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"Toxinology in the 21st century: Public health impact from basic, translational and clinical sciences"

POSTER SESSIONS

ABSTRACTS

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ACADEMIC POSTERS SESSION I

Topic A: Public Health Issues

PURIFICATION OF HYALURONIDASE FROM *NAJA MELANOLEUCA* VENOM AND PRODUCTION OF POLYCLONAL ANTIBODIES.	BINTA	KURFI
THE CHALLENGES AND SPECIFICITIES OF THE PHARMACOVIGILANCE OF ANTIVENOMS	FERNANDO	VAZQUEZ
EPIDEMIOLOGICAL PROFILE OF OPHIDIAN ACCIDENTS IN RIVERSIDE COMUNITIES OF RIOS SOLIMÕES AND JURUÁ RIVERS IN THE STATE OF AMAZONAS: CHARACTERISTICS OF NON-NOTIFIED CASES.	Jacqueline A. G.	Sachett
DEVELOPMENT OF A HANDBOOK FOR PREVENTION AND ATTENTION OF ACCIDENTS BY VENOMOUS ANIMALS IN COLOMBIA	Javier	Rodríguez Buitrago
VULNERABILITY ANALYSIS FOR THE SNAKE BITES IN COLOMBIA	Javier	Rodríguez Buitrago
OPTIMIZATION BY FACTORIAL ANALYSIS OF F(AB´)2 GENERATION FROM HYPERIMMUNE EQUINE PLASMA FOR ANTIVENOM PREPARATION	Matías	Fingermann
EFFICIENT EXPRESSION AND PURIFICATION OF RECOMBINANT SPHINGOMYELINASE D FROM *LOXOSCELES LAETA* IN LEPIDOPTERAN LARVAE AS A CANDIDATE FOR ANTISERUM PRODUCTION	Matías	Fingermann
IMPLICATIONS OF SNAKE VENOM VARIATION ON ANTIVENOM NEUTRALIZATION: THE CASE OF NORTH AMERICAN VIPERS	Alejandro	Alagón

1 PURIFICATION OF HYALURONIDASE FROM NAJA MELANOLEUCA VENOM AND PRODUCTION OF POLYCLONAL ANTIBODIES.

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Snakebite is a worldwide environmental and occupational hazard with significant morbidity and mortality. Snakebite involves subcutaneous or intramuscular injection of venom into prey/human victims. The pathology of snakebite depends upon the concentration and also on the rate at which toxins diffuse into the systemic circulation for transport to their site(s) of action. Hyaluronidase, being ubiquitous enzyme in animal venoms, has not been well studied despite its crucial role in toxins diffusion. This research aimed at purification of hyaluronidase from *Naja melanoleuca* (Forest cobra) venom and production of its polyclonal antibody as a potential vaccine candidate. Hyaluronidase was purified from *Naja melanoleuca* venom through two steps of purification: Sephadex G-75 and DEAE-cellulose ion exchange chromatography. The purified hyaluronidase has molecular weight of approximately 54 KDa on SDS-PAGE. Purified hyaluronidase and *N. melanoleuca* venom were used to produce antiserum in rabbit according WHO guidelines for antivenom production (WHO, 2010). Both the antiserum raised against purified hyaluronidase and N. melanoleuca venom neutralized LD50 concentration of *N. melanoleuca* venom hyaluronidase in vitro in a volume dependent manner. Also In vivo neutralization assay showed that 140 µl of both the antiserum inhibited mouse death 100%, where as 30 and 70µl increased the survival time of envenomed mouse when pre-incubated with LD50 of *N. melanoleuca* venom. Both the antiserum raised against *N. melanoleuca* venom and its purified hyaluronidase were capable of neutralizing hemorrhagic effects induced by *N. melanoleuca* venom in mice. This finding showed that hyaluronidase plays a critical role in snake venom systemic toxicity and can be targeted as potential candidate for snakebite therapy.

2 THE CHALLENGES AND SPECIFICITIES OF THE PHARMACOVIGILANCE OF ANTIVENOMS

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Introduction: Antivenom is the only treatment able to eliminate the venom from the body and thereby to prevent from its toxic effect. Over 95% of animal envenoming, mainly snakes or scorpions, occur in rural areas of the developing world where patients are managed in facilities with very limited infrastructure and resources. This makes safety reporting and evaluation of antivenom quite challenging. It is key to understand the specificities related to envenoming management in order to adequately ensure the pharmacovigilance of antivenoms.

Methods and Results: Clinical manifestations of envenoming are heterogeneous as they depend on many factors including the animal species, the amount of injected venom and the time elapsed from bite or sting. Envenoming can induce adverse events such as allergic reaction similar to those associated to antivenoms making differential diagnosis difficult. In addition, patients are often admitted to hospital emergency or intensive care units which do not have adequate pharmacovigilance system especially in developing countries. Antivenoms often have various potencies and/or different dosage instructions from a product to another, not to mention the worrying issue of counterfeit and substandard drugs. The suspected adverse reactions must be notified and subsequently evaluated for the causality assignment through algorithms. An adaptation of the Naranjo's algorithm specific to antivenoms is proposed here in order to improve the sensitivity and specificity of causality grades. The validity of this proposal will need to be confirmed by prospective clinical study.

Conclusion: Stakeholders from hospitals to manufacturers should take all necessary steps in order to guarantee an adequate pharmacovigilance of antivenoms. A better causality assessment of adverse reactions such as proposed here will contribute to improve safety assessment of antivenoms. This will help to fulfill one the WHO strategic goals which are to ensure access to safe, effective and affordable antivenoms worldwide.

3 EPIDEMIOLOGICAL PROFILE OF OPHIDIAN ACCIDENTS IN RIVERSIDE COMUNITIES OF RIOS SOLIMÕES AND JURUÁ IN THE STATE OF AMAZONAS: CHARACTERISTICS OF NON-NOTIFIED CASES.

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Snakebite accidents are considered an important factor for the severity and mortality of victims from rural areas and with little access to health services, as is the case of the riverside population of the state of Amazonas. The aim was to describe the epidemiological profile of accident victims in riverine populations of two rivers in the state of Amazonas, the "Snowball" technique was used, structured by a questionnaire applied during on-site interviews during the expedition at the Fluvial Basic Unit of January to April 2019. 157 victims of ophidian accidents were interviewed, 82 cases in the Solimões River (52.23%) and 75 cases (47.77%) in Juruá, mainly caused by the genus Bothrops (112 cases, 71.34%), being the majority male (140 cases/89.17%), aged between 16 and 45 years (72 cases/45.86%), with predominance of accidents in the lower limbs (125 cases/79.61%). Of the respondents in the Solimões River, the majority sought medical care (60.98%), unlike those interviewed in the Juruá River, where the majority (74.67%) did not seek care. The main reasons for not seeking care were mainly because the patients did not want or accept care (57 cases, 64.77%), also because they had no idea that they needed help or because they prioritized alternative medicine; and because they did not have the financial conditions or structure for the locomotion until the care (37 cases, 42.05%). The banks of notifications as well as the epidemiological data presented in several studies sometimes do not show the real health conditions of certain populations, especially those coming from places of difficult access such as the riverside. This study can bring data that contribute to the identification of the characteristics of the accidents as well as the influence of the culture and the popular traditions in the life of these people for ophidian accidents.

4 DEVELOPMENT OF A HANDBOOK FOR PREVENTION AND ATTENTION OF ACCIDENTS BY VENOMOUS ANIMALS IN COLOMBIA

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In Colombia, near of 100 cases of snake bite accidents weekly were reported only in 2018. With respect to other kinds of accidents, exist lack of epidemiological records and knowledge by the population, technicians and health professionals. The deficiencies in communitary education against the risk factors for accidents by poisonous animals, their prevention and proper administration of first aids are related with inadequate practices such as suction, the use of tourniquets, application of organic material, incisions, among others that are harmful. By the wide presence of the Military Forces in the Colombian territory, there are vulnerable to these accidents, but also becomes an opportunity to give education for the prevention of this and other risks to population.

A web search was made focused on the handbooks for the prevention and attention of accidents by poisonous animals with emphases on primary health care and community education in Colombia, and on the recommended interventions for the attention of bites and stings by poisonous animals and measures for the prevention of cases through the use of MeSH terms how: Bites and Stings AND First Aid, Bites and Stings AND prevention AND Colombia, including evidence developed by the International Liaison Committee on Resuscitation and the International Federation of Red Cross and Red Crescent Societies.

From the review, the strategies by the prevention and first aid for these accidents were identified and collected and strength of recommendations was measured. Now the Handbook for Prevention and First Aid in Accidents for Poisonous Animals is constructed; in next phase a pilot study with medicine students of Universidad Militar Nueva Granada and members of Military Forces will be conducted to establish the practical application, coherence and easiness of understanding and its impact on the communitarian competences in front of this events, through the application of a semi-structured survey.

5 VULNERABILITY ANALYSIS FOR THE SNAKE BITES IN COLOMBIA

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Due to the global impact of the snake bite, in 2018 a global vulnerability analysis was published. It showed that countries as Colombia have species of medical importance, difficulties for accessing to health services in different regions and limitations in the collection of data that adequately identify the vulnerability of the population.

In Colombia, the snake bite accident was identified as an event of interest in public health by the Ministry of Health since 2004, however, only since 2008 began its mandatory report.

The retrospective analysis of the annual reports on the snake bite accidents of the National Epidemiological Surveillance System SIVIGILA was carried out from 2008 to 2017 to identify vulnerable points in care and registration of the event. The most cases were in Antioquia, Norte de Santander, Meta, Bolívar and Córdoba and the 28% of national population live in this Departments, however the mortality was higher in Departments with less number of incidents such Guainía, Guaviare and Vaupés. Although the most frequent accident was caused by the genus *Bothrops*, *Porthidium*, *Bothriechis*, *Bothriopsis* and *Bothrocophias* (58%), in 26.98% of the cases the kind of accident was not identified, the average of the report was 69% even though in some Departments it may be lower 20% and near of 40% of the first aid measures used are not currently recommended as tourniquets, suction or bleeding. Finally, 85.6% of affected were uninsured or their health care was subsidized by the state and only 11.17 were insured patients.

It's necessary to promote actions aimed to improve the identification of accidents, quality of surveillance, skills for diagnosis and attention by the health providers and encourage initiatives for community participation in the risk management of accidents by fauna and the continuous teaching of first aid for affected populations by the snake bites.

6 OPTIMIZATION BY FACTORIAL ANALYSIS OF F(AB')2 GENERATION FROM HYPERIMMUNE EQUINE PLASMA FOR ANTIVENOM PREPARATION

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Parenteral administration of antivenoms is the only available specific treatment for most animal envenomation situations. Antivenom production from hyperimmune equine plasma is one of the most widespread technologies employed for these purposes. The choice of producing antivenoms based on whole immunoglobulin or its fragments (F(ab')2 and Fab) follows the hypothesis that the Fc portion of IgG could be responsible for early adverse effects observed after antivenom administration due to its complement activation capacity. Production of antivenoms based on F(ab2)' fragments obtained from hyperimmune equine plasma usually follows the procedure described by Harms'. Pepsin digestion of IgG is a critical step in this production process since undigested IgG molecules cannot be easily excluded from the final product by following downstream processing steps. Factorial analysis allows to simultaneously test the effects of different process variables as a first step on process optimization. Hyperimmunised equine plasma used for anti-bothropic antivenom production was employed in these studies. The effect of temperature (T), reaction time (t), saline concentration (S) and enzyme to protein relation (E/P) on IgG digestion were firstly studied with a two-level factorial design of four variables (2^4). The variables selected for T (25ºC and 30ºC), t (30 min and 90 min), S (50 mM and 150 mM) and E/P (1/100 and 1/50) were based on the original Harms protocol. The results obtained evidenced that while T, t and E/P are relevant factors, showing also significant two factor's interaction, S has no appreciable effect. Therefore, a second round of factorial design experiments was performed with a fixed level of E/P (1/50) and S (150 mM) but increasing the temperature to 37ºC and extending the time to 60 min and 180 min. The results of these two sets of experiments were combined and analyzed by regression analysis for process optimization.

7 EFFICIENT EXPRESSION AND PURIFICATION OF RECOMBINANT SPHINGOMYELINASE D FROM LOXOSCELES LAETA IN LEPIDOPTERAN LARVAE AS A CANDIDATE FOR ANTISERUM PRODUCTION

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Spiders from the genus *Loxosceles* are of great health importance for south america and the whole world. Particularly in Argentina, *Loxosceles laeta* is the most abundant species. The bites from *Loxosceles laeta* spiders can cause a wide array of toxic effects ranging from dermonecrosis and complement-dependent haemolysis to disseminated vascular coagulation and renal failure. Current available treatment consists of antiserum produced in horses from whole venom obtained by electrostimulation of spiders which is a complicated process and produces insufficient quantities.

Sphingomyelinase D (Smase D) represents the main toxic component in *Loxosceles laeta* venom and as such constitutes and ideal candidate for recombinant expression for large scale production of antisera. In this work we studied the expression and purification of recombinant Smase D (rSmase D) in baculovirus infected lepidopteran larvae as biofactories.

Expression was successful in both *Spodoptera frugiperda* and *Rachiplusia nu* larvae, obtaining a final yield after IMAC purification of approximately 2529 ± 48 and 1223 ± 120 mU/larvae for each species respectively as measured by commercial colorimetric assay and with a high level of purity. The resulting protein was not glycosylated and inoculation in rabbits demonstrated dermonecrotic lesions comparable to whole venom. Thus, Sphyngomyelinase D represents a good example of the use of insect larvae as a small bioreactor for the production of recombinant proteins in a cost-effective and easily scalable manner.

8 IMPLICATIONS OF SNAKE VENOM VARIATION ON ANTIVENOM NEUTRALIZATION: THE CASE OF NORTH AMERICAN VIPERS

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Mexico is the country with the highest number of venomous snakes in the Americas; 72 species of vipers, grouped in 10 genera, have been described. Four thousand snake bites per year are registered in Mexico, these numbers are likely to be underestimated due to underreporting. There are three antivenoms producers in Mexico: Bioclon, Birmex and Inosan Biopharma. An appropriate snake antivenom in Mexico should be capable to efficiently neutralize all the species of Mexican vipers. Some species have geographic and ontogenetic variation in their venoms which makes the matter of neutralization more complicated. In recent years, our group has focused on the interspecific and intraspecific characterization of different species, including species of *Crotalus* from the *simus complex*, the *molossus complex*, and species of the genus *Ophryacus*. The following ontogenetic variation has been observed in the *simus* and *molossus complexes*: juvenile venoms systematically presented lower LD_{50} than adults. Moreover, juveniles of species such as *C. culminatus*, *C. basiliscus*, *C. tzabcan* and *C. molossus*, presented higher percentages of crotamine, a molecule that is not or only partially neutralized by antivenoms. On the other hand, populations of rattlesnakes (e.g., *C. scutulatus scutulatus* and *C. tzabcan*) have been characterized regarding the presence and absence of crotoxin which is well neutralized by antivenoms. Finally, we have found neurotoxic components in *non-Crotalus* viper venoms that are similar in sequence and lethal potency to crotoxin. These venoms are generally well neutralized by Mexican antivenoms, however the number of vials recommended to treat patients vary from an antivenom producer to another. Venoms variability within snake species needs to be regularly evaluated and its impact on commercialized antivenoms neutralization regarding to lethal, biological and enzymatic neutralization, as well as through well defined clinical monitoring programs.

ACADEMIC POSTERS SESSION I

Topic B: Basic Toxinology

PHOTOBIOMODULATION EFFECT IN C2C12 CELLS AFTER INCUBATION WITH *BOTHROPS JARARACUSSU* VENOM: REDUCTION OF OXIDATIVE STRESS AND INFLAMMATORY MEDIATORS	Stella	Zamuner
NEUROMUSCULAR BLOCKADE BY NXH8, A CHEMICALLY SYNTHESIZED A-NEUROTOXIN FROM *MICRURUS CORALLINUS* (CORALSNAKE) VENOM	Henrique	Ramos
EFFECT OF VANILLIN, SYRINGALDEHYDE, AND PARAHYDROXYBENZALDEHYDE EXTRACTS ON PLASMA RECALCIFICATION TIME OF *NAJA NIGRICOLLIS* VENOM TREATED PLASMA	Hadiza	Abdullahi
PRELIMINAR PROTEOMIC CHARACTERIZATION OF *BOTHRIOPSIS CHLOROMELAS* (BOULENGER, 1912) SNAKE VENOM FROM PER&UACUTE;	Gustavo	Sandoval
TOXICOLOGICAL STUDY OF BEE VENOM (*APIS MELLIFERA*) FROM THE BUENOS AIRES PROVINCE, ARGENTINA.	Jantine	Van Grootheest
NEUTRALIZING CAPACITY OF *BUTEOGALLUS CORONATUS (*"CROWN EAGLE") AND OTHER ANIMAL SERA ON SNAKE VENOM.	Pablo	Regner
TRANSLATIONAL TARANTULA PHYLOGENOMICS: EVOLUTION OF THERAPHOSID SPIDERS AND THEIR DEFENSIVE ARSENAL WITH IMPLICATIONS FOR VENOM BIOPROSPECTING	Tim	Lüddecke
TOXIN TO LEAD: AN INHIBITOR OF FACTOR XIA ENGINEERED FROM BANDED KRAIT VENOM TOXIN FASXIATOR SHOWED SUPERIOR IN VIVO EFFICACY-SAFETY PROFILE COMPARED TO HEPARIN	Cho Yeow	Koh
DEVELOPING TOWARDS ANTI-VENOM DRUGS BY ENDOGENOUS INHIBITOR AGAINST THE METALLOPROTEINASE INDUCED HEMORRHAGE; RATIONAL DESIGN OF DRUG AND THERAPEUTIC POTENTIAL FOR SNAKEBITE	Narumi	Aoki-Shioi
COMPARISON OF THE VENOM COMPOSITION OF *BOTHROPS ASPER, BOTHROCOPHIAS MYERSI, CROTALUS DURISSUS AND LACHESIS MUTA* SNAKES SPECIES, FROM ANDINA REGION IN COLOMBIA.	Stefano	Scovino
POTENTIAL VENOM DIVERSITY HIDDEN BY AUTOMATIC ANNOTATION: MANUAL CURATION OF HIGHLY-EXPRESSED SEQUENCES AUTOMATICALLY ANNOTATED AS "NO HITS"; IN *P. NIGRIVENTER* TRANSCRIPTOME REVEALS NOVEL VENOM COMPONENTS	Ana	Paiva
RESTROSPECTIVE BIBLIOMETRIC ANALYSIS ON COLOMBIAN SNAKE TOXINOLOGY KNOWLEDGE	Augusto Rafael	Acosta Peña
INSIGHTS INTO THE EVOLUTIONARY AND MEDICAL SIGNIFICANCE OF UNIQUE ALPHA-NEUROTOXIN AND PHOSPHOLIPASE A2 COMPOSITIONS IN NAJA SPP. (COBRA) VENOMS	Choo Hock	Tan

1 PHOTOBIOMODULATION EFFECT IN C2C12 CELLS AFTER INCUBATION WITH BOTHROPS JARARACUSSU VENOM: REDUCTION OF OXIDATIVE STRESS AND INFLAMMATORY MEDIATORS

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INTRODUCTION: Bothrops envenomation is often associated with severe local pathological manifestations, with myonecrosis being the most severe damage. The antivenom administration is clinically effective in systemic envenomation but does not neutralize the local manifestations. Previous studies in our group have shown that photobiomodulation (PBM) increase the viability of C2C12 muscle cells when incubated with *B. jararacussu* venom (BjV) and further promotes the proliferation of these cells. **AIM:** To analyze the effect of PBM on oxidative stress and the release of inflammatory mediators caused by BjV on C2C12 myoblast cells. **METHODOLOGY:** Myoblasts cells ($1X10^4$ cells/well) were incubated for 24 hours for cell adhesion. BjV (12.5ug/ml) was applied and the cells were immediately irradiated with PBM (660 and 780 nm, power of 100 mW, irradiated time 10 sec, energy density of 4 J/cm^2). Then, oxidative stress (NO, H2O2, SOD, Gpx and TBARS) were analyzed after BjV incubation. The NO concentration was evaluated by GRIESS and the H2O2 by the phenol red method. The concentration of SOS and Gpx by spectrometry. The analysis of TBARS, IL-1b, IL-6, and TNF-a by the Elisa method. **RESULTS:** C2C12 cells were able to produce both NO and H2O2 after the addition of the venom being statistically different from the control. PBM irradiation significantly reduced NO release, but did not alter H2O2 release. No difference was observed of anti-oxidant enzymes in C2C12 cells incubated to BjV. PBM induced a significant increase of SOD in comparison to the venom group, but not modify Gpx release. Moreover, the release of IL-1b, IL-6, and TNF-a were reduced by 660 and 780 nm irradiation. **CONCLUSION:** PBM resulted in cytoprotection on myoblast C2C12 cells after venom exposure. This protection involves the reduction of reactive species of oxygen and nitrogen and pro-inflammatory cytokines release.

2 NEUROMUSCULAR BLOCKADE BY NXH8, A CHEMICALLY SYNTHESIZED A-NEUROTOXIN FROM MICRURUS CORALLINUS (CORALSNAKE) VENOM

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Coralsnakes of the genus *Micrurus* are the main representatives of the Elapidae in the Americas and envenomation is considered dangerous because of the lethal combination of pre- and postsynaptic neurotoxins in these venoms. In Brazil, most bites are caused by *M. corallinus* and *M. frontalis*, primarily in the southeastern and southern regions of the country. A major limitation to the study of coralsnake venoms is the difficulty in maintaining these snakes in captivity for venom extraction. In addition, most species have very low venom yields. One solution to this problem is to chemically synthesize the major neurotoxins once these have been identified by conventional methods. In the past 20 years, several toxins from *M. corallinus* venom have been characterized, including four potential α-neurotoxins of the three-finger toxin (3FTx) family and a phospholipase A_2 (PLA_2), presumed to be responsible for the presynaptic toxicity of the venom. In this work, we have chemically synthesized NXH8, an α-neurotoxin structurally related to candoxin from krait (*Bungarus candidus*) venom, and examined its neurotoxicity in mouse isolated phrenic nerve-diaphragm preparations. NXH8 (10 mg/ml) caused complete neuromuscular blockade in 20-60 min. This blockade was rapidly and totally reversed by washing the preparations or by adding neostigmine (29 mM, an acetylcholinesterase inhibitor) or 3,4-diaminopyridine (230 mM, a neuronal voltage-gated K^+channel blocker) to the organ bath after complete blockade. In addition, preincubation (30 min, 37 ^oC) of NXH8 with coralsnake antivenom (produced by the Instituto Butantan using venoms from *M. corallinus* and *M. frontalis*; 1 mL neutralizes the lethality of 1.5 mg of *M. frontalis* venom) completely prevented the characteristic blockade produced by the toxin. These results show that NXH8 is a classic α-neurotoxin that produces reversible neuromuscular blockade. The neutralization by antivenom confirms the usefulness of this product for reversing the neuromuscular blockade by NXH8.

3 EFFECT OF VANILLIN, SYRINGALDEHYDE, AND PARAHYDROXYBENZALDEHYDE EXTRACTS ON PLASMA RECALCIFICATION TIME OF NAJA NIGRICOLLIS VENOM TREATED PLASMA

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Several medicinal plants have been used in Nigerian communities as antidotes for snakebite complications without scientific validation. In this study, the effect of vanillin, syringaldehyde, and parahydroxybenzaldehyde extracts on recalcification time of plasma treated with *Naja nigricollis* venom was investigated. The *N. nigricollis* venom was found to increase the plasma recalcification time of all the plasma samples. A dose dependent reduction in venom-associated increase in the plasma recalcification time was observed for the vanillin, syringaldehyde and parahydroxybenzaldehyde extracts in caprine plasma; syringaldehyde extract in the bovine plasma at 1, 10, and 100 µg/ml. Reduction of the plasma recalcification time by the vanillin extract in the ovine plasma and parahydroxybenzaldehyde in the ovine and camelid plasma was also dose-dependent but in a reverse manner. A non-dose dependent reduction was observed for vanillin and parahydroxybenzaldehyde in bovine plasma; vanillin and syringaldehyde extracts in camelid plasma and syringaldehyde extract in ovine plasma. This study shows that extracts of vanillin, syringaldehyde and parahydroxybenzaldehyde could serve as prototypes for an antidote for the reversal of increase in plasma recalcification time posed by *N. Nigricollis* venom.

4 PRELIMINAR PROTEOMIC CHARACTERIZATION OF BOTHRIOPSIS CHLOROMELAS (BOULENGER, 1912) SNAKE VENOM FROM PERÚ

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Bothriopsis chloromelas (Inca Forest-pitviper) is an arboreal snake mainly distributed in the Central Andes of Perú. Its habitat has been severely affected by climate change and urbanization. Together, this situation has contributed to an increase of snakebites in this area. For this reason, it is urgent to get a deep understanding of snake venom composition in order to design appropriate public health strategies to effectively neutralize snake envenomation. The objective of this research was to preliminarily describe the proteome composition of *B. chloromelas* venom using “shot-gun” proteomics approach. Thus, venom from *B. chloromelas* was collected from specimens kept under cautiverium at Serpentarium "Oswaldo Meneses" (Museo de HIstoria Natural-UNMSM). After that, 500 ug of lyophilized venom were dissolved in ammonium bicarbonate 50 mM and then proteins were precipitated in acetone for 3 h at -20 ºC, centrifugated and resuspended in initial buffer. After overnight trypsinization, sample was applied to C18 reverse-phase HPLC column and fractions obtained were analyzed in a mass spectrometer AB SCIEX TOF/TOF^{TM} 5800. Mass of recovered peptides and its amino acid sequences were analyzed using MASCOT (Matrix Science) and searched in GenBank and Uniprot databases. Finally, proteins were identified and functionally annotated. As a result, *B. chloromelas* venom showed a diverse protein composition formed by metalloproteinases (40.1%), serinoproteinases (22.4%) and phospholipases A2 (8.5%). Furthermore, other groups of identified proteins included L-amino acid oxidases, 5’-nucleotidases, phosphodiesterases, glutaminyl cyclases, kallikrein-like and cysteine-rich secretoy proteins (CRISPs). In conclusion, *B. chloromelas* shows a highly complex venom proteome with special characteristics that differs from other snake venoms in Perú

9 TOXICOLOGICAL STUDY OF BEE VENOM (APIS MELLIFERA) FROM THE BUENOS AIRES PROVINCE, ARGENTINA.

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Samples of *Apis mellifera mellifera* venom from different hives in two regions of the Buenos Aires province were analysed for their lethal potency, myotoxic, hemolytic and inflammatory-edematizing activity and the histological alterations they produce in the heart, lungs, kidneys, skeletal muscle and liver of mice. *In vitro* studies focused on the venom´s hemolytic activity in different systems and species (horse, man, sheep and rabbit), and on the proteolytic and coagulant activity in plasma and fibrinogen. In all samples protein content, SDS-PAGE electrophoretic profile and phospholipase activity were determined. The chromatographic profile of the pooled venoms and the hemolytic and phospholipase activity of its fractions were studied as well. Lethal potency in mice ranged from approximately 3.5 mg/kg to 10 mg/kg. Hemolytic activity showed similar toxicity levels when observing hemolysis *in vitro and in vivo*. Statistical analysis did not show differences in toxicity between regions. Erythrocytes of different species varied in their sensitivity to the venom pool, equines being the most sensitive and sheep the most resistant to direct hemolytic action. Local and systemic myotoxicity was evidenced by either the elevation of serum creatin kinase and/or histopathology. All samples caused significant pathological alterations; pulmonary, cardiac, renal and skeletal muscle lesions were substantive and can be related to the physiopathological mechanisms of envenomation. The venoms from different apiaries and regions of the Buenos Aires province showed very similar toxicological and biochemical characteristics. The severity of envenomation in case of a swarming is therefore more related to the number of bees than to the differential toxicity of the venom. The knowledge of antivenom production and its neutralizing capacity needs to be expanded urgently, as there is a worldwide lack of anti-bee antivenoms for the treatment of envenomation due to swarmings.

10 NEUTRALIZING CAPACITY OF BUTEOGALLUS CORONATUS ("CROWN EAGLE") AND OTHER ANIMAL SERA ON SNAKE VENOM.

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Several species of reptiles and mammals possess components in their sera that are able to neutralize toxic components from snake venoms. In this work we studied the neutralizing capacity of the serum of *Buteogallus coronatus* ("crown eagle", *Bc*), a bird that eats snakes. Sera from other birds (*Caracara plancus,* "caracara"*: Cp*; *Saggitarius serpentarius,* "secretario"*: Ss* and *Gallus gallus,* "hen": *Gg*), mammals (*Didelphis albiventris,* "opossum": *Da, Canis lupus familiaris,* "dog": *Cf*) and snakes (*Bothrops alternatus,* "pit viper"*: Ba*) were used for comparison. Serum of Bc partially neutralized the lethal potency of 2.0 MMD of venom in mice, higher than the protection conferred by *Gg*. The Bc serum neutralized the hemorrhagic (1.0 MHD), coagulant (2.0 MPD) and phospholipase activity (5.0 Indirect Hemolytic Doses) of *Ba* venom (p 0.05). With the exception of the *Da* and *Ba* sera , the *Bc* serum showed the highest neutralizing activity in all cases on all the toxic activities tested and the neutralizing capacity was present in the non-immunoglobulin fraction (p0,02 in all cases). The immune fraction and non immune fractions were separated by precipitation and ion-exchange chromatography and studied by SDS-PAGE, the Ouchterlony double immunodiffusion method and immunoelectrophoresis. The results indicate that the neutralizing components possess acidic characteristics and a lower molecular weight than IgY, as has been described for other animal species that present neutralizing components of snake venoms in their serum. Further studies are necessary to elucidate the type of neutralizing components present in this serum, their specific neutralizing potency and the spectrum and mode of action of their inhibitory mechanisms. To our knowledge, this is the first description of the neutralizing capacity of a raptors serum on snake venom.

11 TRANSLATIONAL TARANTULA PHYLOGENOMICS: EVOLUTION OF THERAPHOSID SPIDERS AND THEIR DEFENSIVE ARSENAL WITH IMPLICATIONS FOR VENOM BIOPROSPECTING

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The family Theraphosidae, commonly referred to as tarantulas, represents the most diverse group of mygalomorph spiders. Given their remarkably popular position compared to other spiders, it is rather surprising that theraphosid systematics still largely relies on morphological data, although recent studies demonstrated that theraphosids are affected by high degrees of morphological homoplasy. Their evolutionary history remains as well only poorly understood since reliable phylogenetic trees for its major radiations are lacking so far. Here we used phylogenetic and phylogenomic approaches to unravel the intra-familial relationships within Theraphosidae. For the first time we reconstructed a highly supported backbone phylogeny for the family and by that identified several sub-familial groups in need for taxonomic revision. We discovered a clade inside our phylogeny that is characterized by the presence of urticating setae as a defensive mechanism. Members within this clade are known to comprise less potent venoms than other Theraphosidae and they further contain the most speciose lineages within the family. Based on these observations we discuss that the evolution of urticating setae might have posed a selective pressure onto the theraphosid venom system, leading to the subsequent loss of defensive-toxicity. Therefore the importance of evolutionary costly venom as a means of defense might be reduced in these spiders and, finally, provided those taxa with a more economic alternative that contributed to their outstanding diversification. Lastly, we used the available data of previously identified venom components from publicly available databases. These were subsequently correlated with our phylogenetic tree to identify major genetic lineages within Theraphosidae that have so far been neglected in venom-based bioprospecting attempts. We highlight theraphosid priority groups for future venom surveys and flag phylogenetic studies in general as a useful tool that can be used supportively for the rapid identification of interesting target species for venom biodiscovery in the future.

