during the experimental period.
* Significant difference from control group.
# Significant difference from DENA group. $P < 0.05$.

Keywords: losartan, captopril, perindopril
10.1016/j.toxicon.2012.04.140

140. Identification, Pharmacological and Structural characterization and Engineering of Three-finger Toxins interacting with GPCRs

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Background: To subdue their prey or protect them against predators, venomous animals have selected toxins that primarily interact with voltage-gated and ligand-gated ion channels, targets which play crucial roles in several biological functions. Nevertheless, some toxins are known

to recognize other molecular target families, such as the G-Protein Coupled Receptors (GPCRs). The first part of the toxins interacting with GPCRs can be considered as functional mimetics of natural agonists of the receptor while other toxins display structure and pharmacological profiles unrelated to any natural endogenous ligands.

Results: Here we first presented our last results related to the identification and pharmacological characterization of new three-finger fold toxins interacting with alpha-adrenergic receptors. Two toxins: r-Da1a and r-Da1b, were isolated from the *Dendroaspis angusticeps* snake venom and characterized for their high affinity and specific interaction with a1a- and a2-adrenoceptors, respectively. The particular pharmacological profile of these toxins allows envisioning their exploitation as imaging or therapeutical agents, as for example for r-Da1a in the treatment of benign prostatic hyperplasia. In addition, structure-function studies of various toxin-receptor complexes were performed in order to identify at the molecular level the origin of the specificity of these interactions. Our results reveal how MT7 toxin recognizes the muscarinic M1 receptor and proposes a structural model of this complex in which the MT7 interacts with a dimeric form of the receptor. This model structurally supports the high affinity and selectivity of the MT7-hM1 interaction, shows the atypical mode of interaction of this allosteric ligand with the GPCR receptor and can be used as a starting point to design new ligands with predetermine pharmacological property. Thus, MT7 engineering using block permutations lead to the generation of toxins with new muscarinic/adrenergic functional profiles.

Conclusion: Our results identify molecular determinants involved in the affinity, selectivity and functional property of aminergic toxins and highlight the ability of the three-finger template to support various GPCRs interacting profiles.

References

C. Marquer et al. Structural model of ligand-G protein-coupled receptor (GPCR) complex based on experimental double mutant cycle data: MT7 snake toxin bound to dimeric hM1 muscarinic receptor, J Biol Chem 286 (2011) 31661-31675.
C. Rouget, et al. Identification of a novel snake peptide toxin displaying high affinity and antagonist behaviour for the alpha2-adrenoceptors, Br J Pharmacol 161 (2010) 1361-1374.
L. Quinton, et al. Isolation and pharmacological characterization of AdTx1, a natural peptide displaying specific insurmountable antagonism of the alpha1A-adrenoceptor, Br J Pharmacol 159 (2010) 316-325.

Keywords: three-finger toxins, GPCRs, bio-engineering
10.1016/j.toxicon.2012.04.141

141. Lipid Bilayer Condition Abnormalities Following *Macrovipera lebetina obtusa* and *Montivipera raddei* Snake Envenomation

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Background: Viper bites are an endemic public health problem in Armenia, even in the cities. Venoms produced by snakes of the family Viperidae contain proteins that interfere with the coagulation cascade, the normal haemostatic system and tissue repair, and human envenomations are often characterized by clotting disorders, hypofibrinogenemia and local tissue necrosis.

Methods: Studies on the interaction of snake venom and organized lipid interfaces have been conducted using a variety of systems, including BLMs, SUVs and LUVs. Giant unilamellar vesicles (GUVs) with a mean diameter of 30 μm have a minimum curvature and mimic cell membranes in this respect. GUVs were formed from the total lipid fraction from bovine brain by the electroformation method (Angelova and Dimitrov, 1987). *Macrovipera lebetina obtusa* and *Montivipera raddei* venom was added to the sample chamber before the vesicles were formed. The membrane fluorescence probes, ANS and pyrene, were used to assess the state of the membrane and specifically mark the phospholipid domains. Fluorescent spectra were acquired on a *Varian* fluoremeter instrument.

Results: The membrane fluorescence probes, ANS and pyrene, were used to assess the state of membrane and specifically mark the phospholipid domains. Independent of their lipid composition, all GUVs modified by *Macrovipera lebetina obtusa* venom were enlarged in size as venom-dependent lipid hydrolysis proceeded. In contrast, liposomes modified with *Montivipera raddei* venom demonstrate a so called “oval deformations” and venom-dependent shrinking response. In addition to the visible morphological changes, ANS and pyrene also allows us to quantify the fluidity changes in the membrane by measuring of the fluorescence intensity. The presence of viper venom in GUVs media reveals a noticeable decreasing of membrane fluidity compare the control, while the binding of fluorophores with GUVs modified by venom lead to appearance of channel activity.

Conclusions: These studies emphasize the importance of a membrane surface curvature for its interaction with enzymatic components of venom.

Keywords: BLM, GUV, electroporation, snake venomics, artificial membranes
10.1016/j.toxicon.2012.04.142

142. From alpha-Conotoxins and alpha-Neurotoxins to Endogenous “Prototoxins" and Binding Sites in Nicotinic Acetylcholine Receptors

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Background: Snake venom alpha-neurotoxins helped to isolate a muscle-type nicotinic acetylcholine receptor (nAChR) and more recently the acetylcholine-binding protein (AChBP), an excellent model for the ligand-binding domains of all nAChR subtypes. Alpha-conotoxins from *Conus* snails are more selective in distinguishing neuronal

nAChRs. A breakthrough was the discovery of endogenous "prototoxin " Lynx1, belonging to the same family of three-finger proteins as snake neurotoxins, attached to the membranes via glycosyl phosphatidylinositol (GPI) anchor near nAChRs and modulating their function. The report covers our recent findings, including those in collaboration with European laboratories.

Methods: HPLC analysis of snake venoms, primary structure determination, NMR and X-ray for 3D-structures, *E. coli* heterologous expression of three-finger proteins , solid-phase synthesis of alpha-conotoxins. Computer modeling, molecular dynamics , X-ray analysis, electrophysiology and radioligand analysis for theoretical and experimental studies of alpha-conotoxins and other complexes with AChBPs and nAChRs.

Results: Novel alpha-conotoxin PnIA analogs and radioiodinated derivatives having higher affinity for alpha7 nAChRs and *L. stagnalis* AChBP were designed and synthesized (Kasheverov et al, Mar. Drugs, 2011). Three-finger proteins with an additional disulfide in the loop I, namely WTX *Naja kaouthia* and water-soluble domain of Lynx1 (ws-lynx1) were expressed in *E. coli* and shown to act on nAChRs and muscarinic receptors; ws-lynx1 was demonstrated to bind at the classical site for agonists/competitive antagonists at AChBPs and muscle-type nAChRs, but beyond it at neuronal nAChRs (Luykmanova et al, Biokhimia, 2009; J. Biol. Chem, 2011; Mordvintsev et al, FEBS. J. 2009). A covalent dimer of alpha-cobratoxin, interacting with alpha3beta2 nAChRs, was discovered in *Naja kaouthia* venom and its X-ray structure recently determined (Osipov et al, J. Biol. Chem. 2008, 2012).

Discussion: We demonstrated the advantages of combined approach utilizing novel snake venom protein neurotoxins and synthetic alpha-conotoxins and their analogs as tools in nAChR research. These instruments are supplemented by theoretical and experimental approaches revealing the structure of AChBP complexes which provides information about the binding sites in distinct nAChR subtypes. In particular, with our participation it was demonstrated that binding of alpha-conotoxins (antagonists) produces conformational changes opposite to those induced by agonists (Celie et al, Nature Str. & Mol. Biol., 2005), while for such antagonists as d-tubocurarine and strychnine different orientations are possible within the same AChBP molecule (Brams et al, PloS Biology, 2011).

Conclusion: Peptide and polypeptide neurotoxins provide valuable information about the binding sites in distinct muscle-type and neuronal nAChRs shedding light on functional mechanisms and providing basis for drug design.

Keywords: three-finger toxins, alpha-conotoxins, nicotinic acetylcholine receptors
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143. Exploring 'Labyrinth' of Pain with Scorpion 'Sting'

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Background: Voltage-gated sodium channels (VGSCs), responsible for initiation and probagation of action potentials in most excitable cells, are implicated to play a critical role in the development and maintenance of pain observed in primary afferent neurons following nerve and tissue injury. Scorpion venemation is a severe medical problem in many regions across the world. Victims of envenoming by a scorpion suffer a variety of pathologies, such as fever, convulsion and intense pain.

Discussion: Although the common mechanisms of this pathological pain symptoms have been deduced in part over the years, detail information like pain transmission, integral processing and therapeutic intervention and control have not yet been fully worked out. Currently, it has been demonstrated that the toxic components in scorpion venom are mainly neurotoxic peptides targeting on various ion channels including VGSCs at large.

Conclusions: This review will present a state of the art progress in studying the underlying behavioral phenotypes and peripheral mechanisms of pain responses mimicing by a novel experimental BmK (*Buthus martensi* Karsch) scorpion sting pain model. Through looking in-depth exploration of venom composition, pharmacological binding characteristics, intra-/extra- cellular electrophysiological activities and molecular determinants, a possible cross-talk network regarding the VGSCs-mediated pain initiation and maintanence in overall neuronal sensing and responses pathway by scorpion sting could be approached.

Keywords: pain, scorpion sting, BmK, voltage-gated sodium channels
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144. Analgesic Effect of Crotalphine in a New Model of Rat Bone Cancer Pain

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Background: Crotalphine (CRP), a peptide first identified and isolated from the South American rattlesnake *Crotalus durissus terrificus* venom, induces analgesic effect mediated by opioid receptors. The aim of this work is to characterize the analgesic effect of crotalphine in a new model of bone cancer pain induced by inoculation of Walker 256 tumor cells into the rat femoral cavity.

Methods: Bone tumor implantation and metastasis were determined by histopathological analysis. Bone metabolic alterations were determined by scintigraphy, using ^{99m}Tc-MDP. Femoral images were obtained before and 7, 14 and 21 days after tumor cell injection. Bone cancer pain was characterized by the presence of hyperalgesia (rat paw pressure test) and allodynia (von Frey filaments).

Results and Discussion: Photomicrographs analyzed 21 days after injection of tumor cells, demonstrated the presence of tumor cells in the femur of the animals.

Incorporation of ^{99m}Tc-MDP was significant 7, 14 and 21 days, suggesting the development of tumor on the femoral cavity. Histopathological analysis demonstrated the presence of tumor cells in the lung and spleen, but not in the liver and kidneys of the rats. The results indicate that cells inoculated into femoral bone marrow can spread to some organs, including lymphoid organs. Hyperalgesia and allodynia were detected on days 1, 3, 7, 14 and 21 after cell inoculation. Interestingly, the paw withdrawal threshold in the von Frey test was reduced not only in the ipsilateral hind paw but also in the contralateral one, demonstrating the existence of bilateral allodynia (mirror-image pain). To evaluate the involvement of prostanoids in these nociceptive phenomena, Indomethacin, a cyclooxygenase inhibitor, was administered 3,7,14 and 21 days after tumor cell injection. Indomethacin only partially inhibited hyperalgesia and allodynia induced by bone cancer, indicating the involvement of prostanoids in bone cancer pain. The contribution of prostanoids is more significant within the first 3 days after cell injection. CRP (8μg/kg) administered on day 21, blocked hyperalgesia, allodynia and mirror image pain. The analgesic effect was detected up to 2 days after peptide administration and was blocked by κ-opioid receptor antagonist and partially inhibited by δ-opioid antagonists, indicating the involvement of opioid receptors. Morphine only partially inhibited allodynia and hyperalgesia.

Conclusions: Results indicate that injection of tumor cells causes bone cancer and pain. CRP induces a potent and long-lasting antinociception in this model, with higher efficacy as compared to standard analgesic drugs.

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Keywords: crotalphine, analgesia, bone cancer pain
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145. *In vitro* Vascular Activity of Crude *Bungarus candidus* and *Bungarus fasciatus* Crude Venoms

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Background: The South East Asian region contains a large number of species of venomous snakes that have not been extensively studied. *Bungarus candidus* (Malayan krait) *and Bungarus fasciatus*(Banded krait) are two medically important snakes in this region with highly neurotoxic venoms. Even though considerable work has been done on the neurotoxicity of the venoms, not much is known about other activities of these venoms.

Methods: In this study, we examined the in vitro vascular activity of *Bungarus candidus* and *Bungarus fasciatus* venoms using isolated rat thoracic aorta.

Results: Both venoms were found to produce dose-dependent relaxation (1-50μg/ml) in precontracted endothelium-intact aorta but not endothelium-denuded aorta. The relaxation effect by both venoms was partially inhibited by the cyclooxygenase inhibitor indomethacin (5μM).The muscarinic receptor antagonist atropine (1μM) only partially inhibited the relaxation response produced by *Bungarus candidus* but not *Bungarus fasciatus*. However, the nitric oxide synthase inhibitor L-NOLA (100μM) and pre-treatment of the venom with 4-bromophenacyl bromide (1.8mM) inhibited the relaxation response produced by both venoms. Preliminary screening on the four fractions obtained by size-exclusion chromatography indicated that the relaxation effect was produced by a single fraction. LCMS/MS analysis of this fraction indicated that it consisted mainly of phospholipase A_2 toxins.

Conclusions: These data suggest that vasodilator phospholipase A_2/cyclooxygenase metabolites and endothelium-derived nitric oxide may play important roles in the venoms vascular activity.

Keywords: *Bungarus candidus, Bungarus fasciatus*, blood vessel
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146. An Insecticidal Spider Toxin that Acts as a Positive Allosteric Modulator of Insect Nicotinic Acetylcholine Receptors

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Background: κ-Hexatoxins (formerly κ-atracotoxins) are a family of excitotoxic insect-selective neurotoxins from Australian funnel-web spiders (Hexathelidae: Atracinae) that are lethal to a wide range of agronomically and medically relevant insects, but display no toxicity towards vertebrates. The prototypic κ-HXTX-Hv1c blocks native and heterologously expressed (*pSlo*) cockroach BK_{Ca} channels, but not cockroach Na_V or Ca_V channels or mammalian BK_{Ca} channels. Despite the high affinity and selectivity of κ-HXTX-Hv1c for insect BK_{Ca} channels, we have recently discovered that the insect BK_{Ca} channel does not appear to be the lethal target of the toxin. The aim of this study was therefore to determine the lethal target of κ-hexatoxins.

Methods: Actions of κ-HXTX-Hv1c on voltage- and transmitter-gated ion channels were determined using whole-cell patch clamp analysis of dorsal unpaired median (DUM) neurons from the American cockroach *Periplaneta americana*. Acute toxicity tests were performed in crickets (*Acheta domesticus*) by intrathoracic injection.

Results: Acute toxicity tests revealed that the BK_{Ca} blockers paxilline, charybdotoxin and iberiotoxin, which all block insect BK_{Ca} channels with IC_{50} values < 30 nM, are not lethal in crickets. This suggests that κ-HXTX-Hv1c has additional molecular targets important for its lethality.

Subsequent testing of cockroach K_V channels revealed that κ-HXTX-Hv1c failed to significantly block cockroach K_{Na} and K_{DR} channels, but 1 μM κ-HXTX-Hv1c did cause a 20% block of 'A-type' (K_A) currents. This suggests that κ-HXTX-Hv1c additionally targets either insect K_V1 and/or K_V4 channel subtypes. In support, the non-selective K_A blocker 4-aminopyridine was lethal in insects. However the modest actions at such high concentrations would indicate a different lethal target. Accordingly, we assessed the actions of κ-HXTX-Hv1c on insect GABA ($GABA_AR$), glutamate (GluClR) and nicotinic acetylcholine (nAChR) receptors in DUM neurons. We found that 1 μM κ-HXTX-Hv1c failed to significantly affect $GABA_ARs$ but did produce a modest 21% increase in GluClR currents. In contrast, κ-HXTX-Hv1c produced a concentration-dependent slowing of nicotine-evoked nAChR current decay (EC_{50} = 200 nM) and reversed nAChR current desensitisation. These actions occurred without any alterations to the amplitude of nAChR currents or the concentration-response curve to nicotine. These findings are consistent with a positive allosteric modulation of nAChRs.

Conclusion: κ-HXTX-Hv1c represents the first peptide toxin that selectively modulates insect nAChRs with a mode of action similar to the excitotoxic insecticide spinosyn A. Given the similar phenotype and mechanism of action, we propose that the likely lethal target of κ-HXTX-Hv1c is the insect nAChR.

Keywords: κ-hexatoxins, nicotinic acetylcholine receptor, insecticidal, spider toxin, ion channels, positive allosteric modulator
10.1016/j.toxicon.2012.04.147

147. Isolation and Pharmacological Characterisation of Neurotoxins from the Venom of Three Species of Australian Copperheads (*Austrelaps spp.*) and the Efficacy of Tiger Snake Antivenom to Prevent or Reverse Neurotoxicity

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Background: The venom of the Australian lowlands copperhead (*A. superbus*) produces significant, and potentially lethal, neurotoxic paralysis in cases of clinical envenomation. However, little is known about the neurotoxic components within this venom or the venoms of the related alpine (*A. ramsayi*) or pygmy (*A. labialis*) copperheads. The aim of this study was to isolate and characterize the neurotoxins responsible for these neurotoxic actions at the skeletal neuromuscular junction.

Methods: Neurotoxins were purified using size-exclusion, cation-exchange and reverse-phase liquid chromatography. Masses and primary sequences were determined using ESI- and MALDI-TOF mass spectrometry combined with enzymatic cleavage and Edman degradation. Purified neurotoxins were pharmacologically characterised using the chick biventer cervicis nerve-muscle preparation to determine their site and mechanism of action.

Results: Similar to an expanding number of Australian elapid snakes, all three species displayed potent presynaptic neurotoxicity. This was due to the presence of a slow-acting, high molecular mass (> 40 kDa), multimeric snake presynaptic PLA_2 neurotoxin (SPAN) complex within each venom. Further characterisation of the *A. superbus* SPAN complex, P-EPTX-As1a, revealed classical triphasic alterations to neurotransmitter release and fade in tetanic tension. Interestingly, two of the subunits of P-EPTX-As1a displayed high sequence homology with the α- and β-subunits of the SPAN complex, taipoxin, from the Coastal taipan (*Oxyuranus scutellatus*), an unrelated Australian elapid. Importantly, the activity of all three SPAN complexes could be prevented, but not reversed, by CSL monovalent tiger snake antivenom (TSAV). Muscle paralysis also resulted from the activity of a number of postsynaptic a-neurotoxins present in all three venoms. In the case of *A. labialis* venom, the most potent activity was due to α-EPTX-Al2a (8074 Da), a long-chain α-neurotoxin. α-EPTX-Al2a causes potent pseudo-irreversible antagonism at the skeletal muscle nicotinic acetylcholine receptor (pA_2 = 7.902 ± 0.002). a-EPTX-Al2a contains five disulphide bonds and shares both significant sequence homology and pharmacophore residues of classical long-chain postsynaptic α-neurotoxins. Of clinical importance was the finding that the postsynaptic neurotoxic actions of α-EPTX-Al2a can be prevented, but only partially reversed, by TSAV.

Conclusion: The venoms of all three *Austrelaps* spp. exhibit potent presynaptic and postsynaptic neurotoxicity that may contribute to the clinical picture of envenomation. This is the first study to validate the use of TSAV in cases of clinical envenomation by *Austrelaps* spp. Nevertheless, it highlights the importance of not delaying administration of antivenom, once neurotoxicity becomes evident, due to the lack of antivenom efficacy against presynaptic and irreversible postsynaptic neurotoxins.

Keywords: *Austrelaps*, Australian copperhead, snake presynaptic PLA2 neurotoxin, postsynaptic α-neurotoxin, snake antivenom, neurotoxicity, neurotransmitter release, skeletal muscle nicotinic acetylcholine receptor
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148. Characterization of Inflamin from *Aipysurus eydouxii*: A Novel Class of Toxin that Induces Inflammation

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Background: *Aipysurus eydouxii* (Marbled sea snake) belongs to the Hydrophiidae family. Venom of this snake has not been extensively studied probably due to low venom yield. We sequenced the cDNA clones from its venom gland library and identified several novel proteins. Here, we describe the characterization of a novel protein, named as inflamin.

Methods: Inflamin has 94 residues including six cysteines. We have recombinantly expressed inflamin in *E.coli* cells. Protein was refolded using redox buffer and purified by RP-HPLC. The disulfide linkages were determined by partial reduction and cyanylation-induced chemical cleavage. We examined its effects in mice through i.p. injections. We determined the production of prostaglandins and prostacyclins in the peritoneal exudate at various time points. Edema was monitored after intraplantar injection in rats. The mechanism of action of inflamin was studied by its effect on $cPLA_2$ and COX enzymatic activity in RAW264.7 macrophages. Western blotting was also performed to determine the expression level of these enzymes.

Results: Refolded inflamin showed I-III, II-IV, V-VI disulfide connectivity. In mice it induced writhing, which is probably due to inflammatory pain. Indomethacin suppressed inflamin-induced writhing. Intraplantar injection induced inflammation and edema in rat paws. The peritoneal exudate showed the dose-dependent increase in prostaglandins. Levels of prostaglandins ($PGF_{1\alpha}$, PGE_2, PGD_2, $PGF_{2\alpha}$ and TXB_2) were highest after 10 min and then subsided. The production of these prostaglandins decreased significantly upon indomethacin pre-treatment. We examined its mechanism of action on cultured RAW264.7 macrophages. At sub-cytotoxic concentration of inflamin, the $cPLA_2$ enzymatic activity increased within 5 min of protein administration and becomes 150% compared to saline after 10 min. Western blotting showed that $cPLA_2$ was phosphorylated at Ser505 within 5 min. COX-2 enzyme is induced after 3h which appears to start the second wave of prostaglandin production.

Discussion: The novel protein inflamin induces edema and inflammatory writhing in experimental animals. This inflammation is due to the release of prostaglandins. Inhibition of COX enzyme by indomethacin decreased prostaglandin production and writhing. Inflamin treatment leads to phosphorylation of $cPLA_2$ and increase in its activity and the production of pro-inflammatory eicosanoids.

Conclusions: Inflamin is a novel snake venom protein that induces inflammation and pain. Since *A. eydouxii* depends on eggs-only diet, we speculate that this toxin may be used as a defensive weapon against predators.

Keywords: snake venom, inflammation, prostaglandins
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149. The Role of the Lymphatic System in the Absorption of *Micrurus fulvius* Venom

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Background: Coral snakes have short fixed fangs and a poorly developed system for venom delivery, thereby requiring a chewing action to inject the venom; this physiology suggests that inoculation of the venom is by subcutaneous (SC) route. Venom components in the interstitial space must then pass to blood either directly or through absorption to lymph for systemic distribution.

Methods: The contribution of lymph to the absorption and systemic bioavailability of *M. fulvius* venom after SC administration was determined using a central lymph-cannulated sheep model. As the reference, we also used non-cannulated animals. Also, the kinetics of the venom was followed after intravenous bolus injection. Each of the three groups included four sheep; experiments lasted six hours and the animals were kept under anesthesia during the whole experiment. Venom concentrations in serum and lymph were measured by sandwich enzyme-linked immunoassay.

Results: Venom injected into blood stayed in systemic circulation for 72.8±5 min (MRT), with a half-life ($t_{1/2}$) of 25.3±3.2 min and a volume of distribution (Vss) of 11±0.8%. In contrast, when administered subcutaneously the venom MRT, $t_{1/2}$ and Vss increased 5.5, 9 and 5.6-fold, respectively. The absorption of venom to blood was incomplete when the venom was injected SC, with a recovery of 63±5% of the initial dose during the 6 hours of the experiment. Lymph contributed with 39% of the absorbed venom to blood.

Discussion: The low Vss of venom after IV administration shows that tissues do not retain venom significantly and that the steady state observed after SC injection is the result of a slow absorption process. The kinetics of venom in blood of the thoracic duct cannulated versus non-cannulated groups show that lymphatic absorption contributes to maintain a prolonged steady state in systemic circulation.

Conclusions: These results show that the limiting process in the pharmacokinetics of SC injected *M. fulvius* venom is its absorption and that the lymphatic system plays a key role in the process. Supported by CONACyT (C382-08) and Instituto Bioclón S.A. de C.V.

Keywords: *Micrurus fulvius*, lymphatic system, absorption
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150. Tailoring the Selectivity of Anuroctoxin for Kv1.3 K+ Channels

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Background: The voltage-gated Kv1.3 potassium channel plays a key role in the activation of T lymphocytes by maintaining a negative membrane potential. By blocking these channels the proliferation of T cells can be inhibited. This can result in immunosuppression, which has a great potential in the therapy of certain autoimmune diseases. Anuroctoxin,

a 35-amino-acid peptide isolated and characterized previously from the venom of the scorpion *Anuroctonus phaiodactylus,* blocks Kv1.3 with high affinity (Kd = 0.7 nM). Although with lower affinity, the toxin blocks another K^+ channel, Kv1.2 (Kd = 6 nM), which is expressed in neurons, heart and smooth muscle cells, thus this property of the toxin is not advantageous considering its potential clinical use.

Methods: We examined four variants of anuroctoxin produced by solid-phase synthesis. Besides the wild-type toxin we tested three mutant toxins designed by sequence alignment of published toxins selective for Kv1.2 or Kv1.3. Our expectation was that the mutations would improve the selectivity of the toxin for Kv1.3 over Kv1.2 keeping the high affinity for Kv1.3. We investigated the effect of the toxins with whole-cell patch-clamp technique on activated T lymphocytes endogenously expressing Kv1.3 channels and cells transfected with the gene of hKv1.1, hKv1.2 and IKCa1 channels.

Results: The effect of synthetic wild-type anuroctoxin was similar to that of the natural toxin (Kv1.3: Kd = 0.3 nM and Kv1.2: Kd = 5.3 nM) proving the success of solid-phase synthesis. The first mutant F32T was designed to examine the role of the essential dyad in selectivity. F32T practically lost affinity for Kv1.2 but also showed a slight decrease in affinity for Kv1.3 (Kd = 7.5 nM). We designed the mutant N17A in an attempt to increase affinity for Kv1.3. However, this affinity did not change significantly (Kd = 0.9 nM) and a slight improvement in selectivity could be observed (Kv1.2: Kd = 18.9 nM). Combining the two mutations in one toxin we constructed the mutant N17A,F32T where the advantageous effect of both mutations could be detected. The N17A,F32T mutant is highly selective (no significant effect on Kv1.2 at 100 nM) and a high affinity blocker of Kv1.3 (Kd = 0.6 nM).

Conclusions: With targeted mutations we designed and produced a selective and high affinity blocker of Kv1.3. Our results provide the foundation for the possibility of the production and future therapeutic application of additional, even more selective toxins.

Keywords: Kv1.3, scorpion toxin, selectivity
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151. Immuno-Inflammatory Response after Scorpion Envenomation: Potential Role of Eïcosanoids and Histamine H1-Receptor

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Background: After scorpion envenoming, the massive release of neurotransmitters and inflammatory modulators is mainly due to neurotoxic components of scorpion venoms. This release of mediators leads to a cascade of pathological events, mainly in the cardio-respiratory system, by mechanisms which are not yet well understood.

Methods: The potential role of Histamine H1-Receptor and eicosanoids was investigated in mice using Histamine H-1 antagonist (Prometazine, 5 mg/kg) or phospholipase A(2) inhibitor (dexamethasone, 5 mg/kg) prior to the envenomation with *Androctonus australis hector, (Aah)* venom. Inflammatory markers were measured in sera and tissue homogenates of envenomed mice.

Results: The results obtained showed that the inflammatory process induced by this venom is characterized by hyperleukocytosis associated with elevated production of nitric oxide and pro-inflammatory cytokines in peripheral blood. The migration of neutrophils and eosinophils in lungs and liver was confirmed by the release of myeloperoxidase and eosinophil peroxidase. Hypoalbuminemia and hyper-gammaglobulinemia accompanied by a significant activation of complement system were also the predominant abnormalities observed after envenomation. Our results showed also that Aah venom induced significant increase in tissue lipid peroxidation, concomitant with depletion of antioxidants. Histological analysis revealed the presence of edema, hemorrhage and neutrophil infiltration in lung and liver tissue. The leukocytosis, nitric oxide release and cellular peroxidase activities were inhibited by previous treatment with corticosteroid (dexamethasone). However, fluid accumulation in the organs and tissue damage seem to be partially reduced. Administration of promethazine induces a significant decrease of edema in liver and lung. The myeloperoxidase and eosinophil peroxidase activities decreased also significantly. Histological analysis confirmed the inhibition of edema forming and inflammatory cell recruitment in liver and pulmonary parenchyma compared to envenomed mice. However, high lipid peroxidation rates and lower antioxidative protection were found in the liver and lung homogenates even after inhibition histamine H-1receptor or eicosanoid mediators.

Discussion: Inflammatory response induced by scorpion venom could be mediated by multiple mediators including histamine via H1-receptors. Dexamethasone pretreatment may suppress NF-κB activation and prevent the exacerbation of the inflammatory response after scorpion envenomation.

Conclusions: These results could help to provide safer and selective therapies in the complicated cases of envenomed patients. The use of antioxidants after scorpion envenomation could be useful to produce a protective effect against deleterious manifestations and damages induced by the scorpion venom.

Keywords: inflammation, eicosanoids, histamine, H1-receptor, scorpion venom
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152. Effect of Crotoxin on Secretory Activity of Peritoneal Macrophages Co-cultivated with Tumor Cells. Involvement of Formyl Peptide Receptors

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Background: Crotoxin (CTX) inhibits tumor growth and modulates the functions of macrophages. Macrophages provide a defense mechanism against tumor cells and two distinct polarization states, M1 and M2, have been described for these cells. In the beginning of tumor progression, M1 macrophages release reactive nitrogen/oxygen intermediates, cytokines and lipoxygenase-derived eicosanoids, which may contribute to tumor inhibition. In contrast, during tumor development, the release of these mediators by tumor-associated macrophages (M2 cells) is inhibited, contributing to tumor development. Based on these evidences, the aim of this work is to evaluate if inhibition of macrophage by CTX contributes to the decrease in tumor cell growth caused by this toxin.

Methods: The effect of CTX on the activity of macrophages co-cultivated with LLC WRC 256 tumor cells was evaluated. To determine tumor cell growth inhibition, macrophages ($2x10^5$ cells), obtained from rat peritoneal cavity, were incubated with CTX (0.3μg/mL) for 2 h at 37ºC. After this time, the macrophages were co-cultivated in the presence of LLC WRC 256 tumor cells ($2x10^4$), previously plated in 96-well culture dishes. To determine the levels of cytokines and eicosanoids, macrophages ($5x10^5$) were incubated with CTX (0.3μg/mL) for 2 h and then co-cultivated in presence of the tumor cells ($5x10^4$). The levels of IL-1β, LXA_4 and 15-epi-LXA_4 in culture supernatants were determined by ELISA. The release of nitric oxide (NO) was determined by the quantification of nitrite in the supernatants of cultured macrophages.

Results: Co-cultivation of CTX-treated macrophages together with tumor cells caused a 25% reduction of tumor cell proliferation. A 35% increase in NO production was detected 48 h after co-cultivation. A 273% increase in IL-1β levels was detected 12 and 24 h after co-cultivation. Moreover, an increase in the levels of LXA_4 (35%) and 15-epi-LXA_4 (2.3 fold) was observed 24 and 48 h after co-cultivation. Boc-2 blocked the stimulatory effect of CTX on macrophage asecretory activity and the inhibitory effect of these cells on tumor cell proliferation.

Discussion: Taken together, these results indicate that CTX modifies the secretory activity of M2 cells, which may contribute to the inhibitory action of the toxin on tumor growth. Activation of formyl peptide receptors seems to play a major role in this effect.

Conclusions: These data confirm the actions of CTX on defence mechanisms and open new perspectives for the development of novel substances with therapeutic properties.

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Keywords: crotoxin, macrophage, tumor cells
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153. Biochemical and Pharmacological Characterization of Three-Finger Neurotoxins from the Venom of Eastern Coral Snake (*Micrurus fulvius fulvius*)

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Background: Animal venoms are rich sources of pharmacologically active proteins and polypeptides that exhibit remarkably high potency and specificity for different biological targets, making them excellent probes for studying the distribution and function of ion channels and receptors. Neurotoxins derived from animal venoms in particular are invaluable tools for studying neurotransmission. The primary objective of this work is the identification, isolation and biochemical and pharmacological characterization of novel three-finger neurotoxins from the venom of Eastern coral snake, *Micrurus fulvius fulvius* (*M. f. fulvius*).

Methods: Neurotoxic fractions were purified from *M. f. fulvius* venom using a two-step chromatographic approach guided by *ex vivo* pharmacological screening in isolated avian and mammalian nerve-skeletal muscle preparations. Electrospray-ionization mass spectrometry was used to determine the molecular weight of the purified proteins. Amino acid sequence of the target neurotoxin was subsequently determined by N-terminal sequencing by Edman degradation and circular dichroism spectroscopy was used to determine the secondary structure.

Results: Crude venom (1 - 100 μg/ml) produced time- and concentration-dependent inhibition of nerve-evoked twitch responses in indirectly stimulated chick biventer cervicis muscle with an IC_{50} of 4.5 μg/ml. Bioactivity-guided HPLC purification led to the identification of a 7 kDa neurotoxin, subsequently named MFTx2. Analysis of the amino acid sequence and secondary structure indicated that this is a short-chain three-finger neurotoxin which produced potent postsynaptic neuromuscular blockade with an IC_{50} of 40 nM, that is comparable to erabutoxin-b. However, unlike the poorly reversible neuromuscular blockade produced by erabutoxin-b and other typical α-neurotoxins, MFTx2 produced rapid and near-complete recovery of nerve-evoked twitch responses upon washing.

Discussion: Postsynaptic neuromuscular blockade, as observed by the abolishment of muscle contractile responses to electrical nerve stimulation and to exogenous agonists, indicates that MFTx2 targets muscle nicotinic acetylcholine receptors. Primary sequence comparison of MFTx2 and conventional curaremimetic α-neurotoxins revealed that MFTx2 lacked many functionally important residues for interaction with muscle-type nicotinic acetylcholine receptors, suggesting an alternate mechanism of reversible interaction with postsynaptic nicotinic acetylcholine receptors.

Conclusions: A novel three-finger neurotoxin, MFTx2, which produced postsynaptic neuromuscular blockade with nanomolar affinity, was purified from *M. f. fulvius* venom. In contrast to typical curaremimetic α-neurotoxins, the neuromuscular blockade produced by MFTx2 was rapidly and completely reversible.

Keywords: snake venoms, three-finger toxins, neurotoxins
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154. First Pharmacological Study of the Venom of a Rare African Snake, *Naja multifasciata duttoni*

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Background: The burrowing cobra, *Naja multifasciata duttoni* is a rare snake that has hardly been studied because it is seldom found. Its venom has never been studied scientifically. Two specimens were impounded by UK Customs and transferred to the Liverpool School of Tropical Medicine, allowing the opportunity to collect and study the pharmacological effects of the venom of this species**.**

Methods: Venom was milked from both snakes, pooled and dried. Aliquots were reconstituted immediately before experiments. Effects were determined on the isolated nerve muscle preparation, the chick biventer cervicis, using indirect nerve stimulation and contracture responses to acetylcholine, carbachol and KCl**.**

Results: The venom produced a concentration-dependent reduction in the twitch responses to nerve stimulation. Complete block was reached within 20 min with 30 ug/ml, while 3 ug/ml took about 90 min to cause complete block. After responses to indirect stimulation were abolished by venom, responses to the agonists were tested: there were no responses to acetylcholine or to carbachol, while there were responses to depolarization induced by KCl. Responses to KCl were reduced after exposure to venom at 30 ug/ml and this concentration alos caused a slow contracture of the muscle preparations**.**

Conclusions: Venom of the burrowing cobra, *Naja multifasciata duttoni* has similar pharmacological effects on nerve-muscle preparations as classical cobra venoms. The effects are consistent with the presence in teh venom of postsynaptic neurotoxins and cardiotoxins (or 'cytotoxins').

Keywords: cobra venom, cardiotoxin, neurotoxin
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155. Expression of two endoplasmic reticulum stress markers, GRP78 and GADD153, is involved in the mechanism of action of the Amblyomin-X.

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Introduction: A cDNA library of the *A. cajennense* tick salivary glands was constructed and used to identify a gene encoding a Kunitz-type protease inhibitor. A recombinant protein, named Amblyomin-X was over expressed in *E. coli*. The expressed protein is able to promote apoptosis in murine renal adenocarcinoma (RENCA), decreased proteasomal activity and increased pool of poly-ubiquitinylated proteins in some tumor cell lines, suggesting an endoplasmic reticulum (ER)-stress. However, the mechanistic effects of this protein are still unclear. **Objective:** Evaluate the involvement of ER-stress in tumor cells treated or not with Amblyomin-X**.**

Methods: To evaluate two markers of the ER-stress, GRP78 and GADD153, the genic expression was perfomed by ABI 7500 Real Time PCR System (Life Technologies) using forward and reverse specific genes primers and analysis for Western Blot with specific monoclonal antibodies were performed**.**

Results: RENCA cells treated with Amblyomin-X showed a modest effect in genes related to ER-stress, but significant differences in protein expression was observed**.**

Conclusion: The results suggest that ER-stress is involved in this process pro-apoptotic of the Amblyomin-X.

Keywords: tick, amblyomin-X, ER-stress
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156. Neuromuscular activity of *Bothrops fonsecai* Snake Venom and its Neutralization by Commercial Bothropic Antivenom

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Background: *Bothrops* venoms can produce marked local effects such as hemorrhage and necrosis. Some of these venoms also cause neuromuscular blockade in vertebrate nerve-muscle preparations *in vitro*. In this work, we examined the myotoxicity and neurotoxicity of *Bothrops fonsecai* venom in mouse isolated phrenic nerve-diaphragm preparations. We also investigated the ability of commercial equine bothropic antivenom to neutralize the neuromuscular activity**.**

Methods: Mouse extensor digitorum longus (EDL) preparations mounted in Tyrode solution (37 ^{o}C) were incubated with venom (3-300 μg/ml) or Tyrode solution alone (control) for 120 min, after which the tissues were processed for histological analysis. Venom PLA_2 activity was measured colorimetrically. For neutralization experiments, venom (100 mg/ml) was preincubated for 30 min with bothropic antivenom at ratios of 5:1 and 5:2 (w/v)**.**

Results: *Bothrops fonsecai* produced neuromuscular blockade in EDL preparations, with the time (min) for 50% paralysis by 3, 10, 30, 100 and 300 mg of venom/ml being 84.6±6.5, 76.7±11.8, 65.3±11.9, 41.2±6.4 and 46.6±4.2 min,

respectively (mean±SD; n=5-7; $p<0.05$ compared to control preparations). Histological analysis showed muscle fibers with edema, hypercontraction and vacuolization that resulted in ghost fibers. The PLA_2 activity *of B. fonsecai* venom (100 mg) was 1.75±0.5 mM HCl/min compared to 0.85±0.4 mM HCl/min for *Crotalus durrissus terrificus* (South American rattlesnake) venom (100 μg; positive control; n=5 each; $p<0.05$). Commercial antivenom did not significantly affect venom (100 μg/ml)-induced blockade at the manufacturer's recommended venom:antivenom ratio of 5:1 (74.6±8.6% blockade after 120 min; n=5); partial protection was observed at a ratio of 5:2 (only 37.5±5.5% blockade after 120 min; $p<0.05$; n=5). Venom PLA_2 activity was not inhibited by commercial antivenom (activities of 1.70±0.05 mM HCl/min and 1.3±0.13 mM HCl/min for venom:antivenom ratios of 5:1 and 5:2, respectively; n=3 each).

Discussion and Conclusions: These results indicate that *B. fonsecai* venom is myotoxic and neurotoxic in EDL preparations. These effects may be mediated by venom PLA_2 activity. Commercial bothropic antivenom only partially neutralized the neuromuscular activity, possibly because this venom is not included in the pool used to immunize horses.

Keywords: bothropic antivenom, myotoxicity, neurotoxicity, PLA2 activity.
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157. Validation of an Analytical Method to Measure Neutralizing Potency of Anti *Loxosceles* Plasma

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Background: According to the World Health Organization guidelines "for the production control and regulation of snake antivenom immunoglobulins", a plasma used to produce an antivenom should be checked on its neutralizing potency prior to fractionation to ensure the quality of the antivenom. The validation of the method to measure the neutralizing potency was performed to ensure its reliability.

Method: We validated an analytical method previously developed to measure in BALB/c mice, the neutralizing potency of an anti-*Loxosceles* plasma against recombinant toxins of medical relevant *Loxosceles* species. To the method validation we evaluated different parameters defined in the International Conference of Harmonization guidance: accuracy, precision, inter-assay variation, reproducibility, specificity and selectivity**.**

Results and Discussion: The method is specific, selective, accurate and precise with variation coefficient less than 20 % and was demonstrated its reproducibility inter-assay between different days and inter-analysts. This validation demonstrated that the method is statistically suitable and may be used as quality control test by manufacturers as part of plasma release for the production of the anti *Loxosceles* antivenom**.**

Conclusions: The method is reliable and complies the established acceptance criteria to be used as quality control test through of the determination of neutralizing potency in plasma to produce an antivenom.

Keywords: *Loxosceles*, neutralizing potency, validation, antivenom
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K. Physiology

158. EqT II Causes Endothelial Cell Damage and Intracellular Ca2+ Riske in ECV-304

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Background: Equinatoxin II (EqT II) is one of the actinoporins isolated from the sea anemone *Actinia equina* L. After oligomerization it forms pores in lipid membranes. EqT II is lethal in rat, and the mechanism of this action has been explained by vasoconstriction and cardio respiratory arrest. Endothelium may be responsible for the vasoconstrictory action of EqT II, but the action of EqT II on endothelial cells has not been studied in detail. In this study the effects of EqT II on endothelial-like ECV-304 cells are described**.**

Methods: EqT II was a kind gift of P. Maček and G. Anderluh. ECV-304 cells were cultured in Petri dish and incubated with 1 μM fura-2 AM for 30 min. Fura-2 was then washed out and the cells were mounted in a 200μl chamber on the stage of an inverted Axiovert 100 microscope (Zeiss, Germany) and exposed to EqT II in 1 to 100 nM concentration. To determine the source of cytosolic Ca^{2+} rise a separate set of experiments was performed on thapsigargin pre-treated ECV-304 cells and in Ca^{2+} free medium. Samples were excited alternatively with 340 and 380nm light wavelength from Polychrome II monochromator (T.I.L.L. Photonics, Germany) for ratiometric determination of intracellular Ca^{2+}. Emitted light at 510nm (10nm band-pass) was collected by MicroMAX CCD camera (Princeton Instruments, USA), digitized and stored in the imaging system. Data were recorded every 10 s (0,1Hz) with 250 ms acquisition time. ROIs were selected over the nucleus of cells (central ROI) and surrounding cytoplasm (peripheral ROI). ROI outside cells served for background subtraction**.**

Results: Exposure of cells to EqT II triggered a rise of intracellular Ca^{2+}. Thapsigargin pre-treatment causing depletion of intracellular stores had no influence on the EqT II action. Elimination of Ca ions from the bathing medium by substitution with EGTA abolished the effect of EqT II**.**

Discussion: Results show that EqT II induced rise in cytosolic Ca^{2+} can be explained by influx of Ca^{2+} form the bathing solution and cannot be attributed to the release of intracellular Ca^{2+} stores. It is well known that intracellular Ca^{2+} is a crucial second messenger, and it may be expected that the rise in intracellular Ca^{2+} may cause cell swelling

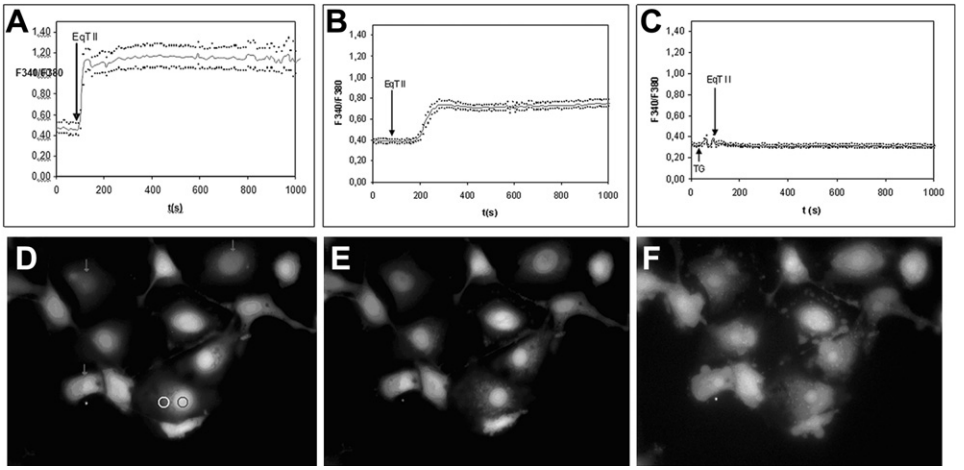

Fig. 1. Top – Changes in intracellular Ca^{2+} concentration induced by equinatoxin II. A) Increase in fluorescence ratio (solid line) of intracellular fura-2 in the central ROI after addition of EqT II (100 nM final concentration) in culture medium containing 2 mM Ca^{2+} ions (dots show +/− standard deviations at the measurement points, $n = 16$). B) Increase in fluorescence ratio of intracellular fura-2 in the peripheral ROI after addition of EqT II (same conditions as in A). C) Pretreatment with 50 nM thapsigargin resulted in a small and transient increase – no increase in fluorescence ratio (solid line) of intracellular fura-2 was observed in the central ROI after addition of EqT II (100 nM final concentration) in culture medium containing 2 nM EGTA – chelating agent selective for Ca^{2+} ions (dots show +/− standard deviation at the measurement point, $n = 13$). Bottom – Consecutive images of ECV-304 loaded with fura-2 in medium containing 2mM Ca^{2+}; D) before; E) immediately after and F) 15 min after addition of 100 nM EqT II. For illustration a peripheral (bright circle) and central (dark circle) ROI is shown in the first image. Note the intensity changes of nuclear regions (arrows) produced by EqT II application. 1–2 min after application of EqT II first blebs of cellular membrane appeared.

and a release of vasoactive substances from endothelial cells. Both effects may contribute to the vasoconstrictory action of EqT II.

