Keywords: Venom, pain, arthropod, ion channel

CURRENT PROGRESS TOWARDS THE NEXT GENERATION OF ANTIVENOMS

A.H. Laustsen. *Department of Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby, Denmark*
E-mail address: ahola@bio.dtu.dk

Background: Snakebite has gained increasing attention as a neglected tropical disease in recent times, and for a good reason. Each year, snakebite affects approximately 5 million people, causing mortality to more than a hundred thousand victims and leaving many more with permanent disabilities. These victims particularly come from poor, rural settings in developing countries. The only effective treatment option currently available is antivenom derived from the plasma of immunized animals. Unfortunately, such antivenoms are expensive to manufacture and may in some cases inflict severe adverse reactions in human recipients due to their heterologous nature, making them incompatible with the human immune system. Plasma-derived antivenoms have existed for more than 100 years. However, in the past few decades, research within novel snakebite envenoming therapies has emerged, including the use of novel immunization techniques, small molecule inhibitors, and antibody discovery. Although, none of these advances have yet reached the clinic, these efforts are likely to provide novel solutions to the ancient problem of snakebite within the foreseeable future.
Method: Development efforts in modern antivenom research have been centered around the use of small molecules and peptide inhibitors, oligonucleotides/aptamers, DNA immunization techniques, toxin-sequestering non-antibody proteins, nanobodies and antibody fragments, and fully human monoclonal antibodies.
Discussion: Although academically interesting results have been obtained in all these areas, several of the reported molecular scaffolds present a number of drawbacks that are likely to hinder their entry in the clinical setting. The main drawbacks include incompatibility with standard manufacturing platforms, high cost of manufacture, poor half-life, lack of versatility for many small molecule inhibitors, safety and immunogenicity for non-human proteins.
Conclusion: For a new generation of snakebite antivenoms to enter the clinic, more focus must be directed on manufacturability, regulatory pathway, adaptability, and translational research. Finally, it may be relevant to focus development efforts on molecular scaffolds that are sufficiently versatile to be able to neutralize several toxin families to avoid being 'one hit wonders' and to allow for streamlined manufacturing processes.
Keywords: Antivenoms, recombinant antivenoms, small molecule toxin inhibitors, toxin-neutralization, aptamers, snakebite, development, manufacture.

EXPLORING THE BASIS OF THE (UNCOMMON) DIRECT HEMOLYSIS CAUSED BY PHOPHOLIPASE A_2 OF *MICRURUS FULVIUS* VENOM

M.L. Fernández [1], P.Y. Quartino [2], R. Arce-Bejarano [1], J. Fernández [1], L.F. Camacho [1], J.M. Gutiérrez [1], D. Kuemmel [3], G. Fidelio [2], B. Lomonte [1,*]. [1] *Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José 11501, Costa Rica;* [2] *Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), Departamento de Química Biológica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina;* [3] *Biology and Chemistry Department, University of Osnabrueck, Osnabrueck, Germany*

* Corresponding author.
E-mail address: bruno.lomonte@ucr.ac.cr (B. Lomonte).