12 TOXIN TO LEAD: AN INHIBITOR OF FACTOR XIA ENGINEERED FROM BANDED KRAIT VENOM TOXIN FASXIATOR SHOWED SUPERIOR IN VIVO EFFICACY-SAFETY PROFILE COMPARED TO HEPARIN

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Coagulation factor XIa (FXIa) plays an important role in thrombosis but not in haemostasis; indeed genetic deficiency of FXIa in humans does not lead to increased bleeding but has a protective effect against stroke and venous thromboembolism. Therefore, FXIa inhibition has recently become one of the most pursued drug targeting mechanisms for the development of anticoagulant therapeutics. We have recently reported that fasxiator from the venom of *Bungarus fasxiatus* inhibits FXIa with moderate potency (IC_{50} = 1.5 µM). Through systemic mutations, we engineered a suitable lead for anticoagulant therapeutics development with enhanced potency and selectivity. The variant, FXI001, has 1000-fold improved affinity towards FXIa (K_i = 0.9 nM). FXI001 is at least 170-fold more selective towards FXIa compared to other blood coagulation serine proteases. In rat thrombosis model, FXI001 dose-dependently protected carotid artery from total occlusion by thrombus induced through $FeCl_3$ application. Intravenous injection of FXI001 at 0.5 mg/kg and above achieved the same antithrombotic efficacy level as clinically used dosage of unfractionated heparin (432 U/kg, human equivalent dose of 70 U/kg). FXI001 has lower bleeding risk than unfractionated heparin as assessed through a tail vein bleeding model. Bleeding time in rats receiving FXI001 increased minimally with increase in dosage. At the clinically relevant antithrombotic level, bleeding time for FXI001 is only 1.7-fold above control (saline) while bleeding time for unfractionated heparin is more than 7-fold above control. Therefore, FXI001 is a promising lead to be developed into a parenteral anticoagulant with low bleeding risk.

13 DEVELOPING TOWARDS ANTI-VENOM DRUGS BY ENDOGENOUS INHIBITOR AGAINST THE METALLOPROTEINASE INDUCED HEMORRHAGE; RATIONAL DESIGN OF DRUG AND THERAPEUTIC POTENTIAL FOR SNAKEBITE

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Crotalinae snake venoms contain a variety of hydrolytic enzymes that contribute to toxicological effects in predator and prey. Metalloproteinase (MP) from the venom of Japanese viper plays an important role in inducing severe hemorrhage in human victims.

The blood of these snakes also contains several endogenous inhibitors that suppress toxin activity and provide protection against the toxins. One such inhibitors, Habu serum factor (HSF) and BJ46a were isolated from the blood of *Protobothrops flavoviridis* and *Bothrops jararaca*, respectively. They inhibit snake venom MP and exhibits anti-hemorrhagic activity. Both inhibitors belong to fetuin protein family which have a common structural feature such as two N-terminal cystatin-like domains (CD1 and CD2) and the third domain at the C-terminal. We isolated and identified two homologs of HSF from Chinese and Japanese mamushi (*Gloydius blomhoffi*) plasma, which were named as Mamushi serum factor (MSF) and HSF like-protein (HLP). Interestingly, despite the high sequence identify (>90 %), only MSF suppressed the MP activity similar to HSF, but HLP did not. Our investigation of physiological activity of HLP resulted that HLP inhibited the precipitation of calcium phosphate as potent as does bovine fetuin.

In this study, we have produced several proteolytically cleaved, truncated forms of HSF to identify the region involved interaction with MP. Subsequently, based on the interaction region, specific inhibitors were developed using phage display libraries and their sequences were evaluated. These results provide insight into structure-activity relationships of HSF and the molecular mechanism of interaction between the endogenous inhibitor and MP toxin.

14 COMPARISON OF THE VENOM COMPOSITION OF BOTHROPS ASPER, BOTHROCOPHIAS MYERSI, CROTALUS DURISSUS AND LACHESIS MUTA SNAKES SPECIES, FROM ANDINA REGION IN COLOMBIA.

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The snake´s venoms are the secretions with higher enzymes and toxins in nature, their composition is variable interspecies and intraspecies, even in the same region. Our purpose was compared the protein composition of *Bothrops asper*, *Bothrocophias myersi, Crotalus durissus and Lachesis muta* venoms, from Andina region from Colombia. The venoms pool was obtained by agreement with the Fundación Zoológico de Cali, by manual extraction, lyophilized and refrigerated. The project was approval of ethical committee of the Universidad El Bosque and the Instituto de Biotecnología of the Universidad Nacional de Colombia. The venoms protein was quantified by spectrophotometry using the Bradford, Lowry and Nanodrop® methods. The protein composition were made by high efficiency liquid chromatography (HPLC), using a Lichosper 100 RP c18 column of dimensions 250X4 mm, pore size of 5um and by polyacrylamide gel electrophoresis (SDS PAGE). The higher protein was found in *Bothrocophias myersi, and Crotalus durissus* venoms with 108,6 mg/mL and 78,1 mg/mL respectively. For the four venoms was found fractions between 35-36 minutes and fractions between 0-5minutes, the lower was 1,7 for *Bothrocophias myersi* and the higher was 3,8 for *Crotalus durissus*. All venoms showing more than 50% of proteins of 15, 20 and 50KDa. We found characteristics peaks for *Bothrops asper* at 25 minute, *Bothrocophias myersi* at 38min and *Lachesis muta* at 62min. Also, *Crotalus durissus* and *Bothrocophias myersi* showed peaks of 100 and 120 KDa, while *Crotalus durissus* and *Lachesis muta* showed peaks of 150KD. We conclude the higher protein quantification was for *Bothrocophias myersi*, the fractions weight was the similar point and they showed few differences in the chromatography characteristics peaks per venom.

15 POTENTIAL VENOM DIVERSITY HIDDEN BY AUTOMATIC ANNOTATION: MANUAL CURATION OF HIGHLY-EXPRESSED SEQUENCES AUTOMATICALLY ANNOTATED AS "NO HITS" IN P. NIGRIVENTER TRANSCRIPTOME REVEALS NOVEL VENOM COMPONENTS

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High throughput methods for transcriptomic analysis combined with computational assembly and annotation of sequence data have allowed rapid characterization of protein components from venom glands. However, in most transcriptomic studies, a high percentage of sequences present no similarity in protein databases when automatically annotated and are classified as "no hits". In a recent transcriptomic study of *Phoneutria nigriventer* spider venom glands, our group showed that 66% of the sequences had no match when automatically annotated against UniProt database. In an attempt to reveal novel putative venom molecules, in this work, we have selected from the *P. nigriventer* transcriptome study, the sequences annotated as "no hits" by automatic annotation which presented FPKM values higher than 100. We used these sequences to perform manual BLASTx and BLASTp against the non-redundant (nr) and Arachnoserver databases, considering an e-value cutoff of 1e-5. SpiderP from Arachnoserver was used to predict signal peptide and propeptide in all sequences and the presence of cysteine frameworks was manually analyzed. Pfam database was used to search for functional domains and the Antimicrobial Peptide database for prediction of potential antimicrobial peptides. After manual annotation of the sequences initially classified as "no hits", we identified 32% of sequences corresponding to putative toxins, 20% of cellular function proteins, 15% of hypothetical/uncharacterized proteins, 13% of putative antimicrobial peptides and only 20% remained as "no hits". From the putative toxins sequences, some were identified as glycine-rich peptides, which had never been reported in *P. nigriventer* venom. Furthermore, we have identified some sequences with two different cysteine frameworks already reported for *P. nigriventer* venom, but with amino acid sequences very dissimilar to previous ones described. In conclusion, our analyses have shown that careful manual analysis makes it possible identification of certain classes of molecules that would remain hidden when using only automatic annotation.

16 RESTROSPECTIVE BIBLIOMETRIC ANALYSIS ON COLOMBIAN SNAKE TOXINOLOGY KNOWLEDGE

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Across human history, venomous snakes have been an important source of attention in arts, engineering, spirituality and, of course, in science and medicine. In part, human interest in snakes is due to the potential threat that they exhibit to human health but at the same time to the ecological, evolutionary and biotechnological relevance of their venoms. Toxinology, as the study of venoms and toxins, has gained attention over the last years but unfortunately, in megadiverse countries like Colombia, it still is an incipient field of research. It is essential to determine the advances for Colombia in this critical field and to identify gaps and future challenges.

In order to quantify the publication dynamics on ophidian toxinology, a bibliometric review of toxinology papers regarding Colombian snake venoms was conducted. Systematic searches were carried out on different academic databases using distinct combinations of search terms into equations. Then, data was extracted on institution affiliation of the authors, year of publication, the focus of the investigation, factor of venom variability analyzed, geographical origin of venoms, taxonomical classification of the species, among others. Some of the results show frequent co-authorship between Colombian and Costa Rican institutions, a tendency on investigations focused on assessment of toxicity neutralization, taxonomy and geography as the factors of venom variability more commonly analyzed, and a comprehensive representation of species such as *Bothrops asper* and *Bothrops atrox* over others from the *Bothriechis*, *Micrurus* and more genera. We found a high frequency of studies on snake venoms of specimens from the western Colombian departments Antioquia and Chocó.

We concluded that studies on the venoms of Colombian snakes, overpassing the 80 papers published since 1896, require more collaborative projects that cover a broader geographic and taxonomic scale, also including new techniques being developed worldwide.

17 INSIGHTS INTO THE EVOLUTIONARY AND MEDICAL SIGNIFICANCE OF UNIQUE ALPHA-NEUROTOXIN AND PHOSPHOLIPASE A2 COMPOSITIONS IN NAJA SPP. (COBRA) VENOMS

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Cobras (*Naja* spp.) are typically Category 1 medically important venomous snakes in Asia and Africa. Three-finger toxins and phospholipases A_2 (PLA_2) are two commonly reported toxin families in cobra venoms. Our recent proteomic and transcriptomic findings, nonetheless, indicate that there are considerable inter- and intra-specific variations across cobra lineages. We found a strong correlation between PLA_2 enzymatic activities with PLA_2 protein abundances in the cobra venoms. High PLA_2 activities were shown in the venoms of Asiatic spitting cobras (*Naja sputatrix*, *Naja sumatrana*), followed by moderate activities in Asiatic non-spitters (*Naja naja*, *Naja atra*, *Naja kaouthia*), African spitters (subgenus *Afronaja*) and forest cobra (subgenus *Boulengerina*). African non-spitting cobras of subgenus *Uraeus* (*Naja haje*, *Naja annulifera*, *Naja nivea*, *Naja senegalensis*) showed exceptionally low venom PLA_2 activities, consistent with the negligible PLA_2 abundance in venom proteomes. The lack of PLA_2 in *Uraues* cobra venoms implies that PLA_2 is not ubiquitous in snake venoms. Meanwhile, the abundance of short- and long alpha-neurotoxins correlates significantly with the lethal potency of cobra venoms from various species and locales in Asia. Cobra venoms containing alpha-neurotoxins >25% of total venom proteins (*Naja philippinensis*, Pakistani *Naja naja*, Thai *Naja kaouthia*) are most lethal (LD_{50} 0.22 μg/g), consistent with the severe neuromuscular paralysis observed in clinical envenomation. With alpha-neurotoxins 15% of total venom proteins, the venoms of Indonesian *N. sputatrix*, Malaysian *N. sumatrana*, Chinese *N. atra*, and *N. kaouthia* from China, Vietnam and Malaysia show higher LD_{50} (0.5–1.0 μg/g) and are less neurotoxic. *N. philippinensis* venom is intriguing as its alpha-neurotoxins are composed solely of short neurotoxins, whereas the Thai *N. kaouthia* venom is dominated by long neurotoxins. The findings support that alpha-neurotoxins, regardless of short- or long-chain subtype, are principal lethal components in cobra venoms. Antivenom production should be tailored toward targeted neutralization of these toxins.

ACADEMIC POSTERS SESSION I

Topic C: Clinical Toxinology

SNAKE BITES: AN OVERVIEW ON VENOM TOXINS AND ANTIENOM IgG RELATED TO NEUROTOXINS ENVENOMATION.	BINTA	KURFI
TOWARD IMPROVED ANTIVENOM MANAGEMENT OF SNAKEBITE ENVENOMATION IN SOUTHEAST ASIA: THE INDONESIAN PERSPECTIVE	TAN	CHOO HOCK
GALLIC AND TANNIC ACIDS POTENTIAL AGAINST LACHESIS MUTA VENOM TOXIC ACTIVITIES	EDUARDO	DE OLIVEIRA
FREE NUCLEIC ACIDS AS PREDICTORS OF SEVERITY IN BOTHROPIC ACCIDENTS	JACQUELINE A. G.	SACHETT
ADVERSE REACTIONS TO ANTIBOTHROPIC SERUM IN A TERTIARY UNIT IN THE BRAZILIAN AMAZON.	JACQUELINE A. G.	SACHETT
EFFICACY AND SAFETY OF SNAKE ANTIVENOMS USED IN URUGUAY IN 2018.	ALBA	NEGRIN
SCORPION ENVENOMING BY *TITYUS (ATREUS) VAISSADEI (BUTHIDAE)* IN SANTANDER, COLOMBIA.	JAVIER	RODRÍGUEZ BUITRAGO

1 SNAKE BITES: AN OVERVIEW ON VENOM'S TOXINS AND ANTIENOM IgG RELATED TO NEUROTOXINS ENVENOMATION.

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Snakebite is a significant cause of death and disability in the rural community of south Asia and sub-Saharan Africa. Clinical patterns of snakebite can be broadly classified into three groups: neurotoxic, cytotoxic and hemotoxic, although myotoxicity can also present in certain cases. Snake venom neurotoxins primarily target the neuromuscular junction of skeletal muscles of which the motor nerve terminal (pre-synaptic) and the nicotinic acetylcholine receptor at the motor-end plate (post-synaptic) are the major targeted sites. This is well supported by clinical observations that neurotoxic snake envenoming almost exclusively results in flaccid paralysis which is due to the blockade of neurotransmission at the neuromuscular junction by venom neurotoxins. Antivenoms have been used for the treatment of snakebite for more than a century. they are polyclonal whole immunoglobulin (IgG) or immunoglobulin fractions (Fab or F(ab')2) raised against venom from one (monovalent) or several (polyvalent) snake species in other animals. These antivenom molecules bind with circulating toxins, forming large venom-antivenom complexes, trapping the venom molecules in the circulation. During snakebite therapy these antibodies likely act via a number of mechanisms, including blocking the active site of the neurotoxin molecules, preventing the toxins from interacting with the target site (neuromuscular junction) by restricting the movement of the neurotoxins to the extravascular target sites, and also increasing the elimination of the toxins. These suggest that antivenom mechanisms in neutralizing snake's venom neurotoxins need to be explored extensively as for minimizing paralysis effect after snake bite

2 TOWARD IMPROVED ANTIVENOM MANAGEMENT OF SNAKEBITE ENVENOMATION IN SOUTHEAST ASIA: THE INDONESIAN PERSPECTIVE

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Snakebite envenomation is a serious but neglected public health problem in Indonesia. The country has a wide diversity of tropical herpetofauna, including various medically important venomous snake species in distinct geographical habitats. The only antivenom product manufactured and distributed for use in Indonesia is a trivalent antivenom called Serum Anti Bisa Ular (SABU), raised against *Naja sputatrix*, *Bungarus fasciatus* and *Calloselasma rhodostoma*. To examine the para-specific utility and composition of SABU, a series of antivenom assessment study was conducted. SABU was effective in neutralizing the lethal effect of the homologous *C. rhodostoma* venom (normalized potency = 121.8 mg venom neutralized completely per gram of antivenom proteins) while weakly effective against *B. fasciatus* venom (normalized potency = 8.5 mg/g) and *N. sputatrix* venom (2.9 mg/g) from Indonesia. The trivalent antivenom lacked cross-neutralization capability against the venoms of distant heterologous snakes, in particular the green pit vipers (e.g. *Trimeresurus albolabris*, *Trimresurus insularis*, *Trimeresurus puniceus*), Russell's viper (*Daboia siamensis*), Malayan krait (*Bungarus candidus*) and king cobra (*Ophiophagus hannah*) which are known to inflict fatal bites in the population. The ineffectiveness of SABU against the heterologous venoms is consistent with its poor immunoreactivity and immunorecognition of the venom protein antigens. We further showed that antivenoms manufactured in Thailand i.e. *Daboia siamensis* monovalent antivenom, *Ophiophagus hannah* monovalent antivenom and *Trimeresurus albolabris* monovalent antivenom exhibited good immunorecognition of the venom protein antigens, and could effectively neutralize the toxic and lethal effects of the venoms of Russell's viper, king cobra as well as major pit viper species in the *Trimeresurus* complex from Indonesia. On the other hand, biochemical and proteomic profiling of SABU revealed the presence of some impurities e.g. serum albumins in the antivenom. A wider species coverage and better purification of the antivenom product are essential for improved snakebite management in the country

3 GALLIC AND TANNIC ACIDS POTENTIAL AGAINST LACHESIS MUTA VENOM TOXIC ACTIVITIES

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Snake venom is composed by a mixture of substances that is responsible for biological effects, such as edema, necrosis, hemostatic disturbs, renal failure and usually death. In Brazil, about 30,000 cases are recorded annually, caused by *Bothrops*, *Crotalus*, *Lachesis*, *Leptomicrurus* and *Micrurus* genera. The snake *Lachesis muta* is the largest venomous snake in Americas, reaching 3.5 meters in length. Besides antivenom, there are many reports in literature describing plants and essential metabolites able to counteract biological activities of snake venoms. So, this work reports the ability of two essential metabolites found in several plants, gallic acid and tannic acid, to neutralize some toxic activities of *Lachesis muta* venom. The molecules were incubated with venom for 30 minutes at room temperature, and then proteolytic, clotting, hemorrhagic and edematogenic activities were performed. The proteolytic activity was performed using azocasein as substrate; the clotting time upon plasma from healthy volunteers; the hemorrhagic and edematogenic activities were realized using Balb/c mice. Gallic acid was able to totally prevent the hemorrhagic activity, inhibited 80% proteolytic activity, increased clotting time in 1.3 fold when compared to control (NaCl) and protected mouse around 10% of edematogenic activity. The Tannic acid was able to protect animals from edematogenic activity in 45%, to inhibit the proteolitic activity in 35% and in 50% the hemorrhage induced by *L. muta* venom. The Tannic acid accentuated in 1.2 fold the clotting time when compared to control (NaCl), showing to be pro coagulant. Our results show that both gallic and tannic acids could be a promising source of inhibitors for enzymes involved in the main toxic activities of *L. muta* venom.

18 FREE NUCLEIC ACIDS AS PREDICTORS OF SEVERITY IN BOTHROPIC ACCIDENTS

Jacqueline A. G. Sachett, Êndila Souza Barbosa, Hiochelson Najibe Santos Ibiapina, Allyson Guimarães Costa, Iran Mendonça Silva, Wuelton Marcelo Monteiro, Gisely Cardoso Melo, Siuhelem Rocha Silva

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Accomplishing millions of people around the world, snake poisonings are considered Neglected Tropical Disease. In Brazil, bothropic accidents are of great importance for gravity being responsible for 90% of the accidents. Studies are needed to identify tools that aid in care since the classification of these accidents is performed by clinical-epidemiological characteristics. Cell-free circulating nucleic acids may act as predictors of prognosis, as demonstrated in studies with other pathologies. The objective of this work was to describe cell-free DNA levels in the severity classification of bothropic poisonings. Observational, descriptive and quantitative study with victims of bothropic accidents, using 5-time plasma samples: day 1, day 2, day 3, day 4 and day 7. DNA extraction and quantitative real-time PCR were performed in triplicate. There were 76 individuals, 82.9% (63) males, mean age 32.7 (± 17.6). The majority were classified as moderate (48.7%) (37). The mean serum ampoules used was 7.8 (± 2.5). In lighter free cell DNA levels were higher on day 1 (1612.5 copies/µl), day 3 decrease to 572.2 copies/µl and increase on day 7 (904.8 copies/µl). In the moderates there was similarity, but they remained higher. At day 1 the median was 1532.9 copies/µl and 1459.65 copies/µl at day 7. In the severe cases the median at day 1 was 2647.1 copies/µl, the highest among the classification levels. On day 2 it was 657.79 copies/µl, increasing to 1842.9 copies/µl on day 4 and median 573.47 copies/µl on day 7. By the frequency and severity of bothropic poisonings, studies for the identification of tools for assistance assistance are important in the management of this problem, in this study it was possible to demonstrate that nucleic acid levels were higher in the victims with greater severity.

19 ADVERSE REACTIONS TO ANTIBOTHROPIC SERUM IN A TERTIARY UNIT IN THE BRAZILIAN AMAZON.

Jacqueline A. G. Sachett, Frandison Gean Souza Soares, Iran Mendonça Silva, Eliane Campos Alves, Samella SIlva Oliveira, Elizandra Freitas Nascimento, Alessandra Santos Santos, Luiz Carlos de Lima Ferreira, Wuelton Marcelo Monteiro

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Snakebites are a global public health problem, especially in countries with tropical and subtropical. Neutralization of the venom occurs through the administration of specific serum therapy with immunoglobulins obtained through the immunization of horses, however, because it is a heterologous serum, patients may present adverse reactions resulting from this therapy. The aim of this study was to evaluate the adverse reactions to the antibothropic serum therapy in patients of a tertiary unit of Manaus - AM. This is a cross-sectional, descriptive and quantitative study, performed at the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado FMT-HVD with patients who suffered from bothropic accidents, attended at the FMT-HVD between 2015 and 2016. Inclusion criteria were: patients older than 18 years, non-indigenous individuals, not having been administered serum therapy in attention prior to the FMT-HVD. The collection instrument included socio-demographic, clinical and laboratory data. A total of 186 patients diagnosed with bothropic accident were evaluated. Of these, 16.1% (30) developed an early adverse reaction to serum; the majority (90.0%) were male; 96.7% (29) occurred in rural areas; the most affected age group was 21 to 30 years (39.3%); most of the bites were in the feet (60.0%); half (50.0%) of the patients were attended between 0-3 hours post-accident (24.4%); only 20% (6) reported a previous accident; 56.7% (17) were moderate cases and the most frequent reactions were urticaria (24.4%), pruritus (17.1%) and vomiting (9.8%). All patients received premedication 30 minutes before with antihistamines and hydrocortisone. The burden of bothropic accidents is high in the Brazilian Amazon and the adverse reactions due to serum therapy can be considered a relevant problem for patients' health. In this study, the most common reactions were urticarias, pruritus and edema, therefore the need to invest in improvements to the prevention of these reactions is perceived.

20 EFFICACY AND SAFETY OF SNAKE ANTIVENOMS USED IN URUGUAY IN 2018.

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Snakebite is mandatory notification event allowing an accurate registry.Poison Control Centre (CIAT) receive all consults and advise in the diagnosis, treatment and antivenom administration. Annually CIAT receives around 60 consults for Bothrops alternatus and B. pubescens accidents, treated with specific antivenom. Until 2018 antivenoms were supplied by Argentina (Instituto Malbran) or Brazil (Instituto Vital Brazil, Butantan and FUNED. They are polyvalent, in liquid form and had a potency above 2,5 mg/mL. Due to a regional shortage of antivenoms Uruguay started to use a new type of antivenom, Lyophilized Suero Antiofídico Polivalente BIOL®. It neutralizes Crotalus durissus, Bothrops alternatus, Bothrops diporus but is formulated in lower potency (≥ 1,5 mg/ml). Snakebite consults were 102 of which 49 were due to Bothrops and need antivenom treatment. Patients treated with BIOL® were 28 and the ones treated with Malbran and Vital Brazil antivenom were 21. All patients who received antivenom have evolved successfully.

Initial dose of BIOL® antivenom were 8 vials instead of 4 used with the others antivenoms and achieved the neutralization of mostly (27/28 cases) Early adverse reactions were detected in 4 patients (3 in children) treated with BIOL antivenom and there were no adverse drug reactions(ADR) in those treated with Malbran and Vital Brazil antivenoms Those ADR were the type B and "Possible" according to Karch & Lasagna Score. All the adverse reactions were clinically moderate and include rush, pruritus, bronchoconstriction and tachycardia. Patients were treated with corticoids, and antihistamines.

In conclusion the lyophilized antivenom BIOL is being used successfully in Uruguay with the adjustment of the administered dose due to its low potency. Records of adverse reactions were under the 10 %. We found a slightly increase in early adverse reactions but we could only confirm this point in the next years with the compilation of more information.

21 SCORPION ENVENOMING BY TITYUS (ATREUS) VAISSADEI (BUTHIDAE) IN SANTANDER, COLOMBIA.

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Scorpionism is a public health problem that affects tropical and subtropical regions of the world. In Colombia stills a lack of awareness about the danger of all scorpion species of Buthidae family, which is reflected in misdiagnoses, administration of inadequate therapeutic measures and the absence of government programs aimed to prevent and report this and other accidents in the country. This work presents the case of a 24-year-old patient from Bucaramanga (Santander), with moderate scorpionism causing progressive alteration of the central nervous system characterized by confusion, dysarthria and sensitive compromise. Was treated by recommendation of toxicology with I.V. fluids, observation in a ICU and the administration of two vials of specific scorpionic antivenom F(ab)2, with adequate response, and brain TC reports in normal parameters.

The scorpion was collected and identified through the application of taxonomic keys of the genus *Tityus* in the Laboratory of Arachnology of the Institute of Natural Sciences of the Universidad Nacional de Colombia, as a female of the specie *Tityus (Atreus) vaissadei* described by Lourenço in 2002. This specie is found at 1500 to 1800 m.a.s.l., located geographically in Santander and Boyacá and is characterized in its adult stage by presenting brown to reddish brown coloration, fingers of pedipalps dark brown to black with yellowish apices and approximate total length of 9 cm. It has sexual dimorphism being males the largest, with pedipalps longer and thinner than females. Due to the morphological characteristics, it can be confused with other species of clinical importance such as *T. (Atreus) pachyurus*, *T (Atreus) forcípula*, among others.

ACADEMIC POSTERS SESSION I

Topic D: Toxinological Technologies

PICHIA PASTORIS AS HOST SYSTEM TO EXPRESS SCFV 6009F	ELBA	VILLEGAS
UTILISATION OF OMIC APPROACHES TO IMPROVE THE IDENTIFICATION, DIAGNOSIS AND TREATMENT OF EXPOSURE TO THE PLANT TOXIN RICIN.	GRAEME	CLARK
HETEROLOGOUS EXPRESSION OF TS16, AN &ALPHA;-KTX FROM *TITYUS SERRULATUS* SCORPION VENOM	ELIANE CANDIANI	ARANTES
CHARACTERIZATION OF MICROVESICLES RELEASED FROM HUMAN WHOLE BLOOD AFTER INCUBATION WITH *BOTHROPS JARARACA V*ENOM: AN IN VITRO PRELIMINARY STUDY	MILENE	MENEZES
TRACING NEGLECTED VENOM SYSTEMS: PREDICTION OF THE INDUSTRIAL POTENTIAL BY A MULTI-DISCIPLINARY DATA NETWORK	KIM	KIRCHHOFF

1 PICHIA PASTORIS AS HOST SYSTEM TO EXPRESS SCFV 6009F

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To date, generation of libraries of single-chain fragment variable (scFv) human antibodies has become an established technique to produce a completely functional antigen-binding fragment in either bacterial and yeast systems. These proteins having antigen-binding capacity can be obtained mainly from genetically transformed bacteria or yeast, and used in immunodetection of biological agents, therapeutic gene delivery, anticancer intrabodies for therapeutic purpose, construction of immunotoxins and antivenom neutralizing animal toxins. Specifically, the human scFv antivenoms have been used to treat the events of scorpion sting and they have been proved to be efficient. The scFv 6009F antibody is capable of neutralizing $2LD_{50}$ of *C. noxius* complete venom, and $2LD_{50}$ of the Cn2 toxin, the main toxic protein component in the complete venom. The scFv 6009F also presents cross-reactivity with other toxins such as the Cn3 from *C. noxius*, Css2 and Css4 from *C. suffusus suffusus*, which makes it a suitable candidate for future therapeutic applications. However, the scFv 6009F protein expression in *E. coli* strain TG1 have yield only up to 1.1 mg/L. Therefore, to improve its yield, we evaluated *P. pastoris* strain KM71 transformed with vectors pPIC9/scFv 6009F and pHILS1/scFv 6009F. The expressed protein was quantified and confirmed by Western-Blot using anti-His. The results showed a yield of 59 mg/L of purified scFV 6009F in *P. pastoris* KM71 pPIC9/scFv 6009F and in *P. pastoris* KM71 pHILS1/scFv 6009F. However, using pPIC9 a glycosylated protein was obtained in contrast with pHILDS1 vector where the protein obtained is not glycosylated. Toxin recognition and neutralizing studies are being conducted.

2 UTILISATION OF 'OMIC APPROACHES TO IMPROVE THE IDENTIFICATION, DIAGNOSIS AND TREATMENT OF EXPOSURE TO THE PLANT TOXIN RICIN.

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Ricin is produced by the seeds (castor beans) of *Ricininus communis* plants. From an evolutionary perspective the production of the toxin is thought to protect castor beans from predation and/or infection. Ricin is a potent ribosome-inactivating protein toxin that inhibits protein synthesis leading to cellular death and exposure to the toxin can be lethal. As a consequence of the toxicity of the molecule, along with the prevalence of castor beans within the environment, has led to ricin being classed as a schedule 1 chemical by the OPCW. Despite this potency the chain of events that occur within the body following exposure to ricin remain poorly understood and as a consequence diagnosis and treatment remain challenging. By using experimental models of exposure our research has focused upon understanding the impact of this toxin following exposure. We have utilised next generation sequencing, cutting edge proteomic and data science techniques to study the transcriptomic and proteomic responses to ricin through time. The toxin appears to have limited measurable effect during the first few hours following exposure however the indirect detection of ricin may be possible through identifiable host biomarkers. The results of this research will aid in the identification of ricin exposures improving diagnosis and potentially treatment of intoxication.