Conclusion: EqT II causes cytosolic Ca^{2+} rise in ECV-304 cells leading to endothelial dysfunction.

Keywords: equinatoxin, endothelium, ECV 304, intracellular calcium, vasoconstriction
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159. Synaptophysin Expression and Neurotoxic Effects of Some Bothropic Venoms and Toxins

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Background: Snake venoms are a mixture of bioactive compounds, displaying a variety of pathological effects, including myotoxicity and neurotoxicity. Some Bothropic venoms as from *B. alternatus* are myotoxic; however, toxin BaTx is neurotoxic. Other venoms as from *B. marajoensis* and its toxin Bmaj-9 are exclusively neurotoxic. In general, the identification of Bothropic toxins with neurotoxic activity has been made through pharmacological approaches, being the molecular mechanism less explored. The aim of this study was to investigate by western blotting the expression of synaptophysin, a synaptic vesicle structural protein, which takes part in the physiology of the neuromuscular junction and allows identifying presynaptically-acting neurotoxins.

Methods: Mouse phrenic nerve-diaphragm (PND) and chick biventer cervicis (BC) preparations were isolated and submitted to conventional myographic, histological and western blotting techniques. PND were treated with *B. jararacussu* venom (100μg/mL) and BthTX-I (from *B. jararacussu* venom, 50μg/mL) and BC were treated with *B. alternatus* venom (200μg/mL), BmjeTX-I and BmjeTX-II (from *B. marajoensis* venom, 10μg/mL). All experiments were maintained at 37°C until the total neuromuscular blockade. After these the tissue was frozen for morphological analyses or homogenate for synaptophysin immunodetection by western blotting.

Results: In PND, *B. jararacussu* venom and BthTX-I caused a total and irreversible neuromuscular blockade (86±13.4 and 30±6.5 min respectively; n=5-6, $p<0.05$ compared to Tyrode controls) as well observed in BC treated with BmjeTX-I and BmjeTX-II (31.2±3.5 and 30±8.1 min respectively; n=7-8, $p<0.05$ compared to Krebs controls). *B. alternatus* did not induce complete neuromuscular blockade (even after 120 min incubation). Qualitative morphological analyses showed that the muscle damage caused by these venoms and toxins leaded the fibres to edema, hypercontraction, vacuolization and, consequently, to ghost fibre. Immunoblotting data showed synaptophysin expression in PND and BC controls preparations, but preparations incubated with *B. jararacussu* venom, BthTX-I, BmjeTX-I and BmjeTX-II toxins did not.

Discussion: Synaptophysin expression is related to synaptic vesicle integrity and its absence in preparations incubated with venoms/toxins is in conformity with the

effect of snake venoms/toxins that affects ACh release at the nerve terminal hence resulting in presynaptic neuromuscular blockade, as per *B. jararacussu* venom, BthTX-I, BmjeTX-I and BmjeTX-II toxins.

Conclusions: These results evidenced that the neurotoxic effects induced by these venom and toxins can be related to the absence of intact synaptic vesicle at the neuromuscular junction.

Financial support: FAPESP (2011/00001-1); CNPq.

Keywords: myotoxicity, neurotoxicity, synaptic vesicles, neuromuscular junction
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160. Role of SVMPs, Matrikines and TLR4 in Snake Venom Induced Edema and Inflammation

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Background: Snake venom metalloproteinases have long been known to be potent agents of extracellular matrix and significant information has been gained in terms of understanding the mechanistic basis for how such degradation leads to hemorrhage and tissue damage. However, little is known regarding whether the products of ECM degradation may also contribute to the pathophysiology of envenomation.

Methods: Both in vitro and in vivo experiments were performed to determine whether SVMP degraded ECM could induce edema and inflammation. Additional studies to elucidate the mechanism of action were also undertaken.

Results: Venom SVMPs were demonstrated to degrade ECM to produce matrikines which could induce edema and inflammation in vivo and up-regulate chemokines in tissue culture. Antagonism of the toll-like receptor 4 (TLR4) could attenuate these activities.

Discussion: It has been recognized that some ECM proteolytic products can activate TLR4 receptors to produce edema and inflammation in certain diseases. The ability of SVMPs to similarly produce ECM matrikines to contribute to the venom-induced edema and inflammation associated with envenomation is a significant novel finding which in turn suggests additional therapeutic routes to attenuate morbidity and dead associated with snake envenomation.

Conclusions: SVMP degraded ECM products can function as matrikines to contribute to envenomation pathophysiology via a matrikine-TLR4 mediated pathway to induce edema and inflammation in the prey. These studies suggest a more complex, nuanced role for SVMPs in venom induced pathophysiology than previously recognized and that novel approaches to therapeutic treatment of envenomation focused on this pathway may attenuate edema and inflammation associated morbidities in patients.

Keywords: SVMPs, matrikines, toll-like receptor 4, edema, inflammation
10.1016/j.toxicon.2012.04.161

L. Plants

161. Unusual Plant Poisoning from Anabasine in an Isolated Community Following Ingestion of Tree Tobacco Leaves (*Nicotiana glauca*)

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Background: Tree or wild tobacco (*Nicotiana glauca*) Solanaceae, native to Argentina, is now established in Australia. All parts of the plant contain anabasine, an alkaloid isomer of nicotine and ingestion can result in severe or lethal poisoning. There are few reports of poisoning.

Case Reports: We were involved in managing a case of severe poisoning after ingestion of tree tobacco leaves and in the course of managing this case discovered there were 5 other cases of poisoning from the same plant. The index case, a previously well 64 year old man, ate a meal of stir fried “bok choy” leaves found by a neighbour in their garden. An hour later he developed leg weakness, blurred vision, dizziness, chest tightness and vomiting and by 3 hours he had profound muscle weakness. He was found by a neighbour after 7 hrs and taken to hospital where he was hypersalivating, with limb twitching, cyanosis and hypertension. He was intubated with rapid improvement in GCS. He showed improvement after 12 hrs and at 24 hrs was extubated successfully and then gave the history of plant ingestion. The plant was identified as Nicotiana glauca at the Botanic Gardens. It was then learned that he and his partner had milder symptoms after eating the plant 2 weeks earlier and 4 other cases of moderate poisoning from eating this plant were also identified, to a total of 6 cases.

Discussion: Anabasine activates then blocks nicotinic acetyl choline receptors centrally and peripherally, resulting in sympathetic and parasympathetic effects plus effects at the neuromuscular junction causing muscle fasciculation, weakness, then paralysis. Death is due to respiratory failure and symptoms generally occur within 4 hrs of exposure. Treatment is supportive, with oral charcoal of doubtful value because of the profuse vomiting and diarrhoea associated with poisoning. If respiratory support is initiated in time in severe cases the outcome should still be good. For patients with suspected exposure, observation for 6 hrs is sufficient, if they remain asymptomatic. This case report is the largest mass poisoning reported for this

Keywords: plant poisoning, *Nicotiana glauca*, anabasine
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162. Membrane-disturbing Properties of Urease and Derived Recombinant Peptides

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Background: Ureases have long been known for their ureolytic activity, dependent on a Ni metallocenter active site. More recently novel toxic properties of ureases were discovered properties that are independent of the enzyme activity. While ureases of plant, fungi, and bacteria align with greater than 50% amino acid identity, toxicity may be due to more divergent domains. Plant ureases have potent toxicity against insects that are not affected by Bt toxins. In the case of jackbean (*Canavalia ensiformis*) urease (JBU), insecticidal activity relies mostly on an internal peptide (jaburetox-2) released upon ingestion by the insect's digestive enzymes. Modeling of Jaburetox-2 revealed a prominent β-hairpin motif consistent with an insecticidal activity based on either neurotoxicity or cell permeation. However, whole ureases display some entomotoxic properties not shared with its peptide. Here we describe membrane-disturbing properties of urease and of its insecticidal peptide and applied site-directed mutagenesis aiming to establish structure X activity relationships.

Methods: the Planar Lipid Bilayer (PLB) and the carboxyfluorescein release assays were used to evaluate the membrane-disturbing properties of JBU and its derived peptides. Jaburetox-2 mutants were obtained by site-directed mutagenesis (Stratagene): deletion of aminoacids 61-75 (Dβ-hairpin); deletion of N-terminal half (D1-44); deletion of the C-terminal half (D45-92).

Results: JBU and Jaburetox-2 (5-10 nM) are able to insert into the PLB forming ionic channels. JBU's channels display four major conductance levels: 1730, 833, 625 and 352 pS and apparently have anion selectivity. Jaburetox-2 also forms channels with different conductance and kinetics. Jaburetox-2 (72 nM) induced leakage of carboxyfluorescein from large unilamellar vesicles composed of acidic lipids. The Dβ-hairpin mutant showed all the properties of the wild jaburetox-2 peptide, indicating that the β-hairpin motif is not relevant for the peptide's membrane-disturbing ability. On the other hand mutants corresponding to halves of the jaburetox-2 molecule (D1-44 and D45-92), although still active on the PLB and carboxyfluorescein leakage assays, showed much decreased activity, suggesting the presence of an active domain that was "split" between the two mutants.

Conclusions: Membrane-disturbing and ion channel forming activities of urease and derived peptides may contribute to their diverse biological activities. These domains are potential targets for manipulation to improve plant defense against herbivores and pathogens.

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Keywords: urease, jaburetox, membrane-disturbing
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163. Antioxidant Effect of *Camellia Sinensis* (Green Tea) Extract Attenuate Acrylamide Induced Testicular Damage in Albino Rats

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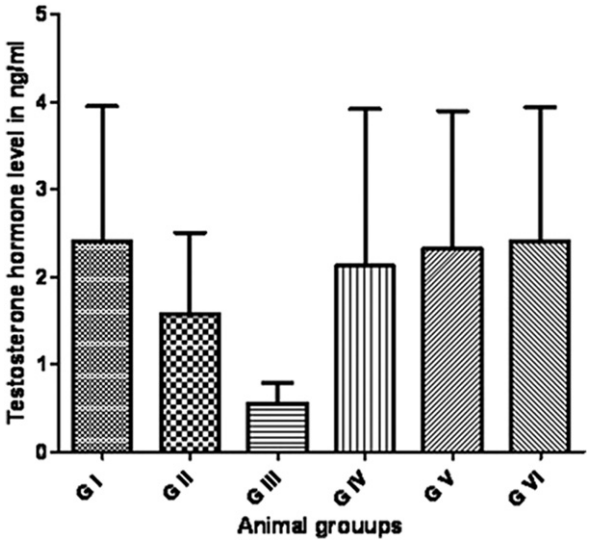

Fig. 1. Testosterone hormone level in different groups of animals.

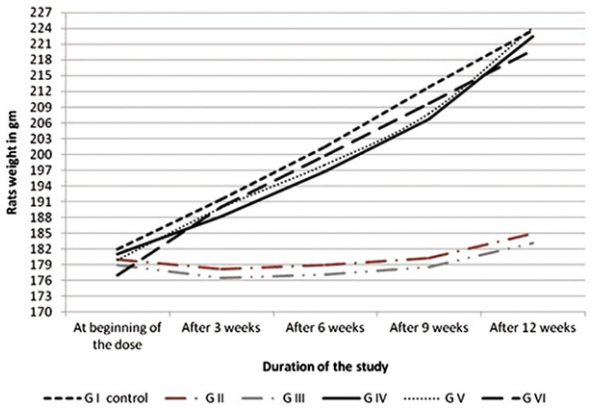

Fig. 2. Effect of acrylamide and protective role of green tea on animal weight.

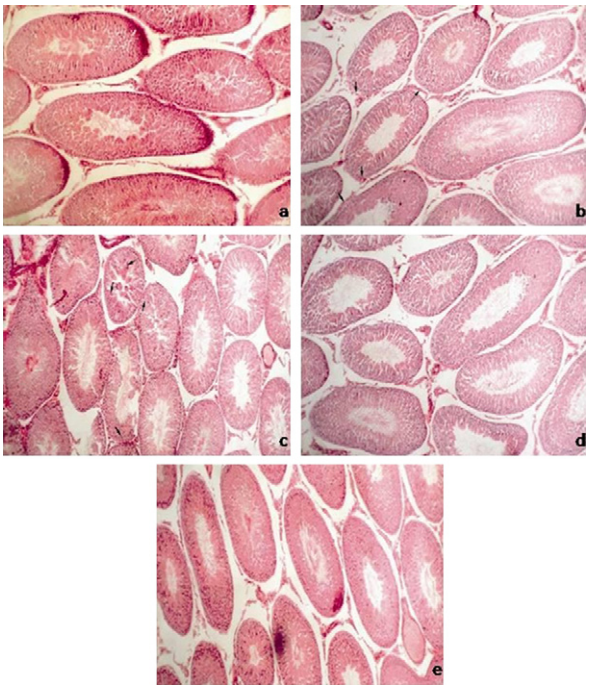

Fig. 3. a) Testicles of control group (Group I). b) Group II showed minimal effects on testicular membrane when compared with the control group. c) Group III showed thickening of the tubular endothelium, degeneration of germ cells and formation of multinucleated giant cells. d) and e) Group IV and Group V showed no changes due to protective effects of green tea.

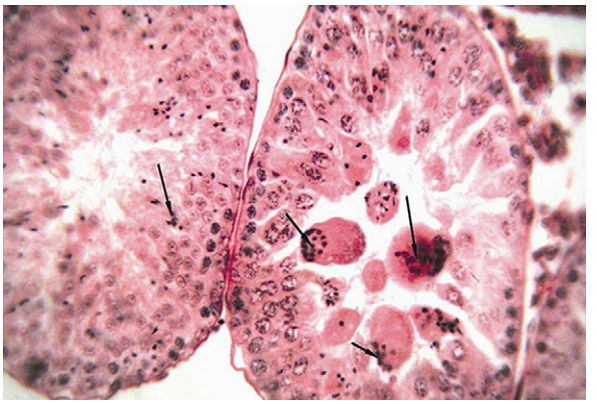

Fig. 4. High power view of testis of Group III showing degeneration of germ cells and the formation of many multinucleated giant cells in atrophied seminiferous tubules (H&E X 400).

Table (2): Effect of acrylamide and protective role of green tea on animal weight in mg:

Animal groups	At beginning of the dose	After 3 weeks	After 6 weeks	After 9 weeks	After 12 weeks
G I	182±2.5	191±1.9	201.5±1.9	212.8±2.2	223.6±2.1
G II	180±2	178.2±1.8*	179±1.4*	180.3±1.5**	185±1.7**
G III	179±2.3	176.5±1.8**	177.2±1.8**	178.6±1.7**	183.2±1.4**
G IV	181±2.7	188.2±1.7**	196.8±1.8**	106.7±1.9**	222.5±1.5**
G V	180±1.9	189.9±2.1**	198.1±1.9**	207.8±2.0**	224.2±1.9**
G VI	177±1.8	190±2.1	199.8±2.0	109.7±2.1	219.9±1.9

Data are represented as mean±S.D. of weight in mg ($n = 10$). Asterisk indicates significant difference between groups and control, $^{*}p < 0.05$; $^{**}p < 0.001$.

Background: Acrylamide is a proved toxin for testicular function, found in food when heated for long period of time. Green tea (Camellia sinensis) is a potent antioxidant; the aim of this study was to investigate the protective effect of green tea extract against the toxic effects of acrylamide in rat testes.

Methods: acrylamide was administered orally to rats in different doses and also the extract of green tea was administered orally to different groups of animals in combination with the acrylamide. The weight of animals, testosterone hormone level and histopathological effect upon testicles were evaluated.

Results: Testosterone hormone level in serum, and histopathological findings were significantly improved with the co administration of green tea extract with the acrylamide. Green tea extract reversed all the toxic effects of acrylamide even in high dose for long period (90 days).

Conclusion: green tea extract is a potent antioxidant antidote for the acrylamide toxic effects upon testicular function.

Keywords: *Camellia sinensis*, testosterone, acrylamide
10.1016/j.toxicon.2012.04.164

164. Evaluation of Anticancer Activity Promoted by Molecules Contained in the Extracts of *Thevetia peruviana*

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Introduction*: Thevetia peruviana* is an evergreen shrub or a small plant in the *Apocynaceae* family, known for

Table (1): Testosterone level in different studied groups.

Groups	Serum testosterone level in ng/ml	No. of animals	No. of dead animals
Group I (control group)	3.95± 0 .87	10	0
Group II (acrylamide 1/10 LD_{50})= 15mg/kg	2.51±0.65*	10	0
Group III (Acrylamide 1/5 LD_{50})= 30mg/kg	0.79±0.32**	10	1
Group IV (acrylamide 15 mg/kg + 70 mg/kg green tea)	3.92±0.33**	10	0
Group V (acrylamide 30 mg/kg + 70 mg/kg green tea)	3.90±0.75**	10	0
Group VI (green tea alone 70 mg/kg)	3.94±0.88	10	0

Data are represented as mean±S.E. of testosterone hormone level ($n = 10$). Asterisk indicates significant difference between groups, $^{*}p < 0.05$; $^{**}p < 0.001$.

highly toxic properties, performed by molecules of cardenolides class. Besides toxicity, these molecules have shown a large potential therapeutic, such as antiparasitic, antimicrobial and also a significant anticancer activity. Cancer disease affect thousands of people and drug therapy is fundamental to increase survival or total cure of the disease. The aim of this study was to analyze the activity of extracts obtained from *Thevetia peruviana* in inhibition capacity of cell replication, important method in the therapy against the cancer.

Method: The extract of *Thevetia peruviana* was obtained by cold maceration using methanol and subjected to reactions of Lieberman-Bouchard and Keddi. Those reactions were performed in the sample for identification of cardenolides. The cytotoxicity assays were performed using tumor cells line HL-60 (CCL-240, Acute Promyelocytic Leukemia Cells), HepG2 (HB-8065, Hepatocellular Carcinoma Human Cells), PC-12 (CRL-1721, Murine Pheochromocytoma Cells) obtained of ATCC (American Collection of Cell Culture). The cells were added in a 96-well plate and treated with different concentrations of extract (DCE) (5, 10, 25, 50, 100 and 200 μg/mL) and incubated for 24 h. The positive control (PC) was done with cisplatin (1mg/mL). After the period, 10 μL of MTT were added to identify the viable cells and again subject in incubation for 3 h. at 37C°, in 5% of CO_2. After that, 100 μL of DMSO were added for solubilization of formazan crystals and the absorbance was measured**.**

Results: The Lieberman-Bouchard and Keddi reactions showed positive results, confirming the presence of cardenolides in the extracts, and the different concentrations of extract inhibited the different tumor cells in the respective sequences : HEPG2 (PC: 18.1%) (DCE: 62.5%, 56.1%, 52.6%, 49.0%, 57.9%, 56.7%); HL60 (PC: 11.6%) (DCE: 90.9%, 61.5%, 55,5%, 52.6%, 38.7%, 26.3%); PC12 (PC: 20.7%) (DCE: 63.8%, 73.6%, 52.0%, 46.2%, 96.4%)**.**

Discussion: The test of cytotoxicity showed inhibition of cell replication in the three tumor cells, more effectively in the type HL-60, showing a dose-dependent correlation with major action in the concentration of 200 μg/mL. In HEP-G2 and in PC-12 the dose-dependence correlation was not observed but obtained significant inhibitions**.**

Conclusions: A large variety of molecules presents in plants brings a huge arsenal of option to development of research in the many areas looking for therapeutics potential against diseases. *Thevetia peruviana* presents molecules that may be used to combat cancer.

Keywords: *Thevetia peruviana*, anticancer activity, cardenolides
10.1016/j.toxicon.2012.04.165

M. Scorpions

165. Molecular Cloning, Expression and Structure-Function Analysis of Neopladine-2, an Antineoplastic Peptide from *Tityus discrepans* Scorpion Venom

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Introduction: *Tityus discrepans* venom gland cDNA may have applications in treating human cancers**.**

Methods: Total RNA was extracted from the venom glands of *Tityus discrepans* and cDNA was obtained by RT-PCR using primers complementary to the nucleotide sequences coding the first six amino acids of the mature protein**.**

Results: The PCR products obtained were cloned and sequenced, establishing the complete *bona fide* sequence of the Neopladine 2. The deduced amino acid sequence comprises 245 residues. The protein was expressed in *E. coli* (XL1 Blue), as a fusion protein for subsequent H_6 purification. The protein was obtained as inclusion bodies and folded *in vitro* to obtain a soluble protein. The folding yield was 80% obtaining about 9.6 mg/L of culture of soluble protein. Its primary structure shows moderate homology with ADAMTS Ca^{2+}-methalloproteinases and it was used to predict the tertiary structure by homology molecular simulation. The SWISS MODEL modeled protein was optimized with the molecular modeling program YASARA, with the YAMBER3 force field. Energy was minimized and binding energies were calculated. Future work will test the anti-neoplastic effect of recombinant and native N2 (1 μg/μL or ∼33 μM) on human breast carcinoma cell line SKBR3 (ATCC# Number: HTB-30#) and normal monkey kidney cell line MA104 (ATCC Number: CRL-2378.1).

Financial support: Partially supported by FONACyT (Venezuela) and FONDEN grant to GDS. Supported in part by a grant provided by DGAPA/PAPIIT, IN-214211, UNAM.

Keywords: recombinant, neopladine 2, *Tityus discrepans*
10.1016/j.toxicon.2012.04.166

166. Toxins of *Tityus Serrulatus* Scorpion Venom Induce Inflammatory Mediators *in vitro*

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Background and Objectives: *Tityus serrulatus* (Ts) is responsible for the majority of cases of human poisoning by scorpions in Brazil. The specific signs of scorpion envenomation are directly related to the venom components. There are studies regarding venom actions, however little is known about the interactions of its toxins with immune cells. This study was designed to evaluate the ability of Ts1, Ts2 and Ts6, in combination or not with lipopolysaccharide (LPS), to induce production of cytokines, nitric oxide (NO) by immortalized alveolar macrophages (MH-S). Lipid bodies (LBs) formation was also investigated. LBs are lipid rich organelles distributed in the cytoplasm of most

eukaryotic cells and are involved in a variety of functions such as lipid metabolism, cell signaling and inflammation. However, nothing is known about the formation and function of LBs in alveolar macrophages stimulated with Ts1, Ts2 and Ts6 from the venom of *T. serrulatus* escorpion.

Methodology and Results: Ts1, Ts2 and Ts6 were not cytotoxic in all concentrations used in MH-S cells. NO concentration was measured by Greiss method and interleukin (IL)-6, IL-10 and tumor necrosis factor (TNF)-α by ELISA. NO, IL-6 and TNF-α production by MH-S cells, stimulated with Ts1 or Ts6, were enhanced under LPS pre-stimulation. On the other hand, Ts2 inhibited the release of these inflammatory mediators and increased IL-10 production. LBs formation increased after toxin stimulation compared to non-stimulated cells.

Discussion and Conclusion: Our results demonstrated that, *in vitro*, individual scorpion toxins possess different properties. Ts1 and Ts6 presented similar effects that were opposite to Ts2 regarding to NO, TNF-α, IL-6 and IL-10. Ts1, Ts2 and Ts6 induced the formation of LBs, which could be related with eicosanoids production. We might suggest that production of cytokine and lipid mediators by toxin-stimulated macrophages is independent of toxin ion channel interactions, since that Ts1 and Ts2 act on Na^+ ion channels, and Ts6 act on K^+ ion channels.

Keywords: MH-S, *Tityus serrulatus*, inflammatory mediators
10.1016/j.toxicon.2012.04.167

167. Novel Potassium Channel Blocker Venom Peptides from *Mesobuthus gibbosus* (Scorpiones: Buthidae)

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Background: Scorpion toxins specific for potassium channels (KTx) have been classified on the basis of the alignment of Cys and other conserved residues into four families, known as alpha-, beta-, gamma-KTx [1] and kappa-KTx [2]. The alpha-KTx family is considered the largest [3]. Until now, twenty-two subfamilies have been reported and several new peptides are continuously being discovered. *Mesobuthus gibbosus* (Brullé, 1832) belongs to the *Buthidae* family. This species is widely distributed in the Eastern Mediterranean region; the geographical range includes the Balkan Peninsula (Albania, Montenegro, Macedonia and Greece) and Anatolia (Turkey, except for the coast of the Black Sea). According to the epidemiological and clinical situation of scorpion envenomations in Turkey, *M. gibbosus* is one of the most important health-threatening scorpion species [4]. Despite the medical importance reported for *M. gibbosus* [5], there is no additional information of toxin and venom components to clarify the toxic effect of a *M. gibbosus* sting.

Methods: Biochemical characterization was performed using different protocols and techniques following a bioassay-guided strategy (HPLC, mass spectrometry and EDMAN degradation sequencing). Venom fractions were tested in electrophysiological assays on a panel of six K^+ channels (K_v1.1-1.6) using the two-electrode voltage clamp technique. A cDNA library from the telson was constructed and specific screening of genes was conducted. Different algorithms and bioinformatics tools were used for the sequences analysis.

Results: In the present study, we report for the first time, the molecular, biochemical and electrophysiological characterization of the components present in the soluble venom from *M. gibbosus*. Three new alpha-KTx peptides were found and called MegKTx1, MegKTx2 and MegKTx3 (*Mesobuthus gibbosus,* K^+ channel toxin number 1 to 3). Biochemical and molecular characterization of MegKTx peptides and genes shows a relation with toxins of three different alpha-KTx subfamilies.

Conclusions: The exploration of the components present in the venom from *M. gibbosus* and the identification of gene sequences are important to understand the biological role of venom components that interact with K^+ channels. Consequently, this information may help in the dissection and knowledge of the noxious effects produced by this scorpion sting.

References

Tytgat *et al.*, 1999 *Trends Pharmacol Sci.* 11:444-7.
Srinivasan *et al.*, 2002 *J. Biol. Chem.* 277:30040-47.
Rodríguez de la Vega and Possani, 2004 *Toxicon* 43:865-75.
Ceraretli and Ozkan 2010 *Rev. Inst. Med. Trop. Sao Paulo* 52(4):215-20.
Adiguzel, 2010 *J. Venom Anim. Toxins Incl. Trop. Dis* 16:198-211.

Keywords: scorpion, alpha-KTx, toxin, gene
10.1016/j.toxicon.2012.04.168

168. *Tityus serrulatus* Venom Induces a Higher Lung Inflammation in Mice Selected for Maximal Inflammatory Response

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Background: *Tityus serrulatus* is the main cause of scorpion envenomations in Brazil. Cardiovascular failure complicated by pulmonary edema is the main cause of death after severe envenomation. *T. serrulatus* venom (TsV) induces a systemic inflammatory response with the release of inflammatory mediators and cytokines both in patients and animal models. Lung alterations have been reported with the presence of inflammatory infiltrating cells and edema. The amount of venom inoculated, age, physical condition and genetic factors of the victims directly influence the severity of symptoms reported by patients. This study aimed to evaluate the action of TsV in the lung inflammation in strains of mice

genetically selected for high (AIRmax) or low (AIRmin) acute inflammatory response and BALB/c and observe if genetic factors are involved in the response to the venom.

Methods: AIRmax, AIRmin and BALB/c mice were inoculated (s.c.) with increasing doses for LD_{50} determination. A subletal dose of 0.75 μg/g of TsV was inoculated in mice and after different periods, the lungs were collected, for H&E histopathological analysis, myeloperoxidase (MPO) quantification, phenotype characterization of infiltrating cells and cytokine and chemokine analysis by ELISA or cytometry analysis.

Results: Lung histology analysis of AIRmax and AIRmin TsV-treated mice showed an increased perivascular infiltrate and the presence of haemorrhage and alveolar edema 1 and 2h after venom inoculation, but no cardiac alterations were observed. MPO activity showed that the venom induced a significant migration of neutrophils in AIRmax when compared to AIRmin and BALB/c mice, 2h after venom inoculation. Phenotypic analysis of lung cell populations of TsV-treated mice showed that after 1 hour, AIRmax presented significantly more $Ly6G^+CD11b^+$ cells and after 2 hours an increase in $F4/80^+CD11b^+$ and $CD3^+$ cells when compared to their controls and to AIRmin strain. In addition, AIRmin mice had an increased number of $Ly6G^+CD11b^+$ cells and $F4/80^+CD11b^+$ only after 2 hours of administration of the venom. Lungs of AIRmax TsV-treated mice presented higher levels of IL-6 and TNF-α and the chemokines MCP-1, MIP-1β and RANTES, compared to AIRmin and BALB/c.

Discussion and Conclusions: Our results suggest that *T. serrulatus* scorpion venom is able to induce significant inflammatory response in the lung with presence of polymorphonuclear cells macrophages and lymphocytes, as well as pro-inflammatory cytokines. This inflammatory response is more pronounced in AIRmax mice, thus suggesting the importance of genetic factors in the inflammatory response to animal venoms.

Financial support: FAPESP, INCTTOX Program – CNPq, Brazil.

Keywords: *Tityus serrulatus*, scorpion venom, lung inflammation, inflammatory response
10.1016/j.toxicon.2012.04.169

169. *Tityus serrulatus* Scorpion Laboratory Breeding and Venom Collection for Antivenom Production and Research

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Background: *Tityus serrulatus* is the most dangerous scorpion in Brazil. The number of scorpion stings due to this specie has sharply increased in the last years as documents from official information health systems. Antivenom therapy is mandatory on mild and severe cases of human scorpionism. Brazilian environmental legal system is very complex and creates serious difficulties to scorpions capture for venom extraction and antigen and research application. In this study we present a successful laboratory breeding protocol for this parthenogenetic scorpion specie and venom obtaining.

Methods: 372 young *T. serrulatus* scorpions were separated immediately after leaving their mothers' back and accommodated in plastic black boxes (2508 cm^2 and 1872 cm^2) with cotton plugs for humidity and paperboard shelters. The animals were kept at 23o C to 25o C and 12 light/shadow period; live crickets (*Gryllus sp.*) were used as food. Young scorpions received 27 %; immature scorpions 38 % and adult scorpions 50 % body weight of food in a 7 days intervals scheme. The venom was milked by electrical stimulation (70 mV, DC) from the animals telsons. The compared toxicity of laboratory breeding animals venom and *T. serrulatus* venom captured in the field was determinated in a mice Lethal Dose 50% (LD50) model by intraperitoneal injection of lyophilized venoms (0.25; 0.5; 1.0 mg/kg, n=8).

Results: The monthly average scorpions weight gain was 37.6 % and after 11 months and 4.5 molts 275 adults (73.9 %) produced 1.7 mg liquid venom/scorpion by electric milking (not different from the field animals average: 2.0 mg liquid venom/scorpion). The DL 50% (48h) from laboratory scorpions, venom was 1.2 mg/kg *i.p.* and the venom from field animals, 1.6 mg/kg *i.p.* All mice showed signs of experimental scorpionism.

Discussion: There is very little information on *T. serrulatus'* field and laboratory biology. The widespread increase in scorpion stings in Brazil, mainly due to *T. serrulatus'* urban colonization, requires basic biologic knowledge of this species.

Conclusions: The present laboratory breeding protocol for this dangerous scorpion species is a very useful tool for standardizing bioterium conditions and venom collection for public health needs and research.

Keywords: *Tityus serrulatus*; laboratory breeding; venom
10.1016/j.toxicon.2012.04.170

170. Identification of a Dynorphin-Degrading Metallopeptidase Releasing Leu-Enkephalin in Brazilian *Tityus spp.* Scorpion Venoms

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Background: Accidents caused by scorpions from the genus Tityus are an important public health problem in Brazil, scorpion envenomings occur more frequently than those caused by other venomous animals, including snakes. In the present study, we have analysed some toxic characteristics of the venoms from three scorpion species of the genus Tityus.

Methods: *T. serrulatus, T. bahiensis* and *T. stigmurus* venoms and antivenoms were provided by Butantan

Institute, São Paulo, Brazil. Hyaluronidase and phospholipase activities were determined by turbidimetric assays. The proteolytic activity was investigated by a fluorimetric method using Abz-FLRRV-EDDnp as substrate. In addition, the human biologically active peptide dynorphin 1-13 (YGGFLRRIRPKLK) was also used as substrate through HPLC assays. The scissile bonds in the dynorphin1-13 were determined by mass spectrometry analysis. Neutralization assays of the protease activity were performed using anti-scorpion, anti-arachnidic and anti-tetanus sera.

Results: The analysis of the enzymatic activity showed that *Tityus* venoms contain a significant hyaluronidase activity, while no phospholipase activity was observed. The presence of proteases with activity on the Abz-FLRRV-EDDnp and dynorphin1-13 substrates were also detected. The protease activity on the substrate Abz-FLRRV-EDDnp was inhibited by 1.10-phenantroline but not PMSF, indicating the presence of metalloproteases in *Tityus* venoms. The peptidase activity on Abz-FLRRV-EDDnp and dynorphin1-13 substrates was just partially inhibited by anti-scorpion and anti-arachnidic sera, but not by anti-tetanus serum, used as negative control. Dynorphin1-13 (YGGFLRRIRPKLK) has one scissile bond for the *Tiyus* venoms metallopeptidase(s), between the residues the R-R, releasing leu-enkephalin.

Discussion: In this study, we have demonstrated the presence of metalloprotease and hyaluronidase molecules in the Braziian *Tityus* spp venoms. The detection of metallopeptidase(s) with specificity for dynorphin1-13 can be important for the understanding of the mechanisms of pain in cases of accidents with scorpion, while hyaluronidases may contribute for the diffusion of other toxins present in these venoms. Furthermore, the partial inhibition of the toxic enzymatic activities by the commercial antivenom indicates a necessity of antivenom production improvement.

Financial Support: CNPq and INCTTox.

Key Words: *Tityus spp* venoms, metallopeptidases; hyaluronidases, dinorphin, leu-enkephalin, serum neutralization
10.1016/j.toxicon.2012.04.171

171. Comments on the Venom Yield of *Tityus trivittatus*, Considering Two Methodologies of Extraction

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Background: *Tityus trivittatus* is the scorpion of highest medical importance in Argentina. At least twenty fatalities during the last two decades are attributed to this species. Despite its medical importance, abundance of this scorpion in the field is scarce, which makes difficult to obtain enough venom for antivenom production.

Material and Methods: In order to study venom yield and its potency, we used two methodologies: electrical stimulation and homogenization of telsons. Twenty-eight collected samples, comprising *circa* 2000 animals were milked for venom (abbreviated MV). Similar number of samples and animals were used for telson homogenates (TH). The protein content and the lethal potency (as median lethal dose in mice) of the venom obtained by these two methodologies, from specimens of different regions of the country and pools constituted by samples of different regions were studied.

Results and Discussion: Protein content (Bradford) was 264 ± 84 μg (TH) or 120 ± 69 μg (MV). When absorbance at 280nm was considered, the values were 0.898/telson (TH) and 0.376/milking. The mean volume by milking was 1.9 ul (minimal 0.8 – maximal 3.8) with a protein content of 46,2 $\pm$ 21,2 μg/ul (minimal 15.1 – maximal 73.1 μg/ul). The lethal potencies from the venoms obtained were 17 μg (95% c.i. 12-21 μg; minimal 8.5 μg - maximal 32 μg) for MV and of 163 μg (106-221 μg) for TH. An important variation was observed in samples from the different regions of the country. Differences in potencies over three fold were observed exclusively in samples from the same province. The lethal potencies obtained from these samples were 3.5 $\pm$ 2.4 LD_{50} / telson (Median 2.6; minimal 1.2 - maximal 9.4 LD_{50}) whereas the milking yield 8.3 $\pm$ 7.1 LD_{50} / milking (Median 7.5; minimal 1.6 – maximal 26.4 LD_{50}). These results indicated that in general, potencies obtained from MV were higher than those obtained from TH ($p < 0.0001$) and the LD_{50}s values were also higher for milking over homogenization (p 0.0102). However, when the source of the scorpions was considered (TH or MV from the same province), no statistical differences were observed.

Conclusions: These results suggest that electrical stimulation is the best method for obtaining higher venom yield (considering the lethal potencies obtained); however, the economic cost for maintaining the colonies of scorpion alive is certainly more expensive.

Keywords: *Tityus trivittatus*, venom yield, telson homogenate, milked venom, lethal potency
10.1016/j.toxicon.2012.04.172

172. Mapping the Receptor Sites of Scorpion Toxins at Voltage-gated Sodium Channels

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Scorpion alpha and beta toxins interact with voltage-gated sodium channels (Na_vs) at two pharmacologically distinct sites. Alpha toxins bind at receptor site-3 and inhibit channel inactivation, whereas beta toxins bind at

receptor site-4 and shift the voltage-dependent activation toward more hyperpolarizing potentials. The two toxin classes are subdivided to distinct pharmacological groups according to their binding preferences and competition for receptor sites at Na_v subtypes. To elucidate the surface of interaction of the two toxin classes with Na_vs and clarify the molecular basis of varying toxin preferences an efficient expression system was established. Mutagenesis accompanied by toxicity, binding and electrophysiological assays, in parallel to determination of the three-dimensional structure using NMR and X-ray crystallography uncovered the bioactive surfaces of toxin representatives of all pharmacological groups. Exchange of external loops between channels that exhibit marked differences in sensitivity to various toxins accompanied by point mutagenesis highlighted channel determinants that play a role in toxin selectivity. These data were used in further mapping of the brain channel $rNa_v1.2a$ receptor sites for the beta-toxin Css4 (from *Centruroides suffusus suffusus*) and the alpha-toxin Lqh2 (from *Leiurus quinquestriatus hebraeus*). On the basis of channel mutations that affected Css4 activity, the known structure of the toxin and its bioactive surface, and using the structure of a potassium channel as template, a structural model of Css4 interaction with the Gating-module of domain II was constructed. This initial model was the first step in identification of part of receptor site-4. In parallel, a swapping and mutagenesis approach employing the $rNa_v1.2a$ mammalian and $DmNa_v1$ insect Na_vs and the toxin Lqh2 as a probe were used to search for receptor site-3. The channel mapping along with toxin dissociation assays and double-mutant cycle analyses using toxin and channel mutants identified the Gating-module of domain IV as the site of interaction with the toxin Core-domain, thus describing for the first time the docking orientation of an alpha toxin at the channel surface.

References

Cohen et-al 2004 *JBC* 279:8206-11; Karbat et-al 2004 *JBC* 279:31679-86; Karbat et-al 2004 *FASEB J* 18:683-9; Cohen et-al 2005 *JBC* 280:5045-53; Cohen et-al 2006 *JBC* 281:20673-9; Cestele et-al 2006 *JBC* 281:21332-44; Karbat et-al 2007 *JMB* 366:586-601; Karbat et-al 2007 *FEBS J* 274:1918-31; Cohen et-al 2007 *JBC* 282:29424-30; Schnur et-al 2008 Biochemistry 47:911-21; Kahn et-al 2009 *JBC* 284:20684-91; Weinberger et-al 2010 *MBE* 27:1025-34; Zhang et-al 2011 *JBC* 286:33641-51; Wang et-al 2011 *PNAS* 108:15426-31; Gur et-al 2011 *JBC* 286:35209-17

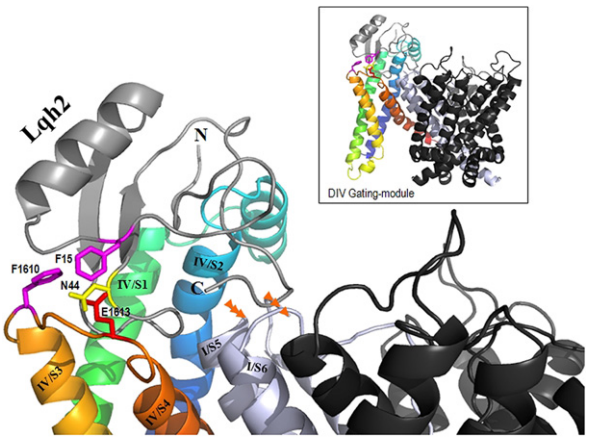

Fig. 1.

Keywords: scorpion toxins, sodium channels, interactions
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173. Characterisation of the Venom of an Australian Scorpion, *Urodacus yaschenkoi*: Proteome and Transcriptome Analysis

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Background: The Urodacus scorpions are the most widely distributed of the four families in Australia and represent half of the species in the continent. However, no comprehensive studies of these venoms have yet been published nor has any molecular phylogenetic analysis of their taxonomic status. In a multidisciplinary approach we have commenced a study of a model of Australia Urodacid species, *Urodacus yaschenkoi.* We present here the first aspect of our studies of this species**.**

Methods: We performed transcriptome analysis of the venom gland by constructing a cDNA library and conducting random sequencing screening of the transcripts. Also proteome analysis of the venom was performed by two complementary approaches: chromatographic separation (HPLC) and mass fingerprinting (LC-ESI-MS) to characterise the venom components**.**

Results: From the cDNA library (prepared from two venom glands) 325 genes were cloned but only 171 with sequence tags (ESTs) were analyzed. These transcripts were further clustered into 120 unique sequences (23 contigs and 97 singlets). The identified putative proteins can be assorted in several groups. One represented precursors similar to gene products implicated in common cellular processes important for venom gland function, others represented putative neurotoxins and antimicrobial peptides. Importantly, those putative neurotoxins specific for sodium channels, known to be major components in Buthidae scorpion venoms, were hardly detected. *U. yaschenkoi* is not known to be dangerous to humans and its venom contains peptides similar to those of *Opisthacantus cayaporum* (antibacterial), *Scorpio Maurus* (maurocalcin), *Opistophthalmus carinatus* (opistoporines) and scorpine-like molecules, amongst others.

By high performance liquid chromatography (HPLC) separation, a total of 74 fractions were obtained. These fractions were subject to high-resolution molecular mass

determination using a hybrid Orbitrap-XL mass spectrometer with a nano-electrospray ionization source (LC-ESI-MS) identifying approximately 210 different molecular masses with molecular weights varying from 291 to 16,290 Da (atomic mass units). The most abundant peptides were those from 3 to 5 kDa representing putative potassium channel toxins.

Conclusion: To the best of our knowledge this report provides the first analysis of Urodacidae scorpion venom and the first full analysis of an Australian scorpion venom proteome and transcriptome. The proteome and the transcriptome analysis will allow the characterisation of a large number of venom molecules and its toxins may provide useful pharmacological tools.

Keywords: Australian scorpion, proteome, transcriptome
10.1016/j.toxicon.2012.04.174

174. *In vitro* Folding of a Recombinant Beta-Scorpion Neurotoxin: The influence of N-Terminal Hydrophobic Regions

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Background: Ts1 is a cysteine-rich 61 amino acid long mammalian neurotoxin from the venom of the scorpion *Tityus serrulatus.* It has an alpha/beta scaffold interlinked by four disulfide bridges. There is a scientific interest in our group to use well-folded recombinant neurotoxins to develop neutralizing antibodies as well as to continue the study of protein-protein interactions between recombinant variants of Ts1 and subtypes of voltage-gated sodium channels. Nevertheless, the heterologous expression and protein folding of the recombinant Ts1 in a bacterial cell as well as *in vitro* conditions do not yield an active neurotoxin. Similarly, folding of the reduced native Ts1 also yields several inactive isoforms. This result in contrast with the successful *in vitro* folding of the related recombinant alpha/beta neurotoxins CssII and CssIV from *Centruroides suffusus suffusus*. Since a well-folded recombinant Ts1 would be valuable to our research, we evaluate the significance of the C-terminal cysteine and of the hydrophobic residues at the N-terminal segment of Ts1.

Methods: Based on bioinformatics, we identified hydrophobic regions in the primary structure of Ts1, and in a similar way, we selected the hydrophilic residues to replace them in order to decrease their hydrophobic character in such region. Seven Ts1 variants, including the parental Ts1 were constructed and inserted into the expression vector, pQE-30. The pQE30-Ts1 variant plasmids were transformed into *E. coli* BL21(DE3), expressed, purified and folded *in vitro* conditions. The toxic effects of the recombinant Ts1 variants when injected intracranial to mice were used as a measure of their proper folding.

Results: The addition of a residue at the C-terminal of Ts1 did not improve the folding of Ts1. Disruption of some hydrophobic patches at the N-terminal region of Ts1 correlated with different folding patterns. Among the Ts1 variants the *in vitro* folding of the recombinant variant I17N resulted in similar toxic symptoms as those of the native Ts1 when injected to mice.

Discussion: Protein hydrophobicity seems to play an important role during protein folding. This factor becomes more significant in cysteine-rich proteins, such as scorpion neurotoxins. Scrambled disulfide bridges produce inactive isoforms. Our results suggest that one way to deal with this problem is to disrupt hydrophobic patches of the protein.

Conclusions: Hydrophilic N-terminal variant of Ts1 I17N was the most toxic to mice suggesting that it favors correctly a folded form of Ts1.

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Keywords: folding, recombinant, neurotoxin
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175. Scorpion Toxins that Cause Human Intoxication

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Review: Scorpion venoms from the family Buthidae contain a great variety of toxic proteins and peptides capable of causing human fatalities (Chippaux and Goyffon Acta Trop 107:71–9, 2008). Among these components are peptides that recognize ion-channels: Na^+, K^+, Ca^{2+} and either block ion conductance of excitable and non-excitable cells or modify their opening-closing kinetics. The most important for human health are toxins specific for Na^+-channels (Na-ScTx), which contain from 59 to 72 amino acid residues, mostly packed by four disulfide bridges (Possani et al., Eur. J Biochem., 264: 287-300, 1999). There are two sub-types, the a-Na-ScTxs that bind to site 3 of the alpha-subunit of the channels prolonging the action potential and the beta-Na-ScTx that bind to site 4 of the same sub-unit shifting the activation mechanism to lower potentials. Several hundreds of such peptides or cloned genes coding for putative peptides have been isolated and characterized. They are the real killers. Antivenom produced against the major Na-ScTx is capable of neutralizing the entire symptoms of intoxication of experimental animals. The K-ScTx are shorter peptides containing from 22 to 42 amino acid residues, mostly packed by 3 or 4 disulfide bridges. Both the Na- and K-ScTxs have a tridimensional folding that comprises a short alpha-helix segment and a triple anti-parallel beta-sheet structure. Four subclasses of K-channel specific peptides were identified: α, β, γ and κ-peptides (Tytgat et al., Trends Pharmacol Sci 20:445-47, 1999). They usually block the channel by binding to the mouth of the channel. They are not real killers, although produce abnormal depolarization of cells, which causes uncoordination of excitable tissues, but also modulate some physiological functions. Calcins are Ca^{2+}-channel specific peptides that affect mainly Ryanodine sensitive calcium channels, but also can affect voltage-gated Ca^{2+}-channels

(Valdivia et al., PNAS 89:12185-89, 1992; Olamendi-Portugal et al., BBRC 299:562-68, 2002). Chorotoxin is the only peptide known to impair chloride permeability. Scorpion venoms might also have cardiotoxins, hemolytic toxins, antimicrobial peptides, enzymes (hyaluronidase, acetylcholinesterase, phospholipase, metalloproteinase and sfingomyelinase D) that cause intoxication. Many other components are present in scorpion venom, from 100 to 600 different molecular masses have been identified in their venoms by mass spectrometry analysis, but for some of them, the structure and function are still unknown; others are being used for their possible therapeutics applications, such as: antibiotics and immunomodulators or analgesic substances.