Background: A unique feature of the venom of *Micrurus fulvius* (Eastern coral snake) is its ability to induce a severe intravascular hemolysis in particular species, such as dogs or mice.
Methods: An experimental model of intravascular hemolysis in mice, after intravenous injection of venom, was developed.
Results: Within one hour, there was a drastic drop in hematocrit, morphological alterations of erythrocytes, hemoglobinemia, and hemoglobinuria together with abundant hyaline casts in kidney sections. These effects were shown to be caused by distinct phospholipase A_2 (PLA_2) isoforms capable of directly lysing erythrocytes *in vitro*, a very uncommon finding for such enzymes. Two PLA_2s, differing in their hemolytic activity, were purified from the crude venom and sequenced. Their structures and functional profiles (PLA_2-17: hemolytic; PLA_2-12: non-hemolytic) were compared, aiming to obtain clues towards understanding the basis for direct hemolysis. The two enzymes differed not only in their ability to cause intravascular hemolysis: PLA_2-17 additionally displayed lethal, myotoxic, and anticoagulant actions, whereas PLA_2-12 lacked all these effects. PLA_2-12 was much more active in hydrolyzing a monodisperse synthetic substrate than PLA_2-17, but the catalytic activity of latter was notably higher on a micellar substrate, or towards pure phospholipid artificial monolayers under controlled lateral pressures. Interestingly, PLA_2-17 could hydrolyze substrate at a pressure of 20 $mN \cdot m^{-1}$, in contrast to PLA_2-12 or the non-toxic pancreatic PLA_2.
Discussion: This finding suggests important differences in the monolayer penetrating power of the enzymes, which could be related to differences in toxicity. Comparative examination of primary structures and predicted three-dimensional folding of PLA_2-12 and PLA_2-17 revealed that differences concentrate in their N-terminal and central regions, leading to variations of the surface properties at the membrane-interacting interface. PLA_2-17 presents a less basic interfacial surface than PLA_2-12, but has more bulky aromatic residues, which could be associated with its higher membrane-penetrating strength.
Conclusion: Altogether, these structural and functional comparative observations suggest that the ability of PLA_2s to penetrate substrate interfaces could be a major determinant of toxicity, perhaps more important than protein surface charge.
Keywords: *Micrurus fulvius*, hemolysis, phospholipase A_2

PROTEOMIC STUDIES ON *MICRURUS* (CORAL SNAKES) VENOM REVEAL A DICHOTOMY OF PHENOTYPES

J. Fernández [1], P. Rey-Suárez [2], D. Pla [3], L. Sanz [3], M. Sasa [1], V. Núñez [2], J.M. Gutiérrez [1], J.J. Calvete [3], B. Lomonte [1,*]. [1] *Instituto Clodomiro Picado, Universidad de Costa Rica, San José, Costa Rica;* [2] *Grupo de Ofidismo y Escorpionismo, Universidad de Antioquia, Medellín, Colombia;* [3] *Instituto de Biomedicina de Valencia, CSIC, Valencia, Spain*

* Corresponding author.
E-mail address: bruno.lomonte@ucr.ac.cr (B. Lomonte).

Background: Nearly half of extant snake species, mainly within the Viperidae and Elapidae families, produce venom secretions of variable toxicities. The application of proteomic tools to the study of these venoms has led to an impressive growth in knowledge about their composition, toxicity, and immunogenicity. About one-third of all 'venomic' studies have focused on elapid species, mainly those inhabiting the Old World. The New World elapids, represented by coral snakes, have been less studied due the more limited availability of their venoms, owing to their difficult maintenance in captivity and very low venom yields.
Method: In recent years, however, a number of venomic studies on *Micrurus* species from North, Central, and South America have been conducted, thanks to the increasing sensitivity of proteomic tools.
Results/Discussion: Some general trends and patterns concerning the compositional, functional, and immunological characteristics of *Micrurus* venoms are emerging from such studies. Results gathered to date, encompassing about one-third of the >70 species of *Micrurus*, have revealed a dichotomy of venom phenotypes regarding the relative abundance of the omnipresent phospholipases A_2 (PLA_2) and 'three-finger' toxins (3FTx): some species express a PLA_2-predominant venom composition, while others display a 3FTx-predominant compositional pattern. These two sharply divergent toxin expression patterns appear to be related to phylogenetic positions and geographical distributions along a North-South axis, but further venom studies encompassing a higher number of species will be relevant to assess this hypothesis. The two venom phenotypes also show correlations to some toxic functionalities, complexity in the diversity of proteoforms, and immunological cross-recognition patterns. Based on the known (but partial) phylogenies, we have proposed the

3FTx-rich venom phenotype to be the ancestral state, with PLA_2-preponderant venoms appearing in more derived species.
Conclusions: Understanding the dichotomy of venom compositions within *Micrurus* snakes, observed in some cases even among sympatric species that inhabit relatively small geographic areas, with probable similar diets, remains a challenging area of research.
Keywords: *Micrurus*, venom, proteomic, phenotypes