3 HETEROLOGOUS EXPRESSION OF AN A-NEUROTOXIN PRESENT IN TITYUS SERRULATUS VENOM IN PICHIA PASTORIS SYSTEM

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Tityus serrulatus is considered the most dangerous scorpion in Brazil. It is responsible for the highest number of accidents with scorpions in the country including those that lead patients to death. Scorpionism in Brazil is a public health issue and the biggest concern is about *T. serrulatus* due to its envenoming severity and the huge expansion of its geographic distribution across the country. Its venom contains many different toxins, including α-neurotoxins, which can interact with ionic channels of excitable cell membranes, stimulating neurotransmitters release. This study aims the transformation of an α-neurotoxin synthetic gene in the yeast *Pichia pastoris*, its heterologous expression and identification. The recombinant vector with the α-neurotoxin gene, designed with affinity tags for purification and identification of the recombinant protein, was linearized with PmeI and integrated into electrocompetent *P. pastoris* cells by electroporation. It was confirmed with PCR of selected colonies and agarose gel electrophoresis. Seven colonies were positively transformed with the α-neurotoxin recombinant vector and they were submitted to a screening expression. The α-neurotoxin expression was induced by methanol in BMMY medium for 144 hours and each colony was tested in two different pHs. Colonies were fed with methanol every 24 hours and a sample from the culture supernatant was collected every 48 hours. Samples were analyzed by Tris-Tricine-SDS-PAGE electrophoresis and seven conditions showed bands with molecular mass similar to the native α-neurotoxin. The toxin identity was then analyzed by an indirect ELISA assay, and the α-neurotoxin was identified in one colony culture supernatant in two expression conditions. Concluding, *P. pastoris* cells were transformed with the α-neurotoxin gene and the toxin was successfully produced. This process will be useful to produce the toxin in large quantity to enable its functional and structural characterization as well as its biotechnological use.

4 CHARACTERIZATION OF MICROVESICLES RELEASED FROM HUMAN WHOLE BLOOD AFTER INCUBATION WITH BOTHROPS JARARACA VENOM: AN IN VITRO PRELIMINARY STUDY

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Cells release different forms of extracellular vesicles, including structures called microvesicles (MVs) that can alter the extracellular microenvironment. Viperid venom toxins induce an acute inflammatory response that contributes to the severity of symptoms observed upon envenomation, possibly involving the release of MVs with different protein contents that may contribute to the pathological context. The goal of this study is to characterize the MVs generated by human whole blood incubated *in vitro* with *B. jararaca* venom in order to understand the role of MVs in venom-induced blood coagulation disorders. To this end, whole human blood was incubated with *B. jararaca* venom (5 and 25 µg/mL), for 15, 30 and 60 min at 37º C, or with PBS (control). Samples were centrifuged at 1200×g for 15 min, plasma was collected, and then centrifuged at 1500×g for 15 min. The supernatant was centrifuged at 14000×g for 35 min, the pellet was resuspended in PBS and submitted to Nanoparticle Tracking Analysis. Moreover, MVs' protein profile was analyzed by SDS-PAGE and mass spectrometry, by in-gel trypsin digestion and LC-MS/MS. The incubation of whole blood with venom induced an increase of MVs release and a change of the SDS-PAGE protein profile of MVs extract, in a dose and time-dependent manner. STRING analysis of protein-protein interaction networks of proteins identified only in MVs from venom-treated blood, showed that in the incubation for 15 min there was an enrichment of biological pathways related to ATP biosynthetic processes. On the other hand, MVs generated after 30 min of incubation contained various proteins involved in exocytosis and vesicle-mediated transport pathways. These preliminary results suggest that venom toxins stimulate the release of extracellular vesicles with specific cargo in the blood.

5 TRACING NEGLECTED VENOM SYSTEMS: PREDICTION OF THE INDUSTRIAL POTENTIAL BY A MULTI-DISCIPLINARY DATA NETWORK

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Animal venoms are highly potent cocktails of confirmed and potential new drugs, research tools, pesticides, and even cosmetics. However, the majority of the estimated 200.000 venomous animals have been neglected in toxinological studies and thus, are inaccessible for applied fields. The difficult access, low amount, lability, and extraction challenges are major reasons for this neglect. In addition, applied fields such as pharmacology depend on novel natural sources to counteract the rapidly evolving drug resistances, lack of new targets, and 90% drug failure in clinical trials.

Therefore, the current drug discovery workflow needs to be strengthened. In order to do so, we fused data from different disciplines into one functional network enabling a more comprehensive prediction of the potential of novel resources with less research effort. Stingrays were chosen as test system as their defensive venom system is highly conserved through 200 Myr of evolution, but exhibits a certain degree of variability among taxonomical levels and lifestyles. Applying our novel concept of a multi-disciplinary network, the evaluation of stingray venom potential and selection of putative high potential candidates occurs stepwise by performing: i) evolutionary studies to comprehend the framework in which the venom system evolved; ii) primary biochemical assessment by chromatography and *in vitro* bioassay to estimate the venom's potency and intraspecific variability; iii) transcriptome analyses to draw the putative venom composition; and iv) high-content screening to characterize the venom's bioactivity. The resulting data network adds basic research data on the venom composition and activity, but also identifies putative candidates in stingray venom and allows first crucial insights into their mechanism of action. This reinforced workflow is supposed to improve the access to the high potential of animal venoms, and finally enhances the rate of success of drug candidates.

STUDENT POSTERS SESSION I

AN ONTOGENETIC AND SEASONAL APPROACH OF THE *BOTHROPS JARARACUSSU* VENOM VARIABILITY	Aguiar	Weslei
EXPRESSION OF AN ALPHA-KTX FROM *TITYUS SERRULATUS* SCORPION VENOM IN *PICHIA PASTORIS* YEAST AND ITS PRELIMINARY ELECTROPHYSIOLOGICAL CHARACTERIZATION	Lopes Pinheiro	Ernesto
EPIDEMIOLOGIC PROFILE OF LATRODECTISM IN BRAZIL AND CLINICAL PREDICTOR FOR NON-MILD ENVENOMATION CASES	Amorim	Filipe
USE OF PHOSPHOPEPTIDE TO PURIFY INDUSTRIALLY IMPORTANT PROTEINS STARTING FROM THE VENOM OF *CROTALUS DURISUSS TERRIFICUS*	Dokmetjian	Christian
PRODUCTION OF AN ALTERNATIVE ANTIVENOM BASED ON EGG YOLK ANTIBODIES AGAINST *CROTALUS DURISSUS TERRIFICUS*	Cangelosi	Adriana
IMPROVING THE BOTHROPIC ANTIVENOM THROUGH A REVERSE ANTIVENOMICS APPROACH	Chiarelli	Tassia
LOXOSCELES GAUCHO VENOM GLAND PROTEOME: A NEW PERSPECTIVE ON LOXOSCELES VENOM BIOCHEMICAL COMPOSITION.	Coelho	Guilherme
HYPANUS AMERICANUS MUCUS: A NEW POINT OF VIEW ABOUT STINGRAY IMMUNITY AND TOXINS	Coelho	Guilherme
SEARCH FOR PROTEASES AND PROTEASE INHIBITORS IN THE WAXY SECRETION THAT COVERS THE TICK *AMBLYOMMA SCULPTUM*'S EGGS (ACARI: IXODIDAE)	Coutinho-Oliveira	Priscila
CROSS-SPECIES AND GEOGRAPHIC POTENTIAL OF B-CELL EPITOPE STRINGS IDENTIFIED FOR GENERATION OF AN AFRICA-SPECIFIC SNAKE VENOM-INDUCED NECROSIS THERAPEUTIC	Dawson	Charlotte
TRANSCRIPTOMIC ANALYSIS OF THE VENOM GLAND FROM THE SCORPION *BOTHRIURUS BONARIENSIS*	de Oliveira John	Elisa Beatriz
CROSS-NEUTRALIZATION ASSAYS OF *TRIMERESURUS PUNICEUS AND CROTALUS ATROX* VENOMS BY PERUVIAN ANTIVENOMS	Electo Oshiyama	Jorge
ISOLATION OF A NEW VEGF FROM *CROTALUS DURISSUS TERRIFICUS* VENOM	Ferreira	Isabela
ARE YOU WHAT YOU EAT? THE INFLUENCE OF DIET ON COMPOSITIONAL AND FUNCTIONAL ACTIVITIES OF *BOTHROPS MOOJENI* SNAKES SUBMITTED TO DIET SHIFT.	Galizio	Nathalia
OCCUPATIONAL OPHIDISM AND ASSOCIATED FACTORS IN BRAZIL	Gomes de Souza	Mariana Brito
BIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF THE EYELASH VIPER *BOTHRIECHIS SCHLEGELII* VENOM FROM SANTANDER, COLOMBIA.	Gómez Robles	Jeisson
BOTHROPS NEUWIDI SNAKE VENOM: ONTOGENETIC CHARACTERIZATION AND IMUNORECOGNITION BY BOTHROPIC ANTIVENOM	Gutiérrez Marín	Juan
TAKING BABY STEPS: INITIAL ONTOGENETIC ANALYSIS OF *BOTHROPS ERYTHROMELAS* SNAKE VENOM	Hatakeyama	Daniela

HARNESSING MONOCLONAL ANTIBODIES FOR DEVELOPMENT OF A SPECIFIC TREATMENT AGAINST *NAJA NIGRICOLLIS* ENVENOMING	Jürgensen	Jonas
CONVERGENT EVOLUTION OF DEFENSIVE VENOM COMPONENTS IN SPITTING COBRAS	Kazandjian	Taline
MOLECULAR ADAPTATION AND RESISTANCE, TO ALPHA-NEUROTOXIN OF ELAPID SNAKES, IN SQUAMATA, AVES AND FISHES.	Khan	Muzaffar Ali
BRAZILIAN BOTHROPS DIPORUS, IN FACT A LINEAGE OF BOTHROPS PUBESCENS:	Kleiz Ferreira	Jessica
DISCOVERY AND EVALUATION OF MONOCLONAL ANTIBODIES FOR STRATIFICATION OF VENOMS FROM BRAZILIAN BOTHROPS, CROTALUS, AND LACHESIS SPECIES	Knudsen	Cecilie
DISCOVERY OF CROSS-REACTIVE AND RECYCLABLE HUMAN MONOCLONAL ANTIBODIES FOR NEW RECOMBINANT ANTIVENOMS	Ledsgaard	Line
PROTEOMIC AND PHOSPHOPROTEOMIC ANALYSES IN HL60 CELLS TREATED WITH THE SCORPION-DERIVED PEPTIDE LUNATIN-1	Lima-Batista	Edleusa
PALYTOXIN-LIKE EFFECT FROM *PALYTHOA CARIBEAORUM* ON *RHINELLA MARINA* OOCYTES ON THE ATLANTIC COAST OF COLOMBIA.	Castaño	Santiago
PHYLOGENY AND TOXICOLOGICAL ASSESSMENTS OF TWO *PORTHIDIUM LANSBERGII LANSBERGII* MORPHOTYPES FROM THE CARIBBEAN REGION OF COLOMBIA	Montoya	Gabriel
SEXUAL DIMORPHISM IN THE VENOM PEPTIDOME OF THE AMAZONIAN SPIDER *ACANTHOSCURRIA JURUENICOLA*: BIOLOGICAL INSIGHTS AND POTENTIAL NEW ANTITUMORAL AND ANTIMICROBIAL AGENTS	Nishiduka	Erika
ABILITY OF TANNIC ACID ADMINISTERED INTRAPERITONEALLY AGAINST THE LETHALITY OF BOTHROPS JARARACA VENOM	Oliveira	Isadora
THE INSECTICIDAL WASP TOXIN &GAMMA;&NDASH;POMPILIDOTOXIN IS A LINEAR PEPTIDE MODULATING VOLTAGE-GATED SODIUM CHANNELS	Peigneur	Steve
IMMUNOENZYMATIC CHARACTERIZATION AND PREDICTION OF ANTIGENIC DETERMINANTS OF A LYSINE-49 PHOSPHOLIPASE A2 FROM *BOTHROPS ATROX* SNAKE VENOM	Proleón Torres	Alex Daniel
REVIEW ON THE EVOLUTION OF ANTIVENOMS FOR SNAKEBITES TREATMENT	Sarmiento	Karen
CLINICAL CHARACTERIZATION OF SNAKESBITES IN A COLOMBIAN HOSPITAL, 2004-2014	Sarmiento	Karen
BIOCHEMICAL PROFILING OF THE *ANTHOPLEURA CASCAIA* AQUEOUS EXTRACT: A SCREEN FOR SERINE PEPTIDASE INHIBITORS	Silva	Daiane
PROTEOMIC ANALYSES OF THE WATER SOLUBLE AND PRECIPITATE FRACTIONS OF *ZOANTHUS SOCIATUS* CRUDE EXTRACT	Silva	Daiane
INTRA-SPECIFIC VENOM VARIATION IN COASTAL TAIPANS	Tasoulis	Theo

HETEROLOGOUS EXPRESSION OF AN &ALPHA;-NEUROTOXIN PRESENT IN *TITYUS SERRULATUS* VENOM IN *PICHIA PASTORIS* SYSTEM	Vilela Bartnick T.	Nádia Mayumi
EXPRESSION AND PRELIMINARY CHARACTERIZATION OF THE FIRST PHOSPHOLIPASE A2 INHIBITOR FROM THE *CROTALUS DURISSUS TERRIFICUS* SNAKE VENOM GLAND	Wiezel	Gisele

1 AN ONTOGENETIC AND SEASONAL APPROACH OF THE BOTHROPS JARARACUSSU VENOM VARIABILITY

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Ontogenetic changes are observed in different snakes' species on many studies. *Bothrops jararacussu* has a peculiarity of a low immunogenicity rate in its venom. Vital Brazil, in 1909, demonstrated that the lethal activity of the venom from *B. jararacussu* is better neutralized by the anticrotalic serum. Despite so many peculiarities in its composition, it is interesting to note the lack of attention that this species has received in the last years of research. OBJECTIVE: This present study aims to perform an ontogenetic and seasonal analysis of venom from *B. jararacussu* snake, following the development of newborn individuals in the Laboratory of Herpetology of Instituto Butantan until adulthood, as well as adult individuals originally from nature. MATERIAL AND METHODS: Venom samples were obtained from *B. jararacussu* snakes from Laboratory of Herpetology of Instituto Butantan. Venom samples were subjected to electrophoresis on SDS-PAGE, followed by Western Blotting (W.B.) method. ELISA assay was performed to determine the antibody titer from different individuals with different antivenom. Venom samples were also analyzed by RP-HPLC. RESULTS: Venom profiles varied between the extractions by SDS-PAGE showing some differences on venom samples patterns in adults. HPLC profiles showed distinct protein profiles for young and adult individuals. W.B. test revealed different immunorecognition patterns when utilized the anti-bothropic or the anti-crotalic serum. ELISA showed similar results among different antivenoms. Curiously, the anti-crotalic showed a high immunorecognition in many different high weight zones, an unexpected result due to the "simplicity" of the crotalic venom. CONCLUSION: Preliminary results showed that there is a variation in the venom composition among individuals of this species. Seasonality also seems to play a role in this variability. Future experiments will help us elucidate better the venom composition of these animals that will be accompanied through the time during the following two years.

2 EXPRESSION OF AN A-KTX FROM TITYUS SERRULATUS SCORPION VENOM IN PICHIA PASTORIS YEAST AND ITS PRELIMINARY ELECTROPHYSIOLOGICAL CHARACTERIZATION

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Scorpions have survived on earth for more than 400 million years and their venoms have several components that act in synergism causing severe symptoms in their victims. *Tityus serrulatus* venom (Tsv), known as yellow scorpion in Brazil, contains mainly neurotoxins, which act primarily on voltage-gated sodium and potassium channels. These toxins can explain the symptoms of envenoming caused by *T. serrulatus*, as well elucidate the mechanism of ion channels functioning. The aim of this study was the expression of Ts15, an α-KTx from Tsv, in *Pichia pastoris* yeast, and its preliminary characterization. The toxin gene was synthetized by GenScript® with the TEV (tobacco etch virus) protease cleavage site before the N-terminal sequence, and cloned into a pPICZαA vector. The recombinant plasmid was transformed in KM71H *P. pastoris* strain and the screening of positive colonies was performed in a deep well plate. For laboratorial scale, the pre-inoculum was first grown in BMGY medium (with glycerol as carbon source), followed by expression induction with BMMY medium (with addition of methanol as carbon source). The expression was analysed by SDS-PAGE (16%) and mass spectrometry. After purification by affinity chromatography, an electrophysiological screening on the voltage-gated potassium channels, Kv1.1, 1.2, 1.3 and 2.1, using the two-microelectrode voltage clamp technique, was performed. The SDS-PAGE revealed two bands corresponding to the rTs15 and rTs15 glycosylated toxin (around 5.5 and 7.6 kDa respectively) and a preliminary electrophysiological screening showed a small inhibition on Kv1.3-type currents. In conclusion, the recombinant α-KTx was successfully expressed in *P. pastoris* yeast and the small inhibition on Kv1.3 is probably due to the recombinant N-terminal portion (poly-His tag and TEV cleavage site). As next steps, the same screening will be performed with glycosylated and cleaved toxins.

3 EPIDEMIOLOGIC PROFILE OF LATRODECTISM IN BRAZIL AND CLINICAL PREDICTOR FOR NON-MILD ENVENOMATION CASES

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Latrodectism is the accident caused by spiders of the genus *Latrodectus* which has a cosmopolitan distribution across the planet. The main component of its venom is the α-latrotoxin, which induces a high release of neurotransmitter, acting in calcium channels and causing pain, paresthesia in the limbs, abdominal pain, tremors and contractures. This work characterize the epidemiologic profile of this accident, identify clinical manifestations and social variables related with non-mild envenomation occurred in Brazil from 2007 to 2015. The cases were obtained from Notification of Injury Information System (Sinan). For the whole period 1025 cases were registered, 833 mild and 149 non-mild. The accidents were distributed in the whole country, and the states with more cases were Minas Gerais (n=221; mild=199/non-mild=18), São Paulo (n=140; mild=188/non-mild=10), Bahia (n=137; mild=107/non-mild=22) and Santa Catarina (n=141; mild=108/non-mild=31). The majority of patients were self-declared white (n=433; 42%), male (n=564; 55%) and between 35 and 59 years old (n=349; 34%). Pearson chi-squared test demonstrated that edema (x^2=19,7; p0,001), ecchymosis (x^2=56,7; p0,001) and systemic manifestations (x^2=72,5; p0,001) had significant difference between mild and non-mild envenomations. Therefore, edema, ecchymosis and systemic manifestations may be good predictor for moderate and severe accidents. Non-mild envenomation cases had 34,3% more edema registered proportionally than mild ones. The same happened for ecchymosis and systemic manifestations with 402,4% and 447,4% more cases, respectively. Also, when latrodectism were registered as work accident, it was observed that it reaches mainly people with lower educational levels (62,2%). In addition, Brazil has been passing through a national problem about anti-*Latrodectus* serum. In Sinan's notification form there is no space to notify whether or not the patient has received anti-*Latrodectus* serum. So it's impossible to know how this serum is been administrated.

4 USE OF PHOSPHOPEPTIDE TO PURIFY INDUSTRIALLY IMPORTANT PROTEINS STARTING FROM THE VENOM OF CROTALUS DURISUSS TERRIFICUS

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Crotalus durissus terrificus (*Cdt*) main toxic compound of its venom is crotoxin (almost 50% of dry venom), is a β-neurotoxin composed by two subunits non-covalently bonded, one inactive and the other a phospholipase A_2. Crotamine (Ctm) is nonenzymatic (about 12% of the dry weight of the crude venom of crotamine-positive *Cdt*) and both have interesting pharmacological uses, as antiviral, antibacterial and against tumor cells.

Peptides have proved to be very useful ligands for the purification of numerous molecules such as antibodies, toxins, among other proteins.

The aim of this work was to design a phosphopeptide (P-Lys) with a phosphoserine in its sequence. Its synthesis was carried out in solid phase on Rink-Amide-ChemMatrix resin. Then, it was immobilized in NHS-agarose to be used as an affinity chromatographic matrix and process conditions were evaluated using SDS-PAGE 15% and SDS-PAGE-tricine gels, and protein concentration and enzymatic activity were evaluated.

For the chromatography, 100 µl of the venom (150 mg/ml) of a 1/20 dilution in the adsorption buffer was applied into the matrix.

In the best conditions, the percentage of adsorption reached 70% and when performing the gels one band was observed in eluate 1 which correspond to the MW of Ctm (5 kDa) and three bands were observed in eluate 2, of which one corresponds to the MW of PLA2 (14 kDa). Said bands were analyzed by mass spectrometry were identified. Only eluate 2 presented enzymatic activity. The yield of the process exceeded 100% (it has been described that free PLA_2 has greater activity than complexed)and with a purity of 90% for PLA_2. For Ctm the purity was 76%.

Through the design of a phosphopeptide it was possible to recover both, PLA_2 and Ctm with an adequate purity from a sample of *Cdt* venom in a single chromatographic step.

5 PRODUCTION OF AN ALTERNATIVE ANTIVENOM BASED ON EGG YOLK ANTIBODIES AGAINST CROTALUS DURISSUS TERRIFICUS

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Snakebite envenoming represents an example of a pathology which effective treatment is the administration of antivenoms. An alternative to mammal polyclonal sera is the use of egg yolk antibodies due to its advantages regarding animal welfare and lower costs of production. The aim if this study is to produce an IgY antivenom against *Crotalus durissus terrificus* at a pilot scale using more simplified methods of purification. A group of laying hens (n=2) were immunized via *i.m.* with 80 µg of *C. d. terrificus* whole venom (pool of venoms of snakes from Argentina) nine times at days 0, 14, 28, 71, 237, 289, 304, 473 and 487. For the first immunization, venom was emulsified with Freund`s complete (1st injection) and incomplete adjuvants (boosters). Eggs were collected during 10 days after the 8th and 9th immunization. In order to choose the optimal method of purification, different methods of purification were evaluated: ammonium sulphate precipitation (24 and 26% w/v), PEG-6000 (12% w/v) and caprylic acid (7% v/v). 0.01% (w/v) thimerosal was added for preservation. Median effective dose (ED_{50}) was assessed in mice by mixing 3 LD_{50} (17 µg) with increasing volumes of IgY antivenom according to WHO guidelines (World Health Organization, 2017). The optimal ED_{50} was obtained by sulphate ammonium precipitation rather than using PEG-8000 and caprylic acid. After 9 immunizations, 1 ml IgY antivenom purified by PEG-8000 neutralized 158 µg of venom. In addition, 1 ml IgY antivenom purified by caprylic acid neutralized 40 µg of venom. However, 1 ml IgY antivenom purified by sulphate ammonium neutralized 395 µg of venom. In conclusion, immunization of hens with sub-lethal doses of *C. d. terrificus* venom produced antivenoms with DE_{50} similar to the obtained in horses. Thus, IgY-technology may allow the production of effective and affordable antivenoms.

6 IMPROVING THE BOTHROPIC ANTIVENOM THROUGH A REVERSE ANTIVENOMICS APPROACH

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Worldwide estimates of snakebite envenoming reach 5 million cases every year, resulting in more than 100,000 deaths and around 400,000 people with permanent disabilities or disfigurements. The severity of the problem and the shortage of antivenoms in some regions of the world, as in sub-Saharan Africa and Asian communities, lead the World Health Organization to add snakebite envenoming to the list of Neglected Tropical Diseases in 2017. Since the end of the XIX century, antivenoms are the only effective treatment for snakebite envenoming. Despite the importance of antivenoms, they also have drawbacks as the high incidence of adverse reactions when injected into the human body. Significant progress has been made in the studies of snake venom composition and of antivenom efficacy, however, few studies have been done to characterize antivenoms at the molecular level. In the present work, we produced an improved bothropic antivenom (iBAv) by affinity chromatography purification of the specific antibodies against the venom toxins of *Bothrops jararaca*. Then, quantitative proteomic analysis of the bothropic antivenom (BAv) and iBAv was performed, and the efficacies of both were compared by surface plasmon resonance (SPR) and *in vivo* assays. The results show that several proteins were highly depleted in the iBAv, producing an antivenom that has a higher venom neutralization efficacy. *In vitro* SPR assays have shown that iBAv produced twice as much response for binding to the venom toxins when compared to the BAv. *In vivo* assays with mice demonstrated that the iBAv is significantly more efficient in neutralizing the venom of *Bothrops jararaca* than BAv. This reverse antivenomics approach may contribute to the characterization of current antivenoms and help in the planning of a new generation of improved antivenoms.

7 **LOXOSCELES GAUCHO VENOM GLAND PROTEOME: A NEW PERSPECTIVE ON LOXOSCELES VENOM BIOCHEMICAL COMPOSITION**.

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In Brazil, Loxosceles are considered to be the most important arthropods for public health, these animals can cause important clinical local or systemic manifestations in human. The cutaneous loxoscelism is characterized by severe inflammation at the site of the bite, that commonly courses with purple colored points forming a marmoreal aspect that spreads gravitationally, that after evolves with a extensive necrotic area. Systemic loxoscelism occurs mainly in children, and presents hemostasis disturbance, renal failure and intravascular hemolysis. The treatment for loxoscelism includes mainly antivenom and corticosteroids for systemic symptoms, and in nowadays tetracycline ointment is used to treat the local lesion. The antivenom efficacy depends on the identification of antigenic toxins that could lead to a neutralization of toxic activities by antiserum use. Proteomic analysis of Loxosceles venom is an very important tool, that bring new strategies to antivenom production and could clarify the differences between envenomation caused by different species of Loxosceles. For this work, the venom gland of three specimens of Loxosceles gaucho were extracted and analysed by 1D-SDS-PAGE and by shotgun proteomics (LC-MS) using three different enzymes (Trypsin, Chymotrypsin and Pepsin). Electrophoresis presented the major bands in the range of 35 kDa characteristic of dermonecrotic D Phospholipases (PDL). On the proteome were observed a wide diversity of Phospholipase D sicaritoxins, belongings to different families: alpha-2, C1, B1, I1 and Beta A1. Specific toxins from L. gaucho were identified LOXN2 and LOXN1/7 and good coverage for LgRec1 protein (69%) and LvSicTox-alpha C1 (54%) were obtained. The proteomic analysis showed 12 differents PDLs, 2 Astacin-Metalloprotease and firstly described the presence of an insecticidal toxin Sicaritoxin-Lia1 on the L.gaucho venom.

8 HYPANUS AMERICANUS MUCUS: A NEW POINT OF VIEW ABOUT STINGRAY IMMUNITY AND TOXINS

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Fish skin plays important biological roles, such as the control of the osmotic pressure gradient, protection against mechanical forces and microorganism infections. The mucus, on the other hand, is a rich and complex fluid, important for the fish acting as innate immunity system, swimming and nutrition. The elasmobranch epidermis is characterized mainly by mucus secretory cells, and marine stingrays have already been described to present secretory glands spread throughout the body. Little is known about the biochemical composition of the stingray mucus, but recent studies denoted the importance of mucus in the envenomation process. Stingrays venom are largely studied due the human medical importance of envenoming caused by sting puncture, that evolve with local inflammation and necrosis, and these toxic events can be correlated to the chemical composition of the sting skin, according to the literature. Aiming to analyse the mucus composition, a new non-invasive mucus collection method was developed that focused on peptides and proteins, and biological assays were performed to analyze preliminary toxic and immune activities of the *Hypanus americanus* mucus. Pathophysiological characterization showed the presence of peptidases on mucus, as well that the induction of edema and leukocyte recruitment in mice. The fractionated mucus improved phagocytosis on macrophages and showed antimicrobial activity against *T. rubrum*¸ *C. neoformans* and *C. albicans in vitro*. The proteomic analyses showed the presence of immune-related proteins like actin, histones, hemoglobin, and ribosomal proteins. This protein pattern is similar to those reported for other fish mucus and stingray venom. This is the first report depicting the *Hypanus* stingray mucus composition, highlighting its biochemical composition and importance for the stingray immune system and the possible role on the envenomation process.

9 SEARCH FOR PROTEASES AND PROTEASE INHIBITORS IN THE WAXY SECRETION THAT COVERS THE TICK AMBLYOMMA SCULPTUM'S EGGS (ACARI: IXODIDAE)

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Ticks are hematophagous mites that can feed on various animal species. The *Amblyomma sculptum* tick is widely found parasitizing animals, including humans, in Brazil, and it is recognized as a vector of important pathogens relevant to public health. For their perpetuation, females of ixodids when engorged, can lay up to 10,000 eggs. Protease inhibitors (PIs) and proteases present in organs and other secretions of ticks have been described, but there is no data in the literature on the presence of these molecules in the wax that covers *A. sculptum* eggs (EW). Therefore, this work had as objective the study and the characterization of PIs and proteases present in EW. The eggs were collected and the EW's aqueous extract was submitted to a 10 kDa molecular weight cut off membrane, and the high molecular weight pool (HMWP) and the low molecular weight pool (LMWP) were obtained. The LMWP was fractionated on a C18-RP-HPLC, and 24 peaks were manually collected and tested as elastase inhibitor, using a FRET substrate. Four peaks inhibited about 50% of the elastase catalytic activity, and their molecular contents and primary sequences are being analyzed by mass spectrometry. Analysis of HMWP by SDS-PAGE showed proteins of 100-260 kDa, in addition to two bands with about 40 kDa. In a proteolytic activity assay, using metallo and serine peptidases inhibitors and FRETs substrates, it was observed the presence of metallopeptidases in the pool. This is an important result and will be deepened, since the inhibition of metalloproteases present in eggs of other species of arthropods prevented their hatching. In addition, the search for bioactive molecules with biotechnological potential in EW's *A. sculptum* showed to be promising, according the results obtained in this work.

10 CROSS-SPECIES AND GEOGRAPHIC POTENTIAL OF B-CELL EPITOPE STRINGS IDENTIFIED FOR GENERATION OF AN AFRICA-SPECIFIC SNAKE VENOM-INDUCED NECROSIS THERAPEUTIC

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Every year 1.8-2.7 million people suffer snakebite envenoming, of these, an estimated 137'000 die and 400'000 are left disfigured or disabled. Fundamental to alleviating this burden is improving antivenom efficacy. Linear B-cells epitopes are short peptides displayed by antigen presenting cells to elicit an immune response. Multiple toxin epitopes can be identified and arranged as 'beads on a string', increasing the probability of a broadly-neutralising immune response. Prior studies have shown epitope-string immunogens are able to generate antibodies capable of neutralising haemotoxic venom proteins. I intend to adopt this approach to create snakebite necrosis specific serotherapies. Snakebite necrosis causes significant physical, mental, and economic distress. Whilst there is a debate over the efficacy of antivenom for necrosis treatment, a targeted therapeutic does not exist to prevent lifelong disability and disfigurement. A necrosis therapeutic requires a focus on the aetiological venom proteins; snake venom metalloproteinases, phospholipase A_2s, hyaluronidases, and cytotoxic three finger toxins. Through bioinformatic interrogation of sequence data from three medically important African snakes (*Echis ocellatus, Bitis arietans,* and *Naja nigricollis*) 6 epitope strings were generated for delivery via a hepatitis B core virus like particle (VLP) carrier. To refine immunisation experiments, I investigated the ability of antivenom used in Africa to recognise the selected epitopes. To understand the geographical utility of those immunogens I also assessed their recognition by antivenom used in Asia and South America. Determining the cross-genera and cross-continent potential of the epitope-string immunogens will provide valuable information on the possibility of a global anti-necrotic. Identifying where cross-specificity is minimal will strengthen the approach, potentially avoiding epitope identification across all necrotic species and their toxin sequences.