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Keywords: ion-channel; scorpion; toxin
10.1016/j.toxicon.2012.04.176

176. Is the Endogenous Opioid System Involved in the Antalgic Effect of Scorpion Toxins in Mice?

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Background: We have hypothesized that pain relief induced by scorpion toxins may be the result of a counter irritation phenomenon due to the activation of “diffuse noxious inhibitory control”. We have examined the alpha- and beta- scorpion toxin effects on the mice behavior during nociceptive tests, after peripheral administration. In particular, we have analyzed the involvement of the endogenous opioid systems in the antinociceptive effects observed after injections of the alpha-anatoxin AmmVIII, a weak modulator of Nav1.2 channel (the muscular Nav1.4 channel remains almost insensitive to its application), and of the depressant insect-selective beta-toxin LqqIT2 previously shown to be devoid of neurotoxicity for mammals. They could be efficaciously injected in mice, even in large amount, without induction of any apparent toxic symptoms.

Methods: The analgesic effects of the two scorpion toxins, administered in mouse at different doses (12 mice each dose) by intraperitoneal route, were observed on hot plate and tail flick latencies. We then compared these effects with those obtained after injection of well-known commercial antinociceptive drugs (ketoprofen and DAMGO) or after acetic acid administration or cold-water tail immersion. These two last tests served as counter irritation tests and are well-known to induce pain relief through the activation of the diffuse noxious inhibitory control (DNIC) and the release of endogenous opioids. We also evaluated the effects of toxins and acetic acid on spinal cord *c-Fos* mRNA expression, a proto-oncogene, which increases after painful stimulus.

Results: Both toxins increased latencies in a dose dependent manner. Also, an increase in latencies obtained with toxins, acetic acid, or cold-water tail immersion was partly reversed by the co-administration of naloxone, a μ opioid receptor antagonist. AmmVIII, LqqIT2, or acetic acid injected alone induced an increase in *c-Fos* mRNA expression.

Discussion: The antalgic effects observed after administration of scorpion toxins could be partly due to a counter irritation phenomenon that implicates the activation of an endogenous opioid system. The toxins could first produce a small dorsal root ganglion neurons (DRG) hyperexcitability which, in a second time, could lead their Nav channels to steady-state inactivation and a decrease in the neuron signaling involved in pain pathways.

Conclusion: To find a pharmacological answer to these observations, we have now to explore the Amm VIII and Lqq IT2 effects, as well as those of the highly lethal “classical” alpha- or beta- toxins, on the voltage-gated sodium currents expressed in DRG neurons.

Keywords: pain, scorpion toxin, analgesia
10.1016/j.toxicon.2012.04.177

177. IgY Antibodies Anti-*Tityus caripitensis* Venom: Purification and Neutralization Efficacy

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Background: *Tityus caripitensis* is responsible for most accidents due to scorpion stings in northeastern Venezuelan regions. Avian antibodies (IgY) isolated from chicken egg yolk represent a new alternative to be applied as antivenom theraphies. In this work we produce IgY antibodies against *Tityus caripitensis* scorpion venom and evaluate its neutralizing capacity both "in vitro" and "in vivo".

Methods: The anti-scorpion venom antibodies were purified by precipitation techniques with polyethylene glycol and evaluated by MABA, an indirect ELISA, and western blot assays. The neutralization of lethality was evaluated by pre-incubation of venom together with antivenom prior to testing.

Results: The specificity of IgY antibodies was demonstrated by a dose-dependent inhibition in western blot assay when antibodies pre-absorbed with the venom did not recognize the venom proteins from *T. caripitensis*. The antivenom was effective in neutralizing 2LD50 doses of *T. caripitensis* venom in vivo (97.8 mg of IgY neutralized 1 mg of *T. caripitensis* venom).

Conclusion: Our results support the future use of avian anti-scorpion *T. caripitensis* venom as an alternative to conventional equine antivenom in our country.

Keywords: *Tityus caripitensis*, anti-venom, IgY antibodies
10.1016/j.toxicon.2012.04.178

178. Development of Nanobodies and Derivatives with High Neutralizing and Protective Capacity against Scorpion Envenoming

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Background: Envenoming following scorpion sting is a public health issue in many parts of the world. During scorpion envenoming, highly toxic small polypeptides of the venom diffuse rapidly within the victim, causing serious medical problems. Nanobodies (Nbs), the recombinant single-domain antigen-binding fragments of camel-specific heavy-chain only antibodies, offer special advantages in therapy over classic antibody fragments due to their robustness and smaller size matching the size of the scorpion toxins (7 kDa). Our aim was to develop antivenom product which more quickly reach the highly diffusible scorpion toxins**.**

Methods: We immunized dromedaries with toxins from *Androctonus australis hector* (Aah) scorpions and cloned the single-domain antibody fragments or nanobodies (15 kDa) from their B cells. Nanobodies against the two most toxic compounds of the venom (AahI and AahII toxins) were retrieved from the libraries, and their neutralization and protective capacity was monitored in mice. Subsequent bispecific and humanized derivatives have been designed and their pharmacokinetics has been studied

Results: It is demonstrated that the retrieved Nanobodies fully protected mice against high lethal doses (7 to 100 LD_{50}) of scorpion toxins administered intracerebroventricularly. Moreover, where current antivenom failed completely to neutralize $2LD_{50}$ of crude venom injected subcutaneously, the designed bispecific NbF12-10 against AahI'/AahII toxins succeeded in neutralizing 5 LD_{50}. Finally, the maximally humanized version of nanobody maintains its high affinity for the toxin without conceding much on expression yield and stability**.**

Discussion: The bispecific NbF12-10 is the best candidate to develop a therapy in human against the most toxic venom compound of one of the most dangerous scorpions. In a challenge assay in which mice were subcutaneously injected with a lethal dose of scorpion venom, the subsequent intravenous injection of 85 microg of NbF12-10 protected all mice, even if the whole procedure was repeated 3 times. Furthermore, the NbF12-10 remained fully protective when mice with severe signs of envenoming were treated a few minutes before the untreated mice died**.**

Conclusions: Nanobodies - derived from HCAbs of camelids and selected after phage display - show great potential to provide a more efficient therapy against scorpion envenoming.

Keywords: nanobodies, scorpion toxins, protective capacity
10.1016/j.toxicon.2012.04.179

179. Automated Mass Fingerprinting of Scorpion Venoms in the Nanogram Range

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Background: Several proteomic approaches were employed recently to assess the diversity of the content of animal venoms. All involve techniques of liquid chromatography and mass spectrometry. But, at some point a tedious manual work of data processing must be performed. We have investigated the possibility to automatically produce a comprehensive venom mass fingerprint (VMF) from nanogram quantities of scorpion venom. A workflow was designed using the most recent advances on ultra-high pressure liquid chromatography (UHPLC) coupled to an automated MALDI-TOF data acquisition and a specially designed data-processing**.**

Methods: Scorpion venoms were extracted by manual stimulation and constituted polled or individual samples. Nano UHPLC analysis were performed using a Dionex UltiMate® 3000 RSLCnano system and 100 μm x 250 mm C18-reversed phase PepSwift Monolithic Nano PS-DVB column. Direct on-line effluent spotting was performed using a LC-Packing Probot and a sampling rate of 3 spots per second with addition of 0.5 μl of twice-diluted CHCA-saturated matrix. MALDI-TOF spectra were recorded on a Bruker Ultraflex II automatically on each spot by using a fuzzy logic feedback control system. A specially designed Excel-macro was used to filter for doubly charged or dimer ions and to combine each individual peak list to a one including the chromatographic retention time. A clustering process was allowed to end with a single list of unique masses at a minimum threshold intensity**.**

Results: The optimal venom quantity to be injected on the nano column was first determined. Few tens of nanograms were the optimum and saturation was observed for hundreds. Special attention was given to the reproducibility of the workflow. Statistics were performed on repetitive sample injections and m/z relative error and normalized relative intensity error were assessed. A very low dispersion for mass value was found, which is crucial for VMF comparison. Dispersion concerning peak intensities was not so helpful, but not surprising since MALDI-TOF/MS is known to be not a good quantitative technique. First, we validated our automated VMF acquisition process using the well known *Androctonus mauretanicus* venom. Then, we were able to study the venom of rare Buthidae collected in Southeast France**.**

Conclusions: Taking into account the results of our study, it is noteworthy that from tens of venom nanograms, comprehensive VMF can be achieved in a timeless and an automatic way. This workflow could help us to consider phylogenic studies based on clades defined from such comprehensive lists of individual masses.

Keywords: UHPLC, venom mass fingerprint
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180. Increased Incidence of *Tityus trivittatus* Envenoming in the City of Buenos Aires

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Background: *Tityus trivittatus* is the scorpion of highest medical importance in Argentina. It is present in the majority of the big cities of the country favouring the contact man – scorpion due its characteristic of adapting to live in human constructions and environments. In recent years, both the number of scorpion sighting reports, as well the number of envenomings increased in the country. We undertook this study to determine if the population of *Tityus trivittatus* has expanded its geographical area during the last decade in the city of Buenos Aires and whether or not there has also been an increase in the frequency of envenoming cases within the affected geographical areas**.**

Methods: the reported cases recorded in our institutions during 2001-2011 were classified by date and location, further geo-referenced in a digital map and analyzed in a geographic information system (GIS). Reported cases were modeled as points and buffer areas were created 250 m around each case to define an arbitrary measure of geographical influence. When reported *Tityus* events occurred close to each other (less than 500m), overlapping areas became merged into a single area containing all points representing those events. These areas were differentially plotted for increasing time intervals from 2001 to 2011. The total area associated with reported findings in square km and the number of events per square km was computed for each time interval.

Time interval (Years)	Compromised Area (km^2)	Reported findings	Reported findings/km^2
2001-2002	0.78	16	20.49
2001-2004	2.43	60	24.68
2001-2006	3.03	82	27.08
2001-2008	3.63	126	34.68
2001-2011	6.00	251	41.81

Results:

Discussion: The geographical area within the city of Buenos Aires, where some *Tityus trivittatus* findings have been reported, has increased at about 0.5 km^2 per year since 2001. The incidence of sightings of scorpions computed by area has also consistently increased since 2001, suggesting the presence of the species has become permanent. Despite the increasing number of sightings of *Tityus trivittatus*, the number of envenoming cases in Buenos Aires city did not increase in the last five years. There have been no deaths and only one moderate envenoming occurred in the city. The toxicity of the venom of these scorpions in the city of Buenos Aires is lower regarding that from other regions of the country. However, the increased geographic area where these scorpions can be found, indicates the need to focus attention to prevent potential envenomings in zones of the city where the presence of *T. trivittatus* has not been historically registered.

Keywords: *Tityus trivittatus,* scorpion, epidemiology, finding, Argentina
10.1016/j.toxicon.2012.04.181

181. Evaluation of a Four-Hour Endpoint for Use in Scorpion Envenomation Studies in Morocco

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Background: Scorpion stings in Morocco are a significant public health issue and children under the age of 15 are the most severely affected. The Moroccan poison center (Centre Anti Poison du Maroc, CAPM) uses a systematic four level envenomation classification system. Scorpion antivenom in North Africa has been controversial in the past and is not currently in use in Morocco. The objective of this study was to characterize a population for which effective antivenom treatment might have the greatest impact and to characterize potential endpoints for use in a subsequent prospective scorpion antivenom trial**.**

Methods: This was a retrospective review of CAPM records representing patients admitted for scorpion envenomation across Morocco. Patients included in the study were 6 months to 10 years old, admitted for a scorpion sting between March 2007 and November 2009. Patients presenting to the hospital more than 4 hours after a sting or with an envenomation class IIa or below were excluded. Indicators of patient outcome were observed hourly for the first five hours after admission seeking evidence of change for these parameters during that time. Final patient outcome at hospital discharge was recorded. No patient received antivenom**.**

Results: Out of 349 cases, 244 met the study inclusion and exclusion criteria. 18.4% ($n = 45$) of the patients progressed to a class III envenomation at some time during their hospital stay. Out of 223 patients for whom final outcome was available, mortality was 11.2% ($n = 25$). Younger patients had the most severe clinical syndrome. Out of the 244 patients, only 3 (1.2%) had clinical improvement documented within 4 hours of admission**.**

Discussion: Our findings are consistent with past reports that scorpion envenomation syndrome without

antivenom persists for greater than 4 hours in North Africa and in North America. Recent clinical trials in North America indicate that severe *Centruroides* envenomation resolves within 4 hours when promptly treated with effective antivenom. Taken together, these findings suggest that the four-hour endpoint in a similar population could be used to test efficacy of an antivenom specific to North African species.

Keywords: scorpions, pediatrics, endpoint determination
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182. Two Case Studies of Pregnancy Outcomes after Scorpion Envenomation and F(ab')2 Scorpion Antivenom Treatment

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Background: The effects of venom and antivenom exposure during Case pregnancy have not been well studied. Clinical trials of scorpion antivenom recently included two pregnant women.

Case Studies: Between 2005 and 2011**,** 1970 patients with presumed *Centruroides* envenomation were enrolled and treated with an $F(ab')_2$ scorpion antivenom in a multicenter treatment protocol in Arizona, USA. After-informed consent, subjects received 3-5 vials of Anascorp®, intravenous. Out of 1970 cases, 2 were eventually recognized as having been pregnant at the time of study enrollment. **Case 1** involved a 28-year-old woman presenting at 8 weeks' gestation with nystagmus, tongue fasciculations and limb paresthesias. Aware that she was pregnant, medical staff reviewed the potential risks and benefits of antivenom treatment with her before deciding to proceed in order to minimize the risk of envenomation itself. She received a total of 4 vials of antivenom, and her signs and symptoms resolved 70 minutes after study enrollment. At term she had a normal labor resulting in delivery of a healthy 9-lb boy. **Case 2** was a 23-year-old woman who was unaware of pregnancy at the time of the scorpion sting. She presented with nystagmus, tongue fasciculations, chest heaviness, and limb paresthesias. She received lorazepam, morphine and 3 vials of antivenom, with complete resolution of her symptoms in less than 2 hours. One week later, she discovered that she was pregnant; and she had a spontaneous abortion approximately 22 days following the scorpion sting**.**

Discussion: Cause and effect cannot be proven by case description alone. It is clear that good pregnancy outcome is possible with first trimester scorpion sting and $F(ab')_2$ antivenom treatment; although pregnancy loss following envenomation and treatment for scorpion sting (here) or snakebite (Langley, 2010) is also a consideration. Fetal loss as in Case 2 could result from unrelated causes; from venom interaction with maternal circulation, placenta or fetus; or perhaps from exposure to equine serum derivatives. Neither of the patients in this series had signs suggestive of type 1 or 3 hypersensitivity; and the rapid resolution of systemic signs in both cases suggests that antivenom use in systemic envenomation was likely more beneficial than harmful**.**

Conclusions: Envenomated patients of childbearing potential should be counseled as to risks and benefits prior to the administration of antivenom. The use of antivenom in many cases is likely to be a safer choice than supportive care alone, but more evidence will be necessary before final conclusions can be made.

Keywords: envenomation, pregnancy, antivenom
10.1016/j.toxicon.2012.04.183

183. Development of Immune Sera Against Algerian Scorpion Venoms: Which Antibodies for Envenoming Treatment in Regions At-Risk?

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Background: The number of dangerous scorpion species (*Androctonus australis: Hector: Aah, Androctonus ammourexi: Amx* and *Buthus occitanus tunetanus: Bot)* raging in some regions At-Risk in the Maghreb remains a real concern for the immune serum producers. The use of one or a mixture of antigens is still subject of controversy. In this order to propose an efficient immune serum three preparations were undertaken, mono-, bi- and tri-valent were tested**.**

Methods: The efficiency of tri-valent sera was compared to that of bi-valent and mono-valent one. Their effects on induced tissue damage and inflammatory response were evaluated

Results: Administration of tri-valent immune sera at 30 min after envenomation, to animals injected with the Aah or Amx or Bot venom neutralized tissue damage (hemorrhage, edema, leukocyte infiltration) in myocardial tissue and hepatic parenchyma. Administration of these antibody treatments also reduced the metabolical perturbations (CPK, LDH, AST, ALT, urea, creatinine and cholesterol) and blood leukocytosis. However, hyperneutrophilia and eosinophilia induced by the venoms were not significantly affected by the antibody treatments. These results showed also the similar efficiency of these three preparations against Aah venom, however, the tri-valent preparation presents a significant neutralizing effect than the mono-valent serum when Amx or Bot venom used for the envenomation. These results are very competitive with those obtained by the already standardized mono-valent immunotherapy**.**

Discussion: Administration of tri-valent antibodies may thus be beneficial in counteracting the whole pathophysiological effects induced by scorpion venoms. The efficiency of this treatment could be due to the neutralization effects of $F(ab')_2$ fragments on circulating of the three venoms**.**

Conclusions: It appears according to this study that this immune serum could be a great contribution to cover all the polymorphisms existing in our country.

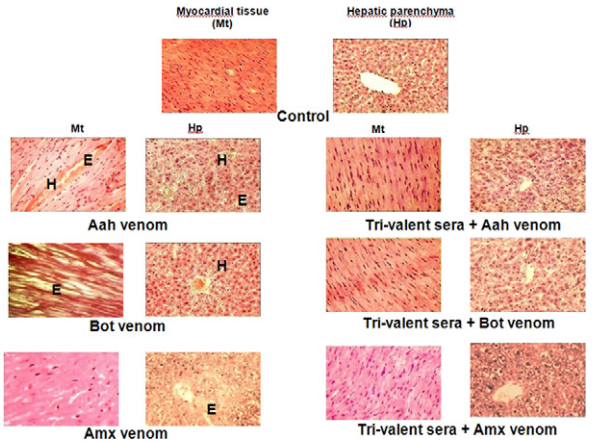

Fig. 1. Hepatic parenchymal and myocardial structure in envenomed mice and treated by trivalent-antibodies (Haematoxylin and eosin coloration, magnification 400, legends: H: hemorrhage, E: edema).

human antibodies (scFv) was constructed and evaluated by means of phage-display and directed evolution.

Methods: A selected scFv (3F) was evolved resulting in a neutralizing antibody (6009F) of the whole soluble venom of *Centruroides noxius* (Riaño et al FEBS J. 272:2591-2601, 2005; Patent US 7,381,802 B2).

Results: New scFv variants like 9004G have been generated. We demonstrated that 6009F and 9004G are capable of protecting mice against venoms from two different scorpion species: *Centruroides suffusus suffusus* and *Centruorides noxius*. A key residue (F101VH) was inserted into scFv 9004G lone improving its neutralizing capacity. The scFv obtained (LR) is the best neutralizing antibody against main toxins and venoms of these two scorpion species obtained to date by our group (Riaño et al. J. Biol. Chem. 286:6143-6151, 2011).

Conclusions: These results show that a single antibody may have multiple neutralizing capacities. Following the same strategy, several scFv antibodies capable of neutralizing the main toxins of *Centruroides limpidus limpidus* have been obtained. These results allow us to propose confidently that a cocktail containing a few optimized human scFv antibodies will become the next generation of scorpion anti-venoms.

Table 1
Effect of tri-valent antibodies ($F(ab')_2$ anti Aah, anti Amx and anti Bot) on metabolical perturbations induced by scorpion venoms.

	CPK (IU/l)	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	Urea (mmol/l)	Crea (μmol/l)
Control	596.5 ± 29.8	87 ± 8.2	17 ± 1.2	73.79 ± 8	7.59 ± 1	1.32 ± 0.09
Aah Venom	1546 ± 77.3	192.6 ± 15.3	56 ± 5	184 ± 15	10.8 ± 0.9	16 ± 0.8
Bot Venom	1543 ± 51.4	180 ± 17	81.2 ± 7	107 ± 10	8.6 ± 1	13 ± 1.1
Amx venom	2608 ± 65.2	247.6 ± 21.3	49.7 ± 5	88 ± 9	7.9 ± 8	13 ± 1.2
Tri-valent-sera+ Aah venom	408 ± 20.1	141.2±14	27.6 ± 3.2	70 ± 7	6.6 ± 7.1	6 ± 0.5
Tri-valent-sera+ Bot venom	1098 ± 36.6	163.7 ± 15.3	22.7 ± 1.8	68 ± 7.1	8.2 ± 0.95	4 ± 0.3
Tri-valent-sera+ Amx venom	934 ± 31	132.8 ± 10.2	44.6 ± 5	61 ± 5.9	6.8 ± 0.5	12 ± 1

TCPK: creatine phospho-kinase; AST: aspartate transaminase; ALT: alanine transaminase; ALP: alkaline phosphatise; Crea: creatinemia.

Keywords: antibodies, valence, scorpion venom, neutralization
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184. Development of Novel Scorpion Anti-Venoms in México

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Background: Scorpions of the family *Buthidae* contain venoms that provoke severe envenoming to humans. Toxic components are short-chain peptides that affect ion-channels (being Na^+-channels the most important), causing an abnormal cellular function. Scorpions of the genus *Centruroides* usually have few highly toxic peptides in their venoms. We have shown that a monoclonal antibody (BCF2) was capable of neutralizing the intoxication caused by both: toxin Cn2 and the whole venom from the scorpion *Centruroides noxius* Hoffmann (Licea *et al.* Toxicon, 34, 843-847, 1996). This finding prompted the hypothesis that anti-venoms against scorpions could be constituted by a few neutralizing antibodies. In order to confirm this idea, a library of single-chain

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Keywords: antivenom; phage display; scorpion; directed evolution
10.1016/j.toxicon.2012.04.185

185. Safety of Equine F(ab')2 Antivenom for Scorpion Envenomation: Results of Prospective Clinical Trials

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Background: Use of serum products including antivenoms is known to involve a risk of type 1 and type 3 hypersensitivity. The technology involved in the production of serum derivatives has advanced during the past century such that third-generation ("fabotherapeutic")

products now have much higher potency relative to total protein content, and the quantity of immunoreactive material injected into patients has been in many cases greatly reduced. Recent prospective clinical trials of equine $F(ab')_2$ scorpion antivenom provided an opportunity to conduct follow-up evaluations of safety outcomes with one of these newer products.

Methods: Children and adults at selected sites in the US and Mexico, envenomated by scorpions between 2004 and 2011, were eligible for enrollment in a series of five prospective clinical trials of Anascorp™ (*Centruroides* (Scorpion) Immune $F(ab')_2$ (Equine) Injection). In all protocols, adverse events were prospectively monitored, including specific observation for symptoms suggestive of serum sickness during the two weeks following enrollment. Adverse events were categorized as to severity and possible cause, all serious adverse events were individually reviewed, and descriptive statistics were applied to the results.

Results: 15 patients were enrolled in a double-blind protocol, 7 of whom received antivenom. 78 patients were enrolled in 3 open label protocols that mirrored the treatment group in the double-blind trial. 1425 additional patients were enrolled in a treatment protocol offered at 28 hospitals across Arizona. The range of antivenom exposure was 1-5 vials, intravenous. In the treatment protocol, 307 adults and 1118 children were treated. Overall, 3 (0.2%) had acute type 1 reactions and 8 (0.6%) appeared to have type 3 reactions; but none had the full syndrome of classical serum sickness. There were 30 serious adverse events, most of which involved secondary respiratory consequences of envenomation or of excessive sedation concomitant with the study. There were no deaths.

Discussion: Acute and delayed immune responses are a risk with any serum derivative; but results of these studies show that refined third-generation fabotherapeutics can have remarkably low adverse event profiles, in contrast with historic descriptions of 60-90% rates of serum sickness using first generation antisera. This study was not designed to prove cause, but it is likely that this improved safety is a consequence of high potency (thus relatively low protein dose), lack of immunogenic Fc with the $F(ab')_2$ fragment, and high purity of this preparation overall.

Keywords: safety, antivenom, hypersensitivity, serum sickness, clinical trial
10.1016/j.toxicon.2012.04.186

N. Snakes

186. Effects of Captivity or Season on Venom Composition in Two Species of Rattlesnakes (*Crotalus atrox* and *C. v. viridis*)

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Background: Snake venoms consist of a variety of biochemical components that serve to immobilize, kill and digest prey. Venom variability within snakes has typically been attributed to factors such as age, season, environment and diet, but studies exploring snake venom composition at fine scales of resolution (within individual snakes) are uncommon. To explore the effects of several of these variables, two separate studies were performed: effects of captivity on the venom composition of adult Western Diamondback Rattlesnakes (*Crotalus atrox*), and seasonal effects on venom composition in snakes collected during spring and fall (free-ranging adult Prairie Rattlesnakes, *Crotalus viridis viridis*).

Methods: Sixteen *C. atrox* were captured from Cochise Co., AZ and maintained in captivity for eight months on a diet of NSA mice. Thirty-three *C. v. viridis* were captured, PIT-tagged, and released in the spring and fall from two well-defined den sites in Weld Co., CO. Venoms were extracted shortly after capture and once every two-three months for the *C. atrox*. Venom samples from both studies were subjected to reducing 1-D SDS-PAGE, reversed-phase HPLC, MALDI-TOF mass spectrometry (MS), five different enzyme assays and a fibrinogen degradation assay.

Results: For both studies, venom composition appeared to remain constant within individuals, as assessed by 1-D SDS-PAGE and fibrinogen digest assay results, while RP-HPLC and MALDI-TOF MS showed only minor differences. For *C. atrox*, venom L-amino acid oxidase (LAAO) and phosphodiesterase (PDE) activity significantly increased over the course of captivity, with no changes occurring in metalloproteinase (MPr), kallikrein-like serine protease (KLSP), or thrombin-like serine protease (TLSP) activities. *Crotalus v. viridis* venoms showed significantly higher PDE activity and lower MPr activity in the spring than in the fall. *Crotalus v. viridis* venom TLSP activity levels also increased significantly with time/age (from spring to fall and fall to spring), with no changes in LAAO or KLSP activity.

Discussion: Since the overall "fingerprint" for each snake's venom remained more/less constant, it can be concluded that major changes in venom composition did not occur within individuals in either species. Small but statistically significant differences were, however, observed for some venom enzyme activities.

Conclusions: These studies indicate that minor differences in venom composition do occur in two rattlesnake species, as a function of season or captivity/diet. Although further testing will be necessary, these differences are likely to be of minimal significance when treating cases of human envenomation or producing antivenom.

Keywords: snake, venom, variation, *Crotalus, atrox, viridis*, season, captivity.
10.1016/j.toxicon.2012.04.187

187. Metalloproteinases from Rear-Fanged ("Colubrid") Snake Venoms: An Under-Utilized Resource for Evolutionary and Structure/Function Studies

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Background: Rear-fanged snakes ("Colubridae") are abundant world-wide, and because their venom proteomes are typically much less complex than those of viperids and

elapids, they represent an excellent group for exploring questions concerning the evolution and biological roles of individual venom proteins. Among the relatively low number of protein families in snake venoms (~20), metalloproteinases are typical in most colubrid venoms, while many other common protein families, such as LAOO, PLA_2, serine proteinases and nucleases, are either uncommonly present or are wholly absent. Snake venom metalloproteinases (SVMPs) have major roles in pathogenesis following envenomations and in the digestion of snake prey, and they are present in all clades of venomous reptiles; however, only a small number of SVMPs from "colubrid" snake venoms have been purified and significantly characterized.

Methods: Venoms were extracted from many rear-fanged snakes using ketamine/pilocarpine as described previously. Venoms were subjected to enzyme analyses, SDS-PAGE, MALDI-TOF-MS, and toxicity assays in several animal models. For select species, metalloproteinases were purified from crude venoms using low pressure LC and HPLC.

Results: Venoms from some species (*Alsophis, Amphiesma*, *Coniophanes*, *Hydrodynastes*) contain SVMP levels similar to viperid snakes, while others (*Ahaetulla*, some *Boiga*, *Drymobius*, *Tantilla*) contain levels comparable to elapids – low to barely detectable activity. Alsophinase, a P-III SVMP from *Alsophis portoricensis* venom, is a single chain protein with a mass of 56 kD. It contains potent fibrinogenolytic and hemorrhagic activities, and it induced subcutaneous and pulmonary hemorrhage in mice and *Anolis* lizards at doses of 4 μg/g and higher. Sequence similarity analyses indicate that most colubrid SVMPs appear to be P-III proteinases with the 3 domain structure typical of these metalloenzymes.

Discussion: SVMP activity is a common component of most colubrid venoms assayed, and while most appear to be P-III enzymes, a putative P-II metalloproteinase was described from *Rhabdophis tigrinus* venom and a matrix metalloproteinase-like enzyme was recently reported from the transcriptome of *Tachymenis*. It is therefore clear that among colubrid snakes, one observes a diversity of classes of metalloproteinases, but at present this diversity is poorly known.

Conclusions: Some general trends are identified from information currently available, and it is likely that the type I/type II compositional dichotomy observed in rattlesnake venoms may be reflected by a larger global pattern of venom composition in advanced snakes. Though "colubrid" metalloproteinases share some significant similarities with other SVMPs, different or novel hydrolytic specificities suggest they may be useful for structure/function studies.

Keywords: alsophinase, enzyme, toxin evolution
10.1016/j.toxicon.2012.04.188

188. The *In vitro* Neurotoxic Effects of the Newly Discovered Central Ranges Taipan (*Oxyuranus temporalis*)

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Background: The Inland Taipan (*Oxyuranus microlepidotus*) and Coastal Taipan (*Oxyuranus scutellatus*) are highly venomous Australian elapids, whose venoms contain potent presynaptic (b) and postsynaptic (a) neurotoxins. Recently a new species of taipan, the Central Ranges Taipan (*Oxyuranus temporalis*), has been discovered (Doughty et al. 2007). Two specimens are held in captivity at the Adelaide zoo. The aim of this study was to characterise and compare the neurotoxicity of Central Ranges Taipan venom with the other taipan venoms.

Methods: Central Ranges Taipan venom was obtained by milking specimens housed at the Adelaide zoo prior to freeze-drying. Venoms from the Inland Taipan, Coastal Taipan, Papuan Taipan (Saibai Island) and Papuan Taipan (Merauke), for comparison, were supplied by Venom Supplies Pty Ltd.

Results & Discussion: Analysis of Central Ranges Taipan venom using size-exclusion and reverse-phase HPLC indicated a markedly different 'profile' compared to the other taipan venoms. In the chick biventer cervicis nerve-muscle preparation, Central Ranges Taipan venom (1 μg/ml) abolished indirect twitches (0.1Hz; 0.2ms, supramaximal V) with a t_{90} value of 24.3 ± 4.0 min (n=5). The venom also abolished responses to exogenous acetylcholine (1 mM, 30 s) and carbachol (20 μM, 60 s), indicating the presence of postsynaptic neurotoxins. Based on t_{90} values, the other taipan venoms were found to be far less neurotoxic with the following rank order of potency: Central Ranges Taipan (15.1 ± 2.0 min, n=5, 3 μg/ml) > Inland Taipan (21.2 ± 0.7 min, n=3, 10 μg/ml) > Coastal Taipan (81.4 ± 6.6 min, n=4, 10 μg/ml) ≥ Papuan Taipan Saibai Island (86.0 ± 2.5 min, n=3, 10 μg/ml) > Papuan Taipan Merauke (129.3 ± 14.9 min, n=3, 10 μg/ml). The inhibitory effects of all taipan venoms (concentrations of either 3μg/ml or 10μg/ml) were neutralised by prior addition of CSL Taipan antivenom (3 Units/ml).

Conclusions: This study suggests that the venom of the Central Ranges Taipan is highly neurotoxic *in vitro* and potentially dangerous to humans.

References

Doughty P., Maryan B., Donnellan S.C., Hutchinson M.N., 2007. A new species of taipan (Elapidae: *Oxyuranus*) from central Australia, *Zootaxa*, 1422, 45-58.

Keywords: taipans, neurotoxicity, antivenom
10.1016/j.toxicon.2012.04.189

189. Neutralization Effect of Glycation on Myotoxic and Nephrotoxic Effects Induced by BthTX-I

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Background: Glycation, a chemical modification of proteins with reducing sugars, indicating a possible explanation for the association between hyperglycemia and the wide variety of tissue pathologies. A good example is glycosiled hemoglobin that can be used as a molecular tool to characterize the severity of diabetes. In this in vitro study, we investigated the effect of glucose, galactose and lactose on the structure and function of BthTX-I, which was chosen as the structural model of sPLA2. Glucose is known and well characterized as one of the most important glycacanting agent of protein. Galactose is another monosaccharide with the same functional groups found on glucose and lactose which is the dimer of galactose and glucose were also evaluated in this study.

Methods: The degree of glycosylation of BthTX-I with the various glycating agents was performed using affinity chromatography phenylboronate, which allowed the identification of glucose and galactose as two main glycants agents and purified the glycated BthTX-I form from the not.

Results: The analysis in reverse-phase HPLC showed that both glucose and galactose significantly reduced hydrophobicity nature of BthTx-I. Moreover, both glucose and galactose significantly changed the spectrum of circular dichroism and intrinsic tryptophan fluorescence of BthTX-I. In addition, glucose and galactose also significantly reduced edema, myotoxicity and nephrotoxicity induced by native BthTX-I.

Discussion: These results indicate the susceptibility of sPLA2 BthTx-I as non-enzymatic glycation by various sugars. It also describes the effects of glycation on the structure and pharmacological activity of BthTX-I.

Conclusion: Moreover, as BthTX-I is an example of sPLA2 both in terms of structural and functional is possible to conclude that nonenzymatic glycation might be affect other types of secretory PLA2 in diabetes for example.

Keywords: sPLA2, glycation, edema, myonecrosis.
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190. First Draft of the Genomic Organization of a PIII-SVMP Gene

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Review: The evolutionary pathway of snake venom toxins remains poorly understood. The origin of SVMPs has been inferred to have occurred following the split of the Pareatidae from the remaining Caenophidians, approximately 54-64 Mya, through recruitment, duplication, and neofunctionalization by positive Darwinian selection of a closely related body cellular ADAM 7 or 28 ancestor gene. The evolutionary history of viperid SVMPs is repeatedly punctuated by domain loss, and minimization of the organization of genes coding for disintegrins, including loss of introns and coding regions, has been documented. However, details on the mechanisms of recruitment and molecular events underlying the origin and evolution of the SVMP multigene family remain elusive. Moreover, the genomic structure of any SVMP gene has not been reported, and thus the changes occurring after recruitment of the ancestral gene into the venom gland remain hidden in the snake genomes. Here we examine the gene structure of the 15652 bp *Echis ocellatus* pre-pro-EOC00089-like PIII-SVMP gene asssembled from PCR-amplified sequences of overlapping genomic fragments. The gene comprises 12 exons interrupted by 11 introns. In a homology model of the EOC00089-like protein, the insertion of introns interrupting coding regions lie between secondary structure elements. LINEs L2/CR1 and RTE/BovB, SINE/Sauria, and a hobo-activator DNA transposon were identified within introns 1, 3, 7 and 8. Pairwise amino acid sequence comparisons between EOC00089-like PIII-SVMP and its closest orthologs, human and *A. carolinensis* ADAM28 showed that their ORFs share 42%/59%, 49%/69%, and 48%/65% (identity/similarity), respectively. The protein-coding positions interrupted by each of the 11 introns of the Echis PIII-SVMP gene are entirely conserved in the *A. carolinensis* and human ADAM28 genes. However, lizard and human ADAM28 genes contain 5 introns not present in *E. ocellatus*. Strikingly, none of the 11 introns shared between EOC00089-like PIII-SVMP and *A. carolinensis* ADAM28 genes exhibit conservation in size. Furthermore, Echis and Anolis introns exhibit quantitatively and qualitatively distinctions in their inserted retroelements. Our finding that retroelements are exclusively located within introns is consistent with the view that introns are added to genes presumably as transposable elements, and identifies introns as possible key elements in the recruitment and amplification process of SVMPs into the venom gland of extant snakes. Reptile genome sequencing projects may shed light on this aspect of the emergence and evolution of venom toxins. Furthermore, the organization of the PIII-SVMP reported here provides a genomic explanation for the emergence of dimeric disintegrin subunits encoded by short messengers.

Keywords: gene organization, SVMP, intronic retroelements
10.1016/j.toxicon.2012.04.191

191. Dexamethasone Antagonizes the Myotoxic and Inflammatory Effect of Bothrops Venoms

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Background: We investigated the toxic activities from *Bothrops* genus snake venom using *in vivo* and *in vitro* experimental protocols in mice muscle and tested the protector effect of dexamethasone (DEXA) in different conditions, comparing it with the polyvalent antivenom. We also expanded the investigations on the antiophidic effect of the *Eclipta prostrata* (EP) crude extract.

Methods: *In vivo* experiments were performed in mouse muscle and in mice with *Bothrops jararaca* and *Bothrops jararacussu* snake venom. We quantified the increase of plasma creatine kinase (CK) activity as well the CK content in the *Extensor digitorum longus* (EDL) of these animals. We measured the edema and inflammatory response evaluated by the presence of inflammatory cells at the inoculation site when we administrated *B. jararacussu* venom (1.0 mg/Kg). *In vitro* we determined the increase of the rate of CK release from the isolated EDL muscle perfused with appropriated nutrition solution. We also observed the amplitude of the indirect evoked twitch-tension at the isolated mouse phrenic-diaphragm preparation.

Results: Treatment with DEXA (1.0 mg/Kg) preserved over 50.0 % of the muscle CK content *in vivo* when evaluated 24 and 72 hours after the injection of *B. jararacussu* venom, and likewise decreased about 20.0 % of the edema induced by this venom. DEXA reduced in 50.0 % the presence of inflammatory cells in the muscle. The EP extract (50 mg/Kg) showed antagonized the edema and preserved the muscle CK content, and its association with DEXA showed an additive effect. EP also antagonized the increase of plasma CK activity induced by the *B. jararacussu* venom in 77 %. The association of DEXA with polyvalent antivenom did not show additive or benefic effect. On the *in vitro* experiments, DEXA did not show ability to antagonize the increase of the rate of CK release from the muscles exposed to 25.0 μg/mL of *B. jararacussu* venom, neither to prevent the fall on amplitude of the indirect evoked twitch-tension at the isolated phrenic diaphragm preparation, while the EP extract showed a 100.0 % protection at concentrations of 50 and 100 μg/mL.

Discussion: Our results are showing that DEXA was able in vivo to decrease the inflammatory response and did not show any protective effect *in vitro*. Otherwise the inflammatory responses were almost completely neutralized by EP.

Conclusion: Our data together are demonstrating that the inflammation is an important element to be neutralized on the envenomation by snake venoms.

Financial support: FAPERJ, CNPq.

Keywords: *Bothrops* venom, dexamethasone, myotoxicity, inflammation, *Eclipta prostrate*
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192. Action of Venom Metalloproteinases on Basement Membranes: Pathogenesis of Hemorrhage and Blistering in Snakebite Envenomings

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Review: Zinc-dependent metalloproteinases are abundant components of snake venoms, especially in viperid species. Snake venom metalloproteinases (SVMPs) are grouped in three classes (P-I, P-II and P-III), with various subclasses, on the basis of their domain composition. SVMPs play a key role in some of the most relevant pathophysiological manifestations of viperid envenomings, such as hemorrhage, blistering, necrosis and coagulopathy, and also induce prominent inflammation. The pathogenesis of hemorrhage by SVMPs is associated with the ability of these enzymes to degrade proteins of the basement membrane (BM) and surrounding extracellular matrix (ECM) in capillary blood vessels. A combination of *in vitro* and *in vivo* experimental approaches has provided novel evidence for understanding this complex phenomenon. Such experimental platforms include electron microscopy, intravital microscopy, immunohistochemistry, electrophoresis, Western blotting, and proteomic analysis of exudate collected in the vicinity of affected tissue. Hemorrhagic and non-hemorrhagic SVMPs of the P-I class have been compared in order to identify differences that might help to understand the basis of this pathological effect. In contrast to a non-hemorrhagic P-I SVMP, a hemorrhagic enzyme is able to hydrolyze perlecan and type IV collagen, as well as types VI and XV collagens, a finding that might have implications for its mechanism of action. On the other hand, P-III SVMPs are, in general terms, more potent hemorrhagic toxins than P-I SVMPs owing to the presence of exosites in their disintegrin-like and cysteine-rich domains, which enable these enzymes to bind to relevant targets in microvessels. Hydrolysis of key structural components in capillary BMs and surrounding ECM, with the consequent mechanical weakening of the microvessel structure, provokes the distention of the capillary wall, due to the action of hemodynamic forces operating *in vivo*, a process that eventually brings mechanical disruption of the vessel wall and extravasation. Similarly, the action of SVMPs on BM components of the dermal-epidermal interphase provokes the formation of blisters. Further studies are necessary to compare the patterns of BM hydrolysis induced by P-I and P-III SVMPs and to identify the structural determinants of their highly variable toxicological profiles.

Keywords: snake, venom, metalloproteinase, hemorrhage, blistering
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193. A Study of Venoms from Individual Snakes of Two Populations of *Rhinocerophis (Bothrops) alternatus* of Argentina

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Background: *Rhinocerophis (Bothrops) alternatus* is the biggest southernmost snake in the world and one of the most medical importances snakes in Southern South America. In previous works we observed variations between venoms of these snakes from different regions in

Argentina, and important individual variations in individual venoms from a region. In addition we could observe that despite some morphological differences in the snake-skin pattern, there were no morphological differences for snakes of different isolated regions. For this reason, we study the individual characteristics of the venom from adult specimens of *Rhinocerophis alternatus* from two isolated regions, considering the amount of venom per animal and their toxicity.

Material and Methods: Specimens from Concordia, Entre Ríos (n= 17) and Olavarría, Buenos Aires (n= 17) were studied. We determined the corporal size, venom yield, lethal (CF-1 Mice) and haemorrhagic (Wistar rats) potencies, and electrophoretic pattern by SDS-PAGE. Immunochemical reactivity of regional pools was studied using experimental specific antivenoms.

Results: The population under study was composed by animals with a mean length of 94 cm (min. 76, max. 121), not showing differences between samples ($p< 0.05$). Venom extracted by animal (vacuum dry) was 152 ± 61 mg, did not show differences between samples ($p< 0.05$). Electrophoretic pattern showed two main groups of components between 15-25 y 30-50 kDa, and major and minor components. Lethal potency of Concordia snakes (Md 64 ug; min. 44 ug, max. 149 ug) was greater than Olavarría snakes (Md 110 ug; min. 67 ug, max. 138 ug) ($p < 0,05$), with a mean potency of 13.9 LD_{50}/mg y de 9.2 LD_{50}/mg of venom respectively. Haemorrhagic potency was also different in both samples. Concordia venom was generally more hemorrhagic (MHD= 108 ± 53 ug) than Olavarría venom (202 ± 94 ug) ($p < 0.002$). No strong relationship between the hemorrhagic and lethal potencies was observed. Immunological patterns were similar.

Discussion: Differences were observed between the activities considered in both samples although with an important dispersion of the values of toxic activities. Analysis of the individual venoms by SDS-PAGE under non-reducing conditions showed very similar patterns although some bands were absent or weaker. The overall disparities in toxicity and venom composition could be due, among other factors, to the variability in climate and food supply under which these populations have to live in two very different regions. Variability of toxicity in individual samples and their pools is discussed.

Keywords: *Rhinocerophis alternatus, Bothrops alternatus*, venom variability, toxicity, hemorrhage, lethality, electrophoresis
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194. Effect of Heparin and L-Arginine on Skin Tissue Regeneration after *Cerastes cerastes* Envenomation

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Background: Tissue necrosis is a relevant local effect in Viperidae envenomation, as it may lead to amputation. The purpose if this study is to investigate the effect of heparin and L-Arginine on tissue regeneration after envenomation.

Methods: Mice were intradermally injected in the dorsal skin with a sublethal dose of *Cerastes cerastes* venom (30 μg/ 20 g of mice body mass). After 72 h, the dermonecrotic activity of the venom was assayed by macroscopic and histopathological studies. For tissue regeneration assay, mice were humanely sacrificed at 1, 2, 3 and 4 weeks after i.d. injection of the venom. Heparin and L-Arginine were used as therapeutic drugs after envenomation. Mice treated with heparin, received an i.v. injection of heparin (10 μg/ g of mice body mass) at 15 min and 4 h after i.d. injection of the venom. Mice treated with L-Arginine, were daily supplemented with 3.75 mg/ ml of water, after i.d. injection of the venom. Treated mice (with heparin and L-Arginine) were sacrificed at 1 and 2 weeks after envenomation.

Results: Results indicated that *Cerastes cerastes* venom induces a strong dermonecrotic activity with a minimum necrotic dose of 19 μg/ 20 g of mice body mass. Histopathological study of skin sections, showed an alteration of the dermis, with hemorrhage, inflammatory infiltration and a destruction of hair follicles and glandular structures. An acanthosis was observed in a disintegrated epidermis and oedema was also observed in the epidermo-dermic junction. Macroscopic and histopathological study of skin regeneration indicated that skin tissue recovery occurred within 2 weeks after envenomation. Treatment of mice with heparin and L-Arginine, enhanced the capacity of skin tissue regeneration, which was markedly observed only 1 week post-envenomation.