VENOM TOXINS TO DRUGS: ANTI-THROMBOTIC AND ANTI-METASTASIS COMPOUNDS FROM SNAKE VENOMS

S.P. Mackessy [1,*], A.J. Saviola [1,2], A.K. Mukherjee [1,3]. [1] *School of Biological Sciences, University of Northern Colorado, Greeley, CO 80639-0017, USA;* [2] *Department of Biomedical and Health Sciences, University of Vermont, Burlington, VT 05405, USA;* [3] *Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur, Assam 784 028, India*

* Corresponding author.
E-mail address: stephen.mackessy@unco.edu (S.P. Mackessy).

Background: Snake venoms contain a variety of components that when deployed trophically result in cascading collapse of numerous regulatory systems of prey. Paradoxically, many of these same components have potential and actual therapeutic utility, largely because they represent co-opted regulatory components of normal vertebrate systems. Notable successful examples include exenatide (Byetta®, derived from *Heloderma* venom) and ziconotide (Prialt®, derived from *Conus* venom), but commercial success of many potential toxin therapeutics has not yet been realized.
Methods: Our lab has purified and characterized several components from venom of the Yucatan Rattlesnake (*Crotalus simus tzabcan*) and from venom of Pakistan Russell's Viper (*Daboia r. russelii*) using several chromatographic steps. A non-enzymatic peptide, named tzabcanin, is a new disintegrin isolated from *C. s. tzabcan* venom (~7105 Da); it contains the canonical RDG domain and is the most prevalent of at least six disintegrins in this venom. Russelobin, a thrombin-like enzyme (~38.7 kDa), was isolated from the venom of *Daboia russelii* and was extensively characterized using numerous *in vitro* and *in vivo* assays.
Results: Tzabcanin shows minimal cytotoxicity toward colon (COLO-205) and breast (MCF-7) cancer cells, but it inhibits binding of these cells to fibronectin and vitronectin at low micromolar concentrations. Tzabcanin further inhibits migration of melanoma (A-375) and lung cancer (A-549) cells in scratch assays. Binding of tzabcanin to these cell lines is dependent on the presence of $\alpha_v\beta_3$ integrins, often greatly overexpressed in cancer cells. Examples of serine proteinases with fibrinogenolytic activities, such as russelobin, have been characterized from *Daboia* and *Crotalus* venoms. These αβ- and α-fibrinogenases may have utility in situations where depletion of patent fibrinogen is desirable, including certain cancers and cardiovascular disorders.
Discussion: Like other disintegrins, tzabcanin may therefore represent a useful model for design of drugs limiting metastatic potential of various cancers. Because it is essentially non-toxic and shows some specificity toward binding $\alpha_v\beta_3$ integrins, non-target effects, unlike standard chemotherapeutics, should be negligible. Venom-derived enzymes also have promise for development as therapeutics or as models for their design, and russelobin was also non-toxic toward mice.
Conclusion: Snake venoms represent a rich source of potent biological activities that can be retargeted for therapeutic uses, and venoms from rear-fanged snakes represent a novel and largely unexplored source of novel compounds. In addition to their use as therapeutics, toxins can have utility in diagnostics and other applications, and so the continued exploration of novel uses for these potent molecules is highly warranted.
Keywords: bioassays, cytotoxic, metastasis, therapeutics, venoms

PURIFICATION AND CHARACTERIZATION OF CYSTEINE RICH-SECRETORY PROTEINS (CRISPS) FROM THE VENOM OF THE SOUTHERN PACIFIC RATTLESNAKE (*CROTALUS OREGANUS HELLERI*): THEIR ROLE ON BLOOD AND LYMPHATIC ENDOTHELIAL CELL PERMEABILITY

J. Marquez [1], V. Parra [1], M. Suntravat [1], E.E. Sanchez [1,2,*]. [1] *National Natural Toxins Research Center (NNTRC), Texas A&M University-Kingsville, MSC 224, 975 West Avenue B, Kingsville, TX 78363, USA;* [2] *Department of Chemistry, Texas A&M University-Kingsville, MSC 161, Kingsville, TX 78363, USA*

* Corresponding author.
E-mail address: elda.sanchez@tamuk.edu (E.E. Sanchez).