11 TRANSCRIPTOMIC ANALYSIS OF THE VENOM GLAND FROM THE SCORPION BOTHRIURUS BONARIENSIS

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Bothriurus bonariensis (Bothriuridae) is responsible for the largest number of envenomation accidents involving scorpion sting in Southern Brazil. Besides the clinical interest in the study of such venom, the characterization of its molecular composition may lead to the discovery of new drug candidates and novel scaffolds for lead compounds. This work intends to provide an analysis of the components in the venom, using a transcriptomic approach, in order to find molecules with possible biotechnological application. For the venom gland *de novo* transcriptome, cDNA libraries were prepared using RNA extracted from milked glands, representing the active state of these organs. Sequencing was performed on Illumina MiSeq System, and the transcriptome reads were assembled using Trinity. The obtained coding sequences were then searched against nonredundant public databases using the Blastp algorithm. The assembly and filtering of the reads lead to the obtainment of circa 11,000 coding sequences with high-scoring hits in the search on the databases. The functional annotation of these sequences revealed the presence of many ion channel modulators, proteases, protease inhibitors, and antimicrobial peptides among the transcripts (alongside with proteins implicated in common cellular processes important for venom gland function), indicating the potential of this venom as a source of molecules with biotechnological relevance. However, a large portion of the transcripts still could not be assigned based on homology, suggesting an abundance of unexplored toxin sequences in this species and further efforts are being made to classify these unknown proteins. For the toxins identified so far, some candidates are being evaluated for experimental functional characterization through different biological assays. These results provided valuable information concerning the diversity of molecules present in *B. bonariensis* venom gland transcriptome and confirmed the potential of this venom in the prospecting of novel compounds.

12 CROSS-NEUTRALIZATION ASSAYS OF TRIMERESURUS PUNICEUS AND CROTALUS ATROX VENOMS BY PERUVIAN ANTIVENOMS

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Snakebite envenomation affects mainly poor and rural populations and is being considered a serious neglected health problem according to the World Health Organization. Currently, the administration of antivenoms is the only scientifically validated therapy to neutralize the effects of snakebite envenoming in humans. Antivenoms are produced by the immunization of animals (horses, sheeps or camelids) either with mixtures of venoms from different viper species (polyvalent antivenoms) or with species-specific venoms (monovalent antivenoms). Previous neutralization efficacy studies of the antivenoms have shown cross-reactivity to venoms from other viper species not used in their production. This feature provides information about the structure similarities of the venoms components and the antivenom efficacy. In this study, three Peruvian commercial antivenoms (lachesic, crotalic, and bothropic INS) were used to test their neutralization effectiveness to *Trimeresurus puniceus* (Asian viper snake) and *Crotalus atrox* (North American viper snake) venoms. Efficacy and cross-reactivity of the Peruvian antivenoms were studied by different assays as enzyme-linked immunosorbent assay (ELISA), Western Blot, and neutralization of enzymatic activities (hyaluronidase, proteolytic, phospholipase, and coagulant). Peruvian lachesic antivenom was very effective in neutralizing the *T. puniceus* venom, while the Peruvian Bothropic antivenom was more effective in neutralizing the proteolytic and phospholipase activities of *C. atrox* venom than the Peruvian crotalic antivenom, which only neutralized the hyaluronidase activity of C atrox. Our findings suggest that the lachesic and bothropic antivenoms have cross-reactivity to *T. puniceus* and *C. atrox* venoms respectively. In vivo studies of the mean effective dose (ED50) of the Peruvian antivenoms will complement their in vitro neutralizing capacity.

13 ISOLATION OF A NEW VEGF FROM CROTALUS DURISSUS TERRIFICUS VENOM

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Snake venoms are rich sources of components with important pharmacological effects. However, many components have not been isolated yet. Vascular endothelial growth factors (VEGFs) are non-enzymatic homodimers of 20-30 kDa with angiogenic properties. Although VEGF have already been identified by omics analysis in the *Crotalus durissus terrificus* (Cdt) venom, it was not isolated so far and its role in the envenoming pathophysiology remains unknown. In the present study, a new VEGF from Cdt venom (CdtVEGF) was isolated. Cdt venom was fractionated by reversed phase FPLC (Fast Protein Liquid Chromatography), using a C-18 column, and all the fractions collected (32) were submitted to an ELISA assay for VEGF identification. The fractions identified as positive for VEGF (19, 20 and 21) were submitted to an anion exchange chromatography (HiTrap QXL column) for CdtVEGF purification. The subfractions obtained were also submitted to the ELISA assay and the CdtVEGF presence was confirmed in the subfractions named 19-2, 20-2, 21-2 and 21-3. Probably they are different isoforms of VEGFs. The CdtVEGFs isolated were characterized by SDS-PAGE as homodimeric proteins with similar molecular masses of approximately 25 kDa for the dimer. This work is pioneer on the isolation and partial characterization of VEGFs from Cdt venom.

14 ARE YOU WHAT YOU EAT? THE INFLUENCE OF DIET ON COMPOSITIONAL AND FUNCTIONAL ACTIVITIES OF BOTHROPS MOOJENI SNAKES SUBMITTED TO DIET SHIFT.

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Bothrops moojeni is a common snake in Brazilian cerrado. This species has a generalist diet, including mammals, amphibians and reptiles. Since comparative studies about venom variation related to diet have not been deeply investigated, the objective of this study is to evaluate the presence of intraspecific variation in venom composition after diet shit from mammal to amphibian and back to mammals. Ten captive *B. moojeni*, fed exclusively with mammals (initial venom), had their diet changed to amphibians for a period of one year (final 1 venom), and then, returned to the diet with mammals for another year (final 2 venom). Venoms were analyzed through RP-HPLC, shotgun proteomics, collagenolytic activity, MCD, MHD, paw edema and LD_{50} in mice and frogs. RP-HPLC showed high individual variability, with no significant differences among venoms after diet shifts. Shotgun proteomic demonstrated some differences between venoms, especially in CTL and SVMP. In general, the collagenolytic activity showed a reduction after one year feeding on amphibians, followed by an increase after the return to mammal's diet. MCD tends to increase after amphibian diet. *In vivo* assays demonstrated that MHD was lower in the initial venoms than in final 1 venoms, and then decreased to a similar initial condition in final 2 venoms. The same pattern was observed in paw edema. However, LD_{50} in mice showed similar values to final 1 and final 2 venoms while initial venom seems to be discreetly more lethal than both. Impressively, the LD_{50} in frogs demonstrated that initial venom is slightly less lethal than final 1 and 2 venoms. Our results evidenced that diet can play an important role on composition and function of *B. moojeni* venom and it demonstrated that venom has a phenotypic plasticity.

15 OCCUPATIONAL OPHIDISM AND ASSOCIATED FACTORS IN BRAZIL

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Occupational snakebite is understood as the ophidism poisoning whose circumstance is a work accident. Despite present on WHO's list of Neglected Tropical Diseases, most of the studies about this condition are limited to describing general epidemiological profiles and reporting characteristics of the injured person. The studies that relate this complaint to characteristics that transcend the individual are few, especially from the occupational perspective. Therefore, the objective of the present study was to investigate the association between the occupational snakebite and Social Determinants variables. To investigate the relationship between occupational snakebite and some Social Determinants Health in the municipalities of Brazil, an aggregate study was conducted. Reported cases of occupational snakebite in Brazilian municipalities (2007-2015) were collected from the Notification of Injury Information System (Sinan). Demographic data were obtained from the 2010 census from the Brazilian Institute of Geography and Statistics (IBGE). Associated factors were investigated by zero-inflated negative binomial regression. Occupational snakebite was associated (p0.05) to: GINI index (IRR=1.078); percentage population employed in agriculture (IRR=1.002); percentage of households with garbage in the surroundings (IRR=1.019); illiteracy index (IRR=1.061); percentage of the municipality earmarked for agriculture (IRR=0.994) and demographic density (IRR=0.999). In view of the above, it is evident that the investigated variables are predictive of occupational snakebite. In addition, it is observed that the problem is structural by nature, and not related to the individual, as commonly reported in the literature. Thus, it is necessary for the government to commit itself to improving the quality of life and working conditions of the population, since it is a health problem that is invisible due to social issues.

16 BIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF THE EYELASH VIPER BOTHRIECHIS SCHLEGELII VENOM FROM SANTANDER, COLOMBIA.

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Bothriechis schlegelii is a venomous snake belonging to the Viperidae family widely distributed from Central America to Northern South America. In Colombia *B. schlegelii* represents one of the species of clinical importance. However, few studies of the venom of this species have been carried out from Colombian populations. In this work we present the biochemical and biological characterization of *B. schlegelii* venom from Santander, Colombia, using reverse phase high resolution liquid chromatography (RP-HPLC), protein electrophoresis (SDS-PAGE) and assays of biological activities *In-vitro* (PLA2, LAAOs, procoagulant and proteolytic activity). In addition, the chromatographic and electrophoretic profiles of venoms from Santander, as well as their in-vitro biological activities, were compared with venoms from two other areas of Colombia. The venom of *B. schlegelii* from Santander showed a chromatographic profile consisting of 35 fractions. The first part of the chromatogram (the first 18 peaks) showed no electrophoretic bands greater than 15 kDa. The electrophoretic bands corresponding to these venoms were mostly distributed in a range of 20-50 kDa. Proteolytic and PLA2 activities had an inversely proportional relation in Santander venoms, showing the highest PLA2 activity among all other areas. No differences were observed in the L-amino acid oxidase activity between the venoms of Santander and the other zones of Colombia. We found that the venom of *B. schlegelii* from Santander presents chromatographic and electrophoretic characteristics similar to those observed in the venoms of other viperids of the Neotropic and is mainly composed of proteins of medium and high molecular weight (20-50 kDa). In addition, our enzymatic assays show the venom of *B. schlegelii* of Santander mainly myotoxic. However, it is necessary to corroborate these observations with in- vivo assays.

17 BOTHROPS NEUWIDI SNAKE VENOM: ONTOGENETIC CHARACTERIZATION AND IMUNORECOGNITION BY BOTHROPIC ANTIVENOM

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INTRODUCTION

Snake venom is modified saliva with a high concentration of active compounds, which displays a strong phenotypic plasticity, it determines both natural history of snake groups and impacts human health. Due to variation on life habits, venom also shows ontogenetic variability by shifting its composition and enzymatic activity among life stages. There are species of snakes that remains lacking comprehensive studies of their venom ontogeny, including *Bothrops neuwiedi*. Here we present the first description and immunorecognition test of *B. neuwiedi* venom across life stages.

MATERIALS AND METHODS

Venom samples were separated by sex and pooled in five life stages. SDS-PAGE at 15% was carried out with 20 µg of each sample and 2-Mercaptoethanol as reducing agent. It also was performed a C18 reversed-phase HPLC to each sample. Collagenolytic activity was performed with Azocoll as substrate. Western blot was carried out for immunorecognition analysis.

RESULTS AND DISCUSSION

HPLC results suggest that juvenile venom has a high peak of serine proteases, while adult venom has a higher one of metalloproteinases. It suggests a higher procoagulant activity for juvenile snakes and a higher proteolytic activity for adults, but collagenolytic activity did not show this correlation. Besides, neonate venom also has three different exclusive bands by SDS-PAGE, which will be identified by mass spectrometry. Western blot reveals that there is a difference on recognition by the bothropic antivenom among protein families of *B. neuwiedi* venom.

CONCLUSION

Divergence between protein composition among life stages may be due to differences in target prey, which involves a different mechanism to subdue and digest the animals. It is possible that bothropic antivenom may not being recognizing the totality of venom protein families of *B. neuwiedi.* Protein identification by mass spectometry.may contribute to elucidate the participation of protein families during ontogenetic development and their possible role on lethality.

18 TAKING BABY STEPS: INITIAL ONTOGENETIC ANALYSIS OF BOTHROPS ERYTHROMELAS SNAKE VENOM

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Introduction: In Brazil, the genus *Bothrops* is responsible for approximately 87% of the snakebites. Bothropic venom is characterized for its proteolytic, hemorrhagic and coagulant effects, resulting in several local and systemic symptoms that may differ, among other variables, according to the age of the snake. *Bothrops erythromelas* is a small snake, which venom differs from other species of this genus: it lacks thrombin-like activity and shows high factor II and X activation. Although some aspects of this species venom were already observed, the ontogenetic variation is not yet completely elucidated. **Objective:** The aim of this study is to analyze the variations that occur in the venom of female and male *B. erythromelas* throughout their lives. **Material and methods:** Newborn *B. erythromelas* venom were milked, pooled into females and males, lyophilized and submmited to initial analysis. SDS-PAGE and protein quantification (Bradford method) were performed to characterize the composition, whilst enzymatic activities of PLA_2, LAAO, casein and collagen were carried out using 4-nitro-3-(octanoyloxy)benzoic acid (NOBA), L-methyonine, azocasein and azocoll as substrates, respectively. **Results and discussion:** Male newborn pool showed significantly higher protein content and PLA_2 activity than female pool, corresponding to the band of approximately 15 kDa in the SDS-PAGE (likely PLA_2) that was more intensely stained in the male venom. LAAO, collagenolytic and caseinolytic activities showed no statistical difference between both sexes. Proteolytic activity over collagen was low in both sexes, and none of them presented LAAO activity. **Conclusion:** So far, we may conclude that the venom of newborn *B. erythromelas* shows no LAAO activity and low collagenolytic activity, and only PLA_2 activity was different between sexes. As next steps, we intend to follow the development of the same snakes to further characterize the changes in the venom.

19 HARNESSING MONOCLONAL ANTIBODIES FOR DEVELOPMENT OF A SPECIFIC TREATMENT AGAINST NAJA NIGRICOLLIS ENVENOMING

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The African black-necked spitting cobra (*Naja nigricollis*) is one of the most notorious snake species found on the African continent. Its venom consists of a highly potent mixture of cytotoxins, which cause severe local tissue damage in victims, who are left with permanent sequalae after a bite. Currently, antivenom is a scarcity throughout the African continent. Furthermore, due to their heterologous origin and low content of therapeutically active antibodies, these antivenoms have a propensity to cause severe adverse reactions, including serum sickness and anaphylaxis, which could lead to death of the patient.

Recombinant antibodies represent a therapeutic alternative. We are developing an antivenom based on recombinant human monoclonal immunoglobulin G (IgG) antibodies, which are predicted to be safer, cost-competitive, and more efficacious than the existing treatment. Here, we present a subset of this work: Through the utilization of phage display technology, it has proven possible to discover and express novel human single-chain variable antibody fragments (scFv) against the five most medically relevant venom toxins from *N. nigricollis*, four being cytotoxins and one being a phospholipase A_2. Out of 486 monoclonal scFvs analyzed, 164 were considered good binders. Of these, 94 were sequenced, resulting in the identification of 31 unique scFvs. The binding properties of these scFvs will be evaluated, and the most promising leads will be converted into the immunoglobulin G format and assessed *in vivo*. It is our hope that the work in this project will help enable radical improvement in the treatment of snakebite envenoming.

20 CONVERGENT EVOLUTION OF DEFENSIVE VENOM COMPONENTS IN SPITTING COBRAS

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Snake venoms are used primarily for prey immobilisation, however three groups of spitting cobras have independently evolved the ability to forcibly eject their venom into the eyes of aggressors as a defence mechanism. This adaptation is underpinned by differences in their fang morphology, while differences in pathology following bites to humans suggests that the molecular composition of venom varies between spitting and non-spitting cobras. To investigate whether the origin of spitting has resulted in parallel molecular evolution of venom composition across spitting cobras, and whether their venoms cause increased pain in comparison with non-spitting cobras, we undertook a multi-disciplinary approach consisting of transcriptomics, proteomics and functional assays for 17 species. We found that spitting cobras have a higher abundance of phospholipase A_2 (PLA_2) toxins in their venom and increased enzymatic PLA_2 activity in relation to non-spitting counterparts. Using a cell-based calcium influx assay as a proxy for the activation of sensory neurons, we show that spitting cobra venoms likely cause significant increased pain than those of non-spitting cobras. Repeating these assays with venom fractions revealed that PLA_2s potentiate this activity. Our findings thus demonstrate that all three spitting cobra lineages have independently increased the abundance of PLA_2 toxins to increase defensive efficacy of their venom, suggesting that defensive adaptations can drive variation in venom composition in snakes. In a wider context, our findings show that the convergent origin of morphological and behavioural adaptations can stimulate convergent evolution at the molecular level, which in turn results in complex functional phenotypes.

21 MOLECULAR ADAPTATION AND RESISTANCE, TO THE A-NEUROTOXIN OF ELAPID SNAKES, IN SQUAMATA, AVES AND FISHES.

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Some animals overcome prey, or protect themselves, using toxins. The species threatened by toxins may evolve resistance to those toxins. This can result in an evolutionary arms race. One example of resistance concerns the α-neurotoxins in elapid venom that binds to the α-subunit of the nicotinic acetylcholine receptor (nAChR). Alteration of an ancestral aromatic residue (position 189) to a glycosylated asparagine (N) is linked to autoresistance in elapids. Similar modifications have been found in mammals, but there are few or no data from other vertebrates. Here, we look for potential resistance-related adaptations to α-neurotoxin in multiple species using PCR and published sequences. A functional assay was performed with *Naja naja* venom on embryonated eggs of *Gallus gallus*, *Pogona vitticeps*, *Danio rerio* and *Gasterosteus aculeatus*. The α- subunit of the nAChR ligand-binding domain was sequenced. We find that 22 different snake species, 2 lizards and one fish have an asparginine at position 187 and at position 189. We found that 11/11 Elapidae, 5/6 Viperinea, 2/3 Natricidae, 1/2 Dipsadidae, 3/15 Colubrinae, Anguidae 1/3, Agamidae 1/4, and *Gasterosteus aculeatus* examined had one of these two adaptations. Interestingly, we found no such changes in birds of prey, or other birds that commonly eat snakes. We also find evidence of secondary loss of the adaptation within clades where it is otherwise present. Further, the functional assays showed *Pogona vitticeps* and *Gasterosteus aculeatus* which have one of the two changes, are far less susceptible to *Naja* venom toxicity than are *Gallus gallus* and *Danio rerio* which lack either change. These findings expand our knowledge of the phylogenetic distribution of the cobra-type adaptation in Squamata, birds and fishes. They show that it has evolved multiple times independently across squamata phylogeny and fish independently. Future work could include studies of the putative functional role of this adaptation

22 BRAZILIAN BOTHROPS DIPORUS, IN FACT A LINEAGE OF BOTHROPS PUBESCENS:MITOGENOMIC, VENOMIC AND ONTOGENETIC STUDIES

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The Neuwiedi group is historically discussed on account of the variability of its species associated to the geographic distribution, being nowadays probably the most complex group of *Bothrops* in Brazil to be taxonomically resolved. The diversity of these snakes requires a deeper exploration in a phylogenetic point of view but also in its venom composition. We performed mitogenomic, venomic and ontogenetic studies of the *Bothrops diporus* and *Bothrops pubescens*, both species from Neuwiedi complex and inhabitants of the Southern region of Brazil. To characterize the venoms and evaluate ontogenetic changes, we used the Venomic approach. It consists in fractionation of crude venom by RP-HPLC, followed by analysis by SDS-PAGE, in gel digestion and mass spectrometry. Our proteomic data showed extreme venom similarity between both species, not yet observed in other Bothrops venoms. We noticed that the venoms undergo ontogenetic changes, mostly in the balance of two types of phospholipases A2 (PLA2), Lys49 and Asp49. To better comprehend these changes, we accompanied a venom pool of an offspring from birth to adult age, with periodic extractions. Interestingly, while the Lys49 increases its expression, the Asp49 decreases, marking a clear ontogenetic regulation since early stages of life. Furthermore, comparing the masses of these PLA2s, we observed that they are identical between species but different between adult and juvenile, confirming this ontogenetic trace and reinforcing the interespecific identity. This notably identity led us to review the relation between these species before other viperids. In addition to a morphological analysis, we assembled the complete mitogenomes for phylogenetic studies. Our data revealed a mitogenomic proximity between these species not yet observed among other viperids, not even between subspecies. Taking together the proteomic and the phylogenetic data, our results strongly indicate that *B. diporus* is in fact a lineage of *B. pubescens.*

23 DISCOVERY AND EVALUATION OF MONOCLONAL ANTIBODIES FOR STRATIFICATION OF VENOMS FROM BRAZILIAN BOTHROPS, CROTALUS, AND LACHESIS SPECIES

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Snakebite envenoming is a neglected disease of the rural tropics, which is as ancient as it is cruel. Although expected to affect millions of victims each year worldwide, the exact extent of snakebite-associated mortality and morbidity is unknown, as accurate epidemiological data is difficult to come by. The only treatment specific for snakebite envenoming is the administration of antivenoms based on antibodies derived from the serum of hyperimmunised animals. The specificity of these antivenoms vary, with some polyvalent antivenoms covering several genera of venomous snakes, while other monovalent antivenoms only cover one or a few species. The choice of which antivenom, if any, to be administered to a snakebite victim is often guided by a syndromic approach to diagnosis. This has the drawback that treatment may be delayed until venom-induced clinical manifestations are visible to the treating clinician, allowing the venom toxins to exert their toxic function for an extended period of time. Here, we present a study focusing on discovery and evaluation of monoclonal murine immunoglobulin G (IgG) antibodies with the purpose of employing these antibodies in the development of an assay for rapid stratification of snake venoms in envenomed patients. Hybridoma technology was used to generate 117 of these antibodies against venom and venom components of *Bothrops*, *Crotalus*, and *Lachesis* species indigenous to Brazil. The antibodies were evaluated with ELISAs to determine their specificity and to identify antibody sandwich pairs for use in a lateral flow assay. Such an assay might be able to support diagnosis of snakebite victims and thereby decrease time to treatment. Furthermore, the assay could find its use in epidemiological studies, where it could help map snakebite incidence and increase awareness of this highly neglected disease.

24 DISCOVERY OF CROSS-REACTIVE AND RECYCLABLE HUMAN MONOCLONAL ANTIBODIES FOR NEW RECOMBINANT ANTIVENOMS

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In 2017, the World Health Organization added snakebite to the list of the world's most Neglected Tropical Diseases, thereby recognizing the huge healthcare impact this indication has worldwide. The only available treatment for snakebite envenoming is antivenom, which is based on polyclonal antibodies derived from the plasma of hyper-immunized animals. Antivenoms have saved countless lives, however, the treatment has several drawbacks associated with its heterologous nature and its manufacturing process. Recombinant antivenoms based on mixtures of human monoclonal antibodies have the potential to become the next generation of envenoming therapies. Such treatments can be designed to have improved therapeutic properties, such as better safety profiles, enhanced efficacy, and improved manufacturability compared to existing polyclonal antivenoms. In the later years, significant scientific developments have been reported within this area, including the development of the first experimental antivenom based on a mixture of fully human monoclonal immunoglobulin G antibodies. Nevertheless, many technical challenges need to be resolved before it is feasible to manufacture recombinant antivenoms at larger scale and evaluate them in the clinical setting. In the early discovery process, these challenges include how to rationally engineer cross-reactive antibodies that target multitudes of toxins, and how to design antibody mixtures that can be administered at very low dose while retaining therapeutic efficacy. Here, we present new strategies for high-throughput discovery of monoclonal antibodies that are cross-reactive and recyclable, with examples of recently discovered antibodies against snake, spider, scorpion, and bee toxins from our lab.

25 PROTEOMIC AND PHOSPHOPROTEOMIC ANALYSES IN HL60 CELLS TREATED WITH THE SCORPION-DERIVED PEPTIDE LUNATIN-1

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Lunatin-1 is a peptide isolated from the scorpion *Hadruroides lunatus* venom with observed antimicrobial and anti-tumor activities, recently described by our group. In order to uncover the molecular mechanisms and signaling pathways triggered by Lunatin-1 in a cancer cell line, we compared the proteome and phosphoproteome of HL60 cells originated from myeloid leukemia and treated with Lunatin-1 (50 µM) for 0, 30 and 60 minutes, combining dimethyl label quantitation, phosphopeptide enrichment by TiO_2, and mass spectrometry. A total of 2094 proteins were identified, being 1550 differentially regulated (p value > 0.05, one-way ANOVA) over time. Of these, 420 were regulated after 30 minutes, 614 were regulated after 60 minutes, and 516 were regulated at both time intervals. Moreover, a total of 638 phosphorylated peptides were identified. Of these, 403 proteins had their phosphorylation states in serine (S), threonine (T) or tyrosine (Y) residues changed significantly after Lunatin-1 treatment. The main downstream molecules and associated signaling pathways triggered by the treatment were PDK1, PKA, PKC, PARP-1 that participate in cell death signaling, apoptosis and DNA damage response. Our data indicate that Lunatin-1 exhibits activity on a human leukemia cell line by triggering critical changes in both levels and phosphorylated status of proteins implicated in a diverse range of cell responses.

26 PALYTOXIN-LIKE EFFECT FROM PALYTHOA CARIBEAORUM ON RHINELLA MARINA OOCYTES ON THE ATLANTIC COAST OF COLOMBIA.

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Palytoxin (PLTX) is a large and complex marine polyether toxin that was originally isolated from *Palythoa* corals, which remain the major known source of this toxin. It targets Na+/K+-ATPase by converting it into an ion channel, destroying the electrochemical gradient of the membrane and causing high toxicity. PLTX can be found in *Palythoa caribaeorum,* an abundant and evenly distributed zoantharian species in the Caribbean Sea. It has been documented, however, that PLTX can be both present and absent from the same *Palythoa* species depending on when and where the observations are being done. An explanation for this unpredictable nature of PLTX is that its presence can be modulated by environmental conditions. This study sought to confirm whether or not PLTX is present in the colonies of *P. caribaeorum* found off the coast of Santa Marta, Colombia. As this had been done by others using HPLC and delayed haemolysis, we used electrophysiology to confirm the presence of this toxin. Using methanol and ethanol as solvents, we took tissue extracts from *P. caribaeorum*. We then evaluated the effect of the extracts on the resting potential and ion currents in anuran oocytes from *Rhinella marina*. This was done using a microelectrode amplifier and measuring the transmembrane potential and ionic conductance over time. The extracts depolarized the cells, confirming the presence of a PLTX-like compound in *P. caribaeorum* in Santa Marta. An advantage of using electrophysiology is that by using ouabain to inhibit the function of Na+/K+-ATPase, we were able to prevent depolarization, demonstrating that this result arises from an interaction with that enzyme, as expected from PLTX. A combined approach of electrophysiology with chemical analysis would be powerful in the screening of PLTX-like molecules.

27 PHYLOGENY AND TOXICOLOGICAL ASSESSMENTS OF TWO PORTHIDIUM LANSBERGII LANSBERGII MORPHOTYPES FROM THE CARIBBEAN REGION OF COLOMBIA

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After a snakebite accident, species identification is of vital importance. However, the existence of intraspecific differences in the body coloration patterns of venomous snakes can generate confusion and delay a convenient and effective treatment. This is the situation for *Porthidium lansbergii lansbergii* from Colombia, for which two distinctive color morphs occur, and the relationship of these morphs with venom toxicity is unknown. Therefore, venom samples from specimens of these two morphs were collected from the Colombian Caribbean region, and their protein profiles compared. Likewise, their venom functional activities were evaluated *in vitro* and *in vivo* in BALB/C mice. Additionally, using sequences of the mitochondrial cytochrome b (Cyt-b) gene, the relationship between these Colombian *P. lansbergii lansbergii* morphotypes was investigated, and their phylogenetic positions were determined for the first time using Bayesian inference. Despite the noticeable coloration divergence between the individuals analyzed, similar protein profiles of their venoms were observed. Additionally, neither their lethality nor biochemical activities were notably different. In general, both venoms were highly proteolytic, lacked a coagulant effect *in vitro*, and extended the clotting time due to the action of venom components, such as disintegrins and proteases, that induce defibrination. These results agreed with the result of our phylogeneticanalysis, suggesting that the two chromatic morphs do not represent isolated populations. The phylogenetic analyses also supported the currently recognized *P. lansbergii lansbergii* subspecies as a monophyletic complex. In conclusion, the results of this investigation suggest similar clinical manifestations regardless of body coloration after a *P. lansbergii lansbergii* envenomation, and pools can therefore be used for antivenom development, medical treatments, and further research efforts.

28 SEXUAL DIMORPHISM IN THE VENOM PEPTIDOME OF THE AMAZONIAN SPIDER ACANTHOSCURRIA JURUENICOLA: BIOLOGICAL INSIGHTS AND POTENTIAL NEW ANTITUMORAL AND ANTIMICROBIAL AGENTS

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Acanthoscurria juruenicola is an Amazonian tarantula spider described for the first time over a century ago. The size and most morphological characteristics of the specimens did not evidence significant differences between genders, however the composition of their venoms remained unknown to date. Here we show the comparative peptidomics of venoms focused on male and female differentiation. To determine these peptidic profiles we performed top-down and bottom-up mass spectrometric strategies, combining analysis of native and digested peptides, venom gland transcriptomics and bioinformatics. Our results revealed a total of 367 features in the peptide fractions of venoms, which were quantitatively compared. The protein concentrations of the female's venoms were about three times higher than the males, and it was observed that the new toxins U2-TRTX-Aj1a and U2-TRTX-Aj1b identified in females were virtually absent in the venoms of males. Seventeen cysteine-rich peptides (CRP) were simultaneously observed in the transcriptome and in the mass spectrometry experiments, from which fourteen were completely sequenced in the mature forms. The masses of mature cysteine-rich peptides covered the 3.7-8.6 kDa range, and the peptides were connected by 3-5 disulfide bonds. In terms of biological activities, the whole venoms induced *in vivo* paralysis in crickets. Moreover, *in silico* analysis revealed that two peptides are potential antitumorals and all mature peptides have potential antimicrobial activity, which could be experimentally verified for the peptide Ap1a against *Micrococcus luteus*, *Pseudomonas aeruginosa* and *Candida albicans*. These results demonstrate the potential of A. juruenicola's CPR as new lead candidates for further pharmacological and antimicrobial studies.

29 ABILITY OF TANNIC ACID ADMINISTERED INTRAPERITONEALLY AGAINST THE LETHALITY OF BOTHROPS JARARACA VENOM

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Introduction: In Brazil most of snakebites are caused by *Bothrops* genus. In late-onset antivenom accidents, acute kidney injury (AKI), death, or even limb amputation can occur. Tannic acid is a protein precipitating agent and could help against the *Bothrops jararaca* effects.

Objective: To evaluate the ability of tannic acid (TA) administered intraperitoneally (i.p.) against the lethality of *Bothrops jararaca* venom (Bjv).

Material and methods: This study was approved by institutional Animal Ethics Committee (CEUA Protocol nº 031/2014). Anesthetized male Wistar rats were randomly divided in 3 groups (n=5) to receive via i.p.: Group 1, 12.5 mg/kg Bjv; Group 2, the supernatant of preincubated (30 min) Bjv (12.5 mg/kg) + TA (0.05 g/mL) mixture; and Group 3, Bjv (12.5 mg/kg) and 2 h after TA (0.05 g/mL). After death, samples of blood and renal tissue were collected to assess hematological, biochemical and oxidative stress biomarkers. Results were expressed by the mean ± E.P.M. (p0.05).