Discussion: This study showed that *Cerastes cerastes* venom induces an important alteration of skin structure, inducing dermonecrosis. Skin regeneration was improved when animals were treated with heparin. Heparin is known to be able to sequestrate growth factors and cytokines that are implied in cell proliferation, fibroblast regeneration and angiogenesis. Skin tissue regeneration was also rescued by exogenous administration of L-Arginine, the precursor of endogenous synthesis of nitric oxide. NO is an activator of angiogenesis, that is involved in tissue wound healing.

Conclusion: These results suggest that heparin and pharmacological activators of the NO pathway may constitute, in association with immunotherapy, a rescuing treatment to avoid loss in tissue mass and function in human envenomation.

Keywords: Cerastes cerastes, venom, dermonecrosis, regeneration, heparin, L- Arginine
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195. A Novel Vascular Endothelial Growth Factor-Like Protein from *Gloydius tsushimaensis* Venom

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Background: Strong vascular permeability enhancing activity ascertained by the Miles assay was found in *Gloydius* tsushimaensis venom when examined together with the same amounts of the venoms of *G. blomhoffii* from several regions of Japan and *G.ussuriensis* in South Korea**.**

Methods: In order to characterize this protein specifically expressed only in the venom of *G. tsushimaensis* of *Gloydius* genus in Far Eastern Asia, this protein was purified using Superdex75, MonoQ columns with ÄKTA purifier chromatography systems. The protein purified migrated on SDS-PAGE as a single band with molecular weights of 27 kD and 13 kD under non-reducing and reducing conditions, respectively, suggesting that it exists as homodimer in native condition. The purified protein was digested with trypsin and the fragments were analyzed by MS on a ESI-Q-TOF**.**

Results: The sequences obtained from Mascot analysis of the MS data showed homology to other snake venom VEGF proteins. Since we found the conserved sequence in the 5' UTR for the snake venom VEGF cDNAs, we performed the 3' RACE using this conserved sequence as the 5' primer and the adapter sequence attached to the 3' oligo(dT) primer to acquire the full sequence of *G. tsushimaensis* VEGF cDNA. The cDNA was 487 bp long and had an ORF of 146 amino acids**.**

Discussion: The protein predicted showed about 80 % identity in sequence to previously published VEGF homologs from Viperidae snake venoms. Since the vascular permeability enhancement by the protein purified was inhibited by VEGF 2 receptor inhibitor but not by bradykinin B2 receptor antagonist or histamine antagonist in the Miles assay, it is certain that the vascular permeability enhancement due to *Gloydius tsushimaensis* venom is caused by its VEGF-like protein possibly through VEGF 2 receptor. It has been reported that the toxicity (LD_{50} value) and hemorrhagic activity of *G. tsushimaensis* venom are about one half and one hundredth those of *G. blomhoffi* venom, respectively**.**

Conclusions: The major activity of *G. tsushimaensis* venom could be ascribed to its VEGF-like protein. This is the first report of the VEGF-like protein from *Gloydius* genus.

Keywords: VEGF like protein, snake venom, regional evolution
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196. Synthetic Peptides from Viperid Phospholipase A2 Myotoxins: Small Structures with Diverse Biomimetic Actions

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Review: Phospholipases A_2 (PLA_2) present in the venoms of viperid snakes are classified within the group IIA of these small (~14 kDa) secreted enzymes. Although not all viperid venom PLA_2s display toxic activities, a large group of isoforms with basic pI 's exert myotoxicity in rodent assays or cell culture models, and have been shown to be largely responsible for the necrosis of skeletal muscle that frequently develops in human envenomings. Among these basic PLA_2s, three types can be recognized: **(a)** the catalytically-active (Asp49) monomeric or homodimeric PLA_2 myotoxins; **(b)** PLA_2s forming a heterodimeric complex (i.e., crotoxin) having both neurotoxic and myotoxic activities; and **(c)** catalytically-inactive PLA_2 homologues presenting the Lys49 substitution, most commonly homodimeric, which display myotoxic action. The highly conserved three-dimensional fold shared by both toxic and non-toxic group IIA PLA_2s represents a fascinating challenge for the study of structure-function relationships, dictated by subtle variations in surface-exposed amino acid residues that were selected under an accelerated evolution process. In the case of myotoxicity, at least two distinct mechanism of action evolved in these proteins, that converge upon sarcolemmal integrity impairment and subsequent cell death. Conventional Asp49 PLA_2s appear to rely on their phospholipolytic activity to induce muscle damage, via the production of fatty acids and lysophopholipids. On the other hand, Lys49 PLA_2 homologues, being catalytically inactive, have been shown to possess a specialized site that plays a key role in myotoxicity. This functional site has been mapped at their C-terminal region, and its exact identity is still being refined by a variety of approaches. Interestingly, short synthetic peptide sequences corresponding to such C-terminal site of the Lys49 myotoxins are capable of reproducing to some extent the diverse activities of their parent proteins, thus acting as small biomimetics. These bioactive peptides present functional properties that might find useful medical applications, or serve as structural leads to develop improvements in their pharmacological characteristics. In addition to their (originally unforeseen) potential applications, these peptides also represent valuable tools for understanding structure-function relationships in PLA_2 myotoxins.

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Keywords: phospholipase A2, myotoxin, biomimetics
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197. Lupane Triterpenoids from *Dipteryx alata* Vogel as Snake Venom Inhibitors

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Background: The antiophidian properties of triterpenoids have not been well-studied**.**

Methods: Triterpenoids from *Dipteryx alata* Vogel (lupeol), lupenone, 28-hydroxylup-20(29)-en-3-one, (28-OH-lupenone), and betulin, were pharmacologically assayed against *Bothrops jararacussu* (jararacuçu, Bjssu, 40 μg/mL, n=19) or *Caudisona durissa terrificus* (cascavel, CDT, 10 μg/mL, n=10) snake venoms using a myographic apparatus.

Results: These venoms showed an irreversible neuromuscular blockade in mice phrenic nerve-diaphragm preparations. However, the preincubated (30 min) mixture of each venom with each triterpenoid (1mg/5 mL) elicited different responses when added into the bath containing the preparation. None of the triterpenoids caused neuromuscular blockade at the chosen concentrations. All triterpenoids were efficacious ($p<0.05$) in counteracting (in %) the blockade of Bjssu venom [betulin (68 ± 7) ~ lupeol (70 ± 8) > lupenone (45 ± 9) ~ 28-OH-lupenone (54 ± 5), n=11, 5, 7 and 4, respectively]; whereas only betulin (39.5 ± 9, n=9) and lupenone (49.5 ± 8, n=4) protected significantly the blockade-induced by CDT venom.

Discussion: The different mechanisms of action of both venoms can explain the superiority of these triterpenoids against Bjssu venom, which is predominantly proteolytic and leads to a progressive myonecrotic process. These agents work less well against CDT venom, which is predominantly neurotoxic.

Conclusions: Betulin is the best phytochemical inhibitor against both snake venoms. The potential use of these inhibitors as therapeutic agents in the treatment of snake bite envenomations, either alone or in combination with other therapies, needs to be evaluated in future studies.

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Keywords: Bothrops jararacussu, Caudisona durissus terrificus, Dipteryx alata
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198. Isolation, Enzymatic Characterization and Action as Spreading Factor of a Hyaluronidase from *Crotalus durissus terrificus* Snake Venom

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Background: Snakebites related to the genus *Crotalus* are responsible for the highest levels of mortality described for ophidic accidents in Brazil. Hyaluronidase (Hyal) from *Crotalus durissus terrificus* snake venom (CdtV) has shown to be a very active enzyme. However, studies on this enzyme, which is known as "spreading factor", are still limited. The aim of this study was the isolation, enzymatic characterization, N-terminal sequencing and evaluation of Hyal from CdtV as a spreading factor of crotoxin and phospholipase A_2 (PLA_2).

Methods: The enzyme was purified through three chromatographic steps (CM-cellulose, Sephacryl S-100 and Heparin-Sepharose). Additionally, Hyal was submitted to a reverse-phase C18 (0.46 cm x 25 cm) high performance liquid chromatography (RP-HPLC) and its initial amino acid sequencing was performed by Edman degradation in a Shimadzu PPSQ-33A Protein Sequencer. The enzymatic activity of fractions eluted from each chromatographic step and the kinetic studies of Hyal were performed by turbidimetric assay. Sodium acetate buffers containing different salts (NaCl, KCl, $CaCl_2$ and $MgCl_2$) were employed to determine ion effects on the enzyme activity. SDS-PAGE containing or not hyaluronan was run and periodic acid-Schiff stain was used to detect glycoprotein. Paw edema was induced in male Swiss mice (28-32 g) by subplantar injection of buffer containing crotoxin or PLA_2 with or without Hyal, in the right hind paw. The volume of the paw was measured with a paquimeter (Mitutoyo, Japan) before and after toxin injections at different intervals of time. Data were analyzed by ANOVA and Student t-test.

Results: Hyal is a monomeric glycoprotein of 66.1 kDa and its first 25 N-terminal amino acids were sequenced. It showed maximum activity at 40 °C, pH 5.5 and in the presence of 0.2 M NaCl. The soluble venom specific activity was 145.1 turbidity reducing units (TRU)/mg, against 5,065.9 TRU/mg for Hyal. Its K_{cat} was 3,780.95 min^{-1} for hyaluronan and about 400 min^{-1} for chondroitin sulphate A, B or C. The pure enzyme decreased the edema caused by subplantar injections of buffer, crotoxin or PLA_2.

Discussion: Hyal exhibited higher specificity to hyaluronan than to chondroitin sulphate A, B or C. Divalent cations and 1M NaCl significantly reduced the enzyme activity. Hyal increased the diffusion of crotoxin and PLA_2 through mice tissues. Hyal potentiated crotoxin action, which was evidenced by mice death.

Conclusions: The reported purification procedure of the Hyal from CdtV (0.02% protein recovery) was able to provide a highly active antiedematogenic enzyme.

Financial support: CNPq, FAPESP

Keywords: *Crotalus durissus terrificus*, hyaluronidase, kinetic studies, isolation, antiedematogenic activity
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199. BaPLA2-IV, an Acidic Phospholipase A2, Isolated From *Bothrops atrox* Snake Venom: Biochemical and Functional Characterization

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Background: Snake venoms are rich sources of compounds displaying a large range of biological and pharmacological activities. As a rule, acidic *Bothrops* PLA_2s do not show experimental toxicity, but induce important pharmacological effects, thus deserving further studies as an attempt to understand the correlation between structure and catalytic/toxic functions.

Methods: Isolation was carried out by three chromatographic steps (molecular exclusion, ion exchange

and reversed-phase chromatography). The myotoxic activity was analyzed using creatine kinase (CK) determination and edema inducing assays in the subplantar region of mice. BaPLA$_2$-IV cytotoxic activity was evaluated by MTT, using HL-60, PC12, HEPG2 and B16F10 tumor lineages.

Results and Discussion: BaPLA$_2$-IV represents about 0.2% of *Bothrops atrox* whole venom. Its N-terminal sequencing showed similarity with other PLA$_2$-like enzymes (myotoxins) and PLA$_2$s from *Bothrops and Crotalus* genera. BaPLA$_2$-IV showed high phospholipase activity even at low doses, with 2 μg as a minimal dose, at different pHs and temperatures, and anticoagulant activity at a dose of 1.5 μg, keeping plasma incoagulable for more than 60 min. It showed a low myotoxic activity when compared with BthTX-I, a basic Lys49 PLA$_2$-like enzyme from *B. jararacussu* snake venom. For the edematogenic activity determination, the enzymes BaPLA$_2$-IV, an acidic PLA$_2$ from *B. jararacussu* and BthTX-I, as well as *B. atrox* venom were used. When the edematogenic activity of BaPLA$_2$-IV was compared with that of *B. atrox* venom (positive control – 100%), it corresponded to 80% (1h after injection) of the whole venom activity. BaPLA$_2$-IV showed a low cytotoxicity in all treatments.

Conclusion: The acidic phospholipase BaPLA$_2$-IV can be isolated by three chromatographic steps from *B. atrox* snake venom. Comparison of its N-terminal sequence revealed similarity with other PLA$_2$-like myotoxins (90%) and PLA$_2$s from *Bothrops* (99%) and *Crotalus* (80%) genera, being an acidic Asp49 PLA$_2$. It showed a high anticoagulant activity even at low doses, low myotoxic and cytotoxic activities on tumor cells, but a high edematogenic activity when compared with BthTX-I.

Financial support: FAPESP, CNPq.

Keywords: acidic phospholipase A2, cytotoxicity, anticoagulant
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200. Antinociceptive Potential of Raw Venom of Egyptian Cobra and Black Tiger Snake: A Functional Magnetic Resonance Imaging Study

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Background: Pharmacological activities of animal venom have been a focal point of interest in toxinology for many years. This study was performed to figure out if raw venom of the Egyptian cobra *Naja haje* and of the Black tiger snake *Notechis ater niger* produces antinociceptive effects in living animals.

Methods: First, we investigated the effects of *Naja haje* and *Notechis ater niger* venom in rats using Hargreaves and tail flick tests for analgesic effects, rotarod test for motoric integrity and open field test for locomotion. Our second and main objective was to determine whether these antinociceptive effects can be measured by functional Magnetic Resonance Imaging (fMRI) using painful topical heat stimuli. In addition, we induced local hyperalgesia by injecting Zymosan A into the left hind paw before the fMRI session. Consequently we could differentiate analgesic effects (right paw) and antihyperalgesic effects (left paw). fMRI measurements were performed on a 4.7 T Bruker BioSpec scanner using a gradient echo EPI (Echo Planar Imaging) technique with 400 x 400 x 1000 μm spatial resolution throughout the whole brain. Raw venom (*Naja haje*: 90 μg/kg; Notechis *ater niger*: 200 μg/kg) or NaCl (control) was injected intraperitoneally inside the scanner. Next a 100 min fMRI experiment was performed. Blood oxygen level dependent (BOLD) signals in the structures of the brain pain matrix were analyzed. BOLD amplitude and activated volume per brain structure were calculated as fractional change with respect to baseline.

Results: A clear analgesic effect was found for *Naja haje* and *Notechis ater niger* venom in behavioral tests: The paw and tail withdrawal latencies were significantly increased. There were no significant changes in rotarod and open field test, indicating that there was no motor impairment due to the neurotoxic activities of the venom. fMRI results showed that *Naja haje* and *Notechis ater niger* venom significantly decreased BOLD amplitude and activated volume in thalamus, cortical structures, limbic system and basal ganglia during nociceptive stimulation, showing differential analgesic and antihyperalgesic effects.

Discussion: Results of behavioral tests and decreases in neuronal activation through *Naja haje* and *Notechis ater niger* venom during nociceptive stimulation argue for analgesic and antihyperalgesic activity of these substances.

Conclusion: Using BOLD fMRI in anesthetized rats is a new valuable method to study antinociceptive effects of venoms without harmful sensations for the experimental animal. Raw Venom of Egyptian Cobra and Black Tiger Snake showed significant antinociceptive potential in this study.

Keywords: antinociception, BOLD-Imaging, fMRI rat brain, snake venom
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201. Molecular Evolution of Snake Venom Phospholipase A2 (PLA2) Genes -Analysis of Group I PLA2 cDNAs from Venomus and Nonvenomous Snakes, and Lizards

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Introduction: Vertebrates universally have the group IB secreted phospholipases A$_2$ (sPLA$_2$s) as digestive enzymes. The venomous snakes in Elapidae family have group IB sPLA$_2$s and group IA sPLA$_2$ as snake venom protein. According to the sequence similarity of groups IA and IB sPLA$_2$s genes, these genes were thought to be evolved from the same ancestral gene. In the erabu sea kraites, *Laticauda semifasciata* (Elapidae), two different types of mRNAs were transcribed from two different promoters on the same PLA$_2$ genes. One was transcribed using a conventional promoter P1 on the 5' upstream region of the genes (4 exons and 3 introns) and the other was transcribed using the novel promoter P2 on intron I (3 exons and 2 introns). The amount of the mRNAs encoding the group IA sPLA$_2$s transcribed from the P1 promoter (IA P1

mRNA) was extremely higher than that from the P2 promoter (IA P2 mRNA) in the venom glands of erabu sea karate. We have compared P1 and P2 IB mRNAs, and P1 and P2 IA mRNAs from the venomous habu snake (*Trimeresurus flavoviridis*), the nonvenomous Japanese rat snake (*Elaphe climacophora*), and the Japanese grass lizard (*Takydromus tachydromoides*) of Reptilia Squamata, and analyzed how group IA PLA_2s acquired the toxic function.

Result and Discussion: The deduced amino acid sequences of P2 IB cDNAs, the Japanese rat snakes, habu snakes and Japanese grass lizard had no signal peptides. In addition, the group IA P2 mRNAs from erabu sea krates and habe snakes had the signal peptide encoding region. Except Japanese grass lizard, the nucleotide sequences of 5' UTR of the P2 IB cDNAs from nonvenomous snakes, were very similar to the signal peptide encoding region of cDNAs from erabu sea krait. Thus the IB P2 cDNAs without encoding signal peptides originally had signal peptides encoding sequences. In the case of the Japanese grass lizard, the length of 5'UTR of the group IB PLA_2 gene was extremely shorter than that from the snakes examined. As a consequence of mutations in a promoter region of the group IB P2 mRNA in the Japanese grass lizard, the transcription initiation site moved to downstream and 5' UTR of P2 mRNA became very short. Thus the group IA PLA_2 gene might possibly be found in the genome of erabu sea kraits and habu snakes before the divergence of these snakes.

Keywords: evolution, snake, phospholipase A2
10.1016/j.toxicon.2012.04.202

202. Brazilian Coral Snake Phospholipases A2 Induce Neuronal Death in Primary Cultures of Hippocampal Neurons

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Background: The venoms from *Micrurus* snakes (Elapidae) are endowed with pre- and postsynaptic neurotoxins. Previously, we showed that the intrahippocampal administration of the PLA2s neurotoxins, MlTx-8 and MlTx-9, isolated from *Micrurus lemniscatus* venom induce seizures and neuronal lesion. In the present work, we study the effects of these neurotoxins on the cell death process in cultured hippocampal neurons (CHpN).

Methods: Rat CHpN were dissociated from hippocampi of E18-E19 Wistar rat fetuses. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2. On the seventh day, cells were incubated with MlTx-08 or MlTx-09 in different concentrations (0.74, 7.4 or 74nM) for 3, 6, 12 or 24 hours, depending on the experiment.

Results: The PLA2-neurotoxins had a potent time and concentration-dependent neurotoxic effects on CHpN. The cell viability determined through MTT assay indicated that CHpN exposed for 12 hr to 74nM MlTx-8 or MlTx-9 reduced the neuronal viability to 70,87±8,56% and 43,87±2,75%, respectively. This assay showed that these PLA2s-neurotoxins reduce the mitochondria dehydrogenase activity. To investigate whether mitochondria lay on the main pathway underlying the neurotoxicity, the mitochondria membrane potential ($\Delta\Psi m$) of CHpNs was evaluated by the fluorescent probe Rodamine 123. After 3 hr of the CHpN stimulation with 74nM MlTx-8 or MlTx-9, we observed a reduction of the $\Delta\Psi m$ in a magnitude of 26 and 39%, respectively. Inspection using fluorescent images of staining with ethidium bromide and ultra structural analysis by scanning and transmission electromicroscopy showed that multiphase injury are characterized by overlapping cell death phenotypes. There were both neurons with apoptosis and necrosis features observed: shrinkage, membrane blebbing, chromatin condensation, nucleosomal DNA fragmentation and the formation of apoptotic bodies. The most striking alteration observed in the electron microscopy was the fragmentation and rarefaction of the neuron processes network. Swollen terminal synapses, cell debris and apoptotic bodies are observed among the fragmented fibers. In the cytoplasm, it was noted numerous large vacuoles, swollen mitochondria and dilated Golgi.

Discussion: This is the first report showing that PLA2s isolated from the *M. lemniscatus* venom induce CHpN death. Our morphological data showed both neurons with apoptosis and necrosis features. We suggest that these neurotoxins could act on mitochondria membrane permeability.

Conclusions: These neurotoxins will be a useful tool for understanding the death pathways in neurotoxic processes and contribute to elucidate the mechanism of action of neurotoxic PLA2s.

Keywords: *Micrurus lemniscatus* , neuronal death, PLA2s-neurotoxins
10.1016/j.toxicon.2012.04.203

203. Cellular and Humoral Immune Responses in Horses Immunized with Crotalus Venom

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Background: The Brazilian Health Ministry reported that 7.7% of 133,391 cases of snakebites between 2007 and 2011 were caused by *Crotalus* rattlesnakes. The *Crotalus durissus* venom is neurotoxic and myotoxic and can cause systemic coagulation disorders. The specific antivenom antibody is the

only effective treatment in cases of accidents. Currently, Butantan Institute is responsible for 60% of the anti-crotalic horse serum produced in Brazil. Horses have been used for anti-venom production by their large size, resistance to toxins and to the production of large amounts of plasma containing effective IgG(T) antibody titles. Despite the well-established efficacy of the rattlesnake antivenom horse serum, there are no data in the literature about the specific cellular immune responses to the venom. The aim of this study was to compare the specific cellular and humoral immune responses to *C. durissus* venom in the peripheral blood of venom immunized horses.

Methods: Three groups of male horses (n=15) were immunized according to a standard protocol for anti-crotalic serum production consisting of different cycles of immunization with whole venom. Heparinized blood and serum samples were collected before and at different periods after immunization. Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll-hypaque gradient separation and stimulated *in vitro* with *C. durissus* venom. Proliferation was measured by thymidine [^{3}H] incorporation, after 5 days culture and considered positive when proliferation index (IP) was $>$ 2.0. Venom specific antibodies titers in sera were determined by ELISA.

Results: There was a significant cell proliferation in the different PBMC samples, which increased after each immunization cycle. In addition, there was a variation in the proliferation index among animals of the same group. All animals presented high titers of antivenom IgG ($>$ 1: 2 x 10^5) with a fluctuation between immunization cycles, and there was also a variability of the response among animals of the same group.

Discussion: Although, there was no individual correlation between the proliferative response and venom specific IgG(T) antibody titers for samples of the same period, there was a correlation between the proliferative response and the IgG titers considering the whole group of animals ($p < 0.05$).

Conclusions: The evaluation of different aspects of cellular immune response like specific proliferative response as well as cytokine production by immunized horses may contribute to improve the protocol for immunization of horses for the production of sera with higher antibody titers.

Financial support: Fundação Butantan.

Keywords: Crotalus venon, horses, cellular immune response, antibodies
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204. *Vipera Lebetina* Venom Nucleases and Nucleotidases

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Background: Snake venoms contain hundreds of biologically active components comprising enzymes (mainly hydrolases), bioactive proteins and peptides. Among hydrolases, nucleases and nucleotidases are ubiquitously present in all snake venoms but there is almost no information about the amino acid and cDNA sequences of these enzymes. Probably due to low abundance these enzymes have not been found during proteomic studies of the venoms. Snake venom nucleases are classified as endo- (DNases and RNases) and exonucleases (phosphodiesterases - PDE). In addition, venoms contain 5`-AMP-hydrolyzing 5`-nucleotidases (5`-NDases). The aim of this study is the isolation of PDE, 5`-NDase and RNase from *V. lebetina* venom and also amplification of these enzymes coding cDNAs from *V. lebetina* venom gland cDNA library.

Methods: The enzymes are isolated using gel filtration on Sephadex G-100 sf, ion-exchange chromatography on CM-cellulose (PDE, 5'-NDase) and affinity chromatography on DNA-agarose (RNase). Enzymes coding cDNAs are separated, cloned and sequenced using standard methods.

Results: *V. lebetina* PDE is a nonspecific exonuclease with molecular mass of 120 kDa and pI in neutral region (6.5-7.5). The pI heterogeneity is probably caused by different glycosylation of the enzyme (vast majority of snake venom enzymes are glycosylated). 5`-NDase has a molecular mass about 80 kDa and the pI in basic region ($\sim$9). Ribonuclease is localized in the fourth gel filtration fraction with nerve growth factor and alkaline protease. Its molecular mass is $\sim$33 kDa and pI $\sim$10. PCR screening of the *V. lebetina* venom gland cDNA library resulted in isolation of 5`-truncated cDNAs encoding PDE and 5`-NDase. The PDE-encoding cDNA comprises 1704 bp including 1644 bp ORF corresponding to 547-amino acid protein sequence. Comparison with the only snake venom PDE represented in the BLAST database, *Crotalus adamanteus* PDE, gives 95% identity in the cDNA and 91% identity in the protein sequence. The 5`-NDase encoding cDNA comprises 1608 bp including 1215 bp ORF corresponding to 404 amino acids. The cDNA is 93% identical to ecto-5`-NDase of *Gloydius blomhoffi brevicaudus*, the protein sequence identity is 92%.

Conclusion: These results point to strong conservation of PDE and 5'-NDase in snake venoms.

Acknowledgement

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Keywords: snake venom, *Vipera lebetina*, phosphodiesterase, 5`-nucleotidase
10.1016/j.toxicon.2012.04.205

205. A Novel Fluorometric Assay for the Evaluation of Substrates for Phospholipase A2 from the Colombian Snakes *Bothrops asper* and *Crotalus durissus cumanensis*

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Background: Phospholipase A_2 (PLA_2) is a versatile enzyme present in all organisms. PLA_2s in snake venom are responsible for multiple systemic effects such as neurotoxicity, myotoxicity, and hemolysis. They hydrolyze the sn-2 ester bond of phospholipids resulting in the formation

of lysophospholipids and free fatty acids. In this study we investigate the specificities of PLA_2s in whole venom samples of the Colombian snakes *Bothrops asper* and *Crotalus durissus cumanensis*.

Methods: Crude venom samples were taken from *B. asper* and *C. durissus cumanensis* in the Guajira region of Colombia. The venom was lyophilized until use. Vesicles composed of 1,2-di-O-octadecyl-sn-glycero-3-phosphocholine (DEPC) carrying the water soluble fluorescent marker calcein at a self quenching concentration (50mM) were used as reporter vesicles, and target vesicles (substrate) made from 1,2-ditetradecanoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DMPG), 1,2-ditetradecanoyl-sn-glycero-3-phosphocholine (DMPC), 1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine (POPE) or sphingomyelin (SM) were used for measuring PLA_2 activity through an indirect calcein release method. We measured the release of calcein encapsulated in DEPC vesicles as triggered by lysophospholipids and free fatty acids produced by the action of PLA_2s on target vesicles. Reporter vesicles were not hydrolyzed by the venom. All measurements were performed in a pc1 Fluorometer (ISS, Urbana, IL) at 37°C with and without calcium (30 μM). Additionally, we evaluated vesicles simulating the outer membrane layer of platelets and red blood cells.

Results: Final concentrations of 0.5 mg/mL whole venom were used in experiments with calcium and 10 mg/mL in absence of calcium. At 37°C PLA_2 activity was only observed with DMPG vesicles, with *C. durissus* venom showing the highest activity and lag time with calcium (%Release 47, lag time 137 s). Experiments without calcium showed that *C. durissus* venom had again the highest activity (%Release 94) but *B. asper* venom showed the highest lag time (71 s). DMPC vesicles showed activity near transition state temperature (24°C) with both venoms showing similar activity.

Discussion: Activity assays showed that PLA_2 present in whole venom has affinity for negatively charged membranes. No activity was observed with any of the other substrates including the vesicles that simulated platelets and red blood cells, thus there must be other elements in the membrane enhancing the affinity or the catalytic activity of PLA_2 in human cells

Conclusions: In this work we present a novel approach to study PLA_2 activity in whole venom which will increase understanding of its effect on the cell and may lead to the development of better treatments against snakebite.

Keywords: PLA2, liposome, fluorometric assay, enzyme activity
10.1016/j.toxicon.2012.04.206

206. Isolation and Characterization of a D49 Phospholipase A2 from *Rhinocerophis (Bothrops) Ammodytoides* venom

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Background: The snake *Bothrops* (*Rhinocerophis*) *ammodytoides* is the southernmost viper in the world. It is generally a small snake which venom resembles generally the characteristics of the venom from vipers of *Bothrops* genus. Although some studies were done on the crude venom and its chromatographic fractions, there are not studies on the components of their venom. In this work we describe the isolation and characterization of a phospholipase A2 (PLA2) from this venom.

Methods: The enzyme was purified from the crude venom by several chromatographic steps: Sephadex G-50, Mono-S in a FPLC system and C18 in a HPLC system and the purity of this protein was verified by mass spectrometry. The toxic characteristics of the enzyme were studied by biological assays.

Results: The enzyme represents the 3% of the total mass of the venom. Close analysis of the sequence shows that belongs to the D49 group of Type II PLA_2. The enzyme has 122 amino acids residues and the full sequence was determined showing a very close similitude with a *Botrhops erythromelas* PLA_2. The theoretical molecular mass was 13,867.65 Da, and the experimentally determined molecular weight was 13,853.65. The difference of 14 Da between these two values corresponds to the fact that the native enzyme is closely packed by 7 disulfide bridges, accounting for this difference. The isoelectric point is 6.13. The enzyme has low toxicity in mice. The LD_{50} was around 117 ug and the MMD was 200 ug. Killed mice showed pulmonary congestion and some hemorrhagic spots in lungs and, hemorrhages in abdominal cavity represented by intraperitoneal bleeding. Mice injected with 300 ug showed congestion and acute edema of lungs with emphysema, atelectasis and thrombosis. The enzyme did not affect *in vitro* or *in vivo* the coagulation although it was observed an inhibition of the clot retraction. A slight dose dependent inflammatory-edematogenic activity from doses of 2 ug was observed. Although CK levels increased after intramuscular injection, values were very close with the controls, despite its statistical significance ($p < 0.05$). Muscles showed lesions of different intensity depending on the dose of enzyme injected, using 10 ug of enzyme after 24 h muscles showed intense inflammation of mononuclear type affecting vessels, inflammatory interfibrillar compromise and focal necrosis of muscular fibers.

Conclusions: The enzyme shows low toxicity although further studies are necessary to elucidate its relation with the systemic bleeding observed at highest doses of the enzyme.

Keywords: *Rhinocerophis ammodytoides*, *Bothrops ammodytoides*, phospholipase, structure, toxicity
10.1016/j.toxicon.2012.04.207

207. Developing of an Antielapidic Sera by Genetic immunization

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Background: *Micrurus corallinus* (coral snake) is a tropical forest snake belonging to the family Elapidae. Its venom shows a high neurotoxicity associated with pre- and post-synaptic toxins, causing diaphragm paralysis, which may result in death. In spite of a relatively small incidence of accidents, serum therapy is crucial for those bitten. However, the adequate production of antiserum is hampered by the difficulty in obtaining sufficient amounts of venom from a small snake that is also difficult to keep in captivity**.**

Methods: Having recently finished the transcriptomic analysis of *M. corallinus*, we have chosen five of the major components of the venom gland and synthesized overlapping pentadecapeptides frameshifted by 3 residues, spanning the entire sequence of the five proteins by the SPOT method on cellulose membranes. The membranes were then screened using an anti *M. corallinus* serum and positive peptides were then used to design two multi-epitope genes that are being used for genetic and recombinant immunization of female BALB/c mice. Five doses of 1 μg DNA will be administered to animals by biolistic gene delivery. At the end of the immunization period, animals will be bled and sera will be subjected to further analysis concerning its neutralizations capabilities**.**

Results: By the time of this abstract submission, the two multiepitope genes were correctly synthesized and are being administered to animals. Further analysis concerning the ability of these sera to neutralize the toxic effects of *M. corallinus* venom will be performed on the next days.

Financial Support: FAPESP, CNPq and Fundação Butantan

Keywords: antielapidic serum, *Micrurus corallinus*, genetic immunization.
10.1016/j.toxicon.2012.04.208

208. Fucose Moieties Are Essential for the Ability of Fucosylated Chondroitin Sulfate to Inhibit Muscle Damage Induced by *Bothrops jararacussu* Venom

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Background: Snakebites by *Bothrops jararacussu* snake induces intense local tissue damage. Phospholipases A_2 are enzymes present in the venom which are responsible for a wide range of activities (Toxicon 45, p.1147, 2005). Some polyanions have been shown to present antivenom properties against this venom (Toxicon 31, p.285, 1993). A new natural polyanion polysaccharide, named Fucosylated Chondroitin Sulfate (fucCS), is involved in many biological activities (JBC 282 (20), p.14984, 2007). We assessed the ability of fucCS and its carboxi-reduced and defucosylated analogues to antagonize the muscle damage induced by *B. jararacussu* crude venom**.**

Methods: *In vitro* CK assays were performed with isolated mouse *extensor digitorium longus* (EDL) muscle bathed with venom alone (25 μg/mL) or incubated with fucCS or analogues (10-50 μg/mL). *In vivo* experiments were performed by i.m. venom injection alone or pre-incubated with fucCS or defucCS (1-10 mg/kg) and the plasma CK activity was evaluated before and 2 hours after injection (1 mg/kg). The phospholipase and hyaluronidase activities were measured using turbidimetric methods. The CK content was evaluated in EDL muscle after a peri-muscular injection of venom (1 mg/kg). Histological sections were performed in EDL muscle after crude venom perimuscular injection. All experiments were approved by the Committee of Animal Use of the Rio de Janeiro Federal University (DFBCICB 026)**.**

Results: It was observed that fucCS inhibits 75% of phospholipase venom activity with $IC_{50} = 10$ μg/mL (n=10) and 100% of hyaluronidase activity with $IC_{50} = 7$ μg/mL (n=5), in concentration-dependent manner. Incubation of fucCS with the venom eliminates the increase of plasma CK, *in vivo* (n=4). The EDL muscle was preserved when exposed to venom with fucCS *in vitro* (30 and 50 μg/mL) (n=4). The reduction of the CK content was prevented by fucCS (1-10 mg/kg) (n=4). DeFucCS was unable to protect the phospholipase activity and miotoxicity *in vivo* and *in vitro*. Light microscopy shows that fucCS can inhibit the muscle damage induced by the venom (n=4)**.**

Conclusions: FucCS was capable to inhibit venom activities related to tissue damage, although defucCS does not have this ability. These results indicate that fucCS presents activity against *Bothrops jararacussu* venom and we believe that this antivenom activity may be due to the interaction of negative charges of fucose moieties of fucCS with positively charges toxins present in this snake venom, like others polyanions.

Financial Support: CNPq, CAPES, FAPERJ and PRONEX.

Keywords: *Bothrops jararacussu*, fucosylated chondroitin sulfate, myotoxicity
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209. Venom-Antivenin Binding and Neutralization of Venom Toxicity: Application of Size-Exclusion Chromatography to Assess Venom-Antivenin Binding

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Background: The World Health Organization is encouraging the development of alternative methods to

animal testing for evaluating venom toxicity and antivenin protection against lethality. Size-exclusion chromatography (SEC) can provide an estimate of venom-antivenin binding based on the formation of venom-antivenin immune complexes, and could provide *a priori* information useful in antivenin protection studies. SEC analysis is relevant to antivenin protection if one assumes that venom-antivenin binding is required for protection. Venom-antivenin binding, however, does not guarantee protection; therefore, both immune complex formation and neutralization of venom toxicity (activity) need to be evaluated with comparable reactants.

Purpose: The purpose of this presentation is to further introduce the concept of SEC analysis as an additional tool in assessing the relationships of venom-antivenin complex formation and neutralization of venom toxicity or lethality. Two articles that address these concepts have been published in *Toxicon* and are the primary sources of data within this presentation[1, 2].

Method: 1) Venom, antivenin, and venom-antivenin mixtures are prepared. 2) SEC elution profiles are obtained for each sample. 3) Regions within the elution profiles are chosen for integration based on comparison of control and venom-antivenin mixture profiles. 4) Profile region areas are determined for each sample. 5) Region area associated with venom-antivenin binding is estimated from the difference between control and venom-antivenin mixture region area (i.e. D Area). 5) Concentration dependant changes in D Area are fit to dose-response functions from which D $Area_{max}$ (maximum D Area) and EC_{50} (effective concentration of reactants at one-half D $Area_{max}$) can be estimated.

Examples: *SEC analysis*. Interaction of *Crotalis atrox* (Western diamondback rattlesnake; *C. atrox*) venom and Crotaldae Polyvalent Immune Fab (ovine) antivenin (FabAV) illustrates the procedure for assessing venom-antivenin interaction. Concentration dependent changes in D Area for FabAV and *C. atrox, C. varidis varidis* (prairie rattlesnake), *Agkistrodon contortrix contortrix* (southern copperhead), and *A. piscivorus leukostoma* (western cottonmouth) venom interactions are compared. *Neutralization PLA_2 Activity*. Concentration dependent changes in D Area and venom phospholipase A_2 activity illustrate concurrent assessment of venom-antivenin binding and neutralization of venom toxicity (activity).

Application: SEC analysis can be used to estimate and compare EC_{50} (which inversely reflects venom–antivenin binding affinity) and maximum D Area (which reflects maximum reactivity) of various venoms and antivenins (or other anti-venoms). SEC analysis parallel to venom activity assays could estimate the concentration of antivenins required for neutralization of venom toxicity (activity).

References

Price, J.A. and Sanny, C.G. *Toxicon* 49:848-854, 2007; 2. Sanny, C.G. *Toxicon* 57:871-881, 2011.

Keywords: venom, antivenin, SE-chromatography
10.1016/j.toxicon.2012.04.210

210. Understanding the Preclinical Efficacy Profile of Antivenoms: From the Lethality Potency Assay to Antivenomics

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Review: The great variability in the biochemical and toxicological profiles of snake venoms poses a great challenge to the selection of venom mixtures for immunization of animals for the production of antivenoms, as well as to the preclinical testing of the neutralizing efficacy of antivenoms. Traditionally, the evaluation of antivenom potency has been based on the neutralization of lethal activity of venoms in mice. Although this test continues to be the gold standard for antivenom potency assessment, advances made in the characterization of snake venoms have paved the way for a more in depth analysis of antivenom preclinical efficacy. Since the venoms of many snake species, particularly of the family *Viperidae*, induce a spectrum of toxic effects, evaluation of antivenoms should include the neutralization not only of lethality, but also of additional toxic activities, such as hemorrhagic, edematigenous, myotoxic, coagulant, and defibrinogenating effects. Moreover, analysis of the neutralization of some enzymatic activities, such as proteinase and phospholipase A_2, has been also implemented. More recently, the proteomic characterization of snake venoms, i.e. 'venomics', has provided a highly detailed view of the complexity and variability of venom composition. An adaptation of the proteomic platform to the analysis of immunoreactivity of antivenoms, a field known as 'antivenomics', now brings the possibility of identifying venom components for which antivenoms contain antibodies. The combination of *in vivo* and *in vitro* neutralization tests, together with antivenomics, allows for a detailed characterization of the reactivity of antivenoms against homologous and heterologous snake venoms. These principles will be illustrated for the case of the polyvalent antivenom manufactured in Costa Rica and used in Central America in the treatment of envenomings by viperid snakes. This type of integrated analysis can be of benefit to manufacturers and regulatory agencies in various parts of the world.

Keywords: antivenoms, neutralization, potency test, antivenomics
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211. Effect of Low Level Laser Therapy (LLLT) on *Bothrops jararacussu* Venom-Induced Myotoxicity in Muscle Cells

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Background: Local myonecrosis is a common consequence in envenoming caused by snakes of the genus Bothrops which occurs through the action of myotoxins that acts directly in the muscle cell membrane. Antivenom therapy and other first-aid treatments do not reverse the local myonecrose. Thus, there is an urgent need to find therapies that can complement antivenoms in the neutralization of local tissue damage. The low level laser therapy (LLLT) is being considered as an alternative treatment for muscle injury situations because its bioestimulation effect.

Objective: The present work was designed to investigate the effect of LLLT in muscle cells submitted to injury by *Bothrops jararacussu* venom (BjssuV).

Methods: C2C12 muscle cell line was used. The cells were grown in culture medium DMEM supplemented with 10% fetal bovine serum, incubated at 37°C with 5% CO2 for 24 hours for cell attachment, after that, the cells received BJssuV venom in the respective concentrations 1, 6, 12.5, 25 and 50 μg/mL and incubated for 15, 30 and 60 min. The cell viability and detachment were analyzed by MTT and crystal violet assay, respectively. Cells were irradiated for 13 s immediately after the venom administration with a semiconductor laser at 635 and 830 nm, dose of 2.5 J/cm^2 and power of 100 mW. The cells that did not receive venom and irradiation served as control.

Results: Our results showed that BjssuV caused a decrease in cell viability and detachment of C2C12 cells that was dose and time dependent. The venom caused a significant decrease in cell viability at doses of 12.5, 25 and 50 mg/mL. Also, results showed an increase in the percentage of cell detachment, this effect was more pronounced at doses 25 and 50 mg/mL promoting 100% of detachment cells at 30 and 60 min. LLLT increased cell viability by 86% and 92% in C2C12 myocytes by 635 and 830 nm, respectively, 30 min after the venom administration. The laser had no effect on cell detachment caused by venom.

Discussion: BjssuV is toxic to muscle cells and LLLT was able to protect the muscle cells against injury caused by venom.

Conclusion: The use of LLLT should be considered as a potentially useful therapeutical approach for treatment of the local effects of *Bothrops* snakebites.

Keywords: muscle cells, myonecrosis, laser therapy, viability, cell detachment
10.1016/j.toxicon.2012.04.212

212. Effect of Light Emitting Diode (LED) in the Inflammatory Response and Myonecrotic Effect Induced by *Bothrops asper* Snake Venom

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Background: In Central America, *Bothrops asper* venom (BaV) is responsible for almost all snakebites. In untreated cases, local necrosis frequently occurs and may require amputation. Additionally, many victims of *Bothrops asper* snakebite suffer risk of death due to sequels and damage to tissues. Currently, the treatment used for snake bite accident is the antivenom therapy (AV). However, AV has been shown ineffective in the neutralization of local effect caused by bothropic venom.

Objective: In the present study, a comprehensive analysis of the effect of red and infrared LED treatment on the outcome of BaV-induced myonecrosis and inflammation was performed.

Methods: Male Swiss mice, was used. Edema was evaluated by pletismograph in different time point: 15, 30 min, 1, 3 and 6 h after the injection of 2.5 μg/paw of BaV or saline (control). Leukocyte influx in the peritoneal cavity was determined by total and differential cell counts at 6 h after the injection of 2.5 μg/cavity of *B. asper* venom. Myonecrosis was induced by injection of 50 μg of *B. asper* in the right gastrocnemius muscle and was evaluated 24 h after venom injection. The myotoxic activity was determined by creatine-quinase assay at 3 h in serum and 24 h in muscle. Mice had received irradiation of Infrared LED (120 mW, 945 nm, 38 s) and red LED (110 mW, 635 nm, 41 s) applied immediately, 1 and 2 h after venom injection.

Results: Our results showed that red LED and Infrared LED treatments had reduced the edema formation in plantar by 70% e 52% and in gastrocnemius muscles by 61% and 86%, respectively. Both LED used had significantly reduced neutrophils migration 6 h after the venom injection. Also, results showed that Infrared LED and red LED treatment significantly reduced serum CK levels by 84% and 70%, respectively. In addition, LED treatment caused a significant increment in muscle CK contents in both wavelengths used, after the venom injection. The histology showed an improvement, at least in part, of myonecrosis, corroborating the biochemistry finding.

Discussion: LED therapy significantly reduces the inflammation and myonecrosis caused by BAV in both 635 nm and 945 nm wavelengths.

Conclusion: Phototherapy should be considered as a potentially therapeutic approach for treatment of the local effects of *Bothrops* species.

Keywords: myonecrosis, inflammation, phototherapy, *Bothrops asper*
10.1016/j.toxicon.2012.04.213

213. Evaluation of Cabenegrin on the Enzymatic Activity and Structure of Basic sPLA2 of *Crotalus durissus terrificus* Venom

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Background: Pterocarpans are naturally occurring plant products carrying a cis-fused benzofuranyl-benzopyran ring system. Many of them are isoflavonoids possessing high antifungal and antibacterial activity and these natural products cabenegrin A-1 (CA-I) are active components of a Brazilian folk medicine used against snake venoms. These potent antidotes have been isolated by Nakanishi and coworkers from the aqueous alcoholic extract of the root of a South American plant called "Cabeca de Negra". On the other hand constitute a basic sPLA2 Fractions more improtante venom of Crotalus durissus ssp with no known effects of CA-I or CA-II on the structure and enzymatic activity of basic sPLA2.

Methods: Reverse HPLC analysis coupled to circular, UV-Vis and fluorescence detector in tanden, ELISA plate reader for enzymatic activity.

Results: In this article we investigated the effect of these compounds on the structures of native basic sPLA2 from the Bothrops jararacussu and examine the extension of the previous incubation of sPLA2 with CA-I and its isoform CA-II on the enzymatic activity of sPLA2, that were performed on the ELISA reader. Both CA-I and CA-II shift the retention time of the isolated sPLA2 in 1.03 and 1.1 minutes in comparison with native sPLA2. Both compound CA-1 and CA-2 decrease the Vmax of the native sPLA2 from Vo=A425nm (0.556 $\pm$ 0.03) for 0.34 $\pm$ 0.02 and 0.28 $\pm$ 0.03, respectively. The analysis performed in wavelength range 260-320 nm did not show a significant shifts of between native sPLA2 to sPLA2: CA I and native sPLA2 to sPLA2: CA II and the results from the analysis performed in the wavelength range 220–260 nm did not allowed to observe a significant increasing of CD profiles of sPLA2: CA I to native sPLA2 and sPLA2: CA II to sPLA2. By other side, the monitoring of CD spectra in the wavelength range from 220 to 260nm showed clear diminishing of the apha-helix and betha strand after incubation of sPLA2 with CA-I or CA-II. The global analysis of the results from the fluorescence scanning assay suggest that increasing of the fluorescence properties of CA I: sPLA2 and CA II: sPLA2 probably involve the summation of the fluorescence spectra of sPLA2 and CA I or CA II residues coupled to sPLA2 and this contributed for the CD spectroscopic analysis of sPLA2:CAI and sPLA2:CAII.