Background: Cysteine-rich Secretory Proteins (CRiSPs) have long been recognized as ubiquitous components of many snake venoms, however, no clear explanation has been provided for the role they play in venoms. Some CRiSPs have been shown to inhibit ion channel activities and have major effects on cell signaling pathways in vascular endothelial cells. We speculate that CRiSPs, via combined effects on cell signaling pathways and ion channel activities, disrupt normal interstitial fluid dynamics adjacent to the snakebite, accelerating the transfer of the macromolecular toxins in the venom into the lymphatic circulation, which plays a critical role in venom absorption and distribution into the systemic circulation. The rapid delivery of these toxins into the circulation contributes to the acute effects of envenomation and the rapid incapacitation and death of the snake's prey. The goal of our study was to characterize the cellular and molecular basis for the effects of Hellerin, a newly identified CRiSP isolated from the venom of the Southern Pacific rattlesnake (*Crotalus oreganus helleri*), on the function of blood and lymphatic endothelial cells.
Method: Crude venom was characterized by reversed-phase HPLC fractionation, followed by analysis of chromatographic fractions by SDS-PAGE and N-terminal sequencing. Preliminary cytotoxicity screening of CRiSP was tested on human umbilical vascular endothelial cells (HUVEC).
Results: svCRiSPs were isolated and characterized from the snake *C. o. helleri.* The N-terminal sequence of a 28 kDa protein band in fraction 13 was determined and it identified the protein as a CRiSP. The preliminary results showed that the purified CRiSP, named Hellerin, dose-dependently inhibited HUVEC cell proliferation with (50% cytotoxic concentration) CC_{50} of 2.3 μM.
Discussion/Conclusion: Our study describes the purification and characterization of Hellerin, the first CRiSP isolated from the venom of *C. o. helleri*. Purified Hellerin had cytotoxic effects on HUVEC cells. Hellerin will be further tested on human dermal blood and lymphatic endothelial cell permeability. Knowledge gained from these studies will contribute to a new level of understanding of the pathophysiology of snakebite.
Keywords: Cysteine Rich-Secretory Proteins, Hellerin, permeability, cytotoxicity

HARNESSING SNAKE VENOMS TO MAKE *T. BRUCEI* FOREVER GO TO SLEEP

A. Martos-Esteban [1,2], M. Carrington [2], A.H. Laustsen [1,*]. [1] *Department of Bioengineering, Technical University of Denmark, Kgs. Lyngby, 2800, Denmark;* [2] *Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QW, United Kingdom*

* Corresponding author.
E-mail address: ahola@bio.dtu.dk (A.H. Laustsen).

Background: *Trypanosoma brucei* is a parasitic protozoan species capable of infecting insects, whose bite transmits African sleeping sickness (trypanosomiasis) in humans. Current treatments are becoming ineffective due to the parasite's ability to avoid the lytic immunogenic response of the host. The parasite achieves this avoidance by modifying the composition of its outer coat, which is mainly composed of Variable Surface Glycoprotein (VSG). Snake venoms are composed of toxic proteins and peptides, with or without enzymatic activity, and a range of other molecules that may influence physiological processes. Previously, it has been demonstrated that viper venoms are able to kill certain parasitic species, but elapid snake venoms have never been investigated. The venom of the elapid *Naja nigricollis* (black-necked spitting cobra) is mainly composed of cytotoxic three-finger toxins (cytotoxins) that interfere with and disrupt cellular membranes with high target specificity. Here, we investigated how *T. brucei* is affected when this parasite is subjected to whole venom of *N. nigricollis*.
Method: The venom of *N. nigricollis* was diluted in HMI-9 media at different concentrations and tested against a genetically engineered *T. brucei* strain expressing GFP attached to glycophosphatidylinositol (GPI),