Results: Animals from Group 2 survived for an observation period of 24 h, while those from Group 1 died 5 h 36 min after envenomation. The supernatant injection did prevent against the platelets reduction (34.6±15.2 to 792.8±39.2 $x10^3 \mu L^{-1}$); did decrease blood urea (129.8 ± 14.1 to 58.5 ± 4.91 mg/dL) and did decrease blood creatinine (2.8±0.38 to 0.46±0.08 mg/dL). Animals from Group 3 survived for 16 h and only platelets number altered from 34.6±15.2 to 247.6±131.5 ($10^3/\mu L$) what could explain the minor longevity than Group 2. Only blood reduced glutathione (GSH) biomarker increased in Group 1.

Conclusion: Tannic acid was able against the lethality of Bjv by the supernatant injection and increased longevity even when administered 2 h after the envenomation. Tannic acid becomes an interesting alternative for treating bothropic envenomation when serum therapy is unavailable.

30 THE INSECTICIDAL WASP TOXIN Γ–POMPILIDOTOXIN IS A LINEAR PEPTIDE MODULATING VOLTAGE-GATED SODIUM CHANNELS

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The fascinating observation that solitary wasps, such as spider wasps (*Pompilidae*), paralyze their prey instead of killing it, suggests their venoms to be interesting reservoirs of components acting on the nervous system. Hence, their venom promises to contain neurotoxins acting on ion channels and receptors. Pompilidotoxins (PMTx) are small (13-15 residue), non-disulfide bridge peptides isolated from solitary wasp venom. PMTxs interact with voltage-gated sodium (Nav) channels. γ-PMTX, isolated from *Cyphononyx fulvognathus* venom, is a novel member of the PMTx family. Electrophysiological characterization indicated that γ-PMTX acts by slowing down channel inactivation. γ-PMTX was screened against a panel of 7 mammalian Nav (Nav1.1-Nav1.6 and Nav1.8), 1 insect Nav from *Blattella germanica* (BgNav1) and 1 arachnid channel from *Varroa destructor* (VdNav1). γ-PMTX displays an interesting specificity for insect Nav channels over mammalian isoforms. Moreover, γ-PMTX induces cytolysis and histamine release from mast cells at higher concentrations. The structure of γ-PMTX was determined by CD and NMR spectroscopies and indicated that the peptide adopts an α-helical, amphipathic conformation in the presence of POPC/POPG lipid vesicles and micelles composed of DPC, in contrast to a disordered state in an aqueous solution. The senolytic and antitumoral properties of γ-PMTX was evaluated in different cell assays. Furthermore, an extensive Ala-scan was performed indicating the relevance of a Phe residue in the middle of its sequence, determinant for the peptide's biological activity. Several cyclized analogs of γ-PMTX were also designed and investigated for their activity on Nav channels. The obtained results indicate that the structural and pharmacological properties of γ-PMTX render this peptide as a potential lead compound for further development of novel insecticides targeting Nav channels. To the best of our knowledge, γ-PMTX is the shortest, Cys-free peptide modulating Nav channel inactivation gating.

31 IMMUNOENZYMATIC CHARACTERIZATION AND PREDICTION OF ANTIGENIC DETERMINANTS OF A LYSINE-49 PHOSPHOLIPASE A2 FROM BOTHROPS ATROX SNAKE VENOM

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Many basic phospholipase A_2 (PLA_2) are myotoxins and they are responsible of myonecrotic effects in ophidic accidents. C-terminal region of these proteins have basic and hydrophobic residues with sarcolemma membrane binding and disruption capacity. In previous works, we have purified myotoxins from Peruvian snakes such as *Lachesis muta*, *Bothrops brazili* and *Bothrops atrox*, which presented a high myonecrotic activity; additionally, these toxins required the highest dose of commercial antiserum to be partially neutralized. Thus, studying the immunogenicity and antigenic determinants of PLA_2 is a fundamental task for the development of better treatments against myonecrosis induced by snakebites. In this study, we have produced rabbit polyclonal antibodies (Anti-BaMtx) using as immunogen a myotoxic lysine-49 PLA_2 of *B. atrox* (BaMtx). Using Enzyme-linked Immunosorbent assay (ELISA), we determinated that Anti-BaMtx recognizes myotoxins isoforms present in venoms of *B. brazili* and *Bothrops pictus* with dilutions of 1: 32000 and 1: 16000, respectively. In addition, isoforms of *L. muta* and *Crotalus durissus* were recognized by dilutions of 1: 8000 and 1: 2000, respectively. By a Western Blot assay, we discovered that Anti-BaMtx recognizes proteins of 15 kDa approximately. Subsequently, we performed an *in silico* prediction and comparisson of conserved epitopes from BaMtx, deducted sequences were obtained from *B. atrox*, *B. pictus* and *L. muta* cDNAs, also including C. *durissus* and *B. brazili* sequences (Uniprot).We found 5 candidate epitopes, revealing a gradual conservation of these antigenic determinants in other PLA_2 isoforms. It is important to note that BaMtx epitope candidates are almost completely conserved in *B. brazili* myotoxin epitopes, which is consistent with our immunoenzymatic assays. To conclude, this report is in the way to obtain antivenoms using only common epitopes sequences of these proteins.

32 REVIEW ON THE EVOLUTION OF ANTIVENOMS FOR SNAKEBITES TREATMENT

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In Colombia, around 5000 cases of snakebites reported annually, the only specific therapy for the treatment of snakebite are antivenoms, complete immunoglobulins purified or (Fab)2 antivenoms. Objective: To present differences between the generations of antivenoms, the importance of the venom´s snake in the antivenom production, compare pharmacokinetic aspects and side effects in patients.

Materials and Methods: A review literature was conducted in databases using combinations of Mesh descriptors and terms, in English and Spanish. Pharmacokinetic parameters were compared in preclinical studies and side effects in clinical studies. Results: Differences were found due to the size of the fraction of the immunoglobulin, so the smaller this is, the greater distribution to the tissues and a shorter half-life is observed, compared with the heavier molecules. At the clinical level, studies were found with a slight decrease in side effects when using F(ab)2 antivenoms compared with the use of antivenom sera, but without statistically significant differences.

Conclusion: the process of elaboration of the antivenoms is determinant to evaluate and compare objectively the parameters measured in vitro and in vivo for this medicine.

33 CLINICAL CHARACTERIZATION OF SNAKESBITES IN A COLOMBIAN HOSPITAL, 2004-2014

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In Colombia snakebite is considered a public health problem issue that causes mortality in 8% and disability in 10% of cases due to inadequate clinical attention. The objective was to describe the clinical-epidemiological characteristics of patients diagnosed with snakebite in a tertiary hospital in Colombia. A review of the clinical charts with diagnosis of ophidism during the 2004-2014 period was made at the La Samaritana Empresa Social del Estado, University Hospital. The frequency of the variables associated with snake bites, previous treatment and in-hospital management was analyzed. Results: 42 medical charts were reviewed. It was found that 98% of the patients were bitten by family Viperidae´s snakes and 1% by coral´s snakes. 76% of cases occurred in male farmers, 53% with bites in lower limbs, 72% initially attended by healers. 63% of the cases were classified as moderate, 28% severe and 7% mild. 95.2% of the patients received antivenom, however, there was a discrepancy between the classification of severity and the antivenom doses required. 92% of patients had an adverse reaction to antivenom serum, 26% anaphylactic shock. Ninety percent of patients had intrainflammatory superinfection, mostly intradermal and skeletal muscles, 30% had wound culture, 74% received antibiotic. 50% of patients received fasciotomy, however, no patient had intracompartimental pressure measurement. The high incidence of infections despite the antibiotic scheme and surgical procedures reevaluated in snakebites. The medical treatment of the snakebites must be continuously updated to reduce disability and mortality in patients.

34 BIOCHEMICAL PROFILING OF THE ANTHOPLEURA CASCAIA AQUEOUS EXTRACT: A SCREEN FOR SERINE PEPTIDASE INHIBITORS

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Sea anemones have recently been receiving particular attention due to the profusion of bioactive molecules that had been described in their tissues. Phospholipases, cytolytic, inhibitory and neurotoxic molecules have been isolated from these animals and their toxins can be grouped into 15 well-established families. On the other hand, only 4% of the 1,100 known anemone species were studied and have had a few toxins isolated. This represents only a small fraction of what still can be explored in these animals. Here, we describe the biochemical characterization and the identification of the presence of serine peptidase inhibitors in the aqueous extract of the Brazilian sea anemone *A. cascaia*. The extract was separated by RP-HPLC and its fractions (F1 to F7), have had their molecular mass profile assessed by MALDI-TOF/MS, ESI-IT-TOF/MS-MS and SDS-PAGE. Moreover, fractions were screened for inhibitory activity over trypsin, using time-course fluorescence-based kinetic assays. The analysis of the RP-HPLC profile revealed that *A. cascaia* aqueous extract is a diverse source of molecules. The SDS-PAGE showed proteins with MM ranging from 97 to 14 kDa, with major bands between 30 and 20kDa. The 1D gel-based proteomic analysis indicates the presence of cell surface glycoproteins; cooper/zinc superoxide dismutase; natterin; histone H4 protein and actitoxins in the extract. Such molecules have already been described for other sea anemone species. Moreover, the MALDI analyses showed that fractions F3 and F4 contains peptides in the 3-7 kDa; in accordance to the molecular masses of known serine peptidases inhibitors. Those fractions also showed strong inhibitory activity over the trypsin. In conclusion, the *A. cascaia* aqueous extract is an important source of bioactive molecules, including, toxins, antimicrobial molecules and serine peptidase inhibitors with possible therapeutic applications.

35 PROTEOMIC ANALYSES OF THE WATER SOLUBLE AND PRECIPITATE FRACTIONS OF ZOANTHUS SOCIATUS CRUDE EXTRACT

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The Cnidaria phylum comprises animals presenting a great diversity of toxins and more than 250 molecules from this taxon have been identified. The Anthozoa class is one major contributor of isolated cnidarian toxins. However, some animals of this class remain poorly studied, in spite of the biotechnological potential already described. Here, we describe the study of the proteome of the crude extract of *Z. sociatus*, a species widely present in the Brazilian shores. The aqueous extract was separated by centrifugation in two fractions (precipitate and supernatant). The supernatant was submitted to a Reversed phase C18 solid phase extraction (SPE), and the analytes were eluted using methanol. The fractions (F1 to F3) obtained from SPE, the extract as well as the precipitate had their molecular mass profile assessed by SDS-PAGE, MALDI-TOF/MS and ESI-IT-TOF/MS-MS. The SDS-PAGE analysis showed that the majority of the protein content of the extract of *Z. sociatus* remains in the precipitate, showing proteins with MM ranging from 97 to 14 kDa. On the other hand, the supernatant and SPE fractions, showed absence of proteins at this MM. When evaluated by MALDI-TOF/MS, the supernatant and F3 displayed mainly peptides ranging from 2 – 6 kDa. The trypsin/pepsin-based proteomic analysis revealed that the precipitate is mainly composed of NBD_sugar-kinase_HSP70_actin, Histone (H4/H2B), SMC and DNA2 superfamilies. These molecules have already been found in other Anthozoans. Additionally, the supernatant and F3 showed the presence of molecules from Ion_transport superfamily, resistin-like molecule (RELM) hormone family, and Trypsin-like serine proteases. The different content of proteins and peptides found between these fractions shows that is possible to further explore the biotechnological properties of these molecules, once that the extract presents both proteins with antimicrobial potential (lysine-rich and arginine-rich histones) and RELM, potentially used for studies of modulation of insulin secretion.

36 INTRA-SPECIFIC VENOM VARIATION IN COASTAL TAIPANS

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Venom Supplies Tanunda S.A.

Background: Snake venom studies typically use pooled venom that obscures geographical and individual variation in venom composition of a species. We investigated the variation in composition and function of venom from a species of medically important Australian elapid. Methods: Four populations of coastal taipans (Oxyuranus scutellatus), spanning 2000km were investigated. Venom profiles were obtained using reverse-phase liquid chromatography, size-exclusion chromatography and reducing gel-electrophoresis. Differences in venom composition were quantified by comparing area under the fraction peaks. Pharmacological activity was compared with phospholipase A2 (PLA2) assays, turbidimetric assays of coagulant activity in human plasma and in vitro neurotoxic activity in chick biventer cervicis nerve-muscle preparations. Identification of toxins of interest was confirmed using mass spectrometry.Results: Intra-specific variation was greater at the intra-population level than inter-population means. This indicates that genetic drift is not an influencing factor in the evolution of coastal taipan venom. Electrophoretic analysis showed that the venom is mostly composed of PLA2. Chromatographic analysis showed that the pre-synaptic toxin is a more abundant toxin (7 to 37%), in the proteome of this species than the post-synaptic 3FTx (0 to 8%). Almost a quarter of all individuals tested lacked post-synaptic neurotoxins (3FTxs). This was confirmed both proteomically and with functional assays. This absence was not correlated to geographical distribution but rather, was random genetic variation within populations. No evidence was found for sexual dimorphism in the venom. All individuals lack LAAO toxin.Discussion: The study provides baseline data for future studies on the evolutionary processes that determine how venoms in different snake populations diverge on independent evolutionary trajectories

37 HETEROLOGOUS EXPRESSION OF AN A-NEUROTOXIN PRESENT IN TITYUS SERRULATUS VENOM IN PICHIA PASTORIS SYSTEM

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Tityus serrulatus is considered the most dangerous scorpion in Brazil. It is responsible for the highest number of accidents with scorpions in the country including those that lead patients to death. Scorpionism in Brazil is a public health issue and the biggest concern is about *T. serrulatus* due to its envenoming severity and the huge expansion of its geographic distribution across the country. Its venom contains many different toxins, including α-neurotoxins, which can interact with ionic channels of excitable cell membranes, stimulating neurotransmitters release. This study aims the transformation of an α-neurotoxin synthetic gene in the yeast *Pichia pastoris*, its heterologous expression and identification. The recombinant vector with the α-neurotoxin gene, designed with affinity tags for purification and identification of the recombinant protein, was linearized with PmeI and integrated into electrocompetent *P. pastoris* cells by electroporation. It was confirmed with PCR of selected colonies and agarose gel electrophoresis. Seven colonies were positively transformed with the α-neurotoxin recombinant vector and they were submitted to a screening expression. The α-neurotoxin expression was induced by methanol in BMMY medium for 144 hours and each colony was tested in two different pHs. Colonies were fed with methanol every 24 hours and a sample from the culture supernatant was collected every 48 hours. Samples were analyzed by Tris-Tricine-SDS-PAGE electrophoresis and seven conditions showed bands with molecular mass similar to the native α-neurotoxin. The toxin identity was then analyzed by an indirect ELISA assay, and the α-neurotoxin was identified in one colony culture supernatant in two expression conditions. Concluding, *P. pastoris* cells were transformed with the α-neurotoxin gene and the toxin was successfully produced. This process will be useful to produce the toxin in large quantity to enable its functional and structural characterization as well as its biotechnological use.

38 EXPRESSION AND PRELIMINARY CHARACTERIZATION OF THE FIRST PHOSPHOLIPASE A2 INHIBITOR FROM THE CROTALUS DURISSUS TERRIFICUS SNAKE VENOM GLAND

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Phospholipase A_2 inhibitors (PLI) are proteins usually present in the blood of snakes. They are believed to be part of an innate defense mechanism against its own or other snake venoms. Besides, they were recently identified in the venom gland of *C. d. terrificus* (Cdt), a venomous snake with high medical relevance in Brazil. The envenoming severity caused by Cdt is mainly related to the high content of phospholipase A_2 (PLA_2) in its venom, specially forming crotoxin (a complex composed of a basic and an acid PLA_2). Therefore, the aim of this study is to produce a recombinant PLI to be further studied as adjuvant in the antivenom therapy. For this purpose, the recombinant vector pPICZαA-PLI containing a synthetic gene with a Cdt PLI coding sequence was used to transform electrocompetent *P. pastoris* cells. The PLI expression was induced by methanol in BMMY medium for 120 h and the recombinant Cdt PLI (rCdtPLI) was purified from the supernatant by three chromatographic steps. Carbohydrate content analysis by PNGase F digestion, SDS-PAGE and periodic-acid Shiff staining revealed ~12% of glycosylation in rCdtPLI. Reduction of the catalytic activity of CB-Cdc (a basic PLA_2 from *C. d. collilineatus* crotoxin) was not statistically significant when tested against the NOB chromogenic substrate. However, rCdtPLI form a complex with CB-Cdc as evidenced by a native PAGE gel. ELISA assay showed rCdtPLI was not recognized by commercial anticrotalic antivenom. Moreover, the recombinant vector pET28(a)-PLI containing the gene described above was used to transform competent *E. coli* cells. The bacterial rCdtPLI was expressed under IPTG induction for 6 h. The soluble fraction from cell lysates showed strong inhibition of CB-Cdc enzyme activity when it was tested against NOB. Concluding, this work paves the way for the heterologous production of a PLI to be studied as pharmacological/therapeutic tool.

ACADEMIC POSTERS SESSION II

Topic B: Basic Toxinology

THE VENOM GLAND TRANSCRIPTOME OF THE WASP SPIDER *ARGIOPE BRUENNICHI*	Tim	Lüddecke
ISOLATION AND CHARACTERIZATION OF HYALURONIDASE FROM NIGERIAN NAJA SPECIES (*NIGRICOLLIS, HAJE, MELANOLEUCA AND KATIENSIS*) SNAKE VENOM	Binta	Kurfi
BATROXIN I, A NEW ANTITUMOR PEPTIDE ISOLATED FROM *BOTHROPS ATROX* SNAKE VENOM	Suely	Sampaio
GENETIC RELATIONSHIPS AND VENOM LETHALITY OF *TITYUS TRIVITTATUS* KRAEPELIN, 1898 FROM URBAN AREAS IN ASUNCIÓN, PARAGUAY	Adolfo	Borges
PRECLINICAL ASSESSMENT OF A NEW POLYVALENT ANTIVENOM (INOSERPTM EUROPE) AGAINST SEVERAL SPECIES OF THE SUBFAMILY VIPERINAE	Alejandro	García-Arredondo
CHARACTERIZATION OF A NOVEL INHIBITOR OF COAGULATION FACTOR XIA IDENTIFIED FROM SALIVARY GLAND TRANSCRIPTS OF THE LONE STAR TICK, *AMBLYOMMA AMERICANUM*	Mrinalini.	
BIOLOGICAL ACTIVITY OF THE RECOMBINANT DISINTEGRINS JARASTATIN AND JARARACIN	Russolina	Zingali
COMPARATIVE CHARACTERIZATION AND SYNERGISM BETWEEN TWO BASIC PHOSPHOLIPASES A2 FROM *BOTHROPS DIPORUS* SNAKE VENOM	Soledad	Bustillo
PRELIMINARY STUDY ON NEUTRALIZING CAPACITY OF MONOSPECIFIC ANTIVENOMS AGAINST *BOTHROPS ALTERNATUS* (VIPERIDAE: CROTALINAE) VENOMS FROM ARGENTINA	Laura	Lanari
COMMENTS ON VENOM PROTEIN CONTENT DURING SEQUENTIAL MILKING OF *TITYUS TRIVITTATUS* SPECIMENS, AS DETERMINED BY DIFFERENT METHODS.	Adolfo	de Roodt
SMALL MOLECULES TARGETING RIBOSOME BINDING SITE OF RICIN TOXIN A SUBUNIT	Xiao-Ping	Li
IDENTIFICATION OF ANTIMICROBIAL COMPONENTS FROM THE VENOM OF THE SCORPION *LIOCHELES AUSTRALASIAE* USING AN INTEGRATED MASS SPECTROMETRIC AND TRANSCRIPTOMIC APPROACH	Masahiro	Miyashita
THE MOLECULAR BASIS OF VENOM RESISTANCE IN A RATTLESNAKE-SQUIRREL PREDATOR-PREY SYSTEM	Juan J.	Calvete
FREE AMINO-ACID COMPOSITION OF A RED ALGAL SPECIES (DIGENEA SIMPLEX) CONTAINING NEUROEXCITOTOXIC KAINIC ACID FROM ISHIGAKI ISLAND, OKINAWA PREFECTURE, JAPAN	Manabu	Asakawa
COMPARATIVE BIOCHEMICAL STUDY OF THE VENOMS OF SCORPIONS *TITYUS ASTHENES* AND *CENTRUROIDES EDWARDSII F*ROM COSTA RICA	Cecilia	Díaz
MYOTOXIC AND HEMORRHAGIC EFFECTS OF FRACTIONS OBTAINED FROM THE VENOM OF LIONFISH (*PTEROIS VOLITANS*) IN MICE AND IDENTIFICATION OF POTENTIALLY TOXIC COMPONENTS	Cecilia	Díaz

*B. NEUWIEDI C*OMPLEX: WHAT THE VENOM OF THESE SNAKE SPECIES HAVE IN COMMON? COMPOSITIONAL AND FUNCTIONAL INVESTIGATION OF VENOMS FROM BOTHROPS NEUWIEDI COMPLEX.	Karen	de Morais-Zani
STRUCTURAL AND FUNCTIONAL COMPARISONS OF THE &GAMMA;PLI FROM VENOMOUS AND NON-VENOMOUS BRAZILIAN SNAKES	Anita	Tanaka-Azevedo
"UNRAVELLING THE PATHOPHYSIOLOGY OF VASCULAR LEAKAGE INDUCED BY *DABOIA RUSSELLI* SNAKE VENOM"	Alexandra	Rucavado
EFFECT OF LOW-LEVEL LASER AND ANTIVENOM THERAPIES OF *PHILODRYAS OLFERSII* (LICHTENSTEIN, 1823) VENOM ON ENDOTHELIAL CELL CULTURE	Stella	Zamuner
ANTICARCINOGENIC POTENTIAL OF BBTX-II ON LARGE LUNG CELL CARCINOMA (NCI-H460)	Salomón	Huancahuire-Vega
USE OF SPLA2 FROM *CROTALUS DURISSUS TERRIFICUS* AS MOLECULAR TARGET IN AFFINITY ULTRAFILTRATION TECHNIQUES FOR THE SCREENING OF SECONDARY METABOLITES FROM LEAF EXTRACTS OF MOQUINIASTRUM FLORIBUNDUM.	Marcos	Toyama
EVALUATION OF A HYDROLYSABLE TANNIN PURIFIED FROM LAGUNCULARIA RACEMOSA LEAVES AGAINST EDEMA AND MYONECROSIS INDUCED BY SECRETORY PHOSPHOLIPASE A2 ISOLATED FROM *CROTALUS DURISSUS TERRIFICUS* VENOM	Marcos	Toyama
FAST AND EFFICIENT PROCEDURE FOR PURIFICATION OF SECRETORY PHOSPHOLIPASE A2 (SPLA2) FROM *CROTALUS DURISSUS TERRIFFICUS* VENOM USING A SOLID PHASE EXTRACTION	Marcos	Toyama
EFFECTS ON MITOCHONDRIAL FUNCTIONALITY OF FLOWER AND LEAF EXTRACTS FROM THE POISONOUS PLANT *SENECIO GRISEBACHII* BAKER ("MARGARITA DE CAMPO", *ASTERACEAE)*	Pedro	Zeinsteger
TRANSCRIPTOMIC AND PROTEOMIC ANALYSIS FROM BLACK WIDOW SPIDER VENOM (LATRODECTUS CURACAVIENSIS)	Marjolly	Caruso
EFFECTS OF A LEAF EXTRACTS FROM THE POISONOUS PLANT *CESTRUM PARQUI* L&ACUTE;HERIT ("DURAZNILLO NEGRO", *SOLANACEAE)* ON GENOTOXICITY	Pedro	Zeinsteger
METATRANSCRIPTOME ANALYSIS OF MICROBIAL COMMUNITY IN VENOMOUS GLANDS OF THREE SCORPIONS OF COLOMBIA.	Santiago	Castano
STUDY ON THE DIFFERENCE BETWEEN THE VENOM OF THE WILD COBRA (*NAJA ATRA*) AND THE ARTIFICIAL FEEDING COBRA	Yu	Xiaodong
VENOMICS OF THE COLUBRID SNAKE *TRIMORPHODON QUADRUPLEX* FROM COSTA RICA	Fabian	Bonilla Murillo
EXPERIMENTAL NEUTRALIZATION OF MICRURUS VENOMS BY AN EXPERIMENTAL POLYVALENT ANTIVENOM AND THERAPEUTIC ANTIVENOMS BASED ON NORTH AND SOUTH AMERICAN MICRURUS VENOMS.	javier	barragan
INTERACTION OF SNAKE VENOM METALLOPROTEINASES WITH TYPE IV COLLAGEN: ROLE OF THE DIFFERENT DOMAINS IN TARGET BIDING	Teresa	Escalante
SNAKE VENOMICS OF *CROTALUS DURISSUS TERRIFICUS* FROM ARGENTINA	Luciano	Fusco
BINDING OF ACIDIC PLA2 BA SPII RP4 FROM *BOTHROPS ALTERNATUS* SNAKE VENOM TO INTEGRIN &ALPHA;V&BETA;3: AN IN SILICO STUDY	Laura	Leiva

IMMUNE RESPONSE INDUCED BY NEW ADJUVANT STRATEGY TO PRODUCE CROTALIC ANTI-PLA2	Luciano	Fusco
AN UNUSUAL PHOSPHOLIPASE A2 FROM *PORTHIDIUM HYOPRORA* VENOM SNAKE: PURIFICATION, STRUCTURAL AND PHARMACOLOGICAL PROPERTIES	Salomón	Huancahuire-Vega
PRECLINICAL EVALUATION OF THE POLYSPECIFIC ANTIVENOM INOSERP^{TM} PAN-AFRICA AGAINST THE VENOMS OF ELAPIDS AND VIPERIDS OF SUB-SAHARAN AFRICA REGION: NEUTRALIZATION OF TOXIC ACTIVITIES.	Henri	Mathe
IDENTIFICATION OF NON-VENOM PROTEIN GENES HIGHLY EXPRESSED IN THE VENOM GLAND OF HABU (*PROTOBOTHROPS FLAVOVIRIDIS*) AND THEIR POTENTIAL CONTRIBUTION TO THE QUALITY CONTROL OF VENOM PROTEINS.	Hiroki	Shibata

1 THE VENOM GLAND TRANSCRIPTOME OF THE WASP SPIDER *ARGIOPE BRUENNICHI*

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The wasp spider *Argiope bruennichi* recently expanded its natural range from southern Europe polewards and is now distributed almost across the whole continent, reaching in Finland its northern distribution border. In Germany, the wasp spider became one of the most common spider species potentially linked to its ability to prey on a variety of insects, which are easily subdued by the spider's venom. The females of *Argiope bruennichi* are larger spiders with a strikingly conspicuous striped pattern that resembles a wasp. These features established the wasp spider as one of the most iconic arachnids in Germany. Given this prominence it is rather surprising that the venom composition of the wasp spider has not been studied yet in more depth. Based on the broad insect diet of this species it can be expected that wasp spider venom might contain several components, which could be exploited as bioinsecticides, agrochemicals or generally could be utilized for applied aspects. We recently sequenced the venom gland transcriptome of German specimens of *Argiope bruennichi* and herewith present our preliminary findings. The venom gland transcriptome of *Argiope bruennichi* contains transcripts which show the typical ICK pattern that is predominant in several spider venoms and a prime candidate for subsequent development into plant protection agents and drugs. It further contains a plethora of enzymatic transcripts. However, in order to validate the transcriptome based findings, we aim to complementary analyze transcriptomic and proteomic data of wasp spider venom with the goal to present the first detailed venom composition and toxin profile of *Argiope bruennichi*.

2 ISOLATION AND CHARACTERIZATION OF HYALURONIDASE FROM NIGERIAN *NAJA* SPECIES (*NIGRICOLLIS, HAJE, MELANOLEUCA* AND *KATIENSIS*) SNAKE VENOM

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Background: Elapid venom is highly valuable and possesses medically and pharmacologically important peptides*, Naja* species venom contains elapid neurotoxins in combination with cytotoxins and cardiotoxins. Venom hyaluronidase contributes to the diffusion of these venom toxins from inoculation site, snake venom hyaluronidases known as "spreading factor" are not extensively studied. The study aimed to biochemically characterize hyaluronidase isolated from four Naja species involved in human accident in Northern Nigeria: *Naja nigricollis, Naja katiensis. Naja melanoleuca* and *Naja haje*. Isolation of the enzyme was done using three steps which include protamine sulphate precipitation, gel filtration on Sephadex G-75 and active fractions were applied to ion–exchange chromatography on DEAE (Diethylaminoethyl) cellulose. Fractions were subjected to SDS – PAGE for molecular weight determination.

RESULTS: Hyaluronidase isolated from the *Naja* species gave a specific activity of 39.129 tru/mg, 20.732 tru/mg and 17.110 tru/mg for *N. nigricollis, N. katiensis, N. melanoleuca* and *N. haje* respectively. Hyaluronidase gave a molecular weight of 44KDa, 29KDa 54KDa and 28KDa for *N. nigricollis, N. haje, N. melanoleuca and N. kateinsis* respectively. The enzyme display optimum pH of 8, 6.5 and 4 for *N. nigricollis, N. haje. N. melanoleuca and N. katiensis* respectively, and optimum temperature was found *to be 37°C, 39°C,40°C, 42°C N. nigricollis N. katiensis* and *N. haje* respectively. The metal ions significantly decreases the activity of hyaluronidase at P 0.005. Initial velocity studies revealed a Km and Vmax of 0.1860mg/ml, 0.1080mg/ml, 0.140mg/ml, 0.3045mg/ml and 0.5698 tru/min, 0.4097tru/min, 0.5469tru/min, 0.6268tru/min for *N. nigricollis, N. katiensis, N.menoleuca* and *N. haje* respectively.

CONCLUSION: The relevance of this findings will be of importance there by using hyaluronidase as a therapeutic agent in the development or design of drug.

3 BATROXIN I, A NEW ANTITUMOR PEPTIDE ISOLATED FROM *BOTHROPS ATROX* SNAKE VENOM

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Introduction: Venom peptides are interesting molecular models that have been used to develop biotechnological approaches to produce therapeutic agents and/or experimental tools for basic and applied research. Objectives: This study aims to characterize Batroxin I, a peptide from *Bothrops atrox* snake venom, as well as to examine its antitumor activity. Methods and Results: Batroxin I was isolated from *B. atrox* venom and characterized using molecular exclusion (Sephacryl S200) and reversed-phase (C18) chromatography. The peptide cytotoxicity was assessed in HepG2 tumor cells (ATCC) and human peripheral blood mononuclear cells (PBMC), using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were treated with chromatographic fractions and Batroxin I at 1 mg/mL, cisplatin at 0.33 mg/mL (positive control), or phosphate buffered saline solution (negative control). The molecular weight of Batroxin I was 1.38 kDa. This peptide was strongly cytotoxic towards HepG2 cells (90% of cell death; IC50= 0.72 µg/mL) but weakly cytotoxic towards PBMC (7% of cell death). The peptide ability to induce apoptosis and necrosis of HepG2 cells was assessed by flow cytometry using annexin V-FITC and propidium iodide. Batroxin I at 0.72 µg/mL induced cell death by both apoptosis and necrosis at the same extent as cisplatin at 8.0 µg/mL – this standard antineoplastic agent is widely used to treat different types of cancer. Conclusion: Batroxin I can be a useful model for the development of new antitumor drugs with potential application to treat cancer or as therapeutic adjuvant of antitumor drugs used in the current clinical practice.