Discussion and Conclusion: Neither CA-I and CA-II did not induced drastic protein unfolding, but strongly decrease the enzymatic activity of native sPLA2.

Keywords: isoflavone, pterocarpans, basic sPLA2, circular dichroism, fluorescence spectroscopy
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214. Evaluation of the Antiophidic Activity of Lapachol and Synthetic Analogues

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Background: Serotherapy against snakebite was discovered more than one hundred years ago, but the antivenin are not available all over Brazil nor in some parts of the world. The use of plants in folk medicine is common mainly in the Brazilian Amazon area. We have investigated the antiophydic activity of Lapachol (Fig.1-1) isolated from *Tabebuia impetiginosa* and analogues (Fig.1) synthesized by using Suzuki-Miyaura coupling methodology, in different experimental protocols against *Bothrops atrox* and *Bothrops jararaca* venoms.

Methods: We investigated the effects of the natural product and its analogues on venom phospholipase, collagenase and proteolytic activity. The antiproteolytic activity was assessed by using azocasein as substrate (Arch of Biochem and Biophy, 315:188, 1978) and the phospholipase activity by a modified turbidimetric method (Biochm Biophys Acta, 554:98, 1965) using a suspension of chicken egg yolk as substrate. The collagenase activity was assessed by the modified method of Chavira et al. (1984). The hemorrhagic lesions were induced in mice by an intradermic injection of *B. jararaca* venom and quantified as previously described (Melo et al., 1994).

Results: The lapachol and synthetic analogues inhibited the proteolytic and collagenase activity of *Bothrops atrox* venom, *and* also showed anti-hemorrhagic activity in vivo against the venom of *Bothrops jararaca*.

Conclusions: Our studies indicate that Lapachol and synthetic analogues present relevant inhibition of collagenase and proteolytic activities of *Bothrops atrox* venom, but do not present Phospholipase activity (data not shown), and also inhibited hemorrhagic activity of *Bothrops jararaca* venom.

Financial Support: CAPES, CNPq, PRONEX and FAPERJ

Keywords: Lapachol, *Bothrops atrox* , *Bothrops jararaca.*
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215. Ability of Polyanions to Antagonize the Cardiotoxic Effect of the *Bothrops jararacussu* Venom

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Background: Previous studies have showed that *B. jararacussu* venom has myotoxic and cardiotoxic effects and the myotoxicity is antagonized by heparin. In this study we investigated the in vitro cardiotoxic activity of *B. jararacussu* crude venom and the antivenom effects of the heparin and dextran on isolated rat hearts.

Methods: We assessed the venom effects in Wistar rat isolated hearts perfused with an appropriated nutritional solution by using the modified Langendorff preparation. We analyzed the tension developed, the electrocardiographic records (EKG), the damaged area and the Creatine Kinase (CK) activity in the perfusate. The preparation was

perfused under control conditions and after 15 minutes of stabilization we added the crude *B. jararacussu* venom (2.5 -10 μg/mL) or the venom associated to the polyanions, heparin or dextran sulfate (10-300 μg/ml). At the end of the experiments, hearts were gently sliced and exposed to 1% triphenyl tetrazolium chloride (TTC) to assess damaged areas (Am Heart J, 593: 101, 1981). Electrical and contractile properties were analyzed by the WINDAQ program.

Results: The crude venom induced a progressive negative inotropic effect (10 μg/mL) decreasing to 0% the heart tension after 15 min, increasing perfusion press, PR interval, decreasing QRS amplitude, with changes on the EKG waves, as well increased the rate of CK release in the perfusate. The addition of heparin 30; 100; 300 μg/ml and dextran 10; 30; 100 decreased in concentration-dependent way the venom cardiotoxic effect in the heart tension reaching 100% of the inhibition with 300 μg/ml (heparin) and 100 μg/ml (dextran), perfusion press and EKG waves changes. The analysis with TTC showed the damaged area as well as the protective effect of the polyanions.

Discussion: The venom induced a significant depression of the cardiac tension, which was antagonized by heparin or dextran sulfate at different concentrations and the functional data were confirmed by the TTC stained tissue and the CK release.

Conclusions: This data confirm that polyanions protect cardiac muscle fibers from the cytotoxic effect of *B. Jararacussu crude venom*.

Financial Support by: CAPES, CNPq, PRONEX e FAPERJ

Keywords: snake venom, cardiotoxic effects, antagonism, polyanions
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216. Experimental, Immunochemical Reactivity and Neutralizing Capacity of *Rhinocerophis (Bothrops) alternatus* Antivenoms, from Distinct Geographical Sources

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Background: Biochemical and toxicological variation in snake venoms can be observed within the same species. We observed significant geographical variation in toxicity in the snake *Bothrops (Rhinocerophis) alternatus* from Argentina, the biggest viper in the South of South America. In addition, variation was also detected in the neutralizing capacity of therapeutic antivenoms for venom from these snakes from different regions, and even in snakes from the same region. In view of these results, we produced experimental antisera against pools of venoms of *Bothrops alternatus* from different regions of the country.

Materials and Methods: Venoms used were from Baradero, San Nicolás, Olavarría and Dock Sud (Province of Buenos Aires), Concordia and Gualeguay (Province of Entre Ríos), the provinces of Córdoba, Misiones and Corrientes. A Pool of venom was generated by mixing equal amounts of each of these regional venoms. Rabbits were immunized with the different pools of venom and the general pool and were bled after a fixed immune response was attained (dilution 1/10 by immunodiffusion). IgG was purified by caprylic acid fractionation, its purity tested by SDS-PAGE and the amount of protein adjusted. The immunochemical cross reactivity was evaluated by direct ELISA, competitive ELISA assays and, additionally, the avidity of antibodies was evaluated. Neutralizing capacity on hemorrhagic, coagulant and thrombin-like activities of the antivenoms were measured.

Results: An important cross-reactivity, without significant differences regarding the specificity of the immunogens, was observed in the majority of the assays. The competitive assays showed similar results and the avidity experiments in presence urea (2 M) did not reveal differences in the avidity of the homologous or heterologous venoms and antivenoms. Neutralization of hemorrhage was very variable, and neutralization of coagulant activities (on plasma and on fibrinogen) was evident in all cases. Some antivenoms neutralized toxic activities strongly present in heterologous venoms but absent or lower in the venom used as immunogen (i.e. thrombin like activity).

Discussion: Although some differences that could be attributed to geographic isolation of snake populations were observed, cross-neutralization of venoms of different regions was widespread. Despite the different toxic potencies of venoms from different regions, antivenoms from snakes of a given region exhibited high immunological cross reactivity and neutralization on the venoms from snakes of very distant and different regions. These findings could be used to improve the generation of pools for antivenom production.

Keywords: *Rhinocerophis alternatus*, *Bothrops alternatus*, venom variability, cross reactivity, immunology, antivenom
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217. IgY Antibodies Anti-*Crotalus durissus cumanensis* Venom: Purification and Neutralization Efficacy

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Review: The Venezuelan rattlesnake *Crotalus durissus cumanensis* (*Crotalus d.c*) is broadly extended across the country, causing more than 33% snakebite cases in Venezuela. Avian antibodies (IgY) isolated from chicken egg yolk represent a new alternative to be applied as antivenom theraphies. In this work we produce IgY antibodies against *Crotalus d.c* venom pooled from different Venezuelan regions and evaluate its neutralizing capacity both "in vitro" and "in vivo".

Methods: The anti-snake venom antibodies were purified by precipitation techniques with polyethylene glycol and evaluated by MABA, an indirect ELISA, and western blot assays. The neutralization of lethality was evaluated by preincubation of venom together with antivenom prior to testing**.**

Results: The specificity of IgY antibodies was demonstrated by a dose-dependent inhibition in western blot assay when antibodies pre-absorbed with the venom did not recognize the venom proteins from *Crotalus d.c.* The antivenom was effective in neutralizing 4LD50 doses of *Crotalus d.c.* venom in vivo (72 mg of IgY neutralized 1.17 mg of *Crotalus d.c.* venom)**.**

Conclusion: Our results support the future use of avian anti-snake *Crotalus d.c.* venom as an alternative to conventional equine antivenom in our country.

Keywords: anti-venom, IgY antibodies, snake, neutralization
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218. Purification of a Metalloprotease from *Naja nigricollis* Venom and Production of Polyclonal Antibodies

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Background: The snakes particularly responsible for serious medical emergencies in Northen Nigeria include *Naja nigricollis*. Bites are common in remote area where accessibility to a hospital is unlikely. There is a need to better understand the pathophysiology of venom components and to explore alternative treatments**.**

Methods: A metalloprotease was isolated from the venom of *Naja nigricollis* and purified to apparent electrophoretic homogeneity by successive chromatography on Sephadex G.75 and DEAE-Cellulose columns. Polyclonal antibodies against the metalloenzyme were recovered from mice vaccinated with purified enzyme**.**

Results: The purified enzyme was optimally active at pH 6.2 and was completely inactivated at pH 4.0. The activity of the enzyme increased progressively with temperature to an optimum of 40°C. The Arrhenius plot from the temperature data gave activation energy of 13.40 kJ/mole. The molecular weight of the enzyme determined by size exclusion chromatography on Sephadex G.75 was 25 kDa. Also the purified enzyme migrated as single 25 kDa band on SDS polyacrylamide gel. Kinetic analysis of the enzyme from initial velocity data gave K_M and V_{max} values of 5.4 mM and 10.6 μmoles/min, respectively when casein was used as a substrate. The enzyme was strongly inhibited by EDTA dose dependently. Delineation of inhibition type revealed non-competitive patterns. The Met-p polyclonal antibodies agglutinated the purified enzyme even at low concentrations. The polyclonal antibodies also inhibited the hemorrhagic activity of the crude enzyme**.**

Conclusions: Metaloprotease from the venom of *Naja nigricolis* is a small sized monomeric protein of 25kDa. Polyclonal antibodies raised against the enzyme have the potential of ameliorating snake envenomation by diminishing the agglutination time of treated blood. The polyclonal antibody inhibition of Cysteine Protease from *Plasmodium berghei* could imply possible homology in the active sites of the enzymes that can be exploited for systemic drug design.

Keywords: metalloprotease, *Naja nicricollis*, venom, polyclonal antibodies, *Plasmodium berghei*
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219. Functional Characterization of a Recombinant Myotoxin Inhibitor from *Bothrops alternatus* Snake Plasma

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Background: Myonecrosis is an important medical complication resulting from snakes bites and, in severe cases, the local myonecrosis may lead to drastic consequences, such as permanent tissue loss, disability, or limb amputation. The venomous and non-venomous snakes have in their blood serum, proteins which inhibit myotoxins, called PLIS**.**

Methods: The ability of the recombinant inhibitor (rBaltMIP) to inhibit the enzymatic activity of PLA_2s Asp49 (BthTX-II, BthA-I-PLA_2, PrTX-III and CB) was evaluated by indirect radial hemolysis method, performed on plates. The myotoxic activity of Lys49 and Asp49 myotoxins (BthTX-I, BthTX-II, PrTX-I and PrTX-III) was carried out by measuring of the Creatine Kinase (CK) levels in mice plasma. Either for phospholipase activity, and for the coagulant activity, PLA_2s and PLA_2-like were previously incubated for 30 min alone or with rBaltMIP in different molar ratios**.**

Results: It was possible to observe a slight reduction in the phospholipase activity of PLA_2s, with maximum inhibitory values of approximately 40% for enzymes BthTX-II and PrTX-III. The recombinant inhibitor was effective in reducing the myotoxicity of myotoxins tested, especially BthTX-I and PrTX-I, both Lys49 PLA_2-like. However, it may be noted that despite a higher efficiency of inhibition against the Lys49 PLA_2-like, rBaltMIP showed a significant inhibitory activity against the Asp49 PLA_2s myotoxins (approximately 50%). It was also performed the inhibition of the activity of different myotoxic PLA_2s myotoxins without the pre-incubation with the inhibitor. rBaltMIP showed considerable ability to inhibit the myotoxic activity of different myotoxins (50%), mainly for Lys49 PLA_2-like myotoxins. Was also tested the viability of serotherapy

complementation by rBaltMIP. In this activity, it was observed that the snake antivenom for veterinary use was capable of neutralizing the myotoxic activity of *Bothrops jararacussu* venom and BthTX-I, moreover, the inhibitor rBaltMIP was more effective to neutralize the myotoxicity of these compounds compared to the antiserum tested. Therefore, when the antiserum was supplemented with rBaltMIP was possible to observe a complementation of its inhibition activity, suggesting a possible commercial application for this inhibitor.

Discussion and Conclusions: It is known that myotoxins has a low immunogenicity, which means that antivenom is not fully effective for those enzymes. rBaltMIP showed high potential for complementation of snake antivenom, however, future studies should be performed.

Keywords: rBaltMIP, PLI, myotoxin inhibitor, myotoxic activity
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220. Evaluation of Extracts and Partitions from Aerial Parts of *Baccharis microdonta* on Enzymatic Activity, Pro-Inflammatory and Myotoxic Activities Induced by Secretory Phospholipase A2 from *Bothrops jararacussu*

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Background: Several species of *Baccharis* have been extensively used in folk medicine for the treatment or prevention of anemia, diabetes and stomach, and other inflammatory diseases. As the inflammation involves the mobilization of arachidonic acid by phospholipase A2, this project used the sPLA2 purified from the *Bothrops jararacussu* as molecular target for the initial studies for characterize the anti-phospholipasic A2 of the methanol extract and its partitions in hexane, dichloromethane, ethyl acetate and Butane -1-ol**.**

Methods: For purification of sPLA2 from *Bothrops jaracussu* used a combination of ion exchange and reverse phase HPLC, for obtantion methanol extraction and their respective partitions. The enzymatic activity was done using a NOB and all experiments were done on the ELISA plate reader. The *Baccharis microdonta* aerial parts were processed for preparation of methanolic was partitioned with hexane, butane-1-ol, dichloromethane and ethyl acetate partitions**.**

Results: The inhibition enzymatic activity of phospholipase A2 was observed only for the partition butane-1-ol while the methanolic extract and the other partitions showed a little or absent. The paw edema test performed in mice were performed with native sPLA 2 from *Bothrops jararacussu*, sPLA2 previously treated with the extract and its partitions, which they have also been previously injected into the animals 30 minutes before the administration of native sPLA2. Under these conditions, we observed that the methanol extract and dichloromethane partition abolished the edematogenic effect of sPLA2 of native *Bothrops jaracussu* PLA2. The partition in Ethyl acetate was injected 30 minutes before abolished the edema induced by native sPLA2. The ethyl acetate partition previously injected 30 minutes before or previously incubated with the native sPLA2 abolished the myotoxic effects of sPLA2. These results suggest that the methanol extract and dichloromethane partitions showed a promising anti-inflammatory activity but did not directly inhibit phospholipase A2. The real-time PCR results showed a decrease in synthesis of COX-1, so that the partition dichloromethane and methanol extract can inhibit the inflammatory activity of sPLA 2 via inhibition of COX-1**.**

Discussion: The previous result analysis showed that *Baccharis microdonta* present some bioactive compound that inhibited the sPLA2 enzymatic activity that seen only found in the butane-1-ol partition that in general allowed the purification of glycosiled flavonoids and tannins that did not extracted by other partition.

Keywords: *Baccharis microdonta*, phospholipase A2, *Bothrops jaracussu*, COX
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221. The Interaction of the Antitoxin DM43 with a Snake Venom Metalloproteinase Analyzed by Mass Spectrometry and Surface Plasmon Resonance

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Background: Natural inhibitors of snake toxins are promising molecules that can contribute to the rational development of new ancillary snakebite envenomation therapies. DM43 is a circulating dimeric antitoxin isolated from *Didelphis aurita*, a South American marsupial naturally immune to snake envenomation. This endogenous inhibitor binds non-covalently to jararhagin, the main hemorrhagic metalloproteinase from *Bothrops jararaca* snake venom, and efficiently neutralizes its toxicity. The aim of this study was to apply mass spectrometry and

surface plasmon resonance to improve the molecular characterization of this heterocomplex.

Methods: Mass spectrometry (nanoESI-Q/TOF MS) was used to analyze jararhagin, DM43 and the stoichiometry of their toxin-antitoxin complex; moreover, the quaternary structure of DM43 was assessed by this methodology. The rate and equilibrium constants of the interaction were determined by surface plasmon resonance. The toxin was captured on a sensor chip derivatized with the anti-jararhagin monoclonal antibody MAJar 2 and the sensorgrams obtained after successive injections of DM43 in a concentration series were globally fitted to a simple bimolecular interaction.

Results and Discussion: The stoichiometry of the interaction was confirmed by MS; from native solution conditions, the complex showed a molecular mass of ~ 94 kDa, indicating that one molecule of jararhagin (50 kDa) interacts with one monomer of DM43 (43 kDa). Although readily observed in solution, the dimeric structure of the inhibitor was barely preserved in the gas phase. This result suggests that, in contrast to the toxin-antitoxin complex, hydrophobic interactions are the primary driving force for the inhibitor dimerization. Regarding the real-time interaction analysis, the following kinetic rates, for the DM43/jararhagin interaction, were obtained: $k_a = 3.54 \pm 0.03 \times 10^4\ M^{-1}s^{-1}$ and $k_d = 1.16 \pm 0.07 \times 10^{-5}\ s^{-1}$, resulting in an equilibrium dissociation constant (K_D) of 0.33 ± 0.06 nM.

Conclusions: The binding stoichiometry data determined by MS unequivocally show that one monomer of DM43 binds to one jararhagin molecule. Moreover, we have shown that DM43 dimers, which are rather stable in solution, are not preserved in the gas phase corroborating that its dimerization interfaces may be due to interaction between hydrophobic residues, a theoretical prediction based on PATCHES analysis. Finally, the kinetic characterization of this interaction indicates that DM43 binds to the referred target toxin with high affinity and may constitute a suitable template for the rational development of novel highly specific therapeutic agents to modulate the activity of SVMPs and their homologues.

Keywords: metalloproteinase, metalloproteinase inhibitor, snake venom, toxin, antitoxin, mass spectrometry, surface plasmon resonance
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222. Molecular Mechanisms Involved in PGE2 Release Induced by the Snake Venom Metalloproteinase BaP1 in Synoviocytes

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Background: Snake venom metalloproteinases (SVMPs) and Matrix metalloproteinases (MMPs) share common domain organization and exhibit identical Zn-binding motif. Studies on SVMPs may thus provide insights into the functions of MMPs. Levels of these enzymes are increased in inflamed articular joints. Articular synovial fibroblasts (B type) are the main cells involved in production and release of inflammatory mediators during joint inflammatory processes, being PGE_2 a major mediator. BaP1 is a 22.7 kDa SVMP from *B. asper* snake venom and contains only the metalloproteinase domain. This enzyme displays potent inflammatory activities both in vivo and in vitro experimental models. In this study we investigated the effects of this SVMP on isolated synoviocytes focusing on the release of prostaglandin E_2 (PGE_2) and the molecular mechanisms involved in this effect.

Methods: B type synoviocytes isolated from rat knee joints synovial membranes (CEUIAB 576/09) were used. Levels of PGE_2 were measured by enzyme immunoassay and protein expression of the PGE_2 receptor EP4 was determined by Western blotting. Participation of both NF-kB and EP4 receptor in the release of PGE_2 and COX-2 protein expression induced by BaP1 was evaluated in cells pretreated with the inhibitors SN50 (50 μg/mL) and AH23848 (30 μM), respectively.

Results: BaP1 induced release of PGE_2 from B type synoviocytes after 1, 3, 6 and 12 h, but not 30 min incubation, in comparison with control cells incubated with RPMI alone. BaP1 induced EP4 protein expression (52 and 65 kDa) by synoviocytes (30min – 6h). Moreover, inhibition of NF-kB or EP4 receptor significantly decreased BaP1-induced PGE_2 release and COX-2 protein expression.

Discussion: These data indicate the ability of BaP1 to induce biosynthesis of PGE_2 and COX-2 expression in isolated B type synoviocytes. These effects are mediated by NF-kB pathway. Moreover, data demonstrated that EP4 receptor regulates BaP1-induced PGE_2 production and COX-2 expression through a positive feedback loop, and strongly suggest that this subtype of PGE_2 receptor contributes for amplification of BaP1-induced release of PGE_2.

Conclusion: BaP1 can directly stimulate synoviocytes for synthesis of PGE_2 and expression of both COX-2 enzyme and EP4 receptor. These findings suggest novel mechanisms of action displayed by metalloproteinases in synoviocytes.

Financial support: FAPESP; CNPq

Keywords: metalloproteinase, prostaglandin E2, synoviocyte
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223. TLR2 and MyD88 Signaling are Required for Efficient Response of Macrophages to MT-III a Phospholipase A2 (PLA2) from *Bothrops asper* Snake Venom

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Background: Toll-like receptors (TLRs) are major components of the innate immune system and primary sensors for noxious stimuli. Upon activation these

receptors initiate an immediate inflammatory response through the myeloid differentiation factor 8 (MyD88) adaptor protein signal transduction. TLRs are highly expressed on macrophages, which are key cells of inflammation. MT-III is a PLA_2 with myotoxic and inflammatory activities. This enzyme induces inflammatory events and activates macrophages functions, including release of inflammatory mediators, phagocytosis and lipid body (LB) formation. However, the role of the TLR system in the inflammatory actions of venom PLA_2s is unknown. In this study we investigated the role of TLR2 and MyD88 adaptor protein in the release of eicosanoids (PGE_2, PGD_2, TXA_2, LTB_4), cyclooxygenase-2 (COX-2) expression and LB formation induced by MT-III in macrophages**.**

Methods: Peritoneal macrophages were obtained from C57BL/6 wild type (WT), $TLR2^{-/-}$ and $MyD88^{-/-}$ male mice and stimulated with MT-III (0.4 μM) or RPMI (control) for 6 h. PGE_2, PGD_2, TXA_2 and LTB_4 levels were quantified by EIA and COX-2 protein expression evaluated by W. blotting. LBs were stained with osmium tetroxide (1%) and counted under phase contrast microscopy**.**

Results: Stimulation of WT macrophages by MT-III caused a marked release of PGE_2, PGD_2, TXA_2 and LTB_4 and increase in numbers of LBs in comparison with respective controls. In MT-III-stimulated $TLR2^{-/-}$ macrophages, formation of LBs and release of PGE_2, LTB_4 and PGD_2, but not TXA_2 were abrogated. In $MyD88^{-/-}$ macrophages, release of PGE_2 and TXA_2 induced by MT-III was not observed in comparison with WT macrophages and the ability of MT-III to induce release of both LTB_4 and PGD_2 was maintained in these $MyD88^{-/-}$ cells. In addition, the ability of MT-III to induce COX-2 protein expression seen in WT macrophages was significantly reduced in both $TLR2^{-/-}$ and $MyD88^{-/-}$ macrophages**.**

Discussion: Obtained data indicate the involvement of TLR2 and MyD88 to the innate immune response to MT-III through activation of macrophages since the absence of these pathways resulted in deficiency in release of inflammatory mediators and LB formation by these cells. Both TLR2 and MyD88 signaling are crucial to PGE_2 biosynthesis, COX-2 expression and LB formation induced by MT-III. Moreover, TLR2 is relevant to LTB_4 and PGD_2 biosynthesis induced by MT-III, whereas MyD88 signal transduction is essential to TXA_2 biosynthesis**.**

Conclusion: TLR2 and MyD88 signaling are relevant in activation of defense functions of macrophages by the snake venom PLA_2 MT-III.

Financial support: INCTTOX, CNPq, FAPESP

Keywords: snake venom phospholipase A2, toll-like receptor, macrophages
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224. Snake Venomics of *Crotalus tigris*. Evolutionary Clues for Generating a Pan-Specific Antivenom Against Crotalid Type II Venoms

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Background: The tiger rattlesnake, *Crotalus tigris*, is a medium-sized ground-dwelling dessert pitviper, that ambushes much of its prey but also active forages small rodents and lizards, juveniles relying heavily on lizards and adults depending more on rodents. In addition, this small rattlesnake has been known to eat fairly large prey, including kangaroo rats, packrats, and even spiny lizards. This is based upon its venom's high lethality, rated the highest of all rattlesnake venoms (LD_{50} value for mice is 0.07 mg/kg intraperitoneal, 0.056 mg/kg intravenous, and 0.21 mg/kg subcutaneous). The comparatively low venom yield (6.4-11 mg dried venom) and short 4.0-4.6 mm fangs of *C. tigris* possibly prevent severe envenoming in adult humans. However, the clinical picture could be very more serious if the person bitten was a child or a slight build individual. We report the proteomic and antivenomic characterization of *Crotalus tigris* venom**.**

Methods: An experimental antiserum was raised in rabbits by subcutaneous injections of subletal amounts of a mixture of venoms from *C.d. terrificus, Crotalus simus and Crotalus lepidus lepidus.* The ability of the experimental antivenom to effectively immunodeplete proteins from the type II venoms of *C. tigris, Crotalus horridus , Crotalus oreganus helleri, Crotalus scutulatus scutulatus, and Sistrurus catenatus catenatus* indicated the feasibility of generating a pan-American anti-*Crotalus* type II antivenom, suggested by the identification of shared evolutionary trends among South and North American *Crotalus* species**.**

Results: This venom exhibits the highest lethality for mice among rattlesnakes and the simplest toxin proteome reported to date. The venom proteome of *C. tigris* comprises 7-8 gene products from 6 toxin families; the presynaptic β-neurotoxic heterodimeric PLA_2, Mojave toxin, and two serine proteinases comprise, 66 and 27% of the *C. tigris* toxin arsenal, whereas a VEGF-like protein, a CRISP molecule, a medium-sized disintegrin, and 1-2 PIII-SVMPs each represent 0.1-5% of the total venom proteome. This toxin profile, in particular, the low metalloproteinase content, the high concentration of Mojave toxin subunits and its high toxicity LD_{50} 0.05(i.v)-0.07(i.p) mg/g of mouse body weight, place a *C.tigris* venom in the type II class defined by Mackessy. The venom composition really explains the systemic neuro and myotoxic effects observed in envenomated animals. In addition, we found that venom lethality of *C. tigris* and other North American rattlesnake type II venoms correlates with the concentration of Mojave toxin A-subunit.

Keywords: North American rattlesnake, *Crotalus tigris*, snake venomics, antivenomics.
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225. Proteomic Analysis and Pharmacological Activities of the Venom of the Moroccan Cobra *Naja haje legionis*.

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Background: In Morocco, envenomation by snake bites poses a serious problem to public health. The cobra *Naja haje legionis* is endemic of the country and one of the most dangerous species known. In this work, we report the proteomic and pharmacological characterizations of biologically active proteins from the venom of this species.

Methods: The various proteins in the crude venom were fractionated and analyzed by a combination of chromatographic separations and proteomic analysis techniques such as gel filtration, RP-HPLC, 1D electrophoresis, in-gel digestion, tandem mass spectrometry and protein database search. The pharmacological properties of *Naja haje legionis* venom were assessed using *in vitro* preparations using rodents and chicks.

Results: Our venomic strategy allowed the identification of 64 proteins and peptides from known database which can classified into 17 families according to their biological activities. We were able to identify cobra venom factor, L-amino-acid oxidases, acetylcholinesterase, metalloproteinase, disintegrin, cysteine-rich secretory proteins, nerve growth factor, phospholipases A_2, vespryns, kunitz-type inhibitor, short neurotoxins, long neurotoxins, weak neurotoxins, neurotoxin like proteins, muscarinic toxins, cytotoxins and cardiotoxins. Pharmacological tests showed that *Naja haje legionis* venom contained neurotoxic activities inducing irreversible blockage of neuromuscular transmission in both rodent and chick nerve-muscle preparations. This venom also exhibit myotoxic and cardiotoxic activities.

Conclusions: These results showed the potency and the complexity of the various proteins present in the venom of *Naja haje legionis*, which present a very similar pattern to other cobra venoms. The contribution of the different components in venom toxicity deserves further investigations.

Keywords: *Naja haje legionis*, characterization, neurotoxins, proteomics, pharmacology
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226. Processing of SVMPs: Detection of SVMP Zymogens and Pro-Domain in Bothrops jararaca Venom and Venom Glands

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Background: Snake Venom Metalloproteinases (SVMPs) responsible for *Bothrops* envenoming cause severe local and systemic complications in humans. They are multidomain enzymes synthesized as zymogens and the enzyme activation is regulated by hydrolysis of the propeptide also known as pro-domain.

Methods: In this work we aim to trace the SVMP processing route by detecting the zymogen molecule or cleaved pro-domain in *Bothrops jararaca* venom glands using anti-pro-domain antibodies and mass spectrometry. The pro-domain was obtained by expression of its cDNA extracted from venom glands in pAE vector/*E. coli*. The recombinant protein (PD-Jar) was used to immunize mice to obtain polyclonal antibodies. The presence of pro-domains in zymogen or processed forms was then evaluated by Western Blotting in venom samples and venom gland extracts collected at 0, 4, 7, 10, 14 and 21 days after milking.

Results: The antiserum was able to recognize bands of 22 and 47 kDa in venom samples collected 7 and 10 days after milking, which contained pro-domain peptides detected by MS/MS. In the extracts of venom glands, antiserum was able to recognize bands of 47 and 76 kDa in all samples. The reactive bands were extracted by pulldown using anti-pro-domain antibodies and subjected to characterization by mass spectrometry.

Discussion and Conclusions: Our results indicate that SVMPs are mainly found as zymogens within the venom-secreting cells and processing is very likely to occur in the lumen of the venom gland, in a time-dependent manner.

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Keywords: zymogen, SVMPs, *Bothrops jararaca*, processing
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227. Proteopeptidome Determination of *Bothrops jararaca* Venom: an Innovative Approach in Snake Venomics

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Background: Aiming for a breakthrough regarding the actual limitations of data volume generated in snake venomics analyses, our group implemented the use of isoelectrofocusing technology (*OFFGEL*) followed by reversed-phase nanochromatography coupled to high resolution mass spectrometry, in order to simultaneously analyze the proteome and peptidome (PROTEOPEPTIDOME) of *Bothrops jararaca* venom.

Methodology: Crude venom was fractionated in high resolution mode (24 wells) using 3-10 or 4-7 pH range strips, for peptidomic or proteomic analysis, respectively. The samples recovered in solution were further desalted and concentrated by ZipTip C_{18} (peptidomics) or digested in solution and desalted by molecular exclusion (7,000 Da cut-off) desalting spin columns (proteomics), followed by mass spectrometric analysis on an LTQ XL-Orbitrap mass spectrometer.

Results: The proteome analysis identified low abundance proteins such as ecto-5'-nucleotidase, phosphodiesterase, hyaluronidase, aminopeptidase A and transferrin and allowed the identification of 320 proteins, 36 of them already described for *B. jararaca* venom. These numbers were eight times higher than those obtained for this venom analyses by previous approaches. The peptidome analysis revealed the existence of a highly complex peptidome, mainly by the identification of peptides related to other groups of proteins (metalloproteinase, phospholipase A_2, serine proteinase, L-amino acid oxidase, vascular endothelial and nerve growth factors, among others) than the traditionally expected bradykinin-potentiating peptide group.

Discussion: The low abundance proteins identified by our proteomic analysis are rarely detected by traditional proteomic approaches; also, the number of identified proteins was significantly higher than previous reports. Regarding the peptidomic approach, our data can initially be viewed as a representation of the snake venom *degradomics*. On the other hand, we hypothesize that some, if not all, of these peptides represent the venom *criptome*, that is: the population of peptides [originated by proteolytic clivage of their criptein(s)] possessing similar or completely different bioactivity from their precursor molecule. Moreover, it is tempting to suggest that they should be chosen as molecules to be tested against an array of biochemical and pharmacological assays, if one is willing to thrive in the search for new bioactive molecules in snake venoms.

Conclusions: The use of *OFFGEL* coupled to high resolution mass spectrometry is an innovative approach to be used for snake venomics studies, in order to contribute to a deeper understanding of the snake biology, the pathophysiology of envenoming, the development of new therapeutic drugs and the improvement of antiophidic therapy.

Keywords: OFFGEL, snake venomics, peptidome, proteome, criptome, *Bothrops jararaca*
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228. Venom Variability and Envenoming Severity Outcomes of the *Crotalus scutulatus scutulatus* (Mojave Rattlesnake) from Southern Arizona

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Background: Snake venoms are well documented as having different venom compositions and toxicity based on taxonomic, geographical locations or intra species variation. The purpose of this study was to determine if the geographical differences in venom of the *C. s. scutulatus* rattlesnake correlate with increased envenoming severity outcomes. We chose the counties of Pima and Cochise AZ, U.S.A. based on the different venom phenotypes documented within each; Pima type B or A+B, Cochise type A. We hypothesized that Cochise County would have more severe envenomations when compared to Pima County.

Methods: Twenty-one *C. s. scutulatus* were collected from Arizona and New Mexico U.S.A. Venom proteome of each specimen was analyzed using reverse-phase HPLC and SDS-PAGE. The toxicity of venoms was analyzed using lethal dose 50 (LD_{50}). Envenoming severity outcomes between Pima and Cochise counties were determined by retrospective chart review of the APDIC database between the years of 2002-2009.

Results: Six phenotypes (A-F) were identified based on three venom protein families; Mojave toxin, snake venom metalloproteinases PI and PIII (SVMP), and myotoxin. Venom changed geographically from SVMP-rich to Mojave toxin-rich phenotypes as you move from south central to southeastern Arizona. Phenotypes containing myotoxins were only found in the transitional zone between the SVMP and Mojave toxin phenotypes. Venom samples containing the largest amounts of SVMP or Mojave toxin had the highest and lowest LD_{50s}, respectively. A significant difference was found when comparing presence of neurotoxic effects ($p = 0.001$). No significant difference was found when comparing severity ($p = 0.32$), number of antivenom vials administered ($p = 0.17$), days spent in a health care facility ($p = 0.23$) or envenomation per 100,000 population ($p = 0.06$). Also noted, there is a 10x and 50x increased risk of death or intubations if envenomated in Cochise County.

Discussion: Our results support and extended the findings of Glenn/Straight. Six venom phenotypes based on neurotoxic (A), hemorrhagic (B), or myotoxic (M) compositions were identified. Types A, B, A+B as previously described, also, A+M, B+M, A+B+M. Despite an increased risk of death or intubations if envenomated in Cochise County or the differences in venom phenotypes identified between Pima and Cochise counties, no significant difference was found in envenoming outcomes.

Conclusion: Clinical manifestations may not have been represented accurately based on the limiting nature of retrospective chart reviews. Following future envenomations on a case-by-case basis may reveal a better correlation with venom proteomics and clinical envenomation manifestations.

Keywords: snake venom, geographic venom variability, venomics, mass spectrometry, snake envenoming, *Crotalus scutulatus*, Mojave rattlesnake
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229. Geographic Variation of Venom Proteins and Neurotoxicity in the Southern Pacific Rattlesnake (*Crotalus oreganus helleri*)

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Background: Snake venoms are comprised of incredibly complex protein arsenals capable of inducing a wide range of physiological effects upon envenomation. Although snakes generally present species-specific venom protein profiles, venom proteins can also vary significantly within a species. Intraspecies venom variation is often seen among geographically separate populations and can also occur among individuals within a population or within individuals over time. The Southern Pacific rattlesnake (*Crotalus oreganus helleri*) has traditionally been characterized as having primarily proteolytic venom; however, neurotoxicity has been documented in a few individuals. A previous study reported Mojave toxin (MT), a heterodimeric phospholipase A_2 (PLA_2) b-neurotoxin found in the venom of the Mohave rattlesnake (*C. scutulatus*), in the venom of some *C. o. helleri* individuals. Mojave toxin, however, fails to account for all *C. o. helleri* neurotoxicity, as neurotoxic envenomations have been documented in individuals shown not to possess MT**.**

Methods: In the present study, 2D SDS-PAGE and liquid chromatography were used to obtain venom protein profiles for individual *C. o. helleri* collected across the species' geographic range. Chick biventer cervicis nerve-muscle preparations were used as non-specific physiological assays of lyophilized venom samples for neurotoxic activity. Biologically relevant crude venom doses (5 mg) were also injected into mice. Chromatography peak fractions were subjected to trypsin digestion and analyzed using liquid chromatography–mass spectrometry. Searches were performed for amino acid sequence similarity in the SwissProt database**.**

Results and Discussion: Mass spectrometry confirmed the presence of MT subunits in several *C. o. helleri* venom samples, and these samples showed neurotoxic activity in the nerve-muscle preparations. However, some *C. o. helleri* venoms lacking the MT subunits also exhibited neurotoxicity in the nerve-muscle assays. Simulated envenomations of mice resulted in immobilization and death in the following order of most rapid to slowest: *C. o. helleri* with MT, *C. o. helleri* lacking MT, *C. s. scutulatus* with MT. *C. o. helleri* venoms express several basic (and some acidic) peptides in the ~4-7 kDa range which appear exceptionally abundant (as % total protein) and variable compared to other rattlesnake taxa. These small basic peptides may account, at least in part, for the non-MT neurotoxicity and increased overall toxicity observed in some *C. o. helleri*. Additional data from on-going investigation of these proteins will be presented. We thank Richard Straight, PhD for his helpful discussions on geographic variability in *C. o. helleri* venoms.

Keywords: *Crotalus oreganus helleri*, venom variability, snake venom neurotoxicity, snake venomics
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230. Determining the Interaction Region Between the Antimyotoxin DM64 and a Snake Venom Myotoxin

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Review: Snake envenomations represent a public health problem in tropical countries. The opossum *Didelphis aurita* is resistant to the toxic effects of snake venoms due to the presence of serum neutralizing factors such as DM43 and DM64. The latter is a glycoprotein able to inhibit the in vivo myotoxicity and the in vitro cytotoxicity of myotoxins I (D49) and II (K49) from the venom of *Bothrops asper*, without inhibiting the phospholipase A2 activity of the first one. Our study aimed to map the region of interaction between DM64 and myotoxin II from *Bothrops asper* venom. The hydrolysis of DM64 by Lys-C generated several peptides that were chromatographed through a myotoxin/NHS-Sepharose affinity column. Three DM64 peptides bound to the column were identified by MS/MS: two located in the third domain and another one in the fifth domain of the inhibitor. Alternatively, DM64 was cross-linked to the myotoxin using BS^3, followed by trypsinization of the complex and nLC-LTQ-Orbitrap analysis. Most observed cross-links occurred between residues K241 (third domain) of DM64 and K15 of myotoxin II, and also between K452 (fifth domain) of DM64 and K60 of myotoxin II. In summary, the interaction of DM64 with myotoxin II seems to involve the second and fifth domains of the inhibitor, as suggested by two complementary methodological approaches. These results contribute to the molecular characterization of this important non-covalent complex and open the perspective that DM64 or its peptides may be used in the treatment of snake envenomations.

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Keywords: DM64, myotoxin, cross-linked
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231. Second Generation Antivenomics: Comparing Immunoaffinity and Immunodepletion Protocols

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Background: The timely parenteral administration of antivenom is the most effective treatment for the

neglected tropical pathology of snakebite envenoming. A deep knowledge of the toxin composition and the immunological profile of venoms are central for developing polyspecific antivenoms exhibiting broad paraspecificity and cross-reactivity against the most medically-relevant snakes of a given geographical area. Preclinical neutralization tests and antivenomic assessments are necessary to demonstrate antivenom safety and efficacy prior to clinical trials. Antivenomics is a proteomic tool for the qualitative and quantitative analysis of the immunoreactivity of antivenoms. The original (first generation, 1G) antivenomics protocol is based on the immunodepletion of toxins upon incubation of whole venom with purified antivenom IgGs, followed by the addition of a secondary antibody or immobilized IgG-binding moiety. Antivenom immunoreactivity is inferred indirectly through the proteomic characterization of the toxin fraction that remains in solution after immunoprecipitation. This antivenomic approach is not appropriate for $F(ab')_2$ antivenoms.

Objective: (i) To design a new method based on affinity chromatography which allows assessing $F(ab')_2$ antivenoms, employing two $F(ab')_2$ monospecific (anti-*Cerastes cerastes* and anti-*Macrovipera mauritanica*) antivenoms; (ii) to compare the 1G, immunoprecipitation protocol, and the new, second generation (2G, affinity capture) antivenomic approaches. These would assess the immunological profile of the pan-African EchiTAb-Plus-ICP® whole IgG antivenom, whose immunoreactivity characteristics have been previously tested towards the venoms of a panel of African viperid snakes and spitting cobras.

Methods: $F(ab')_2$ fragments or whole purified IgG molecules were coupled to a NHS-activated Sepharose®. After incubating the matrix with the venom, the non-retained fraction and the immunospecific venom components were analyzed and quantified by reverse-phase HPLC followed by venomic analysis.

Results: The affinity capture protocol allowed analyzing both types of antivenoms. Furthermore the pan-African EchiTAb-Plus-ICP® antivenom showed qualitatively similar immunoreactivity patterns using either antivenomic approach. Although quantitative departures were noticed between both methods, these may be ascribed to differences in calculating the relative amounts of the non-recognized venom proteins.

Discussion: Our results indicate that both 1G and 2G antivenomic methods can be used interchangeably to investigate the *in vitro* immunoreactivity of antivenoms. An advantage of the affinity approach is the reusability of the affinity columns. Furthermore, the smoother baseline in RP-HPLC chromatograms of affinity column fractions allowed better resolution and a more accurate quantification of the antivenomic outcome than the original 1G protocol. These features contribute to the generalization, economy and reproducibility of the method.

Keywords: snake antivenom, antivenomics, immunodepletion, immunoaffinity protocol.
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232. Death Adder Envenoming Causes Neurotoxicity not Reversed by Antivenom - Australian Snakebite Project (ASP-16)

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Background: *Acanthophis spp* (Death adders) occur in Australia, Papua New Guinea, and parts of eastern Indonesia and envenoming mainly cause neurotoxicity. The objectives of this study were to report the clinical syndrome of death adder envenoming and response to antivenom treatment.

Methods: Definite bites were recruited from the Australian Snakebite Project (ASP) as defined by expert snake identification or detection of death adder venom in blood by enzyme immunoassay. Clinical effects and laboratory results were extracted from the ASP database, including the time course of neurotoxicity and response to treatment. Enzyme immunoassay was used to measure venom concentrations before and after administration of antivenom.

Results: Twenty nine patients had definite death adder bites with a median age of 45 years (Range: 7 to 74y); 25 were male. The species of Death adder was determined in nine cases: four *A. praelongus* (Northern death adder), two *A. antarcticus* (Common death adder), two *A. hawkei* (Barkly Tableland death adder) and one *A. rugosus* (Rough-scaled death adder). Envenoming occurred in 14 patients. Two further patients had allergic reactions without envenoming; both were snake handlers with previous death adder bites. Of 14 envenomed patients, 12 developed neurotoxicity characterised by ptosis (12), diplopia (9), bulbar weakness (7), intercostal muscle weakness (3), limb weakness (6). Two required intubation. Only 2 of the 12 had non-specific systemic symptoms. One patient bitten by a Northern death adder developed myotoxicity and one patient only developed systemic symptoms (abdominal pain and vomiting) without neurotoxicity. No patients developed coagulopathy. The median peak venom concentration in 17 patients with pre-antivenom bloods available was 9.3ng/mL (interquartile range 4.4-24ng/mL, range 0.7-245ng/mL). Antivenom was administered to 14 patients who all received an initial dose of one vial of death adder antivenom. Subsequent doses were administered in eight patients. In eight patients where post-antivenom blood samples were available, no venom was detectable after one vial of antivenom. In all 12 patients treated for neurotoxicity, persistent neurotoxicity occurred for 5 to 168 hours after antivenom.

Conclusion: Death adder envenoming is characterised by neurotoxicity. One vial of death adder antivenom was sufficient to bind all circulating venom. The persistence of neurotoxic effects after antivenom suggests that neurotoxicity may not be reversed by antivenom.

Keywords: Death adder, envenoming, antivenom, neurotoxicity
10.1016/j.toxicon.2012.04.233

233. Hospital Based Retrospective Study of Snakebite Epidemiology in Western Development Region of Nepal

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Background: Snakebite is a common and life threatening public health problem in Nepal. Epidemiological data are fragmentary and sparse in Nepal and accurate, comprehensive epidemiological data on snakebites is still lacking. This study sought to characterize the snakebite epidemiology in Western Development Region of Nepal**.**

Methods: A retrospective study of three years' (2008-2010) snakebite data from medical records of 10 health institutes (1 Zonal Hospital, 3 District Hospital, 1 Private Hospital, 1 Mission Hospital, 2 Primary Healthcare Centers and 2 Army Camps) in the Western Development Region of Nepal was carried out during June 2011 to February 2012 by the use of pretested data sheets. Snakebite data were manually searched from those health institutions where snakebite victims used to be reported and government supplied anti-snake venom freely. Snakebite reported in medical records based on an eye witness and/or claim of snakebite victim, brought snakes, clear signs of snakebite wounds and symptoms of snakebite envenomation were included in the study. Those with a claim of snakebite with no or poor proof of snakebite were considered as suspected snakebite and not included in analysis. Statistical analysis was done by the application of MS Excel and R. The study was approved by the Ethical Clarence Review Board in Nepal Health Research Council**.**

Results: The overall confirmed snakebite reported in three years was 6,993 of which 640 (9%) were envenomed and treated with antivenom (an average of 16 ASVS vials were administered to each victim) and the suspected snakebite cases were 2,562. The overall case fatality rate was 13% (10% in 2008, 16% in 2009, and 13% in 2010). July and August was the highly snakebite risk months (21% of overall snakebites in each month) in this region. The majority of snakebites were reported between 15:00 and 21:00 hours**.**

Discussion: Snakebite and envenomation were greater in 2010 than in the two previous years. But the case fatality rate was greatest in 2009. This study detected decreased mortality associated with anti-snake antivenom use in this region**.**

Conclusion: Snakebite records in the existing snakebite treatment center were poor and prevented complete characterization of the epidemiology of snakebite epidemiology in the Western Development Region of Nepal. Antivenom use was associated with decreased mortality.

Keywords: Envenomation, case fatality, anti-snake venom
10.1016/j.toxicon.2012.04.234

234. Severity of Snakebites in Children in the United States: 2000-2009

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Background: Many of the 10,000 victims of snakebites each year in the US are under 18 years of age. The snakebite management of this population has not been studied as extensively as adults; however, previous studies have demonstrated that less than 2% of the pediatric victims result in major clinical effects. There may be particular characteristics specific to this population that could predict their overall outcomes. Treating physicians could use this information to direct emergency management of the pediatric victims of snakebites.

Objective: Our goal was to determine the distinct characteristic differences of pediatric victims of snakebites between those who had major and those who had minor outcomes**.**

Methods: Observational, case-control study of telephone calls to all US poison centers (National Poison Data System) for human victims of snakebites under 18 years from 2000 to 2009. Major outcome was defined as "significant disability" or death. Minor outcome was defined as "minimally bothersome" or no clinical effect. Those with moderate outcome were excluded**.**

Results: There were 20,285 pediatric snakebites during the 10 year study period. Only 378 (1.9%) had a major outcome, 8,563 (42.2%) had a minor or no effect, and 11,344 (55.9%) had a moderate or unknown effect and were excluded. There were 3 deaths from snakebite. Most victims of major (65.9%) and minor bites (72.3%) were males ($p = .008$). Also, most victims of major (66.9%) and minor bites (78.9%) were over 5 years of age ($p < .001$). Seventeen States did not have a single snakebite that resulted in a major outcome. Four states (Texas, Florida, Georgia and California) represent 53.2 % of major outcomes. Most victims of major (78.8%) and minor bites (52.2%) were bitten by venomous snakes ($P < .001$). Nonvenomous snakes caused 1.5% of major and 9.6% of minor outcomes ($p < .001$). "Unknown snakes" accounted for 20.1% of major and 38.1% minor outcomes ($p < .001$). Rattlesnake bites produced 42.3% of major and 6.1% of minor outcomes ($p < .001$). Surprisingly, many major (44.1%) and minor bites (38.1%) occurred from September to April ($p = .022$).

Conclusions: This is the largest outcome analysis of snakebites in pediatric victims. Almost half of these victims had only minor or no clinical effects. Significant disability or death of these victims was rare. Severity of outcome is associated with victim gender, age, geography, season, and type of

snake. These results may be useful for preventing major outcomes and the planning for snakebite management.

Keywords: Snakebite, pediatrics, outcomes, severity, USA
10.1016/j.toxicon.2012.04.235

235. Antivenom Therapy Following Snakebite: Effectiveness and Strategies for Delivery in West Africa

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Background: In West Africa antivenoms have been useful in clinical management of snakebite envenoming. Response is often dramatic especially following carpet viper envenoming where restoration of blood coagulability and resolution of spontaneous haemorrhage is rapidly achieved. In bites from certain snakes manifesting with similar coagulopathies elsewhere, antivenom effectiveness may be less dramatic and its use has been questioned. We therefore critically evaluated clinical studies on envenoming and antivenom therapy conducted in the region to determine their effects and explored the appropriate strategies for delivery of care. Efficacy of antivenoms developed from the region was explored from preclinical studies.

Methods: All observational, interventional and preclinical studies conducted in the region (or on antivenoms derived from the region) were systematically evaluated to determine the effect of antivenoms used in the studies. Effectiveness of antivenoms in resolving features of envenoming or curtailing mortality was determined where feasible. Different strategies for optimally delivering antivenom therapy in rural savanna populations was explored and compared. Published data was re-analyzed.

Results: There were 23 studies reported with evaluable information on aspects of antivenom therapy from the region. There were 12 observational studies (8 non-comparative and 4 comparative clinical studies), 4 comparative clinical trials and 7 preclinical studies. 13 of the 16 clinical studies determined and strongly suggested effectiveness of antivenom therapy. From 3 of the comparative studies: regionally appropriate antivenom therapy conferred protection against deaths by 87% (95%CI: 52-97%) when compared to inappropriate therapy, by 83% (95%CI: 4-97%) when compared to no therapy, and by 90% (95% CI: 55-98%) when combined with staff education and standardization of clinical management. In preclinical studies most antivenoms used in practice have activity against venoms of local snakes and effect may be broader extending to more species in larger areas of Sub-Saharan Africa. Modality of antivenom delivery usually follows a 'centralized' district-peri-urban approach while 'de-centralized' hub-and-spoke rural model which reduces delay (found to predispose to mortality) found to access has not been used commonly.

Conclusion: Antivenom therapy is effective in management of snakebite especially carpet viper envenoming in West Africa. While a placebo controlled trial will be unethical, antivenoms confer protection of over 80% against death in observational studies. Strategies for improving care should include utilizing regionally appropriate antivenoms, educating staff and standardizing clinical care. Centralized *versus* de-centralized modes of antivenom delivery should be explored further to determine respective benefits, risks, costs and feasibility of the approaches.

Keywords: Antivenom, effectiveness, West-Africa
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236. Determinants of High Cost of Care Among Victims of Snake Bite in Kaltungo, Gombe State, Nigeria, 2009

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Background: Envenomation by *Echis ocellatus* (carpet viper) is an important cause of morbidity and mortality in West Africa. Inaccessibility to care for snake bites results in ineffective and harmful remedies. We conducted a cross sectional study of cases that presented in October 2009 to Kaltungo General Hospital. Our objective was to identify the determinants of high cost of care among victims of snake bite.

Methods: We administered questionnaires to all suspected cases that attended Kaltungo General Hospital in the October 2009. We defined a suspected case as any patient who presented to the hospital with history of snake bite during the study period. We obtained further information from clinical examination and hospital record review. We collected information on patient demographic factors, type of snake, time from bite to presentation, clinical signs and symptoms, first aid given before presentation, duration of hospital stay, estimated cost of care, average family monthly income, and final outcome.

Results: We documented 109 patients; 77% were male. Median age was 24 yrs (range 6-80yrs). The main source of income for the families was farming (78%). Delayed presentation to the snake bite centre of >24hrs was recorded by 26 cases (24%). On univariate analysis, patients with delayed presentation (>24hrs) where more likely to incur high cost of care (OR: 7.4; 95% CI: 1.6 – 20.8), bleed (OR: 3.6; 95% CI: 1.4 – 9.5), ingest herbs (OR:12.7; 95% CI: 1.6 – 98.9), apply traditional ailments at site of bite (OR: 12.1; 95% CI: 1.6 – 93.7), or incise bite (OR: 2.8; 95% CI: 1.1 – 7.5). Risk factors for high cost of care where: delayed presentation, hospital stay >2days (OR: 14.6; 95% CI: 1.9 – 115) and bleeding (OR: 4.0; 95% CI: 1.5 – 20.5). No statistically significant relationship between delayed presentation and sex (OR: 1.7; 95% CI: 0.6 – 4.6), pediatric age group (OR: 1.6; 95% CI: 0.6 – 4.6) or being a farmer (OR: 0.8; 95% CI: 0.3 – 2.1). Multivariate analysis revealed delayed presentation (>24hrs) (aOR: 5.8; 95% CI: 2.0 – 17.0) and

hospital stay >2days (aOR: 19.5; 95% CI: 2.0 – 192.3) as independent risk factors of high cost of care.

Conclusion: Most cases of snake bite occur in young productive farmers and could impact local food production. Delay in presentation associated with unorthodox, harmful practices, could result in prolonged hospital stay and high cost of care.

Fig. 1. A. Haematoma from bite on the buttocks; B. Leg swelling; C. Cellulitis and gangrene; D. Bleeding diathesis.

Keywords: Access, cost of care, *Echis ocellatus*, envenomation, Nigeria
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237. Snakebite Survivors Club: Ten-year, retrospective review of Crotaline envenomations in Central California

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Objective: We investigated clinical patterns of Crotaline envenomation presenting to a tertiary-care academic hospital in Central California over a 10-year period.

Methods: An IRB-approved, retrospective chart review was conducted on all patients diagnosed with snakebite from December 2000 to December 2010. Data abstracted: demographics, anatomic location of bite, comorbid conditions and intoxicants, length of stay, antivenom dose, laboratory results, and complications or procedures.

Results: There were 46 snakebite cases admitted over the study period. Five were “dry bites;” the remaining cases (41/46) received antivenom. There was a male predominance (83% male victims). Upper extremity bites were more common (32/41 upper vs 10/42 lower extremity). One victim sustained bilateral bites to the hands. Thirty-five patients (85%) were admitted, with an average length of stay 2.12 days. The longest hospitalization was 15 days. There were no fatalities. The average time from bite to ED presentation was 164 minutes. Bites occurred during every month except November, with the majority occurring during spring and summer months and peaking in June (12/42 cases). Most bites occurred in the hours between noon and 8 pm. The amount of antivenom given ranged from 2 to 35 vials (average, 9 vials). Interfacility transfers were common in our study population: thirteen (32%) patients were transferred into our emergency department for a higher level of care, and 3 (7%) were transferred out (two because of insurance requirements, and one for higher level of Pediatric ICU care). There were no surgical interventions in our study group. Intoxication did not appear to play a major role in this population as only 3 patients (7%) were found to be acutely intoxicated: one with cannabis and amphetamines, 1 with alcohol, and 1 with opioids.

Conclusions: In Central California, Crotaline envenomations occurred mainly in adult males. Dry bites, or bites not requiring antivenom administration, were uncommon, comprising only 10% of bites in this study population. Contrary to popular and clinical beliefs, substance abuse and/or alcohol intoxication did not appear to play a role in the majority of patients. Care providers and snakebite specialists should also be aware that snakebite patients are often transferred between facilities, a finding that may be useful in informing future first aid protocols and research. We hope these findings add concrete data and help correct some common misconceptions about snakebites in Central California.

Keywords: Snakebites, epidemiology, interhospital transfers
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238. Incidence and Management of Snakebite in Northern Central African Republic

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Background: Snakebite represents a serious public health problem in sub-Saharan Africa.

Methods: A retrospective study was conducted in Paoua hospital (northern CAR) for 27 months to assess the incidence and severity of snakebites. A team of Médecins Sans Frontières (MSF) is working in this hospital the resources of which are better than the average of sub-Saharan facilities.

Results: 842 people were registered for snakebite of which 825 were included in the study. The seasonal

distribution shows a slight increase during rainy season. Only 11 snakes were identified. The sex ratio was 1.56 (503 M/322 F) and the median age [IQ:25;75] was 17 [10;27.5] years. A third of the patients (174/523) reached the hospital within 6 hours after the bite, while 46.5% (243/523) arrived 12 hours after or more. Feet were involved in 70.7% of cases and hands in 24.6%. Immunotherapy was administered to 644 patients (78.1%) who received 1.9 ±0.06 [IC:95%] vials. Antivenom was renewed twice or more for 131 patients (20.3), owing to edema progression (40%), persistent bleeding (30%), abnormal coagulation test (30%) or persistence of neurological disorders (0.2%). Three patients did not benefit from immunotherapy due to lack of antivenom. The average stay was 4.03 ±0.28 days. Seven patients (0.8%) showed clinical signs consistent with envenoming by elapid. Viper-like envenomation was observed in 640 patients (77.6%). Edema was present in 681 of 812 patients for whom the data was specified (83.9%). Edema involving two joints was observed in 399 patients (49.1%) and edema reaching or exceeding the limb's root was present in 114 (14%). A clinical sign of hemorrhage (blisters, persistent local bleeding, epistaxis, gingival bleeding, hemoptysis, hematemesis) was reported in 145 patients (17.6%). Five deaths (0.6%) occurred respectively in 4 children and 1 adult. One of the children died without obvious signs of envenomation probably resulting from poisoning by plants used by traditional healer. Two other children arrived at the hospital with a severe anemia and died despite transfusion and antivenom. The fourth died from a sepsis. The adult died from a severe hemorrhagic syndrome without receiving antivenom due to out of stock. Local complications (necrosis, abscess, flessum) occurred in 68 patients (10.6%). Surgeries were required in 29 for debridement (23 patients), skin graft (4 patients) and amputation (2 patients).

Conclusion: Better management of snakebites and broad use of immunotherapy could explain the good results.

Keywords: Snakebite, antivenom, Africa
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239. Combined Neurotoxicity and Hematotoxicity with Clinically Significant Bleeding after Mohave Rattlesnake (*Crotalus scutulatus*) Envenoming in Southern California

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Methods: Case report. The snake's head was examined by 4 herpetologists with unanimous agreement on speciation.

Results: We describe a confirmed Mohave rattlesnake envenomation with angioedema, hemorrhage, neurotoxicity and rhabdomyolysis. A 31-year-old male presented 1 hour after being bitten by a Mohave rattlesnake encountered in Adelanto, California. On arrival he was tachycardic, hypotensive and altered. He had minimal tissue effects at the bite site. However, he had facial and airway angioedema. He began to complain of progressive difficulty breathing, swallowing and had extraocular muscle weakness consistent with neurotoxicity. He also developed generalized myokymia. He then had two episodes of hematemesis. The patient was intubated and airway was noted to be edematous during the procedure. Because the patient was in shock with serious active bleeding, an initial dose of 12 vials of CroFab ® was initiated. He continued to show signs of hemorrhaging with multiple large bloody bowel movements (approximately 1.5 L), a 8-gram drop in hemoglobin, bloody drainage from foley / gastric tubes and conjunctival hematomas. Initial labs confirmed coagulapathy, INR 3.2, Fibrinogen <50 mg/dl. First platelet count was normal, but severe thrombocytopenia developed shortly thereafter. He was transfused and admitted to the ICU. He ultimately received 6 units of PRBCs, 5 units of FFP and 30 vials of CroFab®, which resolved his bleeding and eventually his coagulopathy as well. He developed rhabdomyolysis, which responded to fluid therapy. He was extubated on day 4, and discharged on day 9 with normal labs and was fully intact neurologically by day 11 follow-up.

Discussion: Until now, only Mohave rattlesnakes with Venom A (neurotoxic) venom have been reported in Southern California. Venom B (hemorrhagic and tissue-destructive) effects have not been previously described after bites by this species from this region. Other areas, such as Arizona and Texas, may have snakes with A, B or A+B venom/effects. In California, this is a novel find.

Conclusion: Hematotoxicity with clinically significant bleeding, together with neurotoxicity and other serious venom effects, can be seen after Mohave rattlesnake envenoming in Southern California.

Keywords: Mohave, Mojave, venom
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240. Development of a Double Sandwich Flourescent ELISA to Detect Rattlesnake Venom In Biological Samples from Horses with a Clinical Diagnosis of Rattlesnake Bite

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Background: The mechanism of cardiotoxicity in horses following envenomation by rattlesnakes endemic to North America is unknown.

Objective: We hypothesized that larger venom doses would cause more severe cardiac damage. The objective of this study was to develop an ELISA to detect rattlesnake venom in equine biological samples in order to test this hypothesis.

Methods: Nineteen horses with a clinical diagnosis of rattlesnake bite were enrolled. Cardiotoxicity was assessed using serum troponin and the presence of arrhythmias on ECG. A double sandwich fluorescent ELISA was developed to detect rattlesnake venom in urine and bite site swabs collected from these horses. A bite site swab was taken at admission and urine was collected at presentation, 24, 48, 72, 96 hours, 1 week and 1 month post presentation. Urine was available from 19 horses however not at every time point. Bite site samples were available from 9 horses. Samples were considered positive if venom concentration was $\geq$2 standard deviations above the negative control.

Results: Venom was detected in urine of 13 of 19 horses and in bite site samples from 5 of 9 horses. No correlation was found between venom concentration and cardiotoxicty.

Discussion: Venom elimination in urine was found to be highly variable. With limited sample sets it was not possible to determine peak elimination or maximum venom concentration.

Conclusions: An ELISA was developed that successfully detected rattlesnake venom in equine biological samples. More consistently timed urine collection may be necessary to further investigate the relationship between venom dose and cardiotoxicity.

Keywords: Rattlesnake, horse, ELISA
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241. Juvenile Rattlesnake Fang as a Retained Foreign Body: Clinical Diagnosis

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Background: An estimated 6,000-8,000 snakebites occur each year in the U.S. with more than half coming from venomous species, prompting many calls to poison centers. The Arizona Poison and Drug Information Center (AZPDIC) lies in a unique location in the southwestern desert region of the United States, handling approximately 200 calls around the state related to rattlesnake envenomation. Recently a surgeon asked AZPDIC regarding the ability to see a juvenile rattlesnake fang in tissue, as he had a patient bitten by a juvenile rattlesnake whose exam suggested a retained fang. To our knowledge it was unknown if this could be diagnosed by traditional diagnostic modalities.

Objective: To determine if a retained juvenile rattlesnake fang is identifiable as a foreign body with traditional clinical imaging techniques.

Methods: Extraction of a fang from a post-mortem juvenile rattlesnake *Crotalus Atrox*, popularly known as the Western Diamondback rattlesnake, aged at less than 1 year of age, followed by implantation in differing media with ultrasound and radiography then performed.

Results: Juvenile rattlesnake fang was visualized with both ultrasound and radiography.

Conclusions: Juvenile rattlesnake fang as a retained foreign body is clinically diagnosable with ultrasound and radiography, and may assist in securing this diagnosis in a clinical setting although small fang size, ultrasound machine resolution, and acoustic windows are limiting factors.

Fig. 1. Fang, *in vivo*

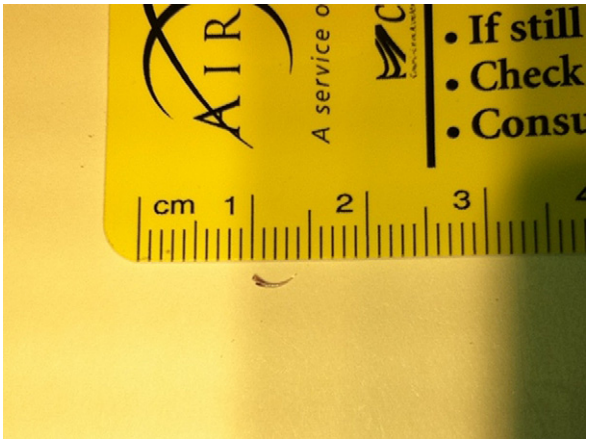

Fig. 2. Fang length

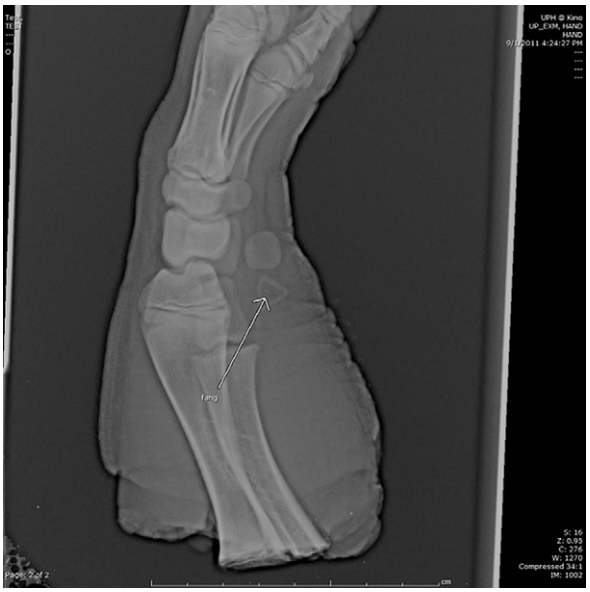

Fig. 3. Radiograph of fang, in pig.

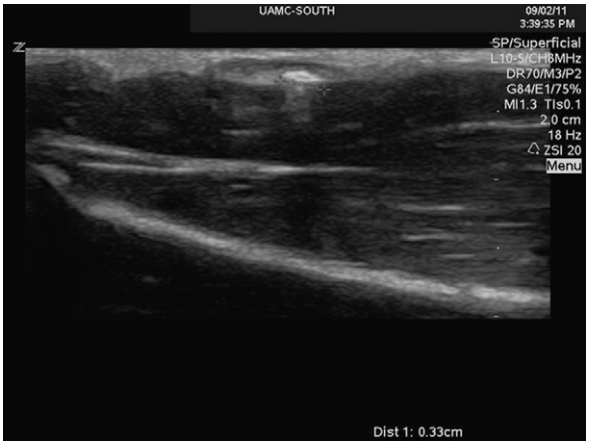

Fig. 4. Fang, long axis.

Keywords: Snake, envenomation, fang
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242. The Effect of Pre-hospital Care for Venomous Snakebite on Outcome in Nigeria

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Background: Snakebite is a common but neglected health problem in rural sub-Saharan Africa. In some rural Nigerian hospitals up to 50% of the total bed capacity may be occupied by snakebite victims at peak times of the early rainy season and harvesting periods. The untreated mortality (without antivenom) is 10-20%. *Echis ocellatus* (carpet viper) is responsible for 66% of bites in the Nigerian savanna. Various pre-hospital care of doubtful efficacy are received by victims.

Methods: We prospectively studied 72 consecutive snakebite victims at a rural north central Nigerian hospital. The primary outcome assessed was death or disability at hospital discharge.

Results: Victims were predominantly male farmers, and in 54 cases (75%) the snake was identified as carpet viper (Echis ocellatus). Most subjects (58, 81%) attempted at least one first aid measure after the bite, including tourniquet application (53, 74%), application (15, 21%) or ingestion (10, 14%) of traditional concoctions, bite site incision (8, 11%), black stone application (4, 5.6%), and suction (3, 4.2%). The majority (44, 61%) presented late (after 4 hours). Most (53, 74%) had full recovery at hospital discharge. Three deaths (4.2%) and thirteen (18%) disabilities (mainly tissue necrosis) occurred. The use of any first aid was associated with a longer hospital stay than no use (4.6±2.0 days versus 3.6±2.7 days, respectively, P=0.02). The antivenom requirement was greater in subjects who used a tourniquet ($P = 0.03$) or presented late ($P = 0.02$). Topical application (Odds Ratio 15, 95% CI 1.4-708) or ingestion of traditional concoctions (OR 20, 95% CI 1.4-963) was associated with increased risk of death or disability. Ingestion and application of concoctions were associated with a longer time interval before presentation, a higher cost of hospitalization, and an increased risk of wound infection.

Conclusion: Traditional first aid measures for viper bites, like use of tourniquets and traditional concoctions, potentially contribute to morbidity and mortality in rural Nigerian communities. Delays in antivenom administration due to late presentation and erratic antivenom supply are also additive factors. Community education on appropriate actions after snake bite, including limb immobilization, cleansing the bite site with soap and water and rapid transport of victims to the hospital could reduce the complications from viper bites in Africa.

Keywords: Viper, envenomation, first aid
10.1016/j.toxicon.2012.04.243

243. Continuous Crotalidae Polyvalent Fab (Ovine) (FabAV) for Selected Snakebite Patients

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Background: In patients bitten by rattlesnakes and treated with Crotalidae Polyvalent Immune Fab (Ovine) (FabAV), persistent or recurrent hematologic effects

(hypofibrinogenemia, prolonged PT/INR, prolonged PTT, and/or thrombocytopenia) are common, difficult to manage, and may result in morbidity and mortality. The optimal management of persistent or recurrent hematologic abnormalities, particularly the use of further treatment with antivenom, has not been well defined.

Objectives: To describe experience using a continuous infusion of FabAV for persistent and/or recurrent hematologic effects in rattlesnake envenomation.

Methods: Retrospective, observational case series. Repeat bolus doses as well as continuous infusions of FabAV were used on an inpatient basis for patient benefit to try to reverse persistent or recurrent hematologic abnormalities and/or associated bleeding complications. Indications, dilution and infusion protocols, and duration of therapy were individualized.

Results: Five cases were identified between July 2010 and September 2011. All patients had profound hematologic abnormalities that persisted, recurred and/or were associated with serious bleeding. Several patients received repeat bolus infusions of FabAV, with or without blood products, with either inadequate or only transient beneficial response. All patients were then managed with a continuous infusion of FabAV and all responded to the continuous infusion of FabAV, titrated to effect, with cessation of progression and, in most cases, improvement in hematologic abnormalities. Rates of infusion varied from 2 to 4 vials per 24 hours (mean = 3.3 +/- 1.0 vials/day) for control or reversal of hematologic abnormalities. The duration of FabAV infusion was between 4 and 8 days from the time of envenomation (mean = 6 +/- 2 days), after which hematologic values were normalized or were normalizing and continued to do so.

Discussion: The use of FabAV as a continuous infusion, particularly after the acute phase of envenomation has passed, provides a continuous source of circulating antibodies to neutralize venom components reaching circulation from tissue stores and allows natural replenishment of hematologic factors such as platelets and/or fibrinogen. This method is the most efficient use of FabAV, avoiding the wasteful excess of a bolus dose, may be more effective, eliminating the potential for destruction of hematologic factors when protective antivenom levels are lost between bolus FabAV doses, and appears to be safe. Further assessment of the stability and sterility of the product is needed and sequential IVs over 4 to 6 hours are currently recommended rather than a single infusion over 24 hours. The need to continue hospitalization for its administration is the major drawback, but that may be needed for other indications (e.g. bleeding) and this method is best suited for event.

Conclusions: A continuous infusion of FabAV between 2 and 4 vials per day, titrated to effect, and continued for 4 to 8 days post-envenomation was associated with reversal of persistent and/or recurrent hematologic effects of rattlesnake envenomation and, combined with blood products, control of significant bleeding. Continuous infusion of Fab antivenom may be safer, more efficacious, and more cost-effective than observation without FabAV treatment or as-needed dosing in high-risk patients.

Keywords: FabAV, continuous IV infusion, recurrence, hematologic effects, *Crotalinae*, envenomation
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244. Mulga snake (*Pseudechis australis*) Bites; A Review of Significant Cases Including an Exceptionally Severe Local Envenoming

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Background: The mulga snake (*Pseudechis australis*) is Australia's largest venomous snake: adults commonly exceed 2m in length. The species is common throughout much of inland and northern Australia. Unlike most other dangerous Australian snakes, mulga snakes cause anticoagulant coagulopathy, but the most prominent feature of envenoming is severe systemic myolysis.

Method: Cases of mulga snake bite treated by one or more of the authors over the last 30 years were reviewed, with regard to circumstances of bites, local and systemic effects, outcomes of treatment, based on prospectively collected data at the time of each bite.

Results: Mulga snake bites occurred in the warmer months of the year, with both diurnal and nocturnal encounters, including bites inflicted while the victim was asleep in bed. Envenoming was systemic in virtually all cases, with predominantly myolysis manifest as massive elevations of plasma CK, usually with macroscopic myoglobinuria. Anticoagulant coagulopathy was less common and was not associated with clinical bleeding. Local pain and swelling of the bite site and adjacent limb was a hallmark feature. Symptomatic response occurred after 1 vial of suitable antivenom in most cases.

Case Report: An 18 yr old woman was bitten on the upper thigh by a large snake while in bed in a rural setting house. The snake was later identified as a mulga snake, confirmed by venom detection. A tourniquet was applied as first aid. Retrieval was organised back to a major hospital, and on arrival she had severe local pain and an area of damaged skin around the bite site, with moderate systemic myolysis and mild anticoagulant coagulopathy. She was given IV Black Snake antivenom (CSL Ltd., Melbourne) and although the systemic features resolved, the local wound progressed to full thickness skin loss requiring debridement and secondary repair. This constitutes an unusually severe local reaction to mulga snake bite.

Discussion: Mulga snake bites are associated with warmer weather, may occur at night and inside dwellings, as well as diurnally, with hallmark features of significant local pain and swelling, severe systemic myolysis, occasional anticoagulant coagulopathy, usually without clinical bleeding, and respond well to a single vial of appropriate

antivenom. They can cause severe local tissue injury, but to date, this is an uncommon phenomenon.

Keywords: Mulga snake, snakebite, envenoming
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245. Pressure Bandaging with Immobilization in *Crotalinae* Envenomation Controversy

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Background: In 2010, the American Heart Association (AHA) and American Red Cross (ARC) published their updated Guidelines for First Aid. Those guidelines, intended to be applied by bystanders or by the victim, included the statement that pressure bandaging with immobilization (PBI) had been demonstrated to be effective for bites by non-neurotoxic (*Crotalinae*) American snakes. This represented a radical change in recommendations in managing *Crotalinae* envenomations pre-hospital in the U.S. In response to this, a number of individuals with expertise in treating *Crotalinae* snakebite wrote to the AHA/ARC, questioning their analysis of existing data, their conclusion that this method had been demonstrated to be effective, and expressing concerns that, based on studies of this method elsewhere in the world, plus limited experimental animal data demonstrating elevated tissue pressures in *Crotalinae* envenomations, it was also potentially harmful. The AHA/ARC defended their process and conclusions, and refused to publish any of the letters or to revise their recommendations.

Methods: A position statement regarding PBI in *Crotalinae* envenomation was drafted and circulated to the American College of Medical Toxicology, the American Academy of Clinical Toxicology, the American Association of Poison Control Centers, the European Association of Poison Control Centres and Clinical Toxicologists, the International Society on Toxinology and the Asia Pacific Association of Medical Toxicology.

Results: The position statement reviewed the current state of the science on PBI and concluded, "The use of pressure immobilization for the pre-hospital treatment of North American Crotalinae envenomation is not recommended". The position statement was endorsed by all six organizations. In addition, a Commentary was written to accompany the position statement. The Commentary noted the extraordinary concurrence of mainstream medical opinion among experts on four continents and concluded that the proper grading of current evidence on PBI was Class III: Evidence and/or general agreement that a procedure/treatment is not useful/effective, and in some cases may be harmful. Both the position statement and accompanying Commentary were published simultaneously in *Clinical Toxicology* and the *Journal of Medical Toxicology*. In response to the impending publication of the position statement, the AHA/ARC engaged in further dialogue, agreed that their guideline was not clear regarding the snake groups, geographic locations and individual circumstances in which PBI might be applicable, and that the data was insufficient to deem PBI safe and effective. They further agreed to revise the guideline, including content experts from the position statement-sponsoring organizations in future guideline development.

Conclusions: Introduction of new treatments for snakebite, particularly first-aid recommendations, should be evidence-based and include considerations of context and real-world application. Collaborative action by toxinologists and toxinology organizations helped to correct a flawed first-aid recommendation for *Crotalinae* envenomations.

Keywords: Pressure bandaging, PBI, *Crotalinae*, envenomation, compartment syndrome
10.1016/j.toxicon.2012.04.246

246. Non-front-fanged Colubroids; A Current Analysis of Medical Significance

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Background: Non-front fanged colubroids (NFFC) comprise about 70% of extant snake species and include several taxa now known to cause lethal or life threatening envenoming in humans. A growing number of other taxa are implicated in medically significant bites. Some of these are increasingly entering the commercial snake trade posing a currently unquantified risk.

Methods: The global literature was searched case reports of NFFC bites. These cases were assessed for evidence-based value, clinical detail and verified species identification. These data were subjected to meta analysis and a hazard index was generated for select taxa.

Results: Case reports meeting the selection criteria were identified for about 120 species. Of these a small subset, designated Hazard level 1, represented those species well documented as having lethal potential and management of these cases included specific therapy for 3 species (antivenom; *Dispholidus typus, Rhabdophis tiginis, R. subminiatus*), whereas others in this group (*Thelotornis* spp.) are treated with replacement therapy (eg cryoprecipitate/fresh frozen plasma/ packed red blood cells etc) and supportive care. Evidence-based analysis positively contraindicates the use of heparin, antifibrinolytics and plasmapheresis/exchange transfusion. Hazard level 2/3 species consisted of several taxa that have mixed quality data implicating these as causing rare systemic effects (eg *Boiga irregularis, Philodryas olfersii, Malpolon monspessulanus*). Recommended management may include use of acetylcholinesterase inhibitors (eg neostigmine) and wound care on a case by case basis. Hazard level 3 species

comprised a larger group capable of producing significant local effects only and this often is associated with a protracted bite (eg *Heterodon nasicus, Alsophis* (= *Borikenophis*) *portoricensis, Platyceps rhodoracis*). Management is restricted to wound care. Bites by Hazard level 4 species consisted of the majority of surveyed taxa and these showed only minor effects of no clinical importance.

Discussion: This study has produced the most comprehensive evidence-based listing of NFFC snakes tabulated against medical significance of bites, together with best-practice management recommendations. This will assist clinicians in managing bites by these snakes. This critical analysis assumes increasing importance concomitant with the growing exposure to lesser known NFFC snakes, particularly in captive collections. This growing exposure may uncover further species of significance in the future. Careful and accurate documentation of bites by verified species of NFFC snakes is required to increase the evidence base.

Keywords: Colubrid snakes, snakebite, envenoming
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247. Envenomation by *Daboia mauritanica* Snakes in Tiznit Province, Morocco: Report of Four Cases

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Background: In Morocco, the incidence of snake bites is estimated at 0.2 for 100 000 inhabitants with an annual average of 5 deaths. The identification of the aggressor snake remains problematic because of the lack of qualified taxonomic expertise in treating practitioners.

Methods: This is the first report in Morocco of four cases of confirmed envenomation by *Daboia mauritanica* occurring in Tiznit province, in the south of Morocco. The identification of the snake was achieved by the Moroccan Poison Control Center and confirmed by the Rabat Scientific Institute.

Results: Two cases of snakebite were severe. Clinical symptoms were characterized by thrombocytopenia, low blood pressure, compartment syndrome, and hemorrhagic syndrome. For one of the two cases, local necrosis of the thumb, thenar and hypothenar areas was observed and both of these cases received fasciotomy. The medical management included vasopressors, antibiotics, analgesics, transfusion and aponeurotomy. Envenomation was moderate (extensive swelling, non-life threatening systemic symptoms) and minor (local swelling, no systemic symptoms) in the third and fourth cases, respectively. Outcomes in these cases were favorable after a short hospitalization. There is currently no antivenom with coverage of this species and no antivenom was given in any of these cases.

Discussion: *Daboia mauritanica* is a venomous viper species listed by the Rabat Scientific institute with a wide native distribution in Morocco. There have been no previous published, documented case reports of envenomation by this snake. Venom-induced toxicity is characterized by its proteolytic, hemorrhagic and phospholipasic activity. It also has fibrinolytic and myolytic (skeletal and cardiac muscle) activity. In order to minimize the number of envenomations, the population in this region should be educated on the risks of bites by *Daboia mauritanica*. To improve outcomes, Medical personnel in this region should be aware of the spectrum of toxicities as well as the management techniques available to treat *Daboia mauritanica* envenomations.

Conclusions: Increased awareness of the population concerning the existence of *Daboia mauritanica* in Tiznit province, the availability of skilled medical providers, and an available, effective serotherapy against this species would decrease the morbidity and mortality due to this envenomation.

Keywords: Envenomation, *Daboia mauritanica*, Tiznit province, Southern Morocco
10.1016/j.toxicon.2012.04.248

248. Venomous Mixtures, Gamma Irradiation and Antivipmyn Africa®

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Background: Antivenoms are the only treatment known to be effective to treat snakebites. Antivipmyn Africa®, produced by Instituto Bioclon, is a polyvalent antivenom against Sub-Saharan snakes with demonstrated safety and efficacy. It is manufactured starting with sera from two groups of horses, one immunized with a mix of viperid venoms (*Bitis arietans, B. gabonica, Echis leucogaster, E. pyramidum* and *E. ocellatus*) and the other to elapid venoms (*Naja haje, N. melanoleuca, N. nigricollis, N. pallida, Dendroaspis polylepis* and *D. viridis*). Those complex venom immunogens can be highly toxic to horses; especially during early phases of immunization. Several papers report that high energy irradiation of venoms could be a practical solution for reducing toxicity of venoms while retaining their immunogenicity. Accordingly, we decided to systematically investigate the effect of gamma radiation on both venom mixes and in their ability to induce protective antibody responses in horses.

Methods: We irradiated, using a Cobalt-60 gamma-ray irradiator, both dry and frozen (dry ice) venom mixes and found that they needed huge irradiation doses to alter venom proteins. However, with venoms in solution

(5 mg/mL, saline) and at room temperature, we found gamma-irradiation doses that decreased the toxicity of venom mixes while the venom protein components remained soluble.

Results: Crude venom mixtures had a LD_{50} (IV route, 18-20 g, CD-1 mice) of 1.15 and 0.48 mg/Kg for viperid and elapid mixes, respectively. The optimal irradiation dose –maximum dose that doesn't cause protein precipitation- for viperid venom mix was 3.1 kGy while that for elapid mix was 5.5 kGy. Under those conditions, the LD_{50} of the viperid venom mix decreased by a factor of 2.8, while that for the elapid mix diminished 3.7 times. Each irradiated immunization mix was used to immunize groups of three horses. None of the horses developed systemic toxicity. Their pooled sera had neutralization potencies comparable to pooled sera collected from horses immunized with untreated venom mixes.

Discussion: Gamma-irradiated detoxified venoms, under well controlled and studied conditions, are a safe and practical alternative for the production of horse serum with high neutralization potency against snake venoms.

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Keywords: African snake venoms, gamma radiation, venoms toxoids
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249. Experience with *Crotalidae* Polyvalent Immune Fab (Ovine) for a non-North American Rattlesnake Envenomation

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Background: There is little experience using CroFab® to manage non-North American pit viper envenomation.

Methods: We report a case using CroFab® for a South American rattlesnake (*Crotalus durissus vegrandis*) envenomation.

Results: A 22-year-old male presented to a southern California hospital after a bite by an Uracoan rattlesnake to his ring finger, which occurred at 01:30 on 08/13/2011. The snake was imported from Venezuela, and herpetologists confirmed species. Initial labs (CBC, PT/INR, fibrinogen, and CK) were normal except fibrinogen, which was 143 mg/dL (reference 200-400). He was treated with CroFab® 5 vials and transferred to our emergency department. Upon presentation to our facility 5 hours post bite, the patient complained of marked pain, swelling and tenderness to the antecubital fossa. He was nauseated and vomited once. Another 4 vials of Crofab® were given at 08:15. Afterwards labs were significant for thrombocytopenia 52 bil/L (reference 140-340) and fibrinogen 98 mg/dL . An additional 4 vials of CroFab® were administered at 12:15 with improvement of both platelet count- and fibrinogen level. At 20:55, the patient complained of increased pain and swelling to the bite area so an additional 4 vials were given at 21:10. At 18:32, he complained of increased swelling and pain to the left forearm and received another 4 vials of CroFab® at 19:31. On day 3, all clinical and laboratory parameters had stabilized and the patient was discharged. He followed up on day 5 and all labs were normal. He returned again on day 7 for a second follow-up, and was found to have thrombocytopenia recurrence, with a platelet count of 111 bil/L. Fibrinogen and hemoglobin were normal. He was admitted to our observation unit and 4 vials of CroFab® were administered. The next morning all lab parameters were improving and continued to do so. Patient was discharged home on day 9. He returned once again on day 11, at which time his labs were normal and local effects had completely resolved.

Discussion: CroFab® is FDA-approved for envenomations by any North American pit viper even though it is made using the venom from only four species. Antivenoms generally have varying degrees of cross-protectivity against venoms not utilized in their manufacture. Although tested in murine LD50 models, there is very little clinical experience with CroFab® for envenomation by anything other than a US Crotalinae.

Conclusion: Further investigation of the use of CroFab® in non-North American vipers is warranted.

Keywords: Crotalidae, exotic, *Crotalus durissus vegrandis*
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250. Critical Shortage of Coral Snake Antivenom is Impacting Patient Care

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Background: Envenomation by Micrurus fulvius is rare. Approximately half the bites reported in the US occur in Florida. Since manufacture of North American Coral Snake Antivenin® was discontinued in 2003, supplies have been dwindling. We report three cases of envenomation with delayed treatment due to supply problems.

Case One: A 39 year old presented approximately 30 minutes following a bite between his thumb and forefinger. He remained relatively asymptomatic for almost 14 hours, whereupon he developed dysphagia and ptosis. Antivenom was believed to be unavailable. He was intubated and placed on a ventilator 19 hours post-bite, at which time 6 vials of antivenom were administered. Attempts to extubate him on days 3, 6 and 12 failed; requiring re-intubation each time. He was successfully extubated on day 24 and discharged home at 30 days post bite.

Case Two: A 6 year old was bitten on the right index finger 45 minutes prior to presentation. He had an episode of emesis prior to arrival, but was asymptomatic at time of transfer to a tertiary facility. Despite administration of 5 vials of antivenom, he was intubated and placed on a ventilator due to difficulty handling oral secretions. The hospital course was complicated by hypotension and aspiration pneumonia. Extubation and discharge occurred on days 13 and 15 respectively.

Case Three: A 41 year-old presented approximately five hours after being bitten by a coral snake on the right 3rd finger. Initial symptoms were interpreted as tongue swelling, potentially an allergic response. He was paralyzed and intubated, and placed on a ventilator; then transferred to a tertiary care facility. The patient was extubated after admission to the intensive care unit but almost immediately needed reintubation due to respiratory failure. He received 5 vials of antivenin. He remained intubated for a total of 9 days, was hospitalized for 19 days and discharged to a skilled rehabilitation facility.

Discussion: Rapid administration of antivenom is important for the prevention of respiratory paralysis. All three patients experienced delays in treatment due to real or perceived shortages of antivenom, necessitating costly intensive care. Early administration of antivenom can prevent the clinical signs of envenomation, and patients may be discharged within 24 hours.

Conclusions: The shortage of antivenom has reached a critical point. Patient care has been compromised due to inadequate supplies. The timely administration of antivenom is life saving and prevents the need for costly intensive care.

Keywords: Coral snake, antivenom, shortage
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251. Acute Hypersensitivity Reaction Following Administration of Crotalidae Polyvalent Immune Fab Antivenom: A Case Report

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Introduction: Crotalidae polyvalent immune Fab (ovine) (*CroFab*®) is commonly used in the treatment of symptomatic North American crotaline snake envenomation. When approved by the U.S. Food and Drug Administration in 2000, the incidence of immediate hypersensitivity reaction rate from *CroFab*® was reported as up to 19%. Recent systematic literature review and meta-analysis showed that it appears to be lower than previously reported, at 0.08. We describe the case of acute hypersensitivity reaction to *CroFab*® in a patient bitten by Copperhead.

Case history: The present case study depicts the envenoming of a 71-year-old female who was bitten on her left index finger. She presented in Emergency Room with complaints of severe extremity pain and puncture wound, surrounded with edema and ecchymosis. Approximately 2 hours after being bitten, edema and ecchymosis had reached patient's shoulder. She complained of the metallic taste in her mouth as well. Patient's past medical history significant for multiple medical problems. Patient had multiple drug allergies. No prior history of anaphylaxis/anaphylactoid reaction. No prior history of envenomation. Our treatment team decided it was in patient's best interest to proceed with *CroFab*®. Patient was premedicated with methylprednisolone and diphenhydramine. Infusion was started with initial dose of 4 vials in 250 ml normal saline. Within seconds of infusion of *CroFab*®, the patient cardiorespiratory arrested. CPR was initiated and endotracheal intubation was performed with great difficulty due to laryngeal edema. Patient was also given epinephrine and pulse returned, although she remained hypotensive for several hours in the Intensive Care Unit. Patient remained intubated for approximately 24 hours and weaned off ventilator without difficulty. She received methylprednisolone, diphenhydramine, and ranitidine drip. The patient was discharged after 48 hours, with clinical improvement and with residual edema and ecchymosis of the left upper extremity but without significant coagulopathy or signs of local infection, and subsequent follow-up revealed no sequelae.

Discussion: Although serious hypersensitivity reactions to *CroFab*® are exceedingly rare, this case highlights the importance of monitoring patients very carefully in critical care setting when giving these type products.

Conclusion: The administration of antivenom is important for saving lives and limbs with an acceptably low risk.

References

Schaeffer, T.H., Khatri, V., Reifler, L.M., Lavonas, E.J. 2012. Incidence of Immediate Hypersensitivity Reaction and Serum Sickness Following Administration of Crotalidae Polyvalent Immune Fab Antivenom: A Meta-analysis. *Acad Emerg Med. 2012 Feb;19(2):121-31* Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22320362.

Keywords: Antivenom, hypersensitivity reaction, envenomation
10.1016/j.toxicon.2012.04.252

252. Coral Snake Antivenin's Deadly Deadline

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Background: The eastern coral snake (*Micrurus fulvis fulvis*) and the Texas coral snake (*M. fulvius tenere*) account for roughly 100 snakebites a year. Antivenin (*M. fulvis*) (Equine Origin), the only antivenom licensed in the U.S. for coral snake bites, is no longer manufactured by Wyeth pharmaceuticals. The expiration date for Lot 4030026 was 10/31/2008 and has been extended multiple times to the current deadline of 10/31/12. Not FDA approved and therefore not readily available, Anticoral (Inst. Clodomiro Pocado, Costa Rica) and Coralmyn (Inst. Bioclon, Mexico) have offered promising results in counteracting effects of *M. fulvius* venom in rodents. Prior to coral snake antivenom, the death rate was approximately 10% due to cardiovascular and/or respiratory failure.

Case Report: A 54-year-old female presented to the Emergency Room after having been bitten by a coral snake 1 hour previously. The patient was alert and in marked

distress secondary to left ankle pain, dysphonia, and difficulty swallowing. The patient's past medical history was pertinent only for hypertension, with no history of snake envenomation or exposure to antivenom. Auscultatory findings and neurologic exam were within normal limits but she subsequently developed respiratory distress. Vital signs were stable. Expired Wyeth Coral Snake Antivenom was acquired through assistance from Poison Control. Patient pre-medicated with diphenhydramine and methylprednisolone. At the completion of the infusion of 5 vials of 50 ml, she required endotracheal intubation and ventilator support. Shortly thereafter, the patient was transferred to a higher-level care facility.

Discussion: With the impending deadline approaching yet again, the already low FDA approved coral snake antivenom availability is soon to run out. Patients will need to be intubated and ventilated while the toxin wears off, leading to increased morbidity and mortality.

Conclusion: While coral snake bites are rare, antivenom implementation is necessary for the patient's safety, as well as cost-minimization.

References

Patient's medical history.
Norris, R.L., 2011. *Coral Snake Envenomation.* Retrieved from http://emedicine.medscape.com/article/771701-treatment#a1126.
U.S. Food and Drug Administration 2011. *Vaccines, Blood & Biologics: Expiration Date Extension for North American Coral Snake Antivenin (Micrurus fulvius) (Equine) Lot 4030026 Through October 31, 2012.* Retrieved from http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/ucm155092.htm.