4 GENETIC RELATIONSHIPS AND VENOM LETHALITY OF TITYUS TRIVITTATUS KRAEPELIN, 1898 FROM URBAN AREAS IN ASUNCIÓN, PARAGUAY

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Tityus trivittatus Kraepelin, 1898 is a scorpion responsible for severe and lethal accidents in children, with a distribution ranging from northern Argentina to Eastern Paraguay and southeast Brasil. Outside of Argentina its medical importance is unknown. Argentinian *T. trivittatus* populations are parthenogenetic while Paraguayan populations exhibit sexual dimorphism and are widespread in the capital city of Asunción. We collected *T. trivittatus* from crevices and pipelines at human dwellings in Asunción to assess its venom toxicity and genetic relationships. Venom was collected by manual stimulation of the telson from a mixture of male and female scorpions. Lethality was evaluated intraperitoneally (i.p.) in female, NIH Swiss mice weighing 25 g. Medium lethal dose was 0.85 mg of venom/kg body weight (0.66—1.22 95% CI), in the range of the toxicity previously assessed i.p. for Argentinian *T. trivittatus* collected in the Argentinian cities of Córdoba, Santa Fé, Cajamarca, and Santiago del Estero in the same mouse strain. Mice injected with lethal doses presented neurotoxic signs suchs profuse sialorrhea, ptosis, and hindlimb paralysis. We sequenced a 560-bp gene fragment encoding the C-terminal portion of the cytochrome oxidase subunit I, amplified from DNA extracted from muscle obtained from pedipalps. Genetic divergence between populations from Paraguay and Córdoba, Argentina, was 7.79%, with a mean divergence time in the Miocene (mean = 11.35 Ma; 95% HPD = 5.0-19.2 Ma), highlighting the existence of a "*Tityus trivittatus*" complex of species of potential medical significance in southern South America. (Financed by CONACyT-Paraguay, Project PRID18-12, to A.B.).

5 PRECLINICAL ASSESSMENT OF A NEW POLYVALENT ANTIVENOM (INOSERPTM EUROPE) AGAINST SEVERAL SPECIES OF THE SUBFAMILY VIPERINAE

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Europe is inhabited by medically important venomous Viperinae snakes. *Vipera ammodytes, Vipera berus* and *Vipera aspis* cause the greatest public health problem in Europe, but there are other equally significant snakes in specific regions of the continent. For example, *Macrovipera lebetina* and *Montivipera xanthina* are the most dangerous species in Turkey. Immunotherapy is indicated for patients with systemic envenoming, of which there are approximately 4,000 annual cases in Europe. In the present study, safety and venom-neutralizing efficacy of InoserpTM Europe, a new F(ab')2 polyvalent antivenom designed to cover envenoming by snakes of the European region, were evaluated. Horse hyperimmune plasmas were produced by immunization with the venoms of the following species: *Vipera ammodytes, Vipera aspis, Vipera berus, Vipera latastei, Montivipera xanthina, Macrovipera schweizeri, Macrovipera lebetina obtusa, Macrovipera lebetina cernovi,* and *Macrovipera lebetina turanica.* F(ab')2 immunoglobulin fragments were obtained by enzymatic digestion with pepsin and precipitation with ammonium sulfate. In accordance with WHO recommendations, several quality control parameters were applied to evaluate the safety of this antivenom and the results showed that it meets those quality requirements such as the low protein content (10 g/dL) and the high level of immunoglobulin fragments F(ab')2 (> 90%). The venom-neutralizing efficacy of the antivenom was evaluated in mice and the results showed that it had appropriate neutralizing potencies against the venoms of several species of the subfamily Viperinae; including *V. ammodytes, V. berus, V. aspis, M. lebetina* and *M. xanthina* in compliance with the European Pharmacopoeia. Paraspecificity of the antivenom was demonstrated as well, since it neutralized venoms of species not included in the immunization schemes. According to this assessment, InoserpTM Europe satisfactorily meets the key WHO criteria from a quality standpoint. This is the first antivenom covering all WHO medical important species from western Europe to Caucasus region.

6 CHARACTERIZATION OF A NOVEL INHIBITOR OF COAGULATION FACTOR XIA IDENTIFIED FROM SALIVARY GLAND TRANSCRIPTS OF THE LONE STAR TICK, AMBLYOMMA AMERICANUM

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In order to facilitate feeding of blood, tick salivary gland secretions typically contain potent and specific inhibitors of serine proteases involved in blood coagulation and fibrinolysis. Structurally, many of these inhibitors contain the well-known protease-inhibiting Kunitz domain. However, potency and selectivity of Kunitz domain against different coagulation factors (serine proteases) may not be readily identifiable through sequence alone. Therefore, we used a yeast surface display screen of several tick salivary transcripts to identify potential binders of blood coagulation factor XIa, a valuable drug target for antithrombotic therapeutics with low bleeding risk. Here we report recombinant expression, purification and functional characterizations of one of the identified transcripts, named P1456, from the lone star tick, *Amblyomma americanum*. P1456 encodes a 127-residue mature protein containing tandem Kunitz domain. The sequences of full-length P1456 and it's two individual Kunitz domains were cloned for overexpression in E. coli and purified from their respective soluble fractions. Full-length P1456 inhibit factor XIa with IC50 of around 13 nM. The N-terminal Kunitz domain of P1456 also inhibit factor XIa to similar extent while the C-terminal Kunitz domain showed no inhibition. Therefore, inhibition of factor XIa by P1456 is mediated exclusively through its N-terminal Kunitz domain. A screen against a panel of 11 blood coagulation serine proteases revealed that full-length and the N-terminal Kunitz domain of P1456 also inhibit plasma kallikrein and plasmin as well as trypsin. We are in the process of improving P1456 selectivity for factor XIa to develop safer anticoagulant therapeutics.

7 BIOLOGICAL ACTIVITY OF THE RECOMBINANT DISINTEGRINS JARASTATIN AND JARARACIN

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Integrins are transmembrane heterodimeric glycoproteins, present in almost all cell types, binding extracellular matrix proteins to cytoskeleton. The activation of the integrin by the interaction with its bind promotes the activation of pathways that regulate adhesion, proliferation, differentiation, migration and apoptosis, thus modulating physiological and pathological processes. For this reason, the integrins has been the target of new antithrombotic drugs. The disintegrins from snake venoms are peptides capable of modulating the activity of integrins, among them the αIIbβ3, responsible for the platelet aggregation and αvβ3, related to angiogenesis. The aim of this study was to express the recombinant disintegrins jarastatin (rJast) and jararacin (rJarc). The secondary structure and their biological activities were analyzed. We performed the expression of these disintegrins in *Pichia pastoris*, using synthetic gene in the vector pPIC9. They were expressed and secreted in the cultured media and were purified using molecular exclusion chromatography. We confirmed the internal sequence and the molecular mass by mass spectrometry and confirmed protein folding by Circular Dichroism and 1H Nuclear Magnetic Resonance spectra. The yield of the rJast and rJarc were approximately 40 and 30 mg/ 1L of culture, respectively. rJast and rJarc inhibited platelet aggregation induced by ADP. Both disintegrins inhibited the adhesion of platelets to collagen under continuous flow. We also evaluated effect of rJast on HMEC-1 cells. The viability of the cells was not altered, even with 10 µM of disintegrin. rJast significantly inhibited the adhesion of these cells to vitronectin as well as HMEC-1 migration Conclusions: We expressed two RGD disintegrins that showed correct folding and inhibition of platelet aggregation similar to the natives proteins. Finally, these proteins can be used as tools to understand the role of integrins in various physiological and pathological systems including thrombosis and angiogenesis.

8 COMPARATIVE CHARACTERIZATION AND SYNERGISM BETWEEN TWO BASIC PHOSPHOLIPASES A2 FROM BOTHROPS DIPORUS SNAKE VENOM

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The vast majority of snakebite envenomings in northeastern Argentina are caused by *Bothrops diporus* (yarará chica), a viperid distributed from southwestern Brazil through Paraguay to central Argentina. The venom of this species causes local tissue damage characterized by myonecrosis, hemorrhage, blistering, and edema. In the present study, two basic phospholipases A2 (PLA2-I and PLA2-II) were isolated from this venom, and their pathological effects upon murine skeletal muscle and myogenic cells in culture were analyzed. Partial amino acid sequencing showed that PLA2-I and PLA2-II are Asp49 and Lys49 PLA2s, respectively. In agreement with this, PLA2-I showed PLA2 activity, whereas PLA2-II did not. Functional assays revealed differences in their myotoxicity, cytotoxicity, and anti-adhesion activity, and in the ability to inhibit cell migration, all of which were greater for the Lys49 variant. Native electrophoresis showed that PLA2-I was less basic than PLA2-II. The two proteins act synergistically to affect the integrity of C2C12 myogenic cells, providing a further example of the concerted action of coexisting snake venom components. Furhtermore, these myotoxins may well play an important role in the development of myonecrosis after envenomation by this species and could be useful for investigating the molecular basis of the synergistic toxic action between Asp49 and Lys49 variants that often co-exist in the venoms of viperids.

9 PRELIMINARY STUDY ON NEUTRALIZING CAPACITY OF MONOSPECIFIC ANTIVENOMS AGAINST BOTHROPS ALTERNATUS (VIPERIDAE: CROTALINAE) VENOMS FROM ARGENTINA

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The intraspecific reactivity of monospecific antivenoms against *Bothrops alternatus* venom was tested in terms of their immunochemical reactivity and neutralizing capacity. The antivenoms, based on venom pools of vipers from Entre Ríos (Concordia, antivenom A-C), the Buenos Aires province (Olavarría, A-O) and a combined pool (A-CO) were assayed against their respective pools and against individual milked venoms (n=5 per region) of vipers from these regions. The three antivenoms reacted similarly on the different venom pools when assessed by ELISA. Nevertheless, the venom from Olavarría was the one with the lowest sensitivity for the three antivenoms. Neutralization experiments showed that the A-O required higher doses to neutralize the venoms pools, while the A-CO presented the highest neutralizing capacity against the three venom pools. When the mice were challenged with 5.0LD50 of the individual venoms, 2.0ED50 of the A-CO protected 100% of the challenged mice in 5 of them (50%). Using 1.0ED50, 100% protection was achieved in 3 individual samples, whereas doses of 2.5ED50 and 3.0ED50 neutralized the remaining 2 samples. In addition, the antivenoms' neutralization of the indirect hemolytic activity was assayed on the two regional venom pools, the combined pool, and on the individual samples. The neutralization conferred by 1.0HED50 (Hemolytic Effective Dose) of each antivenom neutralized the venoms in almost all cases, but to a different degree. In several cases the non-specific neutralization was lower, while the A-CO showed good neutralizing activity in all cases. Despite the observed similarities in immunochemical reactivity, the antivenom produced by immunizing with a mix of venoms resulted in general in a better neutralizing profile of the lethal activity and indirect hemolytic activity of the venom of *B. alternatus*.

10 COMMENTS ON VENOM PROTEIN CONTENT DURING SEQUENTIAL MILKING OF TITYUS TRIVITTATUS SPECIMENS, AS DETERMINED BY DIFFERENT METHODS.

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The protein content (PC) of individual milkings by electrical stimulation of adult specimens of *Tityus trivittatus* (n=71, median length5 cm, min. 3.2, max. 6.2 cm) was determined through their spectrophotometric absorbance at a wavelength of 214, 230 and 280 nm and by the method of Bradford. Scorpions were individually maintained in plastic boxes, fed with a cricket diet and milked after three weeks of starving in all cases. Using BSA as protein standard, the general PC (n=234) determined by Bradford´s method was 131 µg/milking (min. 41, max. 463) and the absorbance values per milking were A214nm=2.717(0.141-5.724), A230nm=1.614(0.015–4.786) and A280nm=0.381 (0.036–1.260). During sequential milking however, the PC of individual milkings varied slightly, depending on captivity duration and the method used to determine PC. Measuring absorbance resulted in decreasing values per milking in all cases (p0.0001). After 30 days of captivity the absorbance was 2.828, 2.297 and 0.514 per milking, at 214, 230 and 280 nm and the PC was 102 µg/milking when determined by Bradford´s method. After 120 days of captivity, changes in protein content were clear in all cases. On day 150 absorbance values of 2,642, 1.422 and 0.338 were recorded at the respective wavelengths mentioned, while a PC of 167 µg/milking was obtained by Bradford´s method (p 0.0001). The size of the surviving scorpions was similar on day 30 and 150 (p0.0001). In addition, when Bradford´s method was used applying IgG as protein standard, readings were 2.17-fold (±0.08) higher when compared to the readings with BSA. More assays are needed to understand the venom yield variation over time. Data on protein content of scorpion venoms measured by different methods, including the same method but with different standard, cannot be reliably compared or extrapolated.

11 SMALL MOLECULES TARGETING RIBOSOME BINDING SITE OF RICIN TOXIN A SUBUNIT

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Ricin toxin A subunit (RTA) interacts with the ribosomal P stalk to access its substrate, the Sarcin/Ricin Loop (SRL). RTA specifically removes a universally conserved adenine from the SRL, a process called depurination, and inhibits protein synthesis. The ribosome binding site on RTA is on the opposite face of the active site. Mutagenesis and x-ray crystallography analysis indicated that both electrostatic and hydrophobic interactions contribute to binding of RTA to the ribosome. We showed that peptides that disrupt the RTA-ribosome interaction inhibit the activity of RTA. There is a well-defined hydrophobic pocket at the interaction site on RTA which could be targeted by small molecules. In order to find small molecules targeting this site, we have screened the Maybridge Ro3 Core fragment library containing 1000 fragments using Biacore T-200. In the initial screening RTA was immobilized on a CM5 chip and fragments at 200 µM single dose were passed over the RTA surface. A peptide corresponding to the last 11 amino acid of ribosomal P protein (P11) and adenine were used as positive controls for the ribosome binding site and the active site of RTA, respectively. From the initial screening, 57 fragments were selected based on their binding levels and sensorgram profiles. Kinetic screening was conducted on the identified fragments. In the kinetic screen, the fragment concentrations were varied from 0 to 500 µM at 5 different concentrations and the KD of each fragment was determined. The fragments were ranked by their affinity. The inhibitory activities of these fragment on the depurination activity of RTA were determined using both yeast and rat ribosomes. We identified 6 fragments with KD lower than 500 µM, which showed 50% of inhibition at concentrations below 300 µM. We are in the process of determining their binding sites on RTA using X-ray crystallography and optimizing their binding affinities and inhibitory activities.

12 IDENTIFICATION OF ANTIMICROBIAL COMPONENTS FROM THE VENOM OF THE SCORPION LIOCHELES AUSTRALASIAE USING AN INTEGRATED MASS SPECTROMETRIC AND TRANSCRIPTOMIC APPROACH

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Scorpion venom is a rich source of bioactive peptides. These peptides in the venom are used to capture prey or to defend themselves against predators. Although neurotoxic peptides are main active components in this regard, a large number of antimicrobial peptides have been identified from the scorpion venom. The primary role of these antimicrobial peptides is assumed to be protection of the venom gland from microbial infections. In this study, we aimed to identify novel antibacterial and antiviral components from the venom of the scorpion *Liocheles australasiae* by combined mass spectrometric and transcriptomic analyses.

The crude venom was initially separated into several fractions using HPLC. Each fraction was subjected to antibacterial and antiviral activity tests using Escherichia coli and hepatitis C virus, respectively. The fractions that showed the activity were further separated by HPLC to obtain single components. Partial amino acid sequences of each active component were determined by MS/MS analysis. Those sequences were used to search the database constructed by a whole transcriptome analysis of the venom gland of *L. australasiae*. As a result, three components were identified as antibacterial peptides consisting of 14-47 amino acid residues without disulfide bonds. On the other hand, an antivirus component was identified as a 13-kDa protein composed of two subunits. We also found that this protein has an N-linked sugar chain consisting of only four saccharide residues.

13 THE MOLECULAR BASIS OF VENOM RESISTANCE IN A RATTLESNAKE-SQUIRREL PREDATOR-PREY SYSTEM

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Venom in predators and venom resistance proteins in prey are coevolving phenotypes, but little is known about the molecular basis of venom resistance. Affinity chromatography on toxin-specific (PIII-SVMP, SVSP, DISI, and PLA2) matrices was applied to capture candidate California ground squirrel serum venom interactive proteins – VIPs) that interact with venom proteins from their main predator, *Crotalus o. oreganus*). Grey squirrel and rabbit sera were used as controls. A whole serum capture assay showed that serum-based resistance is species-specific, with serum proteins from ground squirrels showing higher binding affinities for venom proteins than serum proteins from an allopatric squirrel species. Venom protein specificity assays identified numerous and diverse VIPs representing candidate prey resistance proteins. Resistance may involve multiple serum proteins interacting with multiple venom proteins, likely through a toxin scavenging mechanism. A number of candidate CGS VIPs have been associated with the innate immune system. Expected characteristics of a prey's defense mechanism against envenomation by a snake predator shares functional features of innate immunity: it is a generally nonspecific mechanism, must be very rapid in response, is critical for the recognition of disease-causing agents. A number of candidate VIPs are shared among CGS, grey squirrel and rabbit. Our data suggest that VIPs targeting disintegrins, SVSPs, and PIII-SVMPs may have originated before the divergence of Rodentia and Lagomorpha, approximately 66 Mya. VIPs targeting major *C. o. oreganus* venom PLA2 molecules may have evolved in *O. beecheyi* more recently, presumably through coevolution between the squirrel and its main predator, extant *C. o. oreganus* or an ancestor, during the last 3-6 My. Finally, evolutionary analyses of rates of evolution of VIP protein homologs in related mammals show these proteins evolve under purifying selection. The slower rates of evolution of VIP proteins may constrain the evolutionary responses of prey to rapidly evolving snake venom proteins.

14 FREE AMINO-ACID COMPOSITION OF A RED ALGAL SPECIES (DIGENEA SIMPLEX) CONTAINING NEUROEXCITOTOXIC KAINIC ACID FROM ISHIGAKI ISLAND, OKINAWA PREFECTURE, JAPAn

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Digenea simplex, a red algal species in the family Rhodomelaceae, is widely distributed in tropical and subtropical regions. This species contains kainic acid (C10H15NO4; molecular weight: 213), which is a cyclic analog of glutamate and one of the most potent excitants in the mammalian central nervous system. Specifically, like domoic acid, which is the causative substance of amnesic shellfish poisoning, it is a potent glutamate receptor agonist and exhibits marked neuroexcitatory action and neuroexcitotoxicity. However, little is known about the exact free amino acid composition of this species. This study therefore aimed to determine the free amino acid composition of this alga, and to clarify the production mechanism of kainic acid. Dried powder (0.5 g) of this alga, collected from reefs off the coast of Kabira Bay on Ishigaki Island, Okinawa Prefecture, Japan in June 2017, were placed in vials containing 75% ethanol (50 mL) and subjected to boiling-water treatment for 20 min. The obtained extract was then purified by loading onto a solid-phase cartridge (Sep-Pak C18 Environmental Cartridge) and eluting with methanol (0.1%) and trifluoroacetic acid (3:7). Free amino acid composition was then determined using an amino acid analyzer (JEOL JLC-500/V2). It was possible to distinctly separate kainic acid from other structurally analogous imino acids – hydroxyproline and proline – with a detection limit of 0.67 x 10-3 mg. A total of twenty three free amino acids were detected in the Kabira D. simplex specimens. Of these amino acids, the primary component was kainic acid (114 ± 28.6 mg/100 g dry matter, n=6; maximum: 152 mg; minimum; 89.2 mg), followed by alanine (35.9 ± 22.4 mg/100 g dry matter), glutamic acid (28.5 ± 21.3 mg/100 g dry matter) and α-aminoadipic acid (20.6 ± 8.7 mg/100 g dry matter).

15 COMPARATIVE BIOCHEMICAL STUDY OF THE VENOMS OF SCORPIONS TITYUS ASTHENES AND CENTRUROIDES EDWARDSII FROM COSTA RICA

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In this study, we characterized the venoms of *Tityus asthenes* and *Centruroides edwardsii*, two species of scorpions from Costa Rica, in terms of their biochemical constituents and biological activities. Both venoms are rich in peptides but also contain some higher molecular weight protein components. No phospholipase A2, hemolytic or fibrinogenolytic activities were found, but the presence of proteolytic and hyaluronidase enzymes was evidenced by zymography.

After a 2-step chromatographic approach (size-exclusion followed by reverse phase HPLC) and through Edman N-terminal sequencing, we identified several β-type Na+-channel-modulating peptides with sequence similarity to orthologs present in venoms from other scorpion species of the genera Centruroides and Tityus. It is reported that most of these toxins are mainly effective on invertebrates, but some could cause pain and toxicity also on vertebrates.

By HPLC with an electrochemical detector, we determined the presence of monoamine neurotransmitters in the venoms. We found small amounts of dopamine in both secretions, but also norepinephrine and a higher concentration of serotonin (or a very similar arthropod compound), in *T. asthenes* venom.

16 MYOTOXIC AND HEMORRHAGIC EFFECTS OF FRACTIONS OBTAINED FROM THE VENOM OF LIONFISH (PTEROIS VOLITANS) IN MICE AND IDENTIFICATION OF POTENTIALLY TOXIC COMPONENTS

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Lionfish is a non-native fish that inhabits the Caribbean coast of Central America, which is considered an ecological danger to the coral reefs because of its invasive behavior. Toxicity in humans is associated with strong pain and local symptomatology. In this study, we evaluated the 24 h-in vivo effects of its venom and fractions on the skin and gastrocnemius muscle of mice.

Intraperitoneal injection of lionfish dorsal spines venom (which in this study was not separated from epidermal mucus), is not able to induce lethality up to 250 µg/mouse, but histological analysis shows that 50 µg of venom per mouse, induces myonecrosis and hemorrhage in the gastrocnemius and damage on the skin, by intradermal injection.

Venom was separated by size exclusion-HPLC and five main fractions were obtained, which were injected in mice, and tissue samples were processed for histological analysis.

Fraction 1 (containing the highest molecular weight components), which displays proteolytic activity, was able to induce strong myonecrosis but no alterations on the skin were observed, whereas fraction 2, which contains the hemolysin present in all Scorpaenidae fish family members, did not induce damage on muscle or skin.

Fraction 3, containing the strongest hyaluronidase activity, was very toxic for the skin tissue, inducing a strong lesion, hemorrhage and increased vascular permeability. It also caused red blood cell aggregation towards the vessel walls.

Fraction 4, containing a ~32 kDa very abundant protein with similarity to the secretory cysteine-rich proteins (a Golgi-associated plant pathogenesis-related protein), induced a lesion and hemorrhage on the skin, but no necrosis in muscle. The same effect was observed with fraction 5, which contains a 14.2 kDa protein, similar to gastrotropin and fatty acid binding proteins. These two components have been found recently in the venom of stonefish (*Synanceia horrida*), but their effects have not been characterized yet.

17 B. NEUWIEDI COMPLEX: WHAT THE VENOM OF THESE SNAKE SPECIES HAVE IN COMMON? COMPOSITIONAL AND FUNCTIONAL INVESTIGATION OF VENOMS FROM BOTHROPS NEUWIEDI COMPLEX.

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The snakes that composed the "*B. neuwiedi* complex" underwent a taxonomic revision and as a result of this analysis, it was proposed that this complex is in fact composed by 7 species: *B. neuwiedi (Bn), B. diporus (Bd), B. lutzi (Blu), B. mattogrossensis (Bmt), B. pauloensis (Bpa), B. pubescens (Bpu)* and *B. marmoratus (Bmm).* Since the characterization of the venoms of these species after the taxonomic revision are scarce, the aim of this study is to characterize and compare the protein profile and the enzymatic activities of venoms from this group. For comparative purposes, we have included the species *B. erythromelas* (Be) in our analyses. Protein profile obtained by 1-DE stressed the intraspecies venom variation, showing differences concerning the intensity and the presence of particular protein bands. Concerning proteolytic activity, Bpa, Bd and Bpu venoms displayed the lowest activity upon azocasein and collagen, which is corroborated by the low intensity of bands between 50-100 kDa in 1-DE, that comprehend the SVMP-III area. In contrast, Be showed intense bands in this area and more proteolytic activity. Amongst the species analyzed, Be venoms displayed the lowest thrombin-like activity upon the chromogenic substrate S-2238, followed by the venom of Bmm, while Bn, Bd, Bmt, Bpa and Bpu venoms present similar activity. In vivo assays demonstrate that Bpa, Bd and Bpu are more lethal than the other species. However, Be seems to be the most haemorrhagic of them. Regarding immunerecognition assays, all protein bands resolved by 1-DE of the species analyzed were recognized by bothropic antivenom produced by Instituto Butantan (Western blotting). In addition, venom samples showed comparable immunoreactivity with the same antivenom (ELISA). These results revealed some particular features of the venoms of the seven species under analysis. The next steps include proteomic analysis by mass spectrometry and comparison of their pathophysiological activities.

18 STRUCTURAL AND FUNCTIONAL COMPARISONS OF THE ГPLI FROM VENOMOUS AND NON-VENOMOUS BRAZILIAN SNAKES

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During the snake envenomation, phospholipases A2 (PLA2) are responsible to local symptoms that cannot be totally neutralized by serum therapy. The main natural alternative studied are Phospholipase A2 inhibitors (PLI) from snake blood. Curiously, PLIs are present in venomous snakes, venomous snake and mammals, and their biological role as well as their mechanism of action are not well clarified. So, the aim of the study is to compare the similarities between the BoaγPLI from Boa constrictor (non-venomous snake) plasma and γBjPLI from *Bothrops jararaca* (venomous snake) plasma. Therefore, BoaγPLI and γBjPLI were isolated using two chromatographic steps: an ion exchange column (DEAE), followed by an affinity column (crotoxin coupled to a CNBr-activated Sepharose resin). The purity and biochemical characterization were compared and analyzed by Bradford, SDS-PAGE and circular dichroism. The ability to inhibit PLA2 was determined by enzymatic activity. The purification process showed 0.63% and 1% of recovery of BoaγPLI and γBjPLI, respectively. The differences in this parameter might be caused by the constant contact of γBjPLI with the venom of the B. jararaca, a venomous snake. The SDS-PAGE showed similar patterns, showing a band of 25 kDa in reducing conditions and 20 kDa in non-reducing conditions. In addition, the BoaγPLI showed a band with higher molecular mass, suggesting oligomerization. The secondary structures were similar between the inhibitors. In addition, the inhibitory potential was also similar, about 48% of inhibition of the PLA2 activity. In conclusion, despite the different percentage of recovery, the structural and functional features of these inhibitors were similar. In this context, the study could provide new perspectives for PLA2 inhibitors from snake plasma by characterizing and comparing the PLIs from venomous and non-venomous snakes, which can contribute to the neutralization of the PLA2 effects.

19 UNRAVELLING THE PATHOPHYSIOLOGY OF VASCULAR LEAKAGE INDUCED BY DABOIA RUSSELLI SNAKE VENOM

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We developed an experimental model to study the systemic capillary leak syndrome (CLS) induced by the venom of *D. russelli* from Pakistan, based on the determination of blood hematocrit and plasma albumin in mice. The venoms of *D. russelli* from Pakistan and of *B. asper* induced two different patterns of vascular toxicity in our model. When injected intradermally, *B. asper* venom caused a local hemorrhage, whereas *D. russelli* did not elicit hemorrhage but instead induced an increment in vascular permeability. This reveals two distinct paradigms of vascular toxicity, one based on disruption of microvasculature leading to hemorrhage (*B. asper*) and the other centered in an increment in vascular permeability leading to hemoconcentration (*D. russelli*). The question is whether the hemoconcentration effect induced by *D.russelli* (DR) venom is related to acute kidney dysfunction observed in envenoming. In this work we carried out a set of experiments using different inhibitors and specific antibodies to try to address this question. Varespladib, a small compound that has been tested to inhibit the PLA2 catalytic activity in a wide variety of snake venoms (Lewin et al., 2016), did not inhibit the hemoconcentration effect. Likewise, Varespladib was not able to improve renal function or normalize the albumin level in plasma. sv-VEGFs are known to increase permeability however, anti-VEGF immunoglobulins (anti-human or anti-snake venom VEGF) were not able to neutralize neither capillary permeability nor renal dysfunction. Thus, the role of capillary permeability in kidney dysfunction and sv-VEGF, and PLA-2s remains unclear.

20 EFFECT OF LOW-LEVEL LASER AND ANTIVENOM THERAPIES OF PHILODRYAS OLFERSII (LICHTENSTEIN, 1823) VENOM ON ENDOTHELIAL CELL CULTURE

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The venom from serpents of the species *Philodryas olfersii* cause clinically important ophidian accidents and promote local manifestations such as pain, erythema, edema, inflammation, ecchymosis and also systemic manifestations such as hemorrhage. Thus, the objective of this study was to analyze the effect of antivenom serum and low-level laser therapy (LLLT) in endothelial cells challenged by contact with *Philodryas offersii* venom (PoV) through cell viability and cell detachment assays. tEnd cell line was used. Cells were irradiated for 10 s (area 0.4 cm2) immediately after the venom administration with a semiconductor laser at 660 nm, dose of 4 J/cm2 and power of 100 mW. The cells that did not receive venom neither irradiation served as control. The antivenom used was produced by Inst. Butantan, serie 0710222/D. 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 PoV in the respective concentrations 10, 25, 50, 100, 150, 200 and 250 µg/mL and they were incubated for 3 and 6 h. The cell viability and detachment were analyzed by MTT and crystal violet assay, respectively. Statistical analysis the one-way ANOVA followed by Tukey's post hoc test were employed with a significant level set at $p \leq 0.05$. It was observed that the venom was cytotoxic to the endothelial cells at doses above 100 mg after 6 h of incubation. No effect on cell detachment was observed. Both antivenom and LLLT promoted protection of 79.4% and 61.2% respectively, but when antivenom and LLLT were applied together, the protection observed was 92%. Thus, this study has shown that PoV is cytotoxic to endothelial cells. Both antivenom and LLLT alone promoted partial protection, but when combined, the protection was more effective and reaches values above 90%.

21 ANTICARCINOGENIC POTENTIAL OF BBTX-II ON LARGE LUNG CELL CARCINOMA (NCI-H460)

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The presence of antitumor enzymes in snake venoms were first mentioned during the mid-1960s. Different phospholipases A2 (PLA2s) have been studied for their antitumor properties, among them acidic and basic PLA2s, as well as synthetic peptides derived from K49 PLA2s homologues. Thus, the PLA2 isolated from *Naja naja* venom caused the death of the human neuroblastoma SK-N-SH cell line, similarly the PLA2 isolated from the venom *Bothrops neuweidi*, produced cytotoxic activity in murine melanoma B16F10. BbTX-II is a K49 PLA2 homologous isolated from *Bothrops brazili* snake venom, consists of 121 amino acid residues and has a molecular mass of 13,699 Da. In vivo, it induces local myotoxicity and edematogenic activity, these biological effects occur in the absence of catalytic activity. This work describes the cytotoxic activity of BbTX-II on the large lung carcinoma (NCI-H460) cell line. After formation of the cell monolayer, they were transferred onto plates containing RPMI-1640 medium and 10% FBS, then different concentrations of BbTX-II ranging from 0.008 to 0.5 mg/mL were added. The plates were incubated for 24 and 48 hours in a 5% CO2 oven at 37°C. After each incubation time, the cell viability assay was performed by neutral red staining. The results showed that after 24 and 48 hours of treatment, there was a decrease in cell viability in relation to the control. Thus, after 24 hours from 0.5 mg/mL to 0.15625 mg/mL, between 40 and 50% of the tumor cells were viable. At 48 hours, at the same range of concentrations of BbTX-II, approximately 30% of the cells were viable. This demonstrates the cytotoxic effects of BbTX-II, with approximately 70% dead cells. Both treatment times were statistically significant against the control. We conclude that BbTX-II has antitumor potential, reducing up to 70% the viability of the cells analyzed during 48 hours.

22 USE OF SPLA2 FROM CROTALUS DURISSUS TERRIFICUS AS MOLECULAR TARGET IN AFFINITY ULTRAFILTRATION TECHNIQUES FOR THE SCREENING OF SECONDARY METABOLITES FROM LEAF EXTRACTS OF MOQUINIASTRUM FLORIBUNDUM.

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The capture of molecules by bioaffinity allows the discovery of several compounds with greater or lesser specificity on domains on the molecular target. In addition, there are no methods in literature for identifying and capturing secondary plant metabolites using sPLA2 as a binding molecule for secondary metabolites fishing. Thus, the objective of this work was to employ ultrafiltration and HPLC-MS techniques for screening complex chemical matrices and to identify potential ligands against native sPLA2. Therefore, sPLA2 of *Crotalus durissus terrificus* was incubated with to different extracts obtained from Moquiniastrum floribundum leaves. Moquiniastrum genus is characterized by producing substances from various classes of natural products such as as sesquiterpene lactones, sesquiterpenes, diterpenes, triterpenes, flavonoids, coumarinsand caffeic acid derivatives crude extracts and fractions of this genus present considerable antioxidant activity and anti-inflammatory effects for the most species already studied. Methanol crude extract from M. floribundum was partitioned into five phases: hexane (Hexa), dichloromethane (DCM), ethyl acetate (AcOEt), n-buthanolic (n-But) and aqueous phase. These phases were dissolved with an aqueous solution of ammonia bicarbonate and one by one incubated with aliquots of sPLA2 and the resulting mixture were homogenized and subjected to an ultrafiltration process using a Vivaspin 500 with cut off selection for 10000 Da molecular weight in regenerated cellulose submitted a centrifuge at 10000rcf for 10 minutes. Using this procedure, we were able to isolate two anti-inflammatory compounds which are a caffeoyl quinic acid derivative and hispidulin. Both compounds showed a high anti-inflammatory capacity and significantly reduced the myonecrosis induced by Crotalus durissus terrificus sPLA2. This inexpensive method developed in our laboratory proved to be an excellent method to screening extracts as well as to small-scale isolation of possible compounds that can modulate and attenuate the enzymatic and pharmacological activity of sPLA2.

23 EVALUATION OF A HYDROLYSABLE TANNIN PURIFIED FROM LAGUNCULARIA RACEMOSA LEAVES AGAINST EDEMA AND MYONECROSIS INDUCED BY SECRETORY PHOSPHOLIPASE A2 ISOLATED FROM CROTALUS DURISSUS TERRIFICUS VENOM

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Mangrove is a humid and coastal ecosystem present in tropical and subtropical areas and plays an important role in the preservation of several plant and animal species. It is a threatened environment and very rich in species that produce natural compounds that still need to be investigated. Ellagitannins constitute the largest group of hydrolysable tannins of plants and from this group the casuarictin (Casu) was isolated from some plant species. However, there are not any investigation concerning to inhibition of enzymatic or pharmacological effects induced by secretory phospholipase A2 (sPLA2) from snake venom by this compound. Casuarictin was isolated from chromatographic procedures of n-butanol (n-BuOH) partition from methanol extract of *Laguncularia racemosa* leaves. The pharmacological and biological effects of Casu were performed on isolated sPLA2 from *Crotalus durissus terrificus* (enzymatic, edematogenic and myonecrotic assay) and using a plant bacterial strain. This compound was able to form a protein complex consisting of a stable sPLA2 + Casu complex. Analyzes carried out with MALDI-TOF revealed that sPLA2 molecular mass increased from 14425.62 Da to 1532.74 Da. The enzymatic activity of sPLA2 + Casu complex was significantly lower than native sPLA2, besides molecular interaction of Casu with sPLA2 was able to virtually abolish the native edematogenic effect as well as myonecrosis induced by sPLA2 when the compound was applied 10 minutes after injection of sPLA2. In addition, this compound also showed a significant antimicrobial activity. Therefore, Casu showed a potential therapeutic application on edema and myonecrosis induced by snake venom secretory phospholipase A2 and antimicrobial effect.

24 FAST AND EFFICIENT PROCEDURE FOR PURIFICATION OF SECRETORY PHOSPHOLIPASE A2 (SPLA2) FROM CROTALUS DURISSUS TERRIFFICUS VENOM USING A SOLID PHASE EXTRACTION

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Several methods are used for isolation and subsequent purification of snake venom phospholipase A2 involve at present time a high price liquid chromatographic procedure, including HPLC equipment, columns and other consumables as well as expend a long time for purifying of sPLA2 by conventional methods. Solid-phase extraction (SPE) is a method originally developed for the concentration of synthetic, biological, and environmental samples before analysis in HPLC and mass spectrometry. However, SPE not generally used for protein purification. In this work, we developed a fast and efficient method for sPLA2 purification from snake venom using a solid phase extraction cartridge using cation exchange SPE. Activation of this SPE cartridge followed the protocol described by the manufacturer and after activation of the cartridge two volumes of initial buffer (0.05M ammonium bicarbonate, pH 7.8) and which was also used to dissolve 50mg of total venom of *Crotalus durissus terrificus* in approximately 5mL of solution. The venom solution was centrifuged for 5 min, the supernatant clarified in 0.22 µm filter and the resulting solution of venom was applied to the SPE cartridge. The solution after the passage was collected and reserved for further HPLC analysis, electrophoresis and enzymatic activity for sPLA2, using NOBA as a substrate. The column was then washed with two volumes of 0.15M ammonium bicarbonate buffer, followed by two volumes of the 0.3M and 0.5M and 1M ammonium bicarbonate buffer. The 1M ammonium bicarbonate solution was used to clean the cartridge for a new purification cycle. The electrophoresis reveals the efficient purification and the enzymatic assay assure the sPLA2 activity. This method allowed the sPLA2 purification in large scale and showed a practical, fast and low-cost method of prospection, having an outstanding application for large scale purification.

25 EFFECTS ON MITOCHONDRIAL FUNCTIONALITY OF FLOWER AND LEAF EXTRACTS FROM THE POISONOUS PLANT SENECIO GRISEBACHII BAKER ("MARGARITA DE CAMPO", ASTERACEAE)

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Senecio genus is spread worldwide, being some species poisonous due to the presence of pyrrolizidine alkaloids (PA). Poisoning in humans is associated to the consumption of herbal teas, while in domestic animals to the intake of plants during dry season. PA are alkylating agents with antimitotic effect, causing liver megalocytosis, ductal proliferation and fibrosis. In Argentina, *Senecio grisebachii* Baker ("margarita de campo", SG) grows in Central and Northeastern regions. Aim of this work is to present effects of flower and leaf extracts of SG on cell viability. Extracts were prepared with 15 g of dried flowers or leaves and 200 mL methanol using magnetic stirrer for 24 h. They were rotaevaporated until dryness and re-suspended with 1% HCl, alkalizing with 25% NH3. Further extraction included chloroform:methanol (4:1) and evaporation to dryness under N2, re-suspending with 10 mL DMSO. Presence of PA was performed with Ehrlich reagent and TLC. MTT assay was used to asses cell viability, using bovine lymphocytes stimulated with 100 ug/mL phytohemagglutinin. Cells were cultured in Ham F12 supplemented with fetal bovine serum and antibiotics at 37 °C during 48 h. Treatments were performed on the last 24 h. Absorbance determination was performed with Elisa at 550 nm. Treatments were: 1) negative control 2) positive control (methanol 10%) 3) diluent control (0,1% DMSO); and S. grisebachii extracts 4) 25 µg/mL 5) 50 µg/mL 6) 100 µg/mL and 7) 200 µg/mL. Results indicate that lower concentrations of extracts are innocuous, while flower extract significantly decreases cell viability (p0.001) with concentrations starting from 100 µg/mL, as shown by lower activities of mitochondrial dehydrogenases and cytosolic reductases (100 µg/mL 18.6%; 200 µg/mL 36.9%). Absence of these effects with leaf extract could be attributable to antagonist effects of two PA. Future assays will include experiments to determine DNA damage and oxidative stress.

26 TRANSCRIPTOMIC AND PROTEOMIC ANALYSIS FROM BLACK WIDOW SPIDER VENOM (LATRODECTUS CURACAVIENSIS)

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The female black widow spider (*Latrodectus curacaviensis*) is responsible for cases of envenomation with complex clinical symptoms. Although there are some similarities between venoms from the same genera, the profile of each species is unique. The lack of information regarding the molecules present in the L. curacaviensis' venom and the necessity to better understand the envenomation process leaded us into this investigation. Therefore, we performed complementary approaches using both transcriptome and proteomic analyses. For the transcriptome, a cDNA library from the venom gland was constructed aiming to identify the expressed toxins. So far, we were able to detect 401 toxin sequences using the ArachnoServer database. These molecules were grouped in 24 super families, on which 52.38% of the transcripts belonged to Latrotoxin family (presynaptic neurotoxins), followed by 9.52% from Neprilysin family (neutral endopeptidases), 9.02% from Aranetoxin family and 4.51% from EF-hand family (calcium-binding proteins), indicating that members of the Latrotoxin family are the most abundant molecules expressed at the venom gland. The proteomic analysis was performed by mass spectrometry, after the in-solution digestion of all the components of the venom. This approach allowed the identification of 76 proteins, on which the most part were pooled in three major groups: (i) enzymes, (ii) proteins with binding function, and (iii) neurotoxins (known as typical black widow spider venom proteins). The members of the enzymes and neurotoxins were observed in higher abundance in both analyses. 1D-gel electrophoresis showed that the majority of the molecules present in the venom showed high molecular weight (~ 30-100 kDa) and were recognized by anti-latrodectus serum using western blot technique. Also, a clear proteolytic activity was observed by zimography. Taken together, these data can be considered the starting point along the characterization process of *L. curacaviensis* venom components, providing an overview of the molecules contained in their venom.

27 EFFECTS OF A LEAF EXTRACTS FROM THE POISONOUS PLANT CESTRUM PARQUI L´HERIT ("DURAZNILLO NEGRO", SOLANACEAE) ON GENOTOXICITY

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Cestrum parqui L´Herit ("duraznillo negro", CP) is a poisonous plant native to South America, that affects large animals when consumed during the dry season (June - August). Toxic compounds are atractylosides that inhibit the ADP/ATP carrier leading to ATP depletion, thus affecting cell metabolism, especially in hepatocytes, being coagulative necrosis the consequent lesion. Besides the toxic compounds, other active principles are present in CP that have shown to exert protective effects in cells (with antioxidant and antimicrobial properties). The latter is attributed to the presence of polyphenols, among other compounds, which explains its use as a medicinal plant to heal wounds in some countries of the region. Aim of this study was to analyze the effects of leaf extracts in an in vitro system to determine cell viability in standardized conditions. Extracts were prepared with 10 g dried leaves, 200 mL methanol using magnetic stirrer for 24 h. They were rotaevaporated until dryness and re-suspended with 10 mL DMSO. Phytochemical analysis of an aliquot of the extracts was performed as well. Cytokinesis-blocked micronucleus and comet assays were used to asses genotoxicity, CHO cells were cultured in Ham 's F10 medium supplemented with 10% fetal bovine serum and antibiotics (50 IU penicillin and 50 µg/ml streptomycin) in a humidified atmosphere with 5% CO2. Treatments were as follows: 1) negative control, 2) positive control (bleomycin 1 µg/mL), 3) diluent control (180 µL/mL DMSO), and different concentrations of CP extract, 4) 200 µg/mL 5) 100 µg/mL 6) 50 µg/mL 7) 25 µg/mL and 8) 12.5 µg/mL. Results indicate that higher concentrations of extracts significantly prevent genotoxicity, as shown by statistical analysis. On the other hand, the lowest concentration generated toxicity in CHO cells. It can be concluded that the leaf extracts of CP may act as a genotoxic agent in a dose-dependent manner.

28 METATRANSCRIPTOME ANALYSIS OF MICROBIAL COMMUNITY IN VENOMOUS GLANDS OF THREE SCORPIONS OF COLOMBIA.

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Scorpions present a sting apparatus with venom glands for defense or predation. Venom glands are an exocrine system exposed to external environment and could host a community of microorganisms that can evolve into ecological relationships between microorganisms and the host. These relations could be neutral, parasitic or symbiotic between microorganisms and the host. But first IT is necessary to check the composition of the community of microorganisms in the venomous scorpion glands. To answer this question three species of scorpion from Colombia — *Chactas vandevedenii, Tityus forcípula* and *Tityus asthenes* — were collected. Five venom glands were extracted from five individuals of each species and total RNAseq dataset was analyzed with standard tools for taxonomic annotation: Kaiju and Qiime2, using the NCBI RefSeq and Greengenes databases. The analysis showed that bacteria comprise 93 to 96 % of total microorganisms. The most abundant Phyla (in range) are: Proteobacteria 39 to 52 %, Cyanobacteria 14 to 37 %, Firmicutes 5 to 9%, Actinobacteria 4,6 to 5 %, Bacteroidetes 3,3 to 5 %, Euryarchaeota 3 to 4,7 %, Crenarchaeota 0,83 to 1,2 % and Planctomycetes 0,5 to 1,2 %. Cianobacteria is the most representative phyllum in *Tityus asthenes* whereas Protobacterias are more represented in *Tityus forcípula* and *Chactas vandevedenii*. The most representative species in all scorpions is the *Cianobacteria Leptolyngbya* sp. PCC7336.This is an initial approach to develop models to study relationship for function and coevolution between host organisms on microorganisms and the evolution of antibiotic sequences and other roles that can be involved in the evolution and ecology between scorpions and their microbiotic environment.

29 STUDY ON THE DIFFERENCE BETWEEN THE VENOM OF THE WILD COBRA (NAJA ATRA) AND THE ARTIFICIAL FEEDING COBRA

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The edible and medicinal value of *Naja atra* has been gradually paid more attentions in China, meanwhile, catching the wild snakes (*N. atra*) are prohibited by our government to protect the wild snakes and the ecosystem in China. So, to meet the market demands, as well as to protect the wild snakes, we develop artificial hatching, artificial feeding and no hibernating technologies to produce or culture the snakes. In this study, we investigated the difference between the venoms of the wild adult cobra and the artificial feeding adult cobra at the same age and the same body weight. SDS-PAGE results in the reduction indicated that the protein bands of the wild snake venom are more than ones of the artificial feeding snake venom, and the content of proteins at about 55 k D band of the former was higher than the latter's. RP-HPLC chromatogram peak areas of the former at about 20 min and 50 min, respectively, are larger than the latter's. The results of venom enzyme activities (including PLA2, hyaluronidase, L-AAO, alkaline phosphomonoesterase, acetylcholinesterase, proteinase, 5'-nucleotidase etc.) between the former and the latter showed that there were no obvious difference. The dosages of LD50 between the former venom and the latter venom also showed that there were no obvious difference. In addition, the transcriptome results of their venomous gland cells indicated that there were some slighter difference between the former and latter, which are further being investigated in our lab. The study will contribute to save the snake-bitten workers in the snake farm, and the medicinal development of venom of the artificial feeding cobra.

30 VENOMICS OF THE COLUBRID SNAKE TRIMORPHODON QUADRUPLEX FROM COSTA RICA

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The venoms of snakes with back fangs have been little studied, however they represent a high potential for the discovery of new compounds (Junqueira-de-Azevedo et al., 2016, Mackessy and Saviola, 2016). This work presents the proteome of the Costa Rican lyresnake, *Trimorphodon quadruplex*, as well as the analysis of several of its prominent enzymatic components and, the toxicity of the venom. The poison was investigated by techniques of proteomic, biochemical and biological analysis. The SDS-PAGE fingerprint revealed six prominent bands with approximate masses of 60, 53, 25, 14, 8 and 4 kDa, strongly suggesting toxins in the families of L-amino acid oxidase, snake venom metalloproteinase proteins (P- III), cysteine- rich protein, PLA2 and three fingers toxins of two different size, respectively.

This study showed that the venom of *T. quadruplex* is composed mainly of 3FTxs, which represent almost half of their total proteins. Other types of proteins present in substantial proportions in this venom belong to the MP, CRISP, LAAO and PLA2 families, the last two enzymes are rare venom components in the opisthoglifos species. No myotoxicity or lethality was registered for the main PLA2 purified from the venom of *T. quadruplex* in mice, and therefore, its possible toxic functionality has yet to be determined.

31 EXPERIMENTAL NEUTRALIZATION OF MICRURUS VENOMS BY AN EXPERIMENTAL POLYVALENT ANTIVENOM AND THERAPEUTIC ANTIVENOMS BASED ON NORTH AND SOUTH AMERICAN MICRURUS VENOMS.

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Micrurus comprises the Genus of venomous snakes with the highest number of species in America. The antivenoms used for the treatment envenoming caused by Micrurus species have low or absent cross reactivity against the venoms of geographically non-related species, as was observed in experiments with South and North American Micrurus venoms and their antivenoms. We developed an equine Experimental Polyvalent antivenom(ExPAV) against the venoms of *Micrurus (M.) fulvius*, *M. nigrocinctus* and *M. surinamensis*. The immunochemical reactivity (ELISA and Ouchterlony) and neutralizing capacity on lethality and indirect hemolytic activity (preincubation and rescue assays in mice) of ExPAV and specific North American (NAAV) and South American (SAAV) Micrurus therapeutic antivenoms were tested. The immunochemical reactivity was higher using specific antivenoms or ExPAV, when compared to that of the non-specific anti venoms. ExPAV neutralized all venoms tested, but needed higher doses than the specific antivenom to neutralize the venom of M. pyrrhocryptus, both in pre incubation and rescue experiments. The neutralization by ExPAV was higher than the non-specific neutralization conferred by SAAV or NAAV on the venoms of North or South American Micrurus respectively. The ED50s against 3LD50 of *M. nigrocinctus, M. fulvius, M. surinamensis* and *M. pyrrhocryptus* were 82,112,172 and 52µl for SAAV, 68,39,>250,>250µl for NAAV and 69, 58, 59 and 181 µl for ExPAV, respectively. In rescue experiments the ED50s of ExPAV against *M. nigrocinctus,M. fulvius* and *M. pyrrhocryptus* venoms were 132,173 and 331µl respectively. ExPAVshowed higher neutralization of the indirect hemolysis caused by *M.fulvius* and *M.nigrocinctus* venoms than the one provided byspecific antivenom (p0.05). Despite the neutralization observed, an additional South American coral snake venom should be included in the immunogenic mixture to obtain antibodies able to protect against the venom of the ex-*Micrurus frontalis* group.

32 INTERACTION OF SNAKE VENOM METALLOPROTEINASES WITH TYPE IV COLLAGEN: ROLE OF THE DIFFERENT DOMAINS IN TARGET BIDING

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Snake venom metalloproteinases (SVMPs) disrupt capillary vessels leading to local and systemic hemorrhage. It has been proposed that type IV collagen degradation is one of the key steps in SVMPs-induced hemorrhage, since hydrolysis of this protein results in a significant weakening of the mechanical stability of the capillary wall. However, the sequences in SVMPs ('exosites') which enable them to specifically interact with type IV collagen have not been identified, although it is hypothesized that they are located in the disintegrin-like and cysteine-rich domains characteristic of multi-domain SVMPs. In this study, the interactions of several SVMPs with type IV collagen were investigated in vitro by western blot, ELISA and affinity chromatography. Hemorrhagic PI, PII and PIII SVMPs, in addition to the DC domain, were compared in the different assays in the presence or absence of a metalloproteinase inhibitor. In all the assays, the strongest binding was observed with CsH1, a PIII SVMP, whereas the PI SVMP BaP1 exhibited the lowest binding, and the PII SVMP BlatH1 presented a moderate binding. Interestingly, the binding of the isolated DC domain of CsH1 was lower than the native CsH1 suggesting a role of the metalloproteinase domain in the interaction with type IV collagen.

33 SNAKE VENOMICS OF CROTALUS DURISSUS TERRIFICUS FROM ARGENTINA

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Envenomation by *C.d.terrificus* is a relevant public health problem in South America. To date, there are no proteomic studies of *C. d.terrificus* venom from Argentina, so we here report the venom composition from specimens that inhabit the Northeast Argentina region. The proteins were separated by reverse-phase HPLC and identified by mass spectrometry. *C. d.terrifius* proteome showed the presence of 10-15 main toxins belonging to the following protein families: disintegrins, PLA2s, serine proteinases, vascular endothelial growth factor-like (VEGF), L-amino acid oxidases, C-type lectins-like and phosphodiesterases. The neurotoxic proteins, crotoxin and crotamine, represent the 74.8 % of proteins of the *C.d.terrificus* venome. The crotoxin complex was further analyzed and various PLA2and crotapotine isoforms were observed. The "lethal neurotoxicity coefficient" (LNC: LD50 / neurotoxin concentration) was 1.33 in concordance with a neurotoxic and not hemorrhagic venom, but it is greater than that was reported for *C. d.terrificus* venom from Brazil (0.96). The major LNC value it would be related to the low content ratio of crotamine (8%) in *C.d.terrificus* venom from Argentina. These results provide information about *C.d.terrificus* venom composition from Argentina and they could be considered on defining the mixture of venoms for immunization to produce an effective pan-American anti-Crotalus antivenom.

34 BINDING OF ACIDIC PLA2 BA SPII RP4 FROM BOTHROPS ALTERNATUS SNAKE VENOM TO INTEGRIN AVB3: AN IN SILICO STUDY

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We have previously demonstrated that BaSpIIRP4 (an acidic PLA2 from *Bothrops alternatus* venom), enhance the endothelial cells (EC) detachment effect of a snake venom metalloproteinase. Considering that the binding of cells to their ligands depends on the interaction between extracellular matrix and integral membrane proteins, this effect may be due to interactions between PLA2 and EC integrins. The integrin alpha-v beta-3 (αvβ3) is commonly expressed in endothelial cells. It has been reported that human PLA2-IIA binds to this integrin with high affinity. Moreover, it was demonstrated that arginine residues R74 and R100 are critical for this binding. In addition, the interaction svPLA2-αvβ3 integrin was also described. Considering these previous findings about the interaction of human and snake venom PLA2-IIA with integrin αvβ3, in this work we use an in silico approach to predict whether BaSpIIRP4 would also bind to the same integrin, thus supporting the hypothesis that EC detachment could be a receptor-mediated effect. Since structure of BaSpIIRP4 PLA2 has not yet been elucidated, an enzyme homology model was built with the Modeller software. PLA2 from *Bothrops jararacussu* was selected as template structure. To identify putative sites on the surface of the PLA2 model with capacity to interact with the RGD site on the headpiece of the αvβ3 integrin, the protein-protein docking server ClusPro was employed. The stability of the identified anchoring points was evaluated by Molecular Dynamic simulations with Amber16 and binding free energy calculations with MM-PBSA protocol. Three interaction sites were found, one of them showing a strong resemblance with the previously identified site on human PLA2-IIA. These results suggest that integrin αvβ3 may serve as receptor for PLA2 from *B. alternatus* venom, and this interaction could be a novel therapeutic target.

35 IMMUNE RESPONSE INDUCED BY NEW ADJUVANT STRATEGY TO PRODUCE CROTALIC ANTI-PLA2

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Snake envenomation is a serious medical problem and antivenoms are the main treatment. Antisera are produced by immunization of horses with snake venom using complete Freund's adjuvant and incomplete (booster) but it causes severe local reactions. A new adjuvant strategy is here proposed to increase efficiency in antisera production under much less morbility to immunized animals. Previous works showed that CpG-ODN formulated with a 6-O-ascorbyl palmitate nanostructure (Coa-ASC16) was more efficient as adjuvant than CpG-ODN alone using ovalbumin (OVA) as an antigen model. Here, we evaluated the immune response induced by this adjuvant strategy using crotalic PLA2 enzyme as antigen. Balb/c mice were subcutaneously immunizated on days 0, 15 and 30 with PLA2/CpG-ODN/Coa-ASC16 or PLA2/Freund's Adyuvant (complete first and incomplete-booster) (dose/mice: crotalic PLA2: 10-15µg, CpG-ODN: 30 µg). On day 50, mice were sacrificed. In both immunized mice groups, the plasma antibody titers were high (dilution1/24800), with a similar IgG1/IgG2a ratio. The IgG antibodies were then purified by affinity Sepharose-proteinG column. Indirect hemolytic activity neutralization of the PLA2 (2.5 µg) with specific antibodies (1.7-4.5 µg range; IgG anti-PLA2) were made by radial hemolysis. The evaluation did not show difference in neutralizing capacity of the antibodies produced by CpG-ODN/Coa-ASC16 or Freund's Adyuvant. Macroscopic and microscopic analysis at the site of injection of mice inoculated with Freund's adjuvant showed local damage (with non-infectious abscesses) and hypertrophy of inguinal lymph nodes, whereas mice injected with CpG-ODN/Coa-ASC16 did not. Our results shows that CpG-ODN/Coa-ASC16 produces a humoral response as strong and specific as Freund´s adjuvant, with minor or null local deleterious effect. Thus, this complex emerge as a new adjuvant and a very attractive alternative for anticrotalic sera production.

36 AN UNUSUAL PHOSPHOLIPASE A2 FROM PORTHIDIUM HYOPRORA VENOM SNAKE: PURIFICATION, STRUCTURAL AND PHARMACOLOGICAL PROPERTIES

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Snake venom PLA2 enzymes, in addition to their involvement in the digestion of prey, exhibit a wide variety of pharmacological/toxic effects by interfering in normal physiological processes of prey/victims. Often, single snake venom contains a number of PLA2 isoenzymes, and at times, different isoenzymes induce distinct pharmacological effects. However, not all PLA2 enzymes induce all the pharmacological effects; some mainly express its primary digestive function. This work describes the biochemical/pharmacological characterization of a non-toxic PLA2 designated PhTX-IV, isolated from *Porthidium hyoprora* venom by a single chromatographic step, including reverse-phase chromatography. The purification process employed C4 reverse phase high-pressure liquid chromatography. This enzyme is composed of a unique polypeptidic chain and has a molecular weight of 13.846 Da. Its complete sequence of 121 amino acids was obtained through ESI-MS/MS techniques, showing that it belongs to the Asp49 group of catalytically active enzymes and revealing a high degree of homology sequence (87-49%) with other Bothrops PLA2. PhTX-IV showed Ca2+-dependent enzymatic activity, reaching its maximal activity at pH 8 and 35-45oC. In vivo, did not show myotoxic upon muscular fibers at doses up to 100µg and neurotoxic, cytotoxic, and anti-platelet aggregation activities were absenting. Furthermore, was not lethal to mice at intravenous high doses of 100µg. Induction of local paw edema and weakly inhibited coagulation were the only toxics effects recorded. In conclusion, this enzyme, with the exception of a slight clotting time delay and a moderate edema-inducing effect, did not showed the major toxic actions reported for this type of proteins, as myotoxicity, cytotoxicity and lethality. These pharmacological characteristics suggest that the role of PhTX-IV in the pathophysiology of envenomings by *Porthidium hyoprora* might be restricted to digestive functions.

37 PRECLINICAL EVALUATION OF THE POLYSPECIFIC ANTIVENOM INOSERPTM PAN-AFRICA AGAINST THE VENOMS OF ELAPIDS AND VIPERIDS OF SUB-SAHARAN AFRICA REGION: NEUTRALIZATION OF TOXIC ACTIVITIES.

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Snakebite envenoming has a heavy burden in the public health in sub-Saharan Africa. One of the aspects that limits the distribution and appropriate use of antivenoms in sub-Saharan Africa is the lack of rigorous knowledge on the spectrum of the preclinical efficacy of currently available antivenoms. The World Health Organization (WHO) has published a list of species of medical importance for different countries and territories based partly on the extrapolation of public studies, biological studies of the species in question and epidemiological studies. The venoms of these species must be considered in the development and production of antivenoms that guarantee adequate efficacy for their application in a specific region. For the neutralization of lethal activity, mixtures containing a fixed dose of venom and various dilutions of antivenom were prepared, and incubated at 37ºC for 30 min. Then, aliquots of 0.2 mL of each mixture, containing a dose of venom corresponding to 5 LD50, were injected i.v. into groups of five mice. Mixtures corresponded to various ratios of mg venom/mL antivenom (or mg venom/g antivenom protein). A control group of mice was injected with the same dose of venom incubated with PBS instead of anti- venom. Deaths were recorded for 24 h and the neutralizing ability of antivenom was expressed as the Median Effective Dose (ED50), i.e. the venom/antivenom ratio at which half of the population of mice is protected. InoserpTM PAN-AFRICA shows a neutralization profile ranging from 107.2 to 678.58 LD50 for the venoms of *Echis ocellatus, Echis leucogaster, Echis pyramidum, Bitis arietans, Bitis rhinoceros, Bitis nasicornis, Bitis gabonica, Dendroaspis polylepis, Dendroaspis viridis, Dendroaspis angusticeps, Dendroaspis jamesoni, Naja nigricollis, Naja melanoleuca, Naja haje, Naja pallida, Naja nubiae, Naja katiensis, Naja senegalensis, Naja annulifera, Naja mossambica, Naja anchietae, Dispolidus typus* and *Atractaspis irregularis,* species recommended by the WHO.

38 IDENTIFICATION OF NON-VENOM PROTEIN GENES HIGHLY EXPRESSED IN THE VENOM GLAND OF HABU (PROTOBOTHROPS FLAVOVIRIDIS) AND THEIR POTENTIAL CONTRIBUTION TO THE QUALITY CONTROL OF VENOM PROTEINS.

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Although snake venom proteins have been extensively analyzed, genes for venom protein modification are poorly understood. In our previous study of whole genome sequencing of habu, *Protobothrops flavoviridis,* we identified various venom protein genes and showed their accelerated evolution. In the current study, we focused on non-venom protein genes highly expressed in the venom gland. From the transcriptome data of venom glands of three individuals including one neonate, we identified top 50 genes highly expressed in the venom gland, including 19 venom protein genes and 16 housekeeping genes. From the remaining 16 non-venom and non-housekeeping protein genes highly expressed in the venom gland, we identified four genes encoding proteins involved in protein modifications, two genes of protein disulfide isomerases (PDIA3 and P4HB), selenoproteinM (SELENOM) and calreticulin (CALR). Molecular phylogenic analyses revealed that all four genes are single-copy genes identified in the habu genome, in contrast to the high copy number of venom protein genes. Molecular evolutionary analyses revealed that all these genes are highly conserved so that they can be aligned with human orthologs with high amino acid identities, such as PDIA3 with 78.6% identity and CALR with 79.8% identity. We also observed extremely high conservation of their nucleotide sequences among closely related species (*P. flavoviridis, P. tokarensis, P. elegans and P. mucrosquamatus*). Since all four proteins are known to be involved in disulfide bond formation contributing to protein folding, it is highly likely that these proteins play critical roles in the quality control of venom proteins stored in the venom gland. In contrast to the high copy number and the accelerated evolution of venom protein genes, extremely conservative evolution of these single-copy non-venom proteins suggests the strict and consistent requirement of specific disulfide bond formations in venom proteins underlying their variable and specific functions.