Keywords: Coral snake antivenom, neurotoxicity, respiratory support
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253. Medically Significant Late Bleeding Following Treated Crotaline Envenomation: A Structured Topic Review

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Background: Recurrent and delayed-onset thrombocytopenia and defibrinogenation are well-described following crotaline snakebite treated with crotalidae polyvalent immune Fab (ovine) antivenom (FabAV). However, the likelihood that a patient will develop medically significant bleeding is poorly understood.

Methods: The authors searched PubMed, Ovid MEDLINE and EMBASE from January 1, 1997 to February 6, 2012 to identify all published cohort studies of patients envenomated by North American crotaline snakes who were treated with FabAV. Two content experts independently reviewed full-text articles and extracted data about study design, cohort, treatment, follow-up, and outcomes, which were then reconciled for errors and analyzed using descriptive statistics. In addition, case reports of bleeding that began after initial control of the envenomation syndrome were identified. Late bleeding was defined as bleeding that began or recurred after initial control of the envenomation syndrome. Medically significant late bleeding was defined *a priori* as late bleeding that was treated with blood transfusion, vasoactive drug infusion, or surgery, required re-hospitalization, or was temporally associated with a reduction in serum hemoglobin of $\geq$ 3 g/dL, reduction of hematocrit of $\geq$ 8 percent, permanent or temporary disability, or death. Summary incidence and 95% confidence interval (CI) of late and medically significant bleeding were calculated using a random-effects Poisson regression model.

Results: Eighteen unique cohort studies were identified. Five studies utilized prospective data collection. In 11 studies, patients were followed actively after hospital discharge. A total of 901 subjects were enrolled in these cohort studies. Of these, 8 subjects were reported to have late bleeding with an estimated summary incidence of 0.009 (95% CI 0.003 to 0.023), including 5 subjects with medically significant late bleeding, and an estimated summary incidence 0.006 (95% CI 0.002 to 0.019). Three patients received red cell transfusion; none suffered permanent sequelae. For two patients, detailed clinical manifestations, treatment, and laboratory test results were available for analysis. Three additional cases of late bleeding were identified in case reports; all were medically significant, including one death.

Conclusion: Although recurrent and delayed-onset thrombocytopenia and defibrinogenation are common following treated crotaline snakebite, medically significant late bleeding appears to be very uncommon.

Keywords: Recurrence, bleeding, hematologic effects, antivenom
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254. Failure to Develop Sensitization Despite Repeated Administration of Ovine Fab Snake Antivenom: A Single-Patient, Multi-Center Case Series

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Background: Immediate (anaphylactic and anaphylactoid) hypersensitivity reactions are a known complication of antivenom therapy. Few data exist about repeated exposure to ovine Fab antivenom in the same patient. We report our experience treating a 41-year old man with more than 60 documented instances of envenomation both native and non-native venomous snakes over a 20-plus

year period, including repeated administration of equine IgG, ovine Fab, and various non-native snake antivenoms.

Methods: The poison center and, when available, hospital medical records of snakebites involving this patient were reviewed, with detailed review of those since 2001.

Results: We identified 13 instances since 2001 in which the patient received ovine Fab antivenom (FabAV), beginning in an open-label clinical trial in 1993 and including two separate incidents treated at different hospitals over a 9-day period in 2011. During the most recent treatment episodes, the patient did not receive premedication with antihistamines or corticosteroids. During close observations, no signs or symptoms of acute hypersensitivity were observed. Because the patient was noncompliant with follow-up, data about serum sickness are not available. Two previous episodes of hypotension prior to antivenom administration may reflect Type 1 hypersensitivity to venom.

Discussion: Sensitization to foreign proteins causing Type 1 hypersensitivity reaction is a concern with repeat administration of antivenom to the same patient. Ovine Fab antivenom is produced with a multi-step purification to remove proteins other than venom-specific Fab-fragments. However, each vial contains up to 1 gram of total protein, most of which is 50 kDa Fab fragments. Within the limitations of our data, it appears that this patient has not become sensitized despite serial exposure to FabAV, but may have developed hypersensitivity to venom.

Conclusion: Some patients tolerate repeated administration of ovine Fab antivenom without evidence of hypersensitivity.

Keywords: Antivenins, hypersensitivity, crotalid venoms, snake bites
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255. Local Damage produced by *Vipera* and *Macrovipera* Venoms and Some Immunochemical Characteristics

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Background: The information on the toxic activities is not abundant, by this reason, the toxic activities of *Vipera (V) aspis aspis (Vas), V. ammodytes ammodytes (Vaa), V. amodytes montandoni (Vam), V. berus (Vb), V. xhantina (Vx), Macrovipera (M.) lebetina obtusa (Mlo)* and *M. schweizeri (Ms)* venoms were studied.

Material and Methods: The hemorrhagic, necrotizing and inflammatory-edematogenic activities were determined in rats (Wistar). We also performed a histological study of lesions in animals injected intramuscularly (*extensor digitorum longus*) with sub-lethal doses of the venoms. The reactivity with homologous and heterologous antivenoms was studied.

Results: All venoms exhibited some hemorrhagic activity, the most hemorrhagic being *Vb* (~ 12 MHD/mg) and *Mlo* (~ 6 MHD/mg). Those with lowest hemorrhagic activity were the venoms of *Vama* (~ 2.5 MHD/mg) and *Vamm* (~ 1.6 MHD/mg). The venoms of *Vb, Vx, Vas, Mlo* and *Ms* venoms produced skin necrosis (studied as MND). Inflammation – edema was observed in all venoms in different degrees. The venom with the lowest inflammatory activity was *Vx* (comparable to the controls) and those with the highest activity were *Ms* and *Vb* (two fold over controls). No activity on hemostasis (plasma and fibrinogen) was observed. In all cases venoms were somewhat myotoxic. After 24 h, rats injected intramuscularly were sacrificed and the injected muscle and contralateral control (injected with the same volume of NaCl 0.15 M) were processed for optical microscopy and stained with H & E. Venom of *Mlo* caused acute inflammation, necrosis and hemorrhage of intermediate magnitude whereas that of *Ms* showed lower acute inflammation, hemorrhage and necrosis. *Vaa* caused important acute inflammation, edema and hemorrhage and necrosis while that of *Vam* caused necrosis and acute inflammation but low edema and hemorrhage. *Vb* venom caused hemorrhage, edema and acute inflammation but necrosis was lower. *Vas* venom caused acute inflammation, edema and necrosis but intermediate hemorrhage and that of *Vx* caused considerable edema and hemorrhage but intermediate necrosis. All venoms cross-reacted *in vitro* with anti-*Vipera*, anti-*Bothrops* and anti-*Crotalus durissus terrificus* antivenoms.

Discussion: In some cases the effect observed in muscles could not be detected by the common assays for determining hemorrhage and necrosis. Local lesions caused by the venoms were no correlated with their systemic toxicity (lethal dose). Although intramuscular injection is not the most common inoculation route in viper envenomation, the possibility of muscular lesions should be considered in bites by these species, since in all the cases local damage was observed.

Keywords: *Vipera*, *Macrovipera*, myotoxicity, venom, snakes
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256. A Rapid Reconstitution Method for CroFab® Polyvalent Immune Fab (ovine)

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Background: Reconstitution of CroFab® lyophilized drug product is performed according to its current approved US labeling, which requires the use of 10 mL sterile water for injection followed by up to a minimum of 36 minutes of gentle swirling of the vial. CroFab® has been clinically demonstrated to be most effective when administered within 6 hours of snake envenomation, and improved clinical outcomes are correlated with quicker timing of administration.

Methods: An analytical study was designed to compare the physicochemical properties of 3 separate batches of CroFab® when reconstituted using the standard procedure (10 mL WFI with gentle swirling) and a modified rapid procedure using 18 mL 0.9% saline and manual inversion. The physical and chemical characteristics of the same 3 batches were assessed using various analytic methodologies associated with routine quality control release testing. In addition further analytical methodologies were applied in order to elucidate possible structural changes that may be induced by the changed reconstitution procedure.

Results: Batches A, B, and C required mean reconstitution times of 25 min 51 secs using the label method and 3 mins 07 secs (88.0% mean decrease) using the modified method. Physicochemical characteristics (color and clarity, pH, purity, protein content, potency) were found to be highly comparable. Characterization assays (dynamic light scattering, analytical ultracentrifugation, LC-MS, SDS-PAGE and circular dichroism spectroscopy were also all found to be comparable between methods.

Discussion: When comparing CroFab® batches that were reconstituted using the labeled and modified methods, the physicochemical and biological (potency) characteristics of CroFab® were not significantly changed when challenged by the various standard analytical methodologies applied in routine quality control analysis. Additionally, no changes in the CroFab® molecule regarding degradation, aggregation, purity, structure, or mass were observed.

Conclusion: The analyses performed support the use of the more rapid reconstitution method using 18 mL 0.9% saline in order to allow a significantly reduced time to administration of CroFab® to patients in need.

Keywords: Antivenoms, polyvalent immune fab, crotalid envenomation
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O. Spiders

257. Comparative Analysis of Transcriptomes of *Phoneutria pertyi* and *P. nigriventer* Venom Glands

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Background: Species of the genus *Phoneutria* known as "aranha-armadeira" or armed spiders are responsible for a large number of spider bites in Brazil. Until recently, all studies on the venom of the genus have been restricted to the species *P. nigriventer*. However, some recent proteomic studies revealed that other species venoms also contains a wide variety of proteins and peptides, including neurotoxins which act on the ion channels and chemical receptors of the neuro-muscular systems of insects and mammals. Thus, these venoms have emerged as invaluable tools for research, drug discovery and drug development with application in medicine and agriculture.

Methods: In order to find novel venom components with biological activity and to provide a database for comparative study with the previously described *P. nigriventer* venom gland transcriptome, we constructed a plasmidial cDNA library from the species *Phoneutria pertyi* mRNA venom gland to generate Expressed Sequence Tags (ESTs) data.

Results: After editing, 710 good quality reads were clustered and 295 unique sequences were obtained (106 contigs and 189 singlets). Of these, 197 (67%) had a high degree of homology to spiders toxins deposited in the Uniprot database, most are *P.nigriventer* toxins isoforms. We observed that *P. pertyi* venom gland transcriptome were more abundant in insecticidal toxin sequences than *P. nigriventer* one. We also found new sequences for putative toxins in *P. pertyi* transcriptome, indicating that they can be novel toxins.

Conclusions: These results show that although these spider venoms contain a similar range of toxins isoforms, the expression levels of each type of toxins are different, and also they contain toxins with unique sequences, what can suggest adaptation to different environments.

Keywords: *Phoneutria* venom gland, transcriptome, neurotoxin
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258. Unusual Cases of the Spider *Cheiracanthium Punctorium* Biting in Volgograd Region, Russia

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Background: Beginning in 2009 cases of bites by the spider *Cheiracanthium punctorium*, were reported in the Volgograd region of Russia. There had been no previous reports of bites by this species in that geographic area. The aim of this study was characterization of the epidemiology and clinical picture of such envenomations.

Methods: Retrospective analysis of 19 case reports of inpatients in Volgograd Regional Poisoning Treatment Center in 2010-2011. Only cases of confirmed bites by *Cheiracanthium punctorium* were included. Identification of the spider was made by qualified specialists in the Volgograd State University.

Results: Cases of biting of poisonous animals in Russia are responsible for 1,9% of all poisoning cases in patients admitted to toxicological treatment centers. According to data from 29 centers in 2010 549 (43,4%) patients were admitted with snake bites, 693 (54,8%) with poisonous insect and spider bites, and 23 (1,8%) after contact with poisonous sea animals. Bites of the common viper, *Vipera Berus*, occur over the entire country. In addition, in territories bordering Central Asian countries, including Volgograd region, the bites by tarantulas and black widow spiders are regularly reported. The number of patients admitted to the Volgograd Regional Poisoning Treatment Centre with poisonous spider biting for the last two years was 111,

including 19 with *Cheiracanthium punctorium*. During the first minutes after the bite by *C. punctorium,* 90% of patients reported stabbing and burning pain, with 100% reporting reddening and swelling at the site of the bite. Systemic effects were noted in 70 % of cases, manifested by nausea, vertigo, and general weakness. Pain elsewhere in the body occurred in 30%, including low back pain, extremity pain, and headache. Allergic reactions, manifested as shock and Quincke's edema, was seen in one patient, with urticaria and allergic dermatitis seen in 2 patients. Twelve patients demonstrated insignificant leukocytosis with increased numbers of neutrophils and eosinophils, as well as increased transaminases and amylase in two patients. Therapy was symptomatic and included non-opioid analgesics, antihistamines, and corticosteroids as indicated. Treatment was continued in mild cases for an average hospital stay of 4 days, and for 7 to 9 days in more serious case. Outcomes in all cases were favorable with no skin ulceration and no long-term sequelae**.**

Discussion: Human bites by the spider *Cheiracanthium punctorium* are known in Europe and the Americas, whereas its appearance in Russia has not previously been reported. One possible explanation is anomalously hot weather during the summer months for the past 2 years. The clinical picture demonstrated by patients in our region corresponds to those in the published literature**.**

Conclusion: Poisoning by *Cheiracanthium punctorium* venom could be topical not only for Volgograd region, but also other regions of Russia, if anomalously hot summer temperatures continue to occur. There does not appear to be a significant risk of death and the management is symptomatic and supportive.

Keywords: *Cheiracanthium Punctorium* poisoning
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259. Molecular Basis for the Interaction of Tarantula Toxin Jingzhaotoxin-III (β-TRTX-Cj1α) with the Voltage Sensor of Kv2.1 Channel

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Background: Animal venoms contain a fascinating array of diverge peptide toxins that have cross-activities of different types of voltage-gated ion channels. However, the underlying mechanism remains unknown. Jingzhaotoxin-III (JZTX-III), a 36-residue peptide from the tarantula *Chilobrachys jingzhao,* can selectively inhibit both Nav1.5 and Kv2.1 channels without affecting the majority of other ion channel subtypes. JZTX-III could trap Nav1.5 DII voltage sensor at closed state by binding to DIIS3-S4 linker**.**

Results: In this study, we report that JZTX-III docked on Kv2.1 voltage sensor paddle. Ala-mutation of single residue of Phe274, Lys280, Ser281, Leu283, Gln284 or Val288 can reduce the effects of JZTX-III evidently. Among them, S281 is the most crucial residue and the substitution with Ala, Phe, Ile, Val or Glu increased the IC_{50} value by over 34-folds.

Discussion: Interestingly, the bioactive surfaces of JZTX-III interacting with Kv2.1 and Nav1.5 are very different from each other. Although a hydrophobic patch formed by Trp8, Trp28 and Trp30 is important for JZTX-III interaction with both channels, the patch conserved among other inhibitors of Kv2.1 has been shown to play a critical role in partitioning into lipid membrane. While three residues Asp1, Glu3 and Trp9 are critical only for inhibition of Nav1.5, three charged residues (Arg13, Lys15, and Glu34) and one hydrophobic residue (Val33) are only crucially involved in interaction with Kv2.1**.**

Conclusions: These results strongly supported that animal toxins might use different bioactive surface to target the voltage sensor paddles of different ion channels. Our findings provided new molecular insights for the design of more specific and more potent ion channel inhibitors.

Keywords: Spider toxin; voltage-gated ion channel; ion channel inhibitors
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260. Hainantoxin-III Inhibits Voltage-Gated Sodium Channel Nav 1.7 and Extenuates Inflammatory Pain in Animal Models

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Background: Voltage-gated sodium channels (VGSCs) are important for nociceptive signaling and are potential targets for treating pain. Among nine VGSCs subtypes, Nav1.7 is preferentially expressed in dorsal root ganglion (DRG) and sympathetic ganglion neurons. The important roles Nav1.7 plays in inflammatory pain have been relatively well established**.**

Results: Here, we demonstrated that hainantoxin-III (HNTX-III), purified from the venom of spider *O. hainana,* could selectively inhibit tetrodotoxin-sensitive (TTX-S) in rat DRG cells. Hainantoxin-III is a 33-residue polypeptide with three disulfide bridges and adopts inhibitor cystine knot (ICK) motif, imparting it extraordinary stability. Hainantoxin-III was able to inhibit hNav1.7 expressed in HEK 293 cells through a novel mechanism identical to huwentoxin-IV that could inhibit Nav1.7 by trapping DII S4 voltage sensor on the closed state. Furthermore, nanomolar HNTX-III administrated by intramuscular injection could extenuate inflammatory pain on formalin test and abdominal constriction (writhing) test. Its analgesic effect would compare favorably with that of morphine and wasn't associated with motor side effects even at 20-folder higher dose**.**

Conclusion: HNTX-III would be a novel probe useful in the investigating TTX-S VGSCs including Nav1.7 and an

interesting lead that may be helpful in designing novel drugs for pain treatment.

Keywords: VGSCs, Nav1.7, spider, hainantoxin-III, antinociceptive
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261. Expanding Structural and Functional Diversity of Spider Venom Compounds

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Background: Spiders are masters of venom production. The diversity of both the chemistry and pharmacology of spider venom compounds is striking. In single spider venom, several hundred different components may be identified, varying from inorganic salts to high-molecular-mass proteins. These components aim at a large number of targets in the prey/aggressor organism**.**

Results and Discussion: We shall summarize our recent progress in the field. (i) It becomes obvious that spider venom peptides not only hit the "classical" targets, the vital components of the nervous signaling mechanisms, but also affect many "non-classical" targets, such as sensory receptors. The recently identified purotoxins modulating mammalian purinergic receptors present prospective analgesic drug leads. (ii) The combinatorial libraries of toxins found in spider venom generate novel modes of action. A good example is the family of insect-selective β/δ-agatoxins from the spider *Agelena orientalis*. These toxins possess the ability to modulate both the activation and inactivation processes in insect sodium channels, resembling both α- and β-toxins of scorpions. (iii) "Traditional" peptide toxins from spider venom show neurotoxicity. It seems that evolution created equally effective cytolytic peptides. The "cytolytic champion" spider *Lachesana tarabaevi* produces a host of linear peptides forming a dozen of families. Their activity screening resulted in identification of antimicrobial substances exhibiting potent anti-*Chlamydia* effects. (iv) A new level of complexity is introduced by the so-called "modular" toxins. They combine in their structure two modules, or domains, each corresponding to a "usual" spider toxin. We describe several modular toxins with different domain arrangement**.**

Conclusion: The diversity of spider venom compounds is ever-expanding and further research is greatly anticipated.

Keywords: Spider venom, neurotoxin, cytolytic peptide
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262. Spider Venom Components Affecting the Function of Purinergic Receptors

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Review: Purinergic P2X receptors are membrane ion channels that open in response to the binding of extracellular ATP. These receptors can be involved in pain mechanisms and thereby constitute possible targets for analgesic drugs. Until recently no natural venom or its individual components were known to affect P2X receptors. The first natural peptide, which exerts selective inhibitory action on P2X3 receptors, was purotoxin-1 (PT1) isolated from the venom of the Central Asian spider *Geolycosa* sp. PT1 contains 35 amino acid residues with four intramolecular disulfide bridges. A novel polypeptide named purotoxin-2 (PT2) from the same spider venom consists of 64 amino acid residues with four disulfide bonds and features C-terminal amidation. PT1 and PT2 produce inhibitory effects on P2X3-mediated currents via stabilization of the desensitized state of the P2X3 receptor. Six structural homologues of PT1 were found in EST databases generated from venom glands of various spiders. For functional studies, six recombinant analogs of PT1 from different spiders were produced. PT1 analogs were studied on P2X2, P2X3, and P2X2/3 receptors in rat DRG neurons. Out of six PT1 analogs two peptides were inactive. It was found that one recombinant peptide inhibited P2X2-generated currents, three analogs showed stable inhibitory action on P2X3 receptors, and one of them also produced a small potentiating effect on the current generated by P2X2/3 receptors. So, spider venoms contain a family of polypeptides possessing powerful and selective inhibitory action on P2X receptors. Some of these polypeptides demonstrate marked antinociceptive activity.

Keywords: P2X receptors, spider venom, purotoxins
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263. Molecular Cloning and Characterization of a Sphingomyelinase D from *Loxosceles adelaida*, a Brazilian Brown Spider from Karstic Areas

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Background: Envenomation by spiders belonging to the genus *Loxosceles*, found in temperate and tropical regions of America, Africa and Europe, commonly results in impressive local necrotic skin lesions and can also cause systemic effects, including intravascular hemolysis. *Loxosceles* is the most poisonous spider in Brazil and three different synanthropic species of medical importance are known, *i.e.*, *L. intermedia, L. gaucho, L. laeta*, and more than 6000 cases of envenomation by *L. intermedia* alone are reported each year. We have purified, characterized, cloned and expressed the toxins from *L. intermedia* and *L. laeta* venom that are responsible for all the local and systemic effects induced by the whole venom. Highly homologous proteins with

sphingomyelinase activity (SMase D) were able to induce all the local and systemic effects induced by whole venom. *Loxosceles* species are present in several different habitats, including the karstic environment, and in Brazil it is the most common troglophile arachnid. The aims of the present study were to clone and express SMases D from the spider gland of *L. adelaida*, captured in the caves of PETAR (Brazil), to compare the functional activities of the recombinant proteins with toxins from synanthropic species and to investigate the inter- and intra-species cross-reactivities of antibodies raised against the purified recombinant proteins.

Methods: The gene was amplified by RT-PCR and cloned into a prokaryotic expression vector pRSET*A*. The recombinant protein was compared with the previously characterized SMases D from synanthropic spiders, in terms of their biological, biochemical and structural properties.

Results and Discussion: The *L. adelaida* SMase D A1 cDNA exhibits similarity with previously characterized SMases D. Antiserum produced against the recombinant SMases D from *L. intermedia* and from *L. laeta* was highly cross-reactive against *L. adelaida* SMase D A1, and also exhibit a high level of recognition to SMases present in *L. adelaida and L. gaucho* venoms. SMase D A1 is able to induce a typical dermonecrotic reaction and transform erythrocytes into activators of the autologous complement system, but with lower toxic potential than the SMases D from synanthropic species. Based on sequence and structural similarities, the SMases D can be grouped into two classes depending on the presence of an additional disulphide bridge between the catalytic loop and flexible. *L. adelaida* SMase D A1 is a class II member and conserves all structural features for catalytic activity upon sphingomyelin.

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Keywords: *Loxosceles adelaida*, venom, sphingomyelinase D
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264. Milking and Partial Characterization of *Pamphobeteus spp* (Aranae; Theraphosidae) Venom, from the Colombian Andean Region

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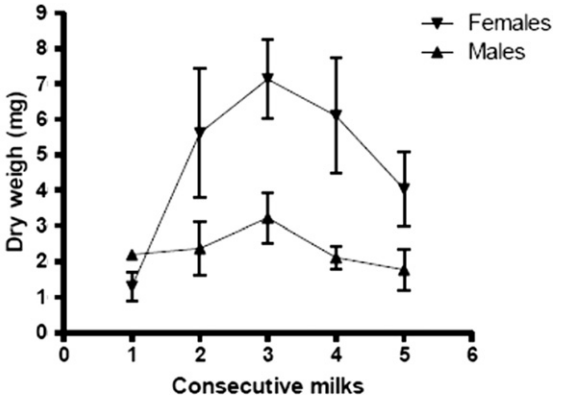
Background: In this work we report the first studies of milking and partial characterization of the *Pamphobeteus* venom made in Colombia using techniques as indirect hemolytic and coagulant activity, electrophoresis, HPLC and peptides identifications. Different reports shows that this venoms are a rich mixture of salts, nucleotides, free amino acids, neurotransmitters, peptides, proteins and enzymes affecting invertebrates and vertebrates. They affect mainly the central nervous system due the content of neurotoxins affecting ionic channels at pre and postsynaptic level and neurotransmitter exocytosis.

Methods: The specimens used in this study come from the Andean region of Colombia (Antioquia province) and where maintained in captivity with food and water ad libitum. 12% PAGE-SDS electrophoretic gels were used runed at 150V for 60 minutes. A HPLC reverse phase system (RP-HPLC) with a C-18 column was used to obtain the chromatographic profile. N-terminals were obtained using a HPLC-nESI-LC/MS system. For partial biochemical characterization, hemolytic (egg yolk method) and coagulant activity were used according with Gutierrez et al and Theakston and Reid respectively.

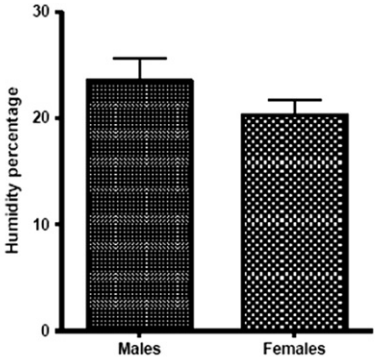
Results: After each sider milking, a low viscosity, colorless, with a density of 1.01 mg/μl and a pH of 5 was obtained. In all cases female quantity of venom obtained were larger regarding male venom quantity (20% wet weigh and 37% dry weigh lager). The humidity percentage did not show a significance difference between males and females (20.45±1.68 %). The electrophoretic profile showed the presence of low molecular compounds (5-15 kDa range) that usually corresponds to peptides and some neurotoxins. In the chromatographic profile we obtained well-defined fractions that allowed a posterior identification of its N-terminal through a HPLC-nESI-LC/MS system. After a BLAST, we found that this fraction has a high homology with ESTx neurotoxins (Eurypelma-spider-toxins) that affect mainly calcium channels and with *Lychasmucronatus* scorpion genus neurotoxins that affect mainly potassium channels. The biochemical assays indicates that this venoms exhibit a catalytic activity more over the neurotoxic activity based the proteins similarity.

Discussion: The Colombian *Pamphobeteus* spider venom shows general content similarities regarding other theraphosinae spiders. The high amount of venom yielded by females may be related with sexual dimorphism expressed in this species. Non-expected catalytic and coagulant activities were showed in this venom.

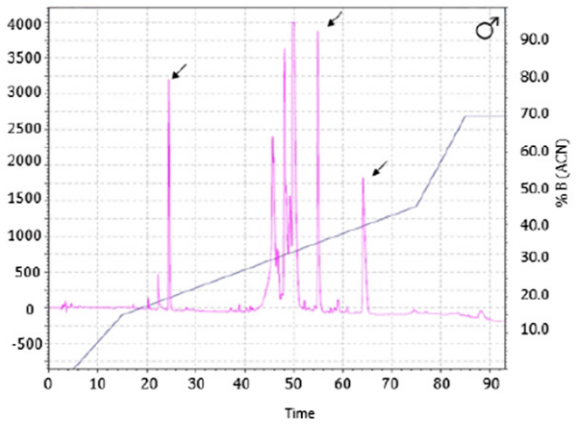
Conclusion: The BLAS indicates that this venom has a neurotoxic effect mediated through ionic channels. The catalytic activity could indicate that spiders uses the venom not only to paralyze they pry but for predigest it as well.



Difference in the venom amount yield between males and females



Difference in the humidity percentage between males and females. P>0.05



Chromatographic profile of the *Pamphobeteus* venom using a RP-HPLC, C18 column, ACN + TFA 0,01% 215 nm

Table

Physicochemical properties	Result
Color	Colorless
Density	1.01 mg/μl
pH	5
Solubility in aqueous solvents	100%

Keywords: Pamphobeteus, neurotoxic, catalytic
10.1016/j.toxicon.2012.04.265

265. Poke but Don't Pinch: Risk Assessment and Defensive Behaviors of the Western Widow Spider (*Latrodectus hesperus*)

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Background: The defensive behaviors of the Western Widow Spider (*Latrodectus hesperus*), a medically relevant, synanthopic species found throughout western North America, are poorly understood.

Methods: To elicit defensive behaviors, wild-caught adult females (N = 43) were either prodded once (low threat), prodded repeatedly (medium threat), or pinched repeatedly (high threat) with gelatin "fingers" in a repeated measures design. We collected data on defensive behaviors that were non-aggressive (retracting limb(s), fleeing, playing dead) and aggressive (silk flicking, biting).

Results: The proportion of spiders whose first response to provocation was non-aggressive was 100% across all threat levels. Besides flight, the most common defensive behavior elicited in low, medium, and high threat conditions was retraction (16%), silk flicking (56%), and biting (59%), respectively. Spiders were significantly more likely to bite ($p<.0001$) "fingers" when pinched (59%) than when prodded singly (0%) or repeatedly (2%). Number of bites delivered (mean +/- SD) was significantly greater for pinched (2.7 +/- 3.6) than repeatedly prodded (0.02 +/- 0.15) spiders. Spiders were significantly more likely to flick silk at fingers when pinched (43%) or prodded repeatedly (56%) than when prodded singly (5%). Number of silk flicks was also significantly greater for pinched (1.8 +/- 2.9) and repeatedly prodded (3.7 +/- 5.5) than for singly prodded (0.05 +/- 0.21) spiders.

Conclusions: We conclude that *L. hesperus* is first non-aggressive, but if compressed between two object will bite defensively. This helps to confirm the idea that the Western Widow Spider is generally non-aggressive. Additional results from studies underway will be presented, including, but not limited to, defensive venom metering and ontogenetic development of defensive behaviors.

Keywords: *Latrodectus hesperus*, defensive behaviors, risk assessment, ontogeny
10.1016/j.toxicon.2012.04.266

266. C5a Receptor is Cleaved by Metalloproteases Induced by Sphingomyleinase D in *Loxosceles* Spider Venom

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Background: Neutrophils are involved in numerous pathologies and are considered to be one of the main factors contributing to the establishment of cutaneous loxoscelism after envenomation by the *Loxosceles* spider. Neutrophils are attracted to the site of envenomation by locally generated C5a and contribute to the tissue destruction. We have investigated the effects of this spider venom on the receptor for C5a: C5aR/CD88, a seven transmembrane G-protein coupled receptor.

Results: We show here that the *Loxosceles* venom induces the cleavage of the C5aR at two sites, resulting in

the release of the extracellular N-terminus, while retaining part of the transmembrane regions. Using specific inhibitors it was shown that the cleavage was induced by activation of an endogenous metalloproteinase of the adamalysin (ADAM) family, which was activated by the sphingomyelinase D in the venom. Although it resulted in the near complete loss of the N-terminus, C5a was still able to interact with the C5aR and induce a small increase in intracellular calcium and the secretion of IL-8.

Discussion: The cleavage of the C5aR may well be a protective response after envenomation, rather than contributing to the pathology of *Loxosceles* envenomation and may represent a general mechanism of how the body protects itself against excess C5a generation in circumstances such as sepsis. Supported by FAPESP and CNPq.

Keywords: C5a receptor, *Loxosceles* spider venom, adamalysin, sphingomyelinase D, dermonecrosis
10.1016/j.toxicon.2012.04.267

267. A Survey of Venom from the Spitting Spider *Scytodes* includes Novel Toxins

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Review: Spiders in the family Scytodidae are found worldwide, include hundreds of species and are known for their unique prey capturing technique – spitting a zig-zag of glue to tether prey to a surface. The effectiveness of this sticky mixture is based on a combination of contraction and adhesion thereby immobilizing prey long enough that the spider can envenomate. We are studying the "venome" of *Scytodes thoracica* with the goals of describing the composition of chemicals involved in this unusual system of prey capture, discovering new toxins and better understanding the phylogenetic breadth of toxins known in other Haplogynes. To do this we have constructed cDNA libraries from venom glands and sequenced over 800 transcripts from this species. We are comparing the transcriptome with the venom proteome of the same individuals using MudPIT analyses of proteins in venom and glue. A comparison of the transcriptomic and proteomic components helps confirm transcripts that code for extruded venom components. We have identified transcripts homologous to astacin metalloproteases, and peptides with significant similarity to U_1-agatoxin-Ao1a from *Agelena orientalis*, U_{12}-lycotoxins from *Lycosa singoriensis* and U_1-nemetoxin-Csp1a from *Calisoga sp.* In addition, we have identified a highly expressed glycine rich peptide that constitutes about 50% of the venom gland transcripts. We have ascertained 13 distinct groups of candidate peptide toxins, each containing signal sequences recognized as sites for cleavage and processing of mature toxins and patterns of cysteine scaffolding motifs characteristic of insecticidal neurotoxins found in spider venoms studied to date. The presence of astacins suggests that the presence of this toxin predates the most recent common ancestor of the superfamily Scytodoidae, while the lack of sphingomyelinase D transcripts is consistent with hypotheses that this medically relevant toxin is unique to sicariids.

Keywords: Venom, transcriptome, proteome, Scytodidae
10.1016/j.toxicon.2012.04.268

268. Biological activities of PnTx2-6, an exciting toxin from spider *Phoneutria nigriventer*

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Background: we previously have shown that PnTx2-6, a peptide toxin (MW 5.291.3Da) purified from the venom of the armed spider *P. nigriventer* potentiates erection function in normotensive rats and also restores the erectile function in hypertensive (DOCA Salt) in diabetic and old rats. These effects are nitric oxide (NO)-mediated. The results presented here show the action of PnTx2-6 in glutamate release from synaptosomes of rat brain and upon ion channels expressed in *Xenopus laevis* oocytes.

Methods: synaptosomes were prepared from rat brain cortices and incubated in the presence or absence of PnTx2-6. The glutamate (L-glu) release was measured after reaction with NADP+ in the presence of the enzyme glutamate dehydrogenase, by a fluorimetric method. The activity on ion channels was assessed by electrophysiological measurements using the two electrode voltage-clamp technique on cloned voltage-gated sodium and potassium channel subtypes, expressed in *Xenopus laevis* oocytes.

Results: PnTx2-6 increases the glutamate release from synaptosomes in a time- and dose- dependent manner (EC_{50} = 20nM). The toxin obtained by heterologous expression in *E.coli* also reproduces the same effect. The increase in L-glu release was totally inhibited by tetrodotoxin and only partially inhibited by EGTA and BAPTA. Calcium channels blockers, such as ω conotoxin MVIIC and GVI A, inhibited only partially L-glu released by PnTx2-6, although nifedipine did not show any inhibition. The toxin, among others, slowed down the inactivation and blocked of sodium currents, with different potency for the different isoforms of sodium channels, being more active on Nav1.3 (EC_{50} = 55nM). However, PnTx2-6 did not show any effect upon K+ channels (Kv1.5, Kv2.1 and Kv3.1).

Discussion: Our previous results suggest that the potentiating effect of PnTx2-6 on erectile function may be

mediated by sodium channels. Here we show that this toxin releases L-glu from synaptosomes of rat brain and has a wide range of action on sodium channels expressed in *X. laevis* oocytes.

Conclusion: PnTx2-6 seems to exert its primary action through sodium channels acting on a wide range of them. This toxin, besides potentiating erectile function, releases L-glu from rat brain synaptosomes. We don't know yet if the release of L-glu and the potentiation of erectile function can be correlated.

Financial support: The research agencies from Brazil: CNPq, CAPES and FAPEMIG

Keywords: Spider toxin, PnTX2-6, sodium channels
10.1016/j.toxicon.2012.04.269

269. Understanding the Chemical Diversity and Evolution of Spider Venoms using Comparative Transcriptomics

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Background: Spiders are the most speciose venomous animal and along with predatory beetles are the most successful terrestrial predators, with over 42,000 extant species described to date. Their evolutionary success is due in large part to the production of a highly complex venom that is used to subdue prey and deter predators. Recent mass spectrometric analyses indicate that the venoms of some spiders contain >500 different peptides but very little is known about how these arachnids evolved such complex chemical cocktails.

Methods: We sampled venom-gland transcriptomes from a taxonomically diverse group of spiders chosen to provide reasonable coverage of the Araneae phylogenetic tree. 454 sequencing using the Roche GS-FLX platform was chosen to provide a good compromise between depth of coverage and accuracy. Our panel included three spiders of medical importance.

Results: The resulting 454 datasets have provided an unprecedented view of the molecular complexity of spider venoms, with >35 different protein superfamilies identified to date. Many of these toxin superfamilies encode 3D scaffolds not previously identified in spider venoms. Comparison of the venom-gland transcriptomes has provided us with the first ever (albeit crude) map of the evolutionary pathway of spider toxins and it has enabled us to identify evolutionarily conserved toxin families.

Keywords: Spider venom, venom proteome, venom evolution, venom peptides, comparative transcriptomics, bioinformatics
10.1016/j.toxicon.2012.04.270

270. Site-Directed Mutagenesis of rPnTx2-6 - A Recombinant *Phoneutria nigriventer* Spider Venom Toxin – Reveals Important Amino Acids for Its Pharmacological Activities

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Background: PnTx2-6 toxin of the *Phoneutria nigriventer* spider venom was cloned and expressed as thioredoxin fusion protein in the cytoplasm of *Escherichia coli*, cleaved from the conjugate and purified by HPLC [Torres *et al.* (2010) *Toxicon 56*, 1172–1180]. As does the native toxin purified from the spider venom, the recombinant toxin - rPnTx2-6 - potentiates erectile function when injected into the rat, and causes glutamate release from rat cortical synaptosomes.

Methods: The cloned gene was used to make site directed mutations, to attempt to pinpoint the residues that are involved in producing this effect.

Results: Six mutant genes were obtained and expressed with specific amino acid substitutions. Five were further purified and their molecular masses were checked. The mutant proteins were tested, and compared with those of the native and recombinant toxins.

Conclusions: The mutant genes were significantly less effective in the release of glutamate from rat cortical synaptosomes than the native and recombinant toxins.

Keywords: *Phoneutria nigriventer* venom, PnTx2-6 toxin, Site-directed mutagenesis ,
10.1016/j.toxicon.2012.04.271

271. Differential Expression of Aquaporin 4 in the Hippocampus of Neonate and Adult Rats after Envenoming by *Phoneutria nigriventer* (*Ctenidae, Araneomorphae*)

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Background: *Phoneutria nigriventer*, popularly known as armed spider, is responsible for most of the cases of araneism in Brazil. Intravenous injection of PNV in rats resulted in the loss of blood-brain barrier (BBB) permeability by impairing transcellular transport and breaking down the paracellular fence barrier of brain microvessels endothelium. As result, vasogenic edema by swelling of astrocytic perivascular endfeet occurs. The swelling resulting from the accumulation of fluid within the brain is main cause of increased intracranial pressure and subsequent traumatic brain injury. AQP4, the predominant brain

water channel, is expressed in astrocyte endfeet facing brain capillaries, perisynaptic spaces and nodes of Ranvier. It is implicated in brain edema formation and resolution because regulates the rapid transport of water within distal feet of the of perivascular astrocytes, having though a key-role in the maintenance of cerebral homeostasy and water flow. The aim of this study is to analyze the expression of AQP4 in the hippocampus, one major target of PNV.

Methods: The time-course analysis (at 2, 5 and 24 h after PNV intra-peritoneal injection, 1.7mg/kg, n=6/time) of the immunohistochemical expression of AQP4 in hippocampal regions (CA1, CA2, CA3) of postnatal day 14 (P14) and 8-10-week (adult) old rats.

Results: Regional analysis of the immunoreactivity was done by GIMP methodology that converts the digitized images to grayscale images after color segmentation allowing determination of pixels density percentage of the anti-AQP4 immunoreactivity. AQP4 was ubiquitously expressed around blood vessel walls; also around neuronal bodies mainly in CA2 and in cell (astrocytes) processes of CA3.

Discussion: The findings revealed that: (i) PNV induced increase of AQP4 expression, as compared with control baseline, in all three hippocampal regions examined, but there were regional differences; (ii) The upregulation of AQP4 was higher in CA3/CA2 than in CA1; (iii) Upregulation of AQP4 was more prominent in adult than in P14 neonate rats; (iv) The peak of AQP4 expression occurred chiefly 24 h after PNV exposure in animals of both ages (with exception of CA3 where AQP4 peaked at 5 h).

Conclusions: The water channel protein aquaporin-4 plays important role in perivascular astrocyte at the blood-brain interface, likely involved in edema resolution; being so a molecule targeted by PNV. The time-course and differential expression distribution of AQP4 in CA1, CA2 and CA3 suggests spatiotemporal dynamics in the water handling in hippocampus after PNV neurotoxic effect and between P14 and adult rats.

Financial support: CAPES, CNPq, FAPESP.

Keywords: Edema, hippocampus region, spider venom
10.1016/j.toxicon.2012.04.272

272. Cellular and Molecular Demonstration that Vascular Endothelial Growth Factor (VEGF) and its Receptor Flt-1 and Flk-1 are Involved in *Phoneutria nigriventer* Envenoming

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Background: VEGF is a major regulator of developmental angiogenesis, vascular permeability, and recently was also recognized as neurotrophic, neurogenic and neuroprotector, hence being upregulated in many neuropathological processes. Evidences that VEGF mediate neurotoxic effect of *P. nigriventer* venom (PNV) in rats was lately done by demonstrating the upregulation of Flt-1 in the CA1, CA2, CA3 and DG regions of hippocampus.

Methods: We investigate the time-course changes (at 2, 5, 24 h following i.p. injection of PNV, 1.7 mg/kg, n=6/group) of the total amount of VEGF and its receptors Flt-1 and Flk-1 proteins by western blotting, as well as their regional distribution by immunohistochemistry (IHC) in the hippocampus of rats (post-natal day 14 (P14) and 8-10 weeks old (adult)). Paired controls were injected with 0.9% saline.

Results: Densitometric analysis of the immunoblots of the proteins in the whole hippocampus provided evidence that VEGF and Flk-1 expression of saline-injected rats is quite the same in P14 animals and that PNV produced no difference relative to baseline values. PNV also did not alter the expression of Flt-1 protein in the hippocampus of P14 rats. However, PNV promoted gradual increase of Flt-1 from 2 to 24 h, which reached statistical significance at 24 h ($p<0.05$). In 8-10 weeks rats, VEGF and Flk-1 expression also showed a trend for increasing in all periods, which was significant just for Flk-1 at 5 h ($p<0.05$). IHC for anti-VEGF showed intense labeling of pyramidal cell bodies and dendrites of CA1, CA2 and CA3 hippocampal regions; anti-Flk-1 labeling mirrowed anti-VEGF labeling with difference that pyramidal cell nuclei are unevenly labeled; anti-Flt-1 reactivity was strongly in pyramidal cell nuclei. Vasogenic edema of venules and capillaries were observed in PNV-treated animals whereas it is not seen in saline-treated ones.

Discussion: Studies describing that VEGF affects epileptiform activities through its receptor Flk-1 and that Flk-1 up-regulation in pyramidal neurons protects these cells from hyperexcitability by inhibiting glutamate release is in conformity with the present finding.

Conclusion: Since PNV produces convulsion in humans and experimental animals by affecting permeability at BBB, the upregulation of Flk-1 at 5 h in the hippocampus of adult animals may be an indication that VEGF augment can have a protective role in PNV envenoming through Flk-1 mediation. The fact that Flt-1 was upregulated in P14 rats exposed to PNV is in line with its known higher expression in neonate rats than in adult rats.

Financial support: CAPES/CNPq/FAPESP/FAEPEX

Keywords: VEGF, spider venom, hippocampus
10.1016/j.toxicon.2012.04.273

273. Heterologous Expression of PaluIT1, A Cysteine Knot Spider Toxin, in *E. coli*.

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Background: Low molecular weight spider neurotoxins have been highly efficient to paralyze or kill insects. Many of the bioactive components specifically act on target receptors in the nervous system of the recipient, such as voltage gate ion channel, among them the Na^+ ion channel is of particular interest for structure and function studies in insects. PaluIT1 is a 37 amino acid long and four disulfides

bridges peptide with an ICK structural motif that consists of a cysteine knot with a triple-stranded beta-sheet. Since PaluIT1 do not recognized mammalian's receptors, it could be employed in binding studies to characterize insect receptors. In addition by labeling the toxin together with specific directed antibodies, it may be employed to clarify relationships between such a ligand and the insect receptor site. Insect-selective neurotoxins such PaluIT1 are not harmful to humans and have the potential to be used to develop bio-insecticides; however, they have to be produced in sufficient amounts first. There are several methods to produce them; however, heterologous expression in bacterial cells would be of increased production but it could be a challenge because of the amount of disulfide bridges to be correctly folded, even though some toxins have been expressed in baculovirus, yeast and bacteria.

Methods: To get sufficient amount of a PaluIT1, a cDNA was constructed and cloned into the expression vector pQE30 containing a 6His-tag and an FXa proteolytic cleavage region. This recombinant vector was transfected into *Escherichia coli* BL21 cells and expressed under induction with isopropyl thiogalactoside (IPTG). Moreover, a tandem construction of PaluIT1 (two PaluIT1 cDNA linked with appropriate nucleotides were inserted for R, 5 G and a S residue to cut expressed toxins), similar plasmids were designed to improve peptide expression and six different primers were constructed to perform tandem construction by PCR.

Results: Several problems associated with the heterologously expressed toxins containing four disulfide bridges are discussed. *Escherichia coli* BL21 frequently mutes the TATAAT sequence inhibiting toxins production, plasmid construction must be sequenced in both directions to correct this problem. The His-tagged recombinant toxin was found exclusively in inclusion bodies. Reduced yields were obtained after oxidation reaction to fold the toxins. To avoid inspecific hybridization of cDNA on tandem construction short specific primers, are required.

Acknowledgements

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Keywords: Heterologous expression, insecticidal spider toxins
10.1016/j.toxicon.2012.04.274

P. Systems & Training

274. A National Serum Depot in the Netherlands; Encountered Antivenom Purchase Difficulties

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Background: Since 2008, a National Serum Depot (NSD) is operational in the Netherlands, guaranteeing antivenom supply during medical emergencies. The NSD is organized by the National Institute for Public Health and the Environment (RIVM), in cooperation with the Dutch National Poisons Information Center (NPIC). The NPIC makes recommendations concerning the content of the National Serum Depot and advices physicians on medical treatment including use of antivenoms. The RIVM is responsible for purchase, storage and delivery of the antivenoms. During establishment and maintenance of the NSD several antivenom purchase difficulties are encountered.

Materials and Methods: Like in many countries, antivenoms are not registered in the Netherlands. There is a permission given by the Netherlands Health Care Inspectorate (IGZ) to the RIVM to purchase, import, store and distribute the antivenoms. Because antivenoms are pharmaceutical products, these activities are performed according to the guidelines of Good Distribution Practice (GDP).

Results: It is a time consuming process to locate and establish a good relationship with the antivenom producers. Some antivenom producers do not respond upon (initial) contact. Others have an international sale embargo because of their code of ethics. A main problem is the availability of antivenoms. Regularly, there are temporarily market failures or the products have only a very short shelf life left from the moment of purchase. Some products arrived in bad shape and did therefore not comply with the guideline of GDP leading to the destruction of the product. The package and even the information leaflet of some antivenoms are only available in the local language like Thai, Chinese or Spanish and not in English. In extraordinary cases the information on the leaflet was incorrect.

Discussion and Conclusions: In order to have sufficient supply during medical emergencies, the establishment of good working relationships with the producers must be taken seriously. To improve the antivenom market, antivenom producing companies must apply to the WHO Guidelines for Production Control and Regulation of Snake Antivenom Immunoglobulins and the general Guideline of Good Manufactory Practice and Good Distribution Practice. Sharing information between various antivenom depots is important to improve the purchase process.

Keywords: Antivenom supply, antivenom market, GxP (GMP and GDP), antivenom acquisition
10.1016/j.toxicon.2012.04.275

275. Safe Utilization of Ketamine as a First Line Induction Agent for Rapid Sequence Intubation (RSI) in the Aeromedical Setting

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Background: Ketamine is a derivative of PCP that acts as a dissociative anesthetic. It has a number of benefits as an anesthetic and in terms of its effects on the physiology of critically injured patients in the prehospital setting requiring RSI.

Objectives: To show that ketamine can safely be used as a first line induction agent for RSI in hypotensive to normotensive patients requiring aeromedical transport.

Methods: Retrospective case series over 6 month period following the introduction of ketamine into aeromedical protocols.

Results: 7 of 18 patients in the case series received ketamine as an induction agent. 100% percent of patients receiving Ketamine avoided hypotensive sequelae. 0% developed hypertension or other adverse effects. Vitals and end tidal CO2 improved in all patients receiving ketamine.

Conclusions: Ketamine was a safe and beneficial alternative to existing induction agents in hypotensive trauma patients being transported by air.

Keywords: Ketamine, aero-medical, RSI
10.1016/j.toxicon.2012.04.276

276. Circus Venomous: An Interactive Tool for Toxinology Education

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Background: Clinical education about envenomations and their treatment may convey clinical and zoological details inadequately or flatly. In recent years, the widespread availability of models and videos of venomous species have created unique opportunities for toxinology education. We share our experiences using a new toolkit for educating a diverse array of clinicians, students, and wilderness medicine enthusiasts.

Methods: We examined the cost, number of participants, and satisfaction data since the initiation of a portable workshop featuring high-fidelity exhibits of venomous species. Termed the "Circus Venomous," this educational toolkit consists of several boxes of props, such as plastic models, photos, and preserved specimens of injurious species (see Table).

The workshop consists of three phases: 1.) participants view all exhibits and answer clinical questions regarding venomous injuries; 2.) short video clips from television and cinema are viewed together, and myths about envenomation injuries are debunked; 3.) debriefing session and wrap-up.

Results: We have utilized the Circus Venomous to teach medical students, residents, practicing community clinicians, nurses, PAs, national and regional parkmedics, and wilderness enthusiasts. The major cost (about $800) was spent on the purchase of highly durable, lifelike models and well-preserved real reptile and arachnid specimens. When formal feedback was solicited, the participants expressed high levels of satisfaction, scoring an average of 4.3, 4.4, and 4.3 out of 5 points in the respective areas of content, presentation, and practical value of the activity. Since we have used this exhibit with approximately 250 participants over 2 years, we estimate the materials cost per participant is approximately $3.

Conclusions: The Circus Venomous is a novel, interactive, flexible, and cost-effective teaching tool about envenomation emergencies. We hope that this concept will encourage other clinical educators towards further innovation. Future directions for our group include greater inclusion of marine species into the Circus Venomous, and formal longitudinal testing to measure knowledge retention based on this approach.

Fig. 1.

Fig. 2.

Fig. 3.

Table: Inventory of Circus Venomous, An Interactive Tool for Toxinology Education

western diamondback rattlesnake—preserved specimen
coral snake—plastic model
non-venomous species—2 plastic model and 1 preserved specimen
coral snake, heloderm lizard, and caterpillars—high-quality images
grizzly and black bear skull – plastic replicas
preserved arachnids—mounted specimens
commercial snakebite "first aid and extraction kit"
splinting and wrapping materials
protective and inadequate footwear for wilderness expeditions

Keywords: Toxicology, instructional tools, envenomation education
10.1016/j.toxicon.2012.04.277

277. Prehospital Management of Envenomations in the State of Queensland, Australia

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Background: Queensland is the second largest state in Australia and covers an area of 1,730,648 km^2. Because of its vast size, there is significant variation in climate across the state and a range of landscapes from coastal environments, deserts, bush, rainforest, mountainous regions and large urban centres. Queensland is home to a variety of species of venomous wildlife. The Queensland Ambulance Service (QAS) provides emergency treatment to patients who experience bites, stings and evenomations. Paramedics are dispatched in road ambulances, aircraft (helicopters and fixed wing) and marine craft to provide care to patients in a range of settings.

Method: This study investigates and profiles bites, stings and envenomations treated by QAS paramedics 2007 2011. Retrospective analysis of QAS patient data was undertaken. A manual audit of box jellyfish stings and Irukandji stings was performed to evaluate the effectiveness of paramedic treatment with antivenom and magnesium sulphate.

Results: The QAS attended 8,741 cases of bites, stings and envenomations 2007-2011. The majority of serious injuries were dog bites, snake bites, spider bites, and fish or jellyfish. Morphine was administered to 582 patients for snake bite (n=117); spider bite (n=77); dog bite (n=61); jellyfish or fish sting (n=55) and other injury types. Of patients who received pain relief (n=1,214 methoxyflurane and /or morphine), a mean reduction in pain of 3.03 points (using the 10 point scale) was observed. Regarding serious marine envenomations, the QAS treated 51 patients with magnesium sulphate for Irukandji stings and achieved a mean pain reduction of 4.66 points in these patients. Eight patients were treated with box jellyfish antivenom, with a mean pain reduction of 2.2 points. An audit of the outcomes for these patients will be presented, with an examination of the efficacy of magnesium sulphate and box jellyfish antivenom in these patients who have suffered a serious and painful injury.

Discussion: The preliminary results show a significant diversity in envenomation types across a range of terrains in Queensland. Given the diversity of environments and envenomation types, paramedic education and training is regionally focused to ensure that paramedics can deliver the most appropriate front-line treatment for patients.

Conclusion: Because the geographic area of Queensland covers a range of environments the delivery of appropriate and standardised ambulance services poses significant challenges. The results of this analysis demonstrate that the QAS, in line with a commitment to evidence-based practice, has reflected the treatment protocols in hospital settings and successfully equipped paramedics to commence definitive treatment for envenomed patients before transporting to hospital.

Keywords: Ambulance, treatment, envenomations
10.1016/j.toxicon.2012.04.278

278. Epidemiologic Situation of Envenomation by Venomous Animals in Argentina. 2007-2011 Period

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Background: Intoxications from venom inoculation by venomous animals are of mandatory notification in Argentina. Major incidence of injuries of toxicological significance, are caused by species of the genera *Tityus* (scorpion), *Lactrodectus*, *Loxosceles* and *Phoneutria* (spiders) and *Bothrops (Rhinocerophis, Bothropoides* and *Bothrops)*, *Crotalus* and *Micrurus* (snakes). The aim of this work is to describe the national situation of envenomation by venomous animals, contributing to the development of the

surveillance network and consolidation of the information available about mortality and morbidity.

Methods: Study design was descriptive and transversal, and data from the period 2007-2011 was collected from the National Surveillance System of Health (Sistema Nacional de Vigilancia de la Salud; SNVS). Analysis was made using Microsoft Office Excel, GeCo C2, Epiinfo 3.5.1 and SIGEpi 1.0 software.

Results: Within the study period 45012 cases of envenomation by venomous animals (9002 cases/year) were registered in the SNVS. 3692 (8.2%) cases were caused by snakes, 6084 (13.5%) by spiders and 35236 (78.3%) by scorpions, corresponding to an incidence rate of 1.8, 3.0 and 17.6 cases/10.000 inhabitants per year, respectively. 15-24 years old was the most affected age group. During the same period 40 deaths were registered, snake bites accounted for 42.5%, scorpion stings for 35.0% and 22.5% were caused by spider bites. Among snake caused deaths, people older than 65 years old were most affected (47.1%), while spiders caused death preferably to the patients older than 45 years old (62.5%). On the other hand, children of 9 years old or less accounted for the majority of deaths by scorpions (96.4%). Geographic distribution of envenomation incidence is not uniform, while scorpions and spiders show the highest incidence in the northwest region (58.5 cases/10.000 inhabitants per year and 7.7 cases/10.000 inhabitants per year respectively), snakes morbidity is higher in the northeast region (8.6 cases/10.000 inhabitants per year). Besides, Northeast and Northwest regions show the greatest notification for snakes (1591 and 1379 cases respectively), while scorpion and spider notification is highest in the center and northwest regions (14033 and 13757 cases for scorpion; 1718 and 1799 for spiders). Envenomation occurs mainly in the summer season for all three poisonous animals.

Conclusions: Envenomation by venomous animals constitutes a significant health problem in some regions of Argentina. These events are always medical emergencies and prevention is made through education. Besides, specific treatment provision and adequate management of patients are necessary to avoid serious damage or death.

Keywords: Health surveillance, snakes, spiders, scorpions, epidemiology
10.1016/j.toxicon.2012.04.279

279. Travel Toxinology: An Illustrative Case of Brown Spotted Pit Viper (*Protobothrops mucrosquamatus*) Bite With Review of Clinical Toxinology Issues in Travel Medicine

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Background: Tourism is emerging as a major economic driver globally and travellers visit ever more exotic places. As a consequence, exposure to Venom Induced Diseases (VIDs) becomes an increasing risk. Lack of resources to adequately diagnose and treat VIDs in many parts of the world increases the risk of suboptimal outcomes.

Case Report: A 12 year old girl on holiday with her family in Vietnam was bitten on her ankle by a snake while on a tour boat in Ha Long Bay. Local suction and a tourniquet were applied as first aid, then she was transferred to the mainland hospital where the tourniquet was removed, but no antivenom given as the snake identity was unknown. The snake had been killed and was brought with the patient. The patient complained of significant local pain which remained untreated, then was transferred to a larger hospital, where again no antivenom was given because of doubt over the snake's identity. By this time, many hours later, the foot and leg, extending to the abdomen, was markedly swollen. A mild coagulopathy was present, with thrombocytopenia and anaemia. The patient continued to be managed conservatively, with no antivenom and after a week was transferred to Australia. There a marked foot drop was evident with MRI evidence of deep foot muscle necrosis. Despite this over the following weeks the patient regained full use of the foot. Clear photographs of the killed snake were identified by experts as *Protobothrops mucrosquamatus*. The only antivenom for this snake, a known inhabitant of northern Vietnam, is made in Taiwan.

Discussion: This case illustrates a number of problems for tourists envenomed in another country where communication and health system issues can adversely affect treatment. Early consultation with a clinical toxinologist might have allowed identification of the snake and sourcing of suitable antivenom. This case is illustrative of a wider problem for "travel toxinology" cases. Local health systems may not have the expected expertise to diagnose and treat envenoming, even by local species. Rapid access to (clinical toxinology) expertise per phone, availability of suitable antivenoms and urgent transfer of stabilised envenomed patients are all required elements in improving patient outcomes.

Keywords: Snakebite, *Protobothrops mucrosquamatus*, envenoming
10.1016/j.toxicon.2012.04.280

Q. Venomous Animal Biology

280. Genetic Regulation of Venom Production during Embryonic Development of the Indochinese Spitting Cobra, *Naja siamensis*

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Background: Snake venoms are a potential source of pharmacologically active components. Since the initial discovery of the ACE inhibitors which were originally derived from the venom of the Brazilian Arrow Head Viper, several compounds have stimulated pharmacological interest although issues relating to venom yield and variability have hampered efforts to isolate these agents.

Ideally it would be beneficial to produce the venom in an *in vitro* model which would allow the desired components to be engineered in suitable amounts. However, to date, fundamental knowledge of the mechanisms underlying venom production and its regulation is not available.

Methods: *Naja siamensis* clutches (n=4) were obtained and embryos removed at varying stages of development. Venom proteins were demonstrated (using therapeutic antivenom as an antibody source) via a direct immunohistochemical technique. Embryos were also screened for gene expression using *in situ* hybridisation with DIG labelled RNA probes targeting sequences responsible for representative proteins within the venom.

Results: Production of venom components was identified in 3 of 4 embryo clutches. Histological examination revealed differences in gland morphology and overall development (between embryos from separate clutches) despite identical post-oviposition times. Gene expression was demonstrated in all *Naja siamensis* clutches at stages when the venom glands displayed similar histological development. A 12 day delay was observed between the initiation of gene expression to the demonstration of venom proteins.

Discussion: The initiation of gene expression was detected at different post-oviposition time points across the embryo clutches. This was likely due to variable development stages within the clutches as major phenotypic differences were observed histologically at the same time post-oviposition preventing correlation between clutches. The 12 day lag between initial gene expression and identification of the venom protein product suggests either post-transcriptional processing or post-translational modification is occurring.

Conclusions: The efforts to elucidate the primary stages of venom production were restricted by the lack of phenotypic correlation between the embryo clutches at the same time points post-oviposition. The need for a correlation strategy is clearly evident however the initial timing of gene expression leading to protein production has been successfully demonstrated. This has lead to several inferences about its importance in venom production, composition and yield.

Keywords: Venom, genes, embryo
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281. The Desert Massasauga (*Sistrurus catenatus edwardsii*): Biology and Venom Biochemistry

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Background: The Massasauga (*Sistrurus catenatus*) is a small rattlesnake which occurs in grasslands of North America, from northern Mexico to southeastern Canada. Although threatened in many parts of its range, the diminutive Desert Massasauga (*S. c. edwardsii*) remains abundant at several locations in more mesic regions of the shortgrass steppe of southeastern Colorado. Our numerous studies of the ecology/natural history and venom biochemistry/genomics make Desert Massasaugas one of the better-characterized species of rattlesnakes, and this summary will examine the interplay of animal biology and venom biochemistry.

Methods: Snakes (750) were collected on a private ranch in southeastern Colorado and were processed in the lab (morphometrics, venom extraction and PIT-tagging; 12 snakes were also implanted with radios for telemetry studies). Massasaugas were radiotracked for two years during the active season to determine spatial ecology and habitat use. Venoms were subjected to a variety of biochemical and proteomic analyses, and two snakes were sacrificed for transcriptomics studies.

Results: Massasaugas make strongly directional migratory movements which are resource-driven. Abundant prey (lizards, centipedes, rodents) and favorable thermoregulatory sites occur in the summer habitat, while stable hibernacula exist in the shortgrass habitat. Long-term mark/recapture studies indicate that Desert Massasaugas are abundant but short-lived, with average adult survivorship of $\leq$4 years. Desert Massasauga venom is quite potent toward both lizards and rodents, and it is much more toxic to a common rodent prey species (*Perognathus*) than toward rodent species not utilized (*Peromyscus*). Proteomic and genomic analyses indicate that a crotoxin homolog, characteristic of other type II rattlesnake venoms, is not expressed in this species, but genes for 5 isoforms of three-finger toxins (3FTXs) are present. Unlike most viper venoms, only one dominant isoform of an acidic PLA_2 is present in venom of *S. c. edwardsii*.

Discussion: The Desert Massasauga is one of only a few species of viperids demonstrated to possess genes for 3FTXs, a protein family which includes the potent α-neurotoxins of elapids and several taxon-specific neurotoxins of some rear-fanged snakes. However, 3FTxs do not appear to be expressed in the venom. Based on 2D SDS-PAGE, over 100 proteins comprise this venom, and serine proteinases (thrombin-like, kallikrein-like) are abundant components which may contribute to high venom lethality in mice and lizards.

Conclusion: The Desert Massasauga in Colorado is an excellent model species for evaluating influences of numerous ecological factors on venom evolution, and continuing studies are investigating population levels of venom and genetic variation.

Keywords: Ecology, toxin evolution, proteome
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282. Sexual Variation in Timing of Egress and Ingress in Tiger Rattlesnakes (*Crotalus tigris*)

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Background: Temperate zone snake species avoid hazardous winter temperatures by overwintering in thermally sheltered hibernacula. The tiger rattlesnake, *Crotalus tigris*, is a summer mating, medium-sized pitviper that

ranges from southern Sonora northward into south-central Arizona.

Methods: With the aid of temperature-sensitive radio transmitters we tracked tiger rattlesnakes in the Rincon (two sites) and Tortolita Mountains (one site) of Pima County, Arizona from 1997-2010.

Results: Based on data pooled across years and sites, we determined that timing of ingress coincides for males and females. However our results revealed that timing of egress varied, with females leaving dens significantly earlier than males. Specifically, the average date of emergence for females was almost a full month (22-23 March) before males (20-21 April). Due to small sample sizes from our Rincon Mountain sites, we were only able to compare interannual variation in the timing of egress at our Tortolita Mountain site from 2004-2006. In each year, females emerged (3/19/2004, 3/16/2005, 3/28/2006) earlier than males (4/12/2004, 3/31/2005, 5/1/2006).

Discussion: Our data provides support for the hypothesis that male rattlesnake species which do not mate in spring should not emerge before females. Unlike females, there is no need to come out to bask in early spring to facilitate the process of gametogenesis. We are also investigating the possibility of sexual variation in overwinter body temperature in *C. tigris* to further our understanding of this species winter ecology.

Keywords: Rattlesnake, ecology, ingress, egress
10.1016/j.toxicon.2012.04.283

283. Relocator Proteins: Identification of the Chemical Component of Venoms Allowing Prey Recovery During Strike-induced Chemosensory Searching

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Background: Among advanced snakes, a chemical mode of dispatching prey is commonly utilized to obtain prey rapidly and with minimal contact. Venoms contain a variety of protein, peptide and small organic compounds, and a persistent issue in the study of venom evolution has been to explain the compositional complexity of venoms. Lethal toxicity toward particular prey has demonstrated the adaptive significance of several taxon-specific venom components, but for most venom proteins, particularly the low toxicity, non-enzymatic fractions, a well-defined role in envenomation and predation has not been established. During predatory episodes, rattlesnakes and other pit-vipers commonly strike, envenomate and release prey, minimizing retaliation. They are then confronted with the additional task of relocating the carcass via chemosensory searching once venom has taken its course, which they do with a high degree of precision. However, the specific component of venom involved in altering the chemical cues of prey, allowing for relocation, has yet to be identified.

Methods: Two-hundred and fifty milligrams of crude *Crotalus atrox* (Western Diamondback) venom was fractionated using a BioGel P-100 size exclusion column. All fractions were tested for enzymatic activity of enzymes common to rattlesnake venoms. Peaks were dialyzed, lyophilized, and frozen at -20 C until behavioral testing. Eight *C. atrox* were tested for vomeronasal chemosensory responses to fractionated peaks. The significant peak (peak III) was further fractionated using RP-HPLC, and protein identification was confirmed by MALDI-TOF mass spectrometry and N-terminal sequencing.

Results: Enzymatic and other major protein components of size exclusion-fractionated *C. atrox* venom had no effect on discrimination of envenomated vs. non-envenomated prey by snakes. Conversely, peak III elicited a statistically significant response to treated prey. Further purification by RP-HPLC and analysis by MALDI-TOF mass spectrometry and N-terminal sequencing confirmed that this peak contained only two monomeric disintegrins, crotatroxins 1 and 2.

Discussion: In the field, this chemical tag on prey will help minimize foraging time and greatly expedite discrimination of a trail left by envenomated prey from the many trails of non-envenomated conspecifics. Our results demonstrate unequivocally that venom components, such as disintegrins, can have important biological roles which extend beyond those that are apparent from their biochemically functional roles.

Conclusion: This is the first study to identify the component of venom allowing for relocation of envenomated prey, and we suggest that a major biological role of venom disintegrins for rattlesnakes is to allow these strike-and-release predators to relocate envenomated prey effectively.

Keywords: Chemosensory searching, disintegrins, prey relocation
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284. The Bio-Logic of Venom Complexity

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Background: The dependency of venomous species on their venom for prey capture has resulted in the evolution of very complex mixtures. Although this strategy is effective, the use of venom has an associated metabolic price tag, and consequently venomous animals tend to use their protein-rich venom sparingly. While complexity appears to stand in contrast to the need for metabolic economy, we still lack knowledge as to the significance of this complexity

for venom use. The aim of the present study was to characterize the molecular complexity of natural envenomations and to examine the contribution of individual venom components to overall venom toxicity.

Methods: We used the Australian funnel-web spider *Hadnyche infensa* and the scorpion *Hottentotta judaicus* as models of two independently recruited venom systems in order to characterize venom use under natural conditions. In both cases, the animals were provoked to repetitively deliver venom, simulating a defensive secretion, leading to the gradual release of multiple venom droplets. The individual venom droplets were then assayed for their toxicity against flesh fly larvae (*Chrysomya megacephala*). The relative abundance of the various components between secretions of each series was assayed using LC-ESI-MS. Additionally, we have examined the distribution of the various components identified previously in the venom, in the venom gland using MALDI imaging.

Results and Discussion: an analysis of the individual secretions, found them to be extremely heterogeneous with regard to their volume, protein content, protein profile, and toxicity. Nevertheless, a pattern was observed whereby the toxicity of the stings peaks with time. This increase correlated with a discernable change of venom composition, whereby the most potent toxins are secreted only in later stings. The correlation between toxicity and peptide abundance reveals that only a fraction of the toxins contribute significantly to venom toxicity. Remarkably, we demonstrate that this manipulation of the venom composition is achieved by a spatial organization of toxin production within the venom gland. Imaging mass spectrometry revealed that the most potent toxins, which are abundantly secreted only late in the secretion series, are stored deeper within the gland than the toxins seen in the initial venom secretions.

Conclusion: We suggest that this arrangement allows venomous arachnids to reduce the selection pressure on their most potent toxins through a reduction in the occasion of their use. This strategy appears to be successful enough to have evolved at least twice, though preliminary evidence exists to suggest this modulation may also occur in spitting cobras, implying an evolutionary favorable strategy

Keywords: Venom complexity, venom optimization, proteomics
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R. Venomous Animal Collections

285. Venomous Workshop: Evolution of a Professional Training Course

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Background: Venomous animals pose a myriad of unique challenges for those who work with them. From propagation to politics, few other animals are surrounded with such mystique and misinformation.

Methods: The Albuquerque BioPark in conjunction with specialists from around the world have funneled their expertise into this week long workshop. Covering topics including handling, exhibit design, fieldwork, envenomations, antivenom, venomics and more, this workshop is a first of its kind. All types of venomous animals will be covered including marine life and terrestrial invertebrates. The workshop is open to zoo staff, field biologists, academic researchers, Federal and State authorities and even the serious private enthusiast.

Results: There has been an appreciable amount of interest in this workshop with attendees expected from countries outside of the United States as well.

Conclusions: A workshop covering all aspects of venomous animal handling has drawn widespread interest. Results will be reported in future presentations.

Keywords: Venomous animals, professional training
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286. Legal Conundrums Impeding Patient Safety Initiative to Prevent Exotic Envenomation in the United States

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Background: During 2011 In New York state there were fifty-seven venomous snakes confiscated comprising forty-nine species of world-wide origin after the possible suicide of their owner. The owner was found with puncture wounds consistent with a snakebite and her black mamba's (*Dendroaspis polylepis*) enclosure was unlocked. The large collection was brought to the Bronx Zoo and placed in quarantine. This presentation will discuss how current federal state and local law affects the availability of venomous reptiles and the clinical relevance of that availability.

Discussion: There is currently limited federal law regarding the importation, possession and interstate transport of these animals. A great variability between states in this area exists. An example pertinent to this case is the relatively strict regulations of New York State as compared to nearby states–especially Pennsylvania. To possess a venomous reptile in New York State, a permit from the New York Department of Environmental Conservation is required. Outside of a few local municipalities there is no regulation in Pennsylvania. There are large snake expos in Western Pennsylvania that have been responsible for multiple envenomations in the New York Metropolitan area based on feedback from snakebite patients. This inconsistent regulatory situation makes primary preventative measures against snake envenomation difficult. Recently, federal regulations banning the interstate transport of large constrictor snakes have been adopted. A proposal for similar

federal regulation regarding venomous reptiles is likely to be controversial.

Keywords: Venomous snakes, envenomation, regulation
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287. Logistics and Problems in Managing a Large Confiscation of Venomous Snakes

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Background: The New York Department of Environmental Conservation (DEC) contacted the Bronx Zoo on June 14th 2011, requesting assistance for the removal and temporary maintenance of a large confiscation of venomous snake held at a private residence. Local police in Putnam County were investigating an apparent suicide and discovered a collection of 17 elapids, 10 vipers, 29 pit vipers and one rear fanged colubrid).

Methods: Bronx Zoo Herpetology Department Staff confirmed identification of species and removed collection to a secure quarantine facility at the Bronx Zoo. DEC provided chain of custody document granting transfer while enforcement officials worked to resolve the case. Dr Michael Touger, Jacobi Medical Center's Snakebite Trauma Unit and Bronx Zoo consultant, was apprised of the confiscation. He then began identifying appropriate antivenom and locations for products we might lack. As staff identified snakes with potential health problems our veterinary staff began medical work up. Routine quarantine diagnostics were started.

Results: During the quarantine period fecal samples revealed several endoparasites; *Strongyles*, rhabditid nematodes, flagellated protozoa and *Cryptosporidium*, these were treated with appropriate drugs. Snake feces were screened for the presence of *Cryptosporidium* through indirect immunofluorescent antibody staining and when positive, repeat fecal samples were sent for cryptosporidial species identification, as determined through PCR. Several specimens were found to have serious health problems that resulted in euthanasia. These snakes underwent complete necropsies. A portion of the live collection was screened for ophidian paramyxovirus, using PCR on lung wash samples. Because many of these snakes were fastidious eaters and only defecated occasionally, collection of enough fecal samples to meet routine quarantine testing requirements was a long arduous task. When the quarantine period was complete we began the process of distributing the snakes to other AZA zoos.

Discussion: When wildlife agencies confiscate venomous snakes they are often not able to care for these reptiles and must turn to zoos for assistance. An alternative option is euthanasia of the confiscated specimens which has occurred in some instances. The commitment of staff time to care for these additional snakes was significant. The benefit was the opportunity it created for staff to gain additional training and experience with venomous species not in our collection. A few specimens were incorporated in our collection. The commercial availability of these venomous snakes and illegal private ownership resulted tragically in this case.

Keywords: Confiscation, quarantine, zoo
10.1016/j.toxicon.2012.04.288

288. Unique Challenges Faced During the Creation of a New Herpetology Department with New Venomous Animal Species and Safety Policies within the City of Virginia Beach's Virginia Aquarium and Marine Science Center

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Background: Our 25-year-old, city-owned aquarium initially housed 1 native venomous snake species "Canebrake rattlesnake" [Crotalus horridus atricaudatus]. Over time, we added two additional, native exhibits featuring "Cottonmouths" [*Agkistrodon contortrix*] and "Copperheads" [*Agkistrodon piscivorus*]). We had no dedicated/skilled herpetology staff and only two individuals capable of working directly with venomous vipers. In 2003 we began planning an expansion that would house Tomistoma Crocodiles (*Tomstoma Schlegelii*), Komodo dragons (*Varanus komodoensis*), Egyptian cobras (*Naja haje*), Fat-tail scorpions (*Adroctonus australis hector*), Green Pitvipers (*Crytelytrops albolabris*), a walkthrough Red Sea aquarium tunnel and numerous other exhibits.

Challenges and Responses: Adding exotic venomous species to our native vipers required the development of a new institutional herpetology department, with new systems and protocols. This included the recruitment and training of animal care staff, the acquisition of non-native antivenoms, as well as issues related to their storage and potential use, event preparedness protocols, and EMS and healthcare provider outreach. An initial training session was held with the assistance of the regional poison center and an EMS consultant from the Miami-Dade Antivenom Unit. Regional physicians, local universities, EMS response teams, animal control and aquarium officials were invited, with CME made available to participating physicians. A second training program was presented to the local EMS providers and envenomation management protocols developed and approved for use. Acquiring non-native antivenoms can be difficult and labor intensive. We acknowledged that antivenoms, if available, should be obtained prior to venomous animal acquisition. Particular difficulties were encountered in acquiring SCORPIFAV, which would provide coverage in the event of a sting from the Fat-tail scorpion. Suppliers for this antivenom were limited and very difficult to contact. Acquisition of this antivenom required more than two years to accomplish. Despite a hobbyist collection of this species, no U.S. zoos stocked this antivenom and, to our knowledge, the only other available antivenom in the U.S. is through Miami-Dade Antivenom Unit.

Conclusions: The development of a venomous animal collection, particularly one housing non-native venomous species, presents challenges in animal selection, antivenom acquisition, staff recruitment, training and safety policies, envenomation management protocols, and the involvement of regional medical professionals.

Keywords: Herpetology, protocol development
10.1016/j.toxicon.2012.04.289

289. 2011 Putnam County Venomous Snake Confiscation: Antivenom Coverage Decisions

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Background: In 2011, Putnam County, NY, confiscated 57 highly venomous snakes of world-wide origin**.**

Methods: Descriptive case series**.**

Results: For seven animals, adequate available species-specific antivenom supply was lacking. Forty-four animals are to be dispersed to other institutions. Ten animals are to be kept as part of the permanent collection. Three animals died of causes unrelated to the confiscation**.**

Discussion: Confiscation of highly venomous snakes results in scenarios that require clinical decision making, guided by availability of exotic antivenoms that are required for staff safety.

Conclusion: Overall the antivenom stocks at the Bronx Zoo provided excellent coverage for the majority of the confiscated animals.

Keywords: Snake confiscation, antivenom supply
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290. Microbiological Evaluation of Different Strategies for Management of Snakes in Captivity

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Background: Keeping snakes in captivity to produce venom for scientific research and production of inputs is now a worldwide practice. Maintaining snakes in captivity involves capture, infrastructure investments, management techniques and appropriate qualified personnel. Furthermore, the success of the project requires knowledge of habitat, nutrition and reproduction, and control of opportunistic infections**.**

Methods: This study evaluated the management of snakes in three types of captivity (quarantine, intensive and semi-extensive) and diagnosed bacterial and fungal contaminants. A bacteriological profile was obtained by swabbing the oral and cloacal cavities, scales and venoms of healthy adult snakes from *Bothrops jararaca (Bj)* and *Crotalus durissus terrificus (Cdt)***.**

Results: There was predominance of *Enterobacteriaceae*, especially non-fermenting Gram-negative bacilli excluding *Pseudomonas* spp, Gram-positive bacteria and non-fermenting Gram-negative bacilli. Statistically, intensive captivity resulted in the highest number of bacterial isolates, followed by recent capture (quarantine) and by semi-extensive captivity. No statistical difference was found between *Bj* and *Cdt* bacterial frequency. *In vitro* bacterial susceptibility testing found the highest resistance against the semi-synthetic penicillins (amoxicillin and ampicillin) and highest sensitivity to amicacin and tobramicin amynoglycosides. To evaluate mycological profile of snakes from intensive captivity, collections were obtained from two healthy *Bj* and one *B. moojeni*, one *B. pauloensis* and one *Cdt* showing whitish lesions on the scales suggestive of ringworm. Using conventional methods and DNA-based molecular procedures, five samples of *Trichosporon asahii* were identified**.**

Conclusions: Despite the traditional role of intense captivity in ophidian venom production, semi-extensive captivity was more effective in the present study by virtue of presenting superior control of bacterial and fungal transmission, easier management, cheaper cost and decreased mortality; therefore, it should be considered a good alternative for tropical countries.

Keywords: *Bothrops jararaca*, *Crotalus durissus terrificus*, *Trichosporon asahii, Enterobacteriaceae,* management in captivity
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291. The Dilemma: Balancing Antivenom Cost vs. Investment in Conservation

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Background: As the cost of antivenom continues to rise and zoo budgets become more restricted it is more difficult to justify the costly expense of antivenom. The number of critically endangered species is on the rise and tangible *in-situ* and *ex-situ* conservation projects may be the only way to ensure their sustainability. Though both are critical components of an institution's collection and mission, they unfortunately compete for the same funding. As zoo managers, how do we make the decision as to where we channel funding and when confronted with that choice how do we defend it?

Methods: Zoological managers prepare an annual budget which includes the allocation of funds for the purchase of antivenom. When venomous reptiles are in the collection, the antivenom provides safety for the zoo staff,

as well as a broader resource for bites in the community. These same funds could be used to sustain new or established conservation programs. Some of the factors that influence how the decisions are made include:

- Adherence to the institutional mission statement
- Collection diversity and uniqueness
- Commitment of the institution to *in-situ* and *ex-situ* conservation projects
- Influence on annual attendance provided by a species
- Capabilities of staff that will handle venomous species
- Educational program tie-in
- Individual interest of the manager

Results: After all factors are carefully reviewed and discussed by managers and staff, the decision whether to purchase antivenom or to fund conservation programs must be made.

Discussion: Individual philosophical opinions will play a significant role in the process. Institutions have a commitment to the conservation of species but must generate revenue to finance these commitments. A unique and diverse collection can ensure continual public financial support and help fund conservation work.

Conclusions: Each institution and individual manager must determine the balance within their collection based on their mission. It will always be a challenge for zoo managers to determine whether the cost of keeping venomous species has greater value to the institution than supporting conservation efforts. The overall value determines how funds are allocated to antivenom costs and conservation support.

Keywords: Cost, antivenom, conservation
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292. How Do Komodo Dragons Kill Their Prey?: Lack of Role for Oral Flora in Predation

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Background: While the assumption continues to circulate that the oral flora of the Komodo dragon (*Varanus komodoensis*) exerts a lethal effect on its prey, supportive bacteriological evidence is sparse. One previous report was based on field observation, while a single subsequent study suggested *Pasteurella multocida* as the putative culprit pathogen despite its recovery from only 2/39 wild and captive dragons studied.

Methods: A new study of both the aerobic and anaerobic oral flora of 16 captive Komodo dragons (including 10 adults and 6 neonates) using saliva and gingival samples collected by zoo personnel, inoculated into anaerobic transport media and couriered to a reference laboratory revealed different results. Strains were identified by standard methods and 16S rRNA gene sequencing when required. Adult dragons grew 128 unique organisms including 37 aerobic gram-negative rods (1–8 per specimen); 50 aerobic gram-positive bacteria (2–9 per specimen), especially *Staphylococcus sciuri* and *Enterococcus faecalis*, as well as 41 anaerobes (1–6 per specimen), especially clostridia. Hatchlings grew aerobes but not anaerobes. There was some strain variation by zoo, likely reflective of different dietary components. No virulent species were recovered. These data correlate with bacteriologic studies of the oral flora of other reptiles and suggest that *V. komodoensis* oral flora is simply reflective of the gut and skin flora of their recent meals and environment, and is unlikely to cause rapid fatal infection.

Discussion: This suggests prey capture is largely due to mechanical damage alone for smaller prey, and mechanical damage plus venom induced anticoagulation for larger prey. Any wound sepsis to prey such as water buffalo are due to this animal living outside its natural ecology as a result of introduction by man, with attempted predation by komodo dragons operating outside evolutionary selection pressure. Fatal infection could be due to seeking refuge in local feces-laden water holes rather than their native fresh water pools.

Keywords: Komodo dragon, microbiology, oral flora
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293. Coral Snakes, Antivenoms, Hospitals and Zoos: How Can One Little Snake Cause So Many Problems?

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Background: The Texas coral snake, *Micrurus tener*, is the only elapid snake found in the state of Texas. Although they are relatively common in the Houston area, they are only rarely encountered, being very shy and secretive in their habits, and almost never bite. Less than 1% of venomous snake bites in the United States are attributed to Coral snakes. However, due to the scarcity of the FDA approved Wyeth Coral snake antivenom, the Houston Zoo often receives calls requesting antivenom from area hospitals when a Coral snake envenomation occurs in southeast Texas.

Problem: The Houston Zoo has provided Bioclon Coralmyn and Instituto Clodomiro Picado Anti Coral antivenoms to area hospitals in the past upon request. However, there have been numerous problems associated with this policy such as antivenom delivery, use, reimbursement or replacement, return, and accountability which has resulted in a considerable expenditure of time, money, and angst for the zoo and zoo staff.

Discussion: These difficulties led to the development of a standardized protocol and associated documentation with the delivery of Coral snake antivenom to area hospitals. The new procedures have, so far, proven successful and have increased not only accountability (on the part of the hospitals involved) but also zoo willingness and ability to provide antivenom in a timely and efficient manner when requested. These new protocols are now used whenever the zoo is requested to provide antivenom for any exotic species and not just for Coral snakes.

Keywords: Coral snake, antivenoms, zoos, hospitals
10.1016/j.toxicon.2012.04.294

S. Veterinary Toxinology

294. A Randomized Multicenter Trial of Crotalidae Polyvalent Immune Fab Antivenom for the Treatment of Rattlesnake Envenomation in Dogs

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Objective: To determine clinical efficacy of the Crotalidae polyvalent immune Fab (ovine) (Crofab®) antivenom (OPAC) against progressive Crotalid envenomation in the dog as reflected in stabilization or improvement of snakebite severity scores (SSS). Additionally, due to the potential decreased half life of the Fab antibodies in dogs we compared SSS between dogs receiving two different dosing regimes.

Design: Prospective, clinical trial.

Setting: Five veterinary emergency and critical care facilities one in each city Tucson, Az; Phoenix, Az; Austin, Tx; Escondido, Ca; and Denver, Co .

Animals: 115 client owned Crotaline (rattlesnake) snake-bitten dogs in whom worsening of the envenomation syndrome was observed before OPAC treatment.

Interventions: In a multicenter randomized clinical trial a single dose (1 vial) of OPCA alone was compared with two doses (1/2 vial each) administered 6 hours apart. Standard supportive care was provided in all cases.

Measurements and Main Results: Data was available for 115 patients, nine of which were fatalities. All patients' clinical condition was documented with a standardized SSS system accounting for each major body system. Each fatality received maximum severity scores of 20. The mean severity score of the 115 patients decreased from 4.19 to 3.29 points and there was no difference between the two treatment groups. The mean severity score of the 107 patients without fatalities decreased from 4.16 to 2.15. Antivenom related acute reactions occurred in 6 patients (6%), and no serum sickness occurred within the 95 cases contacted at the two week post treatment follow up.

Conclusions: In the first canine randomized trial of antivenin in the United States, Fab AV effectively stabilized or terminated venom effects. There were no statistical differences between treatment groups within the study time frame.

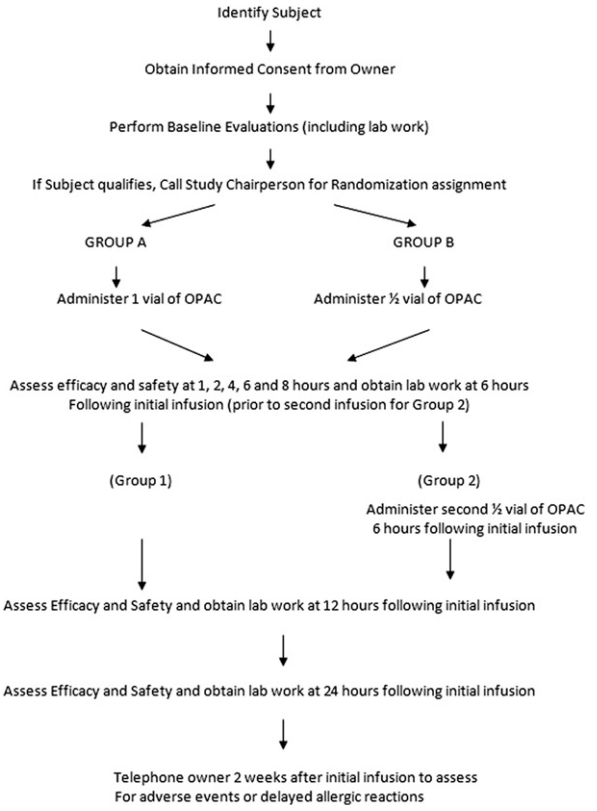

Fig. 1. Study schematic.

Keywords: Antivenom, snakebite, dogs
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295. F(ab')2 Antivenom in Dogs Envenomated by Pit Vipers

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Background: A previous clinical study using a $F(ab')_2$ antivenom was performed in pit viper envenomation in dogs. Some of these data were previously presented at 2010 International Veterinary Emergency and Critical Care Symposium. Pit viper envenomation in dogs is a common situation and represents an excellent comparative clinical model to study the effects of antivenoms owing to clinical

pathology, natural envenomation, and diagnostic capture methods.

Methods: This review is from a prospective study in 74 dogs presenting at 10 veterinary emergency hospitals in Arizona, Southern California, Texas and Florida which were naturally envenomated by pit vipers. Dogs were enrolled based on inclusion and exclusion criteria and were randomized into two $F(ab')_2$ antivenom treatment groups; Group A (n=37) received 4 vials at baseline and Group B (n=37) received 2 vials at baseline and 2 vials at 3 hours. Clinical scores and blood were collected at baseline (pre-treatment) and at hours 1, 3, 6, 12 and 24 after treatment.

Results: 73 of 74 dogs survived, with one small dog (<7kgs) succumbing to an intra-ocular envenomation. Over time, all dogs (Groups A and B) had statistically significant decreases in pain ($p<0.001$), extension of pain ($p<0.001$), discharge amount ($p<0.001$), discharge type ($p<0.001$), and overall clinical morbidity scores ($p<0.001$). Both groups had significant improvements in prothrombin time (PT) ($p<0.005$), and echinocytosis ($p<0.001$). Group A had significant reductions ($p=0.025$) in partial thromboplastin time (PTT) and Group B dogs had significant improvement ($p=0.016$) in platelet counts over time.

Discussion: The use of pit viper antivenom in veterinary medicine is popular as the propensity for envenomation is remarkably higher than for human medicine. In veterinary medicine, the use of $F(ab')_2$ antivenoms appears to have considerable advantages compared to whole IgG or Fab products, primarily related to affordability, ease of use, handling, and preferred distribution and elimination profiles.

Conclusions: This study demonstrated that administration of a $F(ab')_2$ pit viper antivenom is well tolerated and associated with a significant improvement in various clinical and hematologic abnormalities associated with pit viper envenomation.

Keywords: Antivenom, veterinary, dogs
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296. Crotalidae Venom Levels in Dogs Before and After Administration of $F(ab')^2$ Antivenom

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Background: Some of these data were previously presented at 2011 International Veterinary Emergency and Critical Care Symposium. The dog is an excellent comparative model to study envenomation owing to their similarity to human envenomation. Serum venom levels provide information to assess the neutralization properties of antivenoms.

Methods: This study evaluated the venom neutralizing effects of a $F(ab')_2$ antivenom (Instituto Bioclon) in 55 dogs naturally envenomed by pit vipers. Dogs received 4 vials of antivenom in the first 3 hours and serum was collected pre-treatment and at 1, 3, 6, 12, and 24 hours after antivenom administration. Venom levels (ng/mL) were determined using an enzyme-linked immunosorbent assay. Statistical analysis was performed using 1-way ANOVA repeated measures.

Results: The average venom level before antivenom administration was 158 +/- 22.3 ng/mL; average venom levels were significantly decreased ($p<0.001$) at T1 (0.07 +/- 0.07 ng/mL), T3 (0.12 +/- 0.11 ng/mL), T6 (0.00 ng/mL), T12 (1.74 +/- 0.81 ng/mL), and T24 (5.09 +/- 1.67 ng/mL).

Discussion: Venom level measurements are clinically valuable for a variety of reasons. First, venom levels can confirm envenomation. Serum venom level measurements can determine if circulating venom was neutralized by antivenom and if more antivenom is required. In addition, venom levels are valuable in understanding venom and antivenom pharmacokinetics.

Conclusions: This study demonstrates that administration of $F(ab')_2$ pit viper antivenom is associated with a rapid and prolonged neutralization of circulating pit viper venoms.

Keywords: Venom, dogs, antivenom
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297. A Retrospective Review of Coral Snake Envenomation in the Dog and Cat: 20 cases 1996 to 2011

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Objective: To expand the current limited data base and describe clinical signs, treatment, and outcomes of dogs and cats after envenomation by *Micrurus fulvius fulvius* and to report our clinical experience with the use of Coralmyn.

Animals: 16 dogs and 4 cats with *Micrurus fulvius fulvius* encounter or envenomation evaluated at a university teaching hospital.

Methods: Retrospective study.

Results: Medical records meeting the inclusion criteria were reviewed and evaluated for signalment, date and time of the snake encounter, elapsed time between encounter and hospital examination, initial physical examination results, antivenom type, length of hospitalization, and outcome. Initial physical examination findings included: quiet mentation, tetraparesis, ptyalism, tachypnea, shallow breathing, decreased to absent gag reflex, ataxia, muscle fasciculations, and decreased spinal reflexes. Laboratory findings in dogs included proteinuria, bilirubinuria, hemeproteinuria, increased AST, increased ALT, and hemolysis. Four dogs and 2 cats received Coralmyn and 4 dogs received North American Coral Snake Antivenom (NACSA). Adverse reaction to antivenom was suspected in 1 dog that received NACSA. Eight of 11 envenomated dogs were survivors with a median length of hospitalization of 4.5 days. Two of 3 envenomated cats were survivors with a median length of hospitalization of 4 days. Two dogs were euthanized, 1 dog suffered respiratory arrest, and 1 cat developed tachycardia that

progressed to pulseless electrical activity. Five dogs and 1 cat in the encounter group survived to discharge.

Conclusions: *Micrurus fulvius fulvius* envenomation is likely in the dog that is found to have concomitant lower motor neuron neuropathy, bulbar palsy, and hemolysis. Early diagnosis is crucial as antivenom administration can reduce morbidity and perhaps mortality. Prognosis is considered good with 71% of the envenomated patients in this study surviving to discharge. Supportive care which may include ventilator assistance and antivenom administration are the mainstays of therapy.

Keywords: *Micrurus fulvius fulvius*, Coralmyn, Veterinary toxinology
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