STUDENT POSTERS SESSION II

ISOLATION AND CHARACTERIZATION OF COMPONENTS WITH PROTEOLYTIC ACTIVITY PRESENT IN FRACTION II-III FROM *TITYUS SERRULATUS* VENOM	Acosta	Letícia
CONTRIBUTION TO *B. ATROX* VENOM INFLAMMATORY REACTION OF TWO SVMPS AND THE HYDROLYSIS PRODUCT OF THESE ENZYMES ON BASEMENT MEMBRANE COMPONENTS	Almeida	Michelle
ANTIPLATELET EFFECT OF A METALLOPROTEINASE RHOMBETLYSIN-I, FROM *LACHESIS MUTA RHOMBEATA* VENOM IS MEDIATED BY AFFECTING THE BINDING OF VWF AND GPIB	Alvarenga	Valeria
SYNERGISTIC EFFECT BETWEEN THE PEPTIDE LYETX1-B AND CISPLATIN TO KILL TRIPLE NEGATIVE BREAST CANCER CELLS, MDA-MB-231	Avelar Júnior	Joaquim
PARTIAL PROTEOMIC CHARACTERIZATION OF THE *PHYLLOMEDUSA MEGACEPHALA* SECRETION AND THE DIFFERENTIAL ACTIVITY OF SOME PEPTIDES AGAINST TUMOR AND NON-TUMOR CELL LINES	Avelar Júnior	Joaquim
INVOLVEMENT OF SNAKE VENOM NATRIURETIC PEPTIDES IN VASCULAR RELAXATION AND MICROVASCULAR PERMEABILITY	Barroso Pereira	Beatriz
SNAKE VENOM MATRIX METALLOPROTEINASES: A NEW MAJOR CLASS OF PROTEOLYTIC TOXINS ACROSS DIPSADIDAE.	Bayona Serrano	Juan
SUBSTITUTIONS OF RESIDUES IN A LOOP SURROUNDING THE ACTIVE SITE OF A P-I SNAKE VENOM METALLOPROTEINASE ABROGATES ITS HEMORRHAGIC ACTIVITY	Camacho	Erika
RATIONAL DESIGN OF ANALOGS PEPTIDES FROM *TITYUS SERRULATUS* SCORPION TOXIN AGAINST PATHOGENIC BACTERIA	Flores	Taylla
IMPORTANCE OF METALLOPROTEINASES IN ENVENOMING BY *BOTHROPS ATROX*, IN THE BRAZILIAN AMAZON: LOCAL DAMAGE APPROACHES	Gimenes	Sarah
PHYSICAL-CHEMICAL CHARACTERIZATION OF CARBON NANOTUBES-HYDROXYAPATITE NANOCOMPOSITES WITH FIBRIN SEALANT DERIVED FROM SNAKE VENOM	GOUVEIA	DANIEL
DISTRIBUTION OF METALLOPROTEINASES AND PHOSPHOLIPASES A2 IN SNAKE VENOMS AND RELATIONSHIP TO VENOM TOXICITY	Guimarães	Noranathan
HEMORRHAGIC METALLOPROTEINASE HF3 FROM *BOTHROPS JARARACA* VENOM: OBTENTION OF RECOMBINANT DOMAINS DC USING A CELL-FREE EXPRESSION SYSTEM, AND INTERACTION WITH ENDOTHELIAL CELLS	Ferreira	Jessica
BRAZILIAN THREE-FINGER TOXINS: WHAT CAN WE LEARN? FROM MOLECULAR FRAMEWORK TO INSIGHTS IN BIOLOGICAL FUNCTION	Kleiz Ferreira	Jessica
PHOTOBIOMODULATORY PROPERTIES OF THE ALGAAS LASER DIODE ON LOCAL TOXIC ACTIONS INDUCED BY *BOTHROPS LEUCURUS* VENOM	Santana Sales Lauria	Pedro
USE OF ALTERNATIVE ADJUVANT SYSTEMS TO PRODUCE NEUTRALIZING IGY ANTIBODIES AGAINST BOTHROPS ALTERNATUS VENOM	Leiva	Carlos

EVALUATION OF AN IGY-BASED ANTIVENOM AGAINST APITOXIN FROM HONEYBEES	Leiva	Carlos
BETA-CARDIOTOXIN, A NOVEL COMPOUND FROM KING COBRA VENOM, SUPPRESSES CARDIAC FUNCTION VIA NON-BETA-ADRENERGIC PATHWAY	Lertwanakarn	Tuchakorn
PEGYLATING TOXINS: A NEW TREND IN TOXINOLOGY? A SUCCESSFUL EXAMPLE OF A PEGYLATED SNAKE VENOM SERINE PROTEASE	Lopes Pinheiro	Ernesto
EXTRACELLULAR VESICLES FROM *BOTHROPS JARARACA* VENOM: COMPOSITION AND INITIAL ASSESSMENT OF BIOLOGICAL FUNCTIONS	Machado	Larissa
BOTHROPS MOOJENI WHOLE VENOM ACTIVATES PREADIPOCYTES TO RELEASE PROINFLAMMATORY MEDIATORS	Marques	Rodrigo
EXPOSURE OF LACTATING WISTAR RATS TO *TITYUS BAHIENSIS* SCORPION VENOM: EFFECTS ON CYTOKINE AND GROWTH FACTOR (BDNF) LEVELS OF OFFSPRING.	Beraldo Neto	Emidio
ANTI-TUMORAL POTENTIAL OF A NOVEL DISINTEGRIN ISOLATED FROM *PORTHIDIUM LANSBERGII LANSBERGII* VENOM ON BREAST CANCER CELLS	Montealegre-Sanchez	Leonel
DISCOVERY AND CHARACTERISATION OF NOVEL PEPTIDES FROM AMAZONIAN STINGING ANT VENOMS WITH ANTIPARASITIC ACTIVITY	Nixon	Samantha
INVESTIGATING THE NEUROMODULATORY EFFECTS OF CORAZONIN IN EMERALD JEWEL WASP VENOM	Nolan	Cebrina
POLYVALENT ANTINEUROTOXIC ARACHNIDIC ANTIVENOM. PRELIMINARY REPORT.	Oliveira	Vanessa
SOME MOLECULAR PROPERTIES OF AN ACID PHOSPHOLIPASE A2 FROM THE VENOM OF *BOTHROPS ATROX* SNAKE AND ITS RELATIONSHIP WITH MYOTOXICITY	Quispe Cerón	Edwin
METALLOSERRULASES 3 AND 4 FROM THE *TITYUS SERRULATUS* SCORPION VENOM AND ITS INFLAMMATORY PROPERTIES.	Silva	Cristiane
ACTIVITY IN TWO KEY TOXIN GROUPS IN AUSTRALIAN ELAPIDS SHOW A STRONG CORRELATION TO PHYLOGENY BUT NOT TO DIET	Tasoulis	Theo
THREE-FINGER TOXINS AND AUSTRALIAN ELAPID VENOMS: ARE THEY IMPORTANT?	Tasoulis	Theo
SYSTEMIC AND VASCULAR ACTIONS OF *LEPTODEIRA ANNULATA* (BANDED CAT-EYED SNAKE; DIPSADIDAE) VENOM	Torres-Bonilla	Kristian
BIOCHEMICAL CHARACTERIZATION OF THE MAJOR *TITYUS BAHIENSES* VENOM TOXINS	Beraldo Neto	Emidio
CHARACTERIZATION OF INSECTICIDAL PEPTIDES FROM THE VENOM OF THE NORTH AFRICAN SCORPION, *BUTHACUS LEPTOCHELYS*	Yoshimoto	Yusuke
COMPARISON OF THE EFFICACY OF TWO MONOVALENT EXPERIMENTAL ANTIVENOMS FOR BOTHROPS ASPER FROM COLOMBIA	Sarmiento	Karen

CLINICAL MANIFESTATIONS IN OPHIDIAN ACCIDENTS BY SERPENTS OF THE BOTHROPS GENUS IN THE STATE OF AMAZONAS: DIFFERENCES BETWEEN THE SOLIMÕES AND JURUÁ RIVERS.	Altair	Farias

1 ISOLATION AND CHARACTERIZATION OF COMPONENTS WITH PROTEOLYTIC ACTIVITY PRESENT IN FRACTION II-III FROM TITYUS SERRULATUS VENOM

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Scorpionism is a public health problem in Brazil. Scorpions are responsible for most accidents involving venomous animals in the country, causing serious symptoms, which can lead to death. *Tityus serrulatus* venom (Tsv) is composed of neurotoxins, lipids, inorganic ions, nucleotides, vasoactive amines, proteases, protease inhibitors, muco-polysaccharides, amino acids, peptides, some of which are hypotensive, and hyaluronidase. To date, there are few studies on its proteases. Therefore, the aim of the present study was twofold: 1) to isolate and characterize the components with proteolytic activity present in fraction II-III from *Tityus serrulatus* venom (Tsv), and 2) to determine the proteases' relevance on the venom toxicity and on the post-translational modifications of venom neurotoxins. To this end, fractions II, III and a pool of fraction II-III were submitted to reversed-phase chromatography using a C18 column (1 x 25 cm, 5 µm, 300 Å). Some of the eluted peaks were submitted to Edman degradation sequencing, being identified as fragments of Ts19, PAPE fragment and venom α-amylase. The pool of fraction II-III showed several components within the range from 30 to 70 kDa by SDS-PAGE under reducing conditions. Fractions II and III degraded starch, confirming the presence of an active α-amylase. Both fractions also showed proteolytic activity upon fibrinogen, even in the presence of the inhibitors EDTA and PMSF, revealing that they contain serine and metalloproteases. Additionally, the Ts19 fragments were submitted to an electrophysiological assay on voltage-gated potassium channels using the two-electrode voltage clamp technique. The C-terminal fragment of Ts19 showed about 40% of inhibition on the Kv1.2 channel, while the N-terminal fragment showed no activity. In conclusion, this study revealed that fractions II and III from Tsv contain: serine and metalloproteases, a C-terminal fragment of Ts19 which blocks the Kv1.2 channel, a PAPE fragment and an active venom α-amylase.

2 CONTRIBUTION TO B. ATROX VENOM INFLAMMATORY REACTION OF TWO SVMPS AND THE HYDROLYSIS PRODUCT OF THESE ENZYMES ON BASEMENT MEMBRANE COMPONENTS

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Metalloproteinases (SVMPs) are the key toxins for local and systemic signs observed in patients in accidents with viper snakes. The mechanisms involved in SVMPs action are related to the hydrolysis of blood vessels basement membrane components (BM) as well as direct action to platelets, endothelial and inflammatory cell receptors. Several evidences indicate that endogenous factors derived from hydrolysis of BM enhance local effects induced by SVMPs and consenquently, the severity of snakebites. This work aimed to evaluate the ability of Atroxlysin-Ia (P-I) and Batroxrhagin (P-III) SVMPs isolated from *Bothrops atrox* venom and their hydrolysis products of BM, in inflammation models. Balb/C mice received 2 µg of SVMPs intraplantar or intraperitoneal route for assessment of edema and leukocyte accumulation, respectively. The edema induced by the two toxins increased the size of the paw in 70%. The leukocyte infiltrate reached levels of 5x106 with P-III, 6x106 by P-I. Matrigel was incubated with the toxins (1:10) for 1 h at 37 °C, the hydrolysis products were isolated in centrifugal filter devices (cut off at 10 kDa), and identified by LC/MS/MS. Laminin, collagen IV, nidogen and BM-specific sulfate proteoglycan fragments were observed after hydrolysis. The isolated peptides were injected into mice, and the edema induced by the fragments generated by both enzymes increased paw size by 30%. Leukocyte accumulation was 5x106 and 6x106 with hydrolysis products from Atroxlysin-Ia and Batroxrhagin, respectively. The results suggest that SVMPs and basement membrane fragments contribute to the inflammatory response observed in envenomings.

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3 ANTIPLATELET EFFECT OF A METALLOPROTEINASE RHOMBETLYSIN-I, FROM LACHESIS MUTA RHOMBEATA VENOM IS MEDIATED BY AFFECTING THE BINDING OF VWF AND GPIB

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Introduction: Snake venom metalloproteinases (SVMPs) are zinc-dependent endopeptidases grouped into P-I to P-III classes, according to their domain structures. They are abundant proteins in most viperidae venoms and are the key toxins involved in snake venom-induced pathogenesis, such as local hemorrhage, edema, inflammation and necrosis as well as the drastic systemic effects on hemostasis. They are associated with bleeding in several organs, coagulopathies and renal failure by cleaving target factors involved in coagulation, platelet function and extracellular matrix (ECM). Here, we report a 23-KDa SVMP, termed rhombetlysin-I (rhomb-I), from *Lachesis muta rhombeata* venom. Rhomb-I showed proteolytic activity on several plasma and ECM proteins including, fibrinogen, fibronectin, laminin among others and exhibited low hemorrhagic effect (MHD=43 µg/22g). Rhomb-I was purified from L. m. rhombeata venom using molecular exclusion chromatography on Sephacryl S-200 and Sephadex G-50. Some biochemical features associated with its effect on hemostasis and platelet aggregation was assessed. The in vivo experiments were conducted according to the guidelines established by the Brazilian College for Animal Experimentation and approved by CEUA/FUNED (94/2016). Rhomb-I is an α-fibrinogenase that preferentially cleaves the α-chains of fibrin and fibrinogen, as well as fibronectin and vitronectin. It was not detected proteolytic activity on type I and IV collagens, but digests laminin from matrigel at molar ratio of 1:50. Rhomb-I dose-dependently inhibit platelet aggregation on vWF-induced platelet aggregation of washed platelets. Moreover, the proteinase digests the recombinat A1-domain of vWF (rvWF-A1) and glycoprotein (GP)Ib as demonstrated by western blotting using antibodies against rvWF-A1 domain and anti-GPIb complex, respectively. Taken together our data indicate that rhomb-I impairs platelet aggregation by affecting GPIb-vWF interaction. Thereby, rhomb-I may be used as a tool to identify the binding motifs that are involved for the vWF-GPIb and vWF-ECM interaction. Financial support: CAPES/FAPEMIG.

4 SYNERGISTIC EFFECT BETWEEN THE PEPTIDE LYETX1-B AND CISPLATIN TO KILL TRIPLE NEGATIVE BREAST CANCER CELLS, MDA-MB-231

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The breast cancer is the most common and the biggest cause of women cancer death, being responsible by 25% of total cancers in women. Among them, triple negative breast cancer type is the most difficult to treat, because it is less responsive to hormone- based treatments. In this research we propose a combination between a new synthetic antitumoral peptide we have studied, LyeTx I-b, derived from a natural peptide (LyeTx I) from the spider Lycosa erythrognatha, and cisplatin, a known anticancer drug, as an approach to kill the MDA-MB 231 cells. Firstly, a trial was performed to evaluate the compounds IC50 in separate, resulting in IC50 of 2.47 µM and 94.69 µM to LyeTx I-b and cisplatin, respectively. After that, isobolographic analysis was performed taking in account the IC50 obtained when three different proportions of LyeTx I-b and cisplatin were tested, 1:1, 3:1 and 1:3. It was shown that proportion 1:1 showed a synergistic effect. Cell cycle analysis was performed to each proportion used in isobolographic analysis showing a G2 stop in cell cycle that could be indicative of autophagy. Western blot analysis of AKT and ERK-proteins showed that in the proportion 1:1 of the compounds, there was a decrease of the AKT phosphorylation, although phosporylated ERK did not exhibit any change, compared to untreated cells. These founds suggest that a combination between LyeTx I-b and cisplatin, in the proportion of 1:1, which exhibited a synergistic effect, could be a good candidate as a new therapy against breast cancer.

5 PARTIAL PROTEOMIC CHARACTERIZATION OF THE PHYLLOMEDUSA MEGACEPHALA SECRETION AND THE DIFFERENTIAL ACTIVITY OF SOME PEPTIDES AGAINST TUMOR AND NON-TUMOR CELL LINES

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Phyllomedusa megacephala is a Brazilian frog from Minas Gerais' cerrado (tropical savanna), which skin secretion was never studied. In this work, we fractionated the skin secretion of *P. megacephala* by reverse phase HPLC chromatography, followed by a MALDI-TOF mass spectrometry and Edman degradation analyses. A total of 15 peptides had their primary structures determined by de novo sequencing, and were synthesized and tested against bacteria (according to CLSI norm with modifications) and four lineages, MDA-MB231, MCF-7, HCT-116 (solid tumor cells lines) and HEK-293 (normal cell line). Of these peptides, eight similar to phylloseptin family were found, being three complete and five truncated versions of them. In addition, we found one peptide similar to dermaseptin and a short orphan sequence similar to a peptide named "bioactive peptide 1" from *Phyllomedusa hypochondrialis* that has no activity described. Three novel orphan peptides were also described. These orphan peptides were tested against bacteria (Escherichia coli and Staphylococcus aureus strains), three cell cancer lines and one normal cell line. No antimicrobial activity was found, by using an agar trial assay. When tested against MDA-MB-231, MCF-7, HCT-116 lineages (by sulforhodamine B assay), two of the peptides (named peptide 1222 and peptide 1223) increased cell proliferation. No effect was observed against the HEK-293 (normal kidney line). These results suggest that these peptides could be used as tools to investigate the signaling pathways involved in cell survival and proliferation that contribute to carcinogenesis.

6 INVOLVEMENT OF SNAKE VENOM NATRIURETIC PEPTIDES IN VASCULAR RELAXATION AND MICROVASCULAR PERMEABILITY

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The role of snake venoms' natriuretic peptides (NPs) in vascular reactivity is poorly understood. We examined the involvement of NPs in vascular responses to venoms of *Bothrops jararaca* (BJ), *Crotalus durissus terrificus* (Cdt), *Lachesis muta* (LM) and *Micrurus spixii* (MS). Rat thoracic aortic rings were mounted in organ baths containing aerated Krebs solution (37 °C, tension: 10mN). After stabilization, vascular reactivity and endothelial intactness were assessed with KCl (80mM), phenylephrine (1µM) and acetylcholine (1µM). Rings were contracted with phenylephrine and concentration-relaxation curves were obtained for each venom. A single venom concentration was used to screen for NPs in presence of A71915 (3nM), an NP GC-A receptor antagonist. Vascular permeability was assessed based on 125I-albumin extravasation in the dorsal skin of thiopental (40mg/kg, i.p.)-anesthetized rats. Venom was injected without or with A71915 (100µL/site). Results (mean±SEM) were compared using one-way ANOVA and Tukey-Kramer tests; p0.05 indicated significance. ANP relaxed aortic rings by 56.5±7.6% (10-9M). Without endothelium, the relaxation was 14.7±1.6% and A71915 attenuated it to 26.5±1.6%. All venoms caused endothelium-dependent vasorelaxation; MS was the most potent. Vasorelaxations by each venom (without vs. with A71915) were 49.1±1.1% vs. 20.1±2.9% (BJ 100µg/ml), 46.4±1.4% vs. 29.6±2.7% (Cdt 300µg/ml), 23.9±2.8% vs. 18.7±1.7% (LM 300µg/ml) and 41.1±3.5% vs. 29.2±0.8% (MS 10µg/ml). ANP (30µg/site) caused plasma extravasation (120.6±11.6µl) that was reduced (68.6±7.5µl) by A71915. Cdt was the most potent venom. A71915 attenuated all responses: 125±7 vs. 74.4±7µl (BJ), 193.2±23 vs. 64.4±7µl (Cdt), 133±14 vs. 57.8±7µl (LM) and 149.8±13 vs. 56.4±13µl (MS) (n=6; p0.05). The venoms caused vasorelaxation and enhanced vascular permeability, which were partially attenuated by A71915, indicating NPs involvement.

7 SNAKE VENOM MATRIX METALLOPROTEINASES: A NEW MAJOR CLASS OF PROTEOLYTIC TOXINS ACROSS DIPSADIDAE.

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An unexpectedly diverse amount of different toxins, some resembling those described in viperids and elapids, has been found in the venom and venom glands of several Dipsadidae species transforming these historically neglected groups into a great model to search for new toxin families. Snake Venom Matrix Metalloproteinases (svMMPs) have been reported in both the venom gland transcriptome and venom proteome of *Thamnodynastes strigatus*, and are thought to occur in more groups across Dipsadidae. In this work, we sequenced and de novo assembled the venom gland transcriptomes of several species across the Dipsadidae family in order to screen for svMMPs transcripts and verify their distribution across snake phylogeny. svMMPs appear to be the main component of the venom gland transcriptomes of two tribes, Tachymenini and Xenodontini, within Dipsadidae and are practically absent in all other groups. We found two types of svMMPs resembling endogenous MMP-9 and MMP-7 domain organizations. In order to understand the evolution of this family of proteins we performed a ML tree search using endogenous MMPs and our annotated toxin sequences. svMMPs appear to have originated from an endogenous MMP-9 and then suffered a series of domain losses similar to that observed in Snake Venom Metalloproteinases (SVMPs) toward a more simple organization. As SVMPs are present in most basal groups, svMMPs appear to be a derived acquisition of the venom of these two tribes, apparently emerging independently in both of them. Moreover, we found a shifting pattern between SVMPs and svMMPs in which their expression levels are inversely proportional, indicating that the proteolytic function may be retained but the proteins in charge of it are changing. Proteomic and functional analyses and a more in-depth curation of more svMMP sequences are needed in order to fully elucidate the evolutionary history of this family of toxins.

8 SUBSTITUTIONS OF RESIDUES IN A LOOP SURROUNDING THE ACTIVE SITE OF A P-I SNAKE VENOM METALLOPROTEINASE ABROGATES ITS HEMORRHAGIC ACTIVITY

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Snake venom metalolloproteinases (SVMPs) are the main toxins responsible for the hemorrhage in viper snakebite envenomation. SVMPs are classified into three classes, according to the presence or absence of different domains (metalloproteinase domain, disintegrin domain, cysteine- rich domain and disintegrin-like domain). The active site of the metalloproteinase domain present in PI, PII and PIII has a consensus zinc-binding sequence (HEXXHXXGXXH). In the different classes of SVMPs there are hemorrhagic and non-hemorrhagic toxins, even though their sequences are highly conserved. The understanding of the structural determinants that predict the hemorrhagic potential of the SVMPs of the PI is relevant given the limited knowledge that exists on this subject. Previous studies by molecular dynamic simulation showed higher flexibility in the first part of the Ω loop surrounding the active site (156-163) in hemorrhagic SVMPs, as compared to non-hemorrhagic SVMPs. In the present work, an abrogation of the hemorrhagic activity of BaP1, a PI SVMP from the venom of *Bothrops asper*, was achieved by the substitution of residues in the first part of the Ω loop by the corresponding residues of a structurally-similar non-hemorrhagic PI SVMP from a related venom. The results suggested that the Ω loop is critical for protein-protein interface and may be involved in the interaction with extracellular matrix proteins, hence influencing the ability of the toxin to bind and hydrolyze basement membrane components. The SVMP with the site mutation completely lost the hemorrhagic activity, and only loss partially the proteinase activity, indicating that this region in the loop plays a key role in the ability to induce hemorrhage. Our findings demonstrate a structural determinant of the hemorrhagic capacity of PI SVMPs.

9 RATIONAL DESIGN OF ANALOGS PEPTIDES FROM TITYUS SERRULATUS SCORPION TOXIN AGAINST PATHOGENIC BACTERIA

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Nosocomial infections have been an emerging and comitant problem for the negative impact on public health. Since the loss of efficiency of pre-existing antibiotics and the number of deaths increasing exponentially and significantly, it is necessary to seek new tools to control these pathogens. The present work focuses on the study of butylatus-1 and -2 based on TsAP-1 antimicrobial peptides sequence from scorpion *Tityus serrulatus*. The analogs peptides were constructed based in repetitive pattern h+HH (h+: positively charged hydrophilic amino acid residues; H: hydrophobic amino acid residues), observed in the TsAP-1 primary sequence studied. After the rational design, the peptides were synthetized by F-moc solid phase strategy and then purified by RP-HPLC C18. For bioassay, the peptides were quantified by absorbance and serially diluted. The peptides were actives against pathogenic bacteria, it were observed that the synthetic peptide TsAP-1 did not present antimicrobial activity against *E. coli* (ATCC 25922) and for *E. faecalis* (ATCC 19433) until 73 µM, whereas the butylatus-1 and -2 presented antimicrobial activity against *E. coli* (ATCC 25922) and for *E. faecalis* (ATCC 19433) in the concentrations of 5.3 and 43.1 µM, respectively. Regarding the hemolytic activity, butylatus-2 presented cellular hemolysis in 43.1µM, however butylatus-1 and TsAP-1 not shown cytotoxicity. Despite the reported data on the good performance of analogs peptides, due to the high action potential of butylatus-1 at low dosages and not shown hemolysis, it stands out as an excellent candidate in the development of a new alternative against infections caused by pathogenic bacteria.

10 IMPORTANCE OF METALLOPROTEINASES IN ENVENOMING BY BOTHROPS ATROX, IN THE BRAZILIAN AMAZON: LOCAL DAMAGE APPROACHES

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Brazil's northern region has registered highest prevalence of snakebites, and *Bothrops atrox* is responsible for majority of accidents with reports of severe local damage. Its venom presents a rich composition of metalloproteases P-III and P-I class, which hydrolyze and bind to extracellular matrix proteins (ECM). According to the lower capacity of antivenom act on local damage, we seek to understand the local complications triggered by envenoming, analyzing the presence of venom/antivenom and investigate the protein profile on the blister content. Five patients who had suffered *B.atrox* snakebite and were attended at Tropical Medicine Hospital, Manaus, Brazil were included in this study. The venom/antivenom presence in the blisters was quantified by ELISA/Western Blotting, the proteomics technique was used to analyzed the blister content. The blisters were collected after 48hrs, and it was possible to identify the presence of venom in the blister. Interestingly, at it this same time we also identified the presence of the antivenom, which could recognize by Western Blotting the region of SVMPs. The antivenom concentration was higher than venom on the blister content, and the venom/antivenom levels had not correlation with the severity of envenoming. Even the antivenom being present in the blister, patients bitten by *B.atrox* suffer severe local damage. These data suggest that other factors are exacerbate in the local damage. Then, we analyzed the blister proteomic profile, and approximately 647 proteins were identified, these include proteases, ECM fragments, which could amplify the proinflammatory effect, such as DAMPs; and immunodulators, such as protein S-100 and C-reactive protein, which are described exacerbated in ulcers due to tissue damage. Thus, our studies contribute to understanding the blister environment of B. atrox envenoming and may add to the development of new strategies to improve the local treatment.

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11 PHYSICAL-CHEMICAL CHARACTERIZATION OF CARBON NANOTUBES-HYDROXYAPATITE NANOCOMPOSITES WITH FIBRIN SEALANT DERIVED FROM SNAKE VENOM

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The Fibrin Sealant developed from Crotalus durissus terrificus (rattlesnake) venom is a heterologous derivative, a thrombin-like enzyme which converts fibrinogen into fibrin. It is a natural, gel-shaped, biodegradable, biosorbent, non-toxic and non-immunogenic product. The mixture of fibrin sealant with multiwalled carbon nanotubes (MWCNT) and nanohydroxyapatite (nHAp) forms a compound with possible potential to accelerate bone regeneration. Here, we prepared a series of nHAp/MWCNT nanocomposites aimed at producing materials that combine similar bone characteristics (nHAp) with high mechanical strength (MWCNT). As first stage, the physical-chemical characterization of this compound mixture was performed. The following groups were analyzed: Hydroxyapatite + PBS (HA); Hydroxyapatite + fibrin sealant (HAS); HAS + Carbon nanotubes 1% (HAS1%); HAS + Carbon nanotubes 2% (HAS2%) and HAS + Carbon nanotubes 6% (HAS6%). Analysis of mass loss, pH and morphological analysis by scanning electron microscopy (SEM) after immersion in PBS in the period of 7, 14 and 21 days were perfomed to physical-chemical characterization of samples. It was verified that the pH of samples remained constant for all treatments and times studied. Fifteen percent mass loss was found in HAS compound during the first 7 days and it was maintained on days 14 and 21. Mass loss of 8% was observed in the groups HA, HAS1%, HAS2% and none for the group HAS6%. Morphological analysis showed greater homogeneity of the samples with nanotubes (HAS1-6%) compared to HAS. Our findings revealed from physical-chemical characterization that the samples HAS1% and HAS2% are most appropriate composites for in vitro and in vivo tests considering the possibility of cytotoxicity (to be evaluated) of the HAS6%.

12 DISTRIBUTION OF METALLOPROTEINASES AND PHOSPHOLIPASES A2 IN SNAKE VENOMS AND RELATIONSHIP TO VENOM TOXICITY

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Crotalus venoms are classified as Type I and Type II according to the expression of SVMPs and PLA2. In Bothrops snakes, SVMPs are predominate in most venoms, but some are rich in PLA2s. In this study, we analyzed the distribution and function of SVMPs and PLA2s in venoms from two Bothrops species presumably representatives of Type I and Type II. We compared the venoms of *Bothrops atrox* and *Crotalus atrox*, with high expression of SVMPs (Type I) and *Bothrops jararacussu* and *Crotalus durissus terrificus*, PLA2 venoms (Type II). B. atrox and C. atrox showed a similar electrophoretic and chromatographic profiles, predominantly with SVMPs, corresponding to 56.47 and 43.11%, respectively, in proteomics analysis. In contrast, PLA2s were predominant in C. d. terrificus and B. jararacussu venoms representing 51.28 and 24.19%, respectively, of isoforms detected by proteomics. Functionally, B. atrox and C. atrox, the type I venoms, showed higher SVMPs enzymatic activity when compared to type II venoms. However, only C. d. terrificus venom showed higher enzymatic activity on PLA2 substrate, which was not observed in *B. jararacussu* venom. Also, C. d. terrificus was the only venom with pronounced lethal activity, probably due to the neurotoxic action of their major PLA2. Our results indicate that the differences in distribution of SVMPs and PLA2s in Bothrops venoms is not as pronounced as in Crotalus. Also, the functional differences observed in Type I and Type II Crotalus venoms is not observed in Bothrops and the role of the predominant PLA2 in *B. jararacussu* has not yet been elucidated. The differential expression of these proteins may be related to the ecological function of the venoms and possible adaptive advantages regarding the toxicity and digestion of prey in nature, which is currently under investigation in our lab.

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13 HEMORRHAGIC METALLOPROTEINASE HF3 FROM BOTHROPS JARARACA VENOM: OBTENTION OF RECOMBINANT DOMAINS DC USING A CELL-FREE EXPRESSION SYSTEM, AND INTERACTION WITH ENDOTHELIAL CELLS

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HF3, a snake venom metalloproteinase (SVMP) from *Bothrops jararaca*, is an extremely hemorrhagic toxin, which degrades extracellular matrix proteins and inhibits platelet aggregation. Various studies have shown the functionality of the non-catalytic domains of SVMPs, and the role of the C-domain as a potential protein-protein adhesive interface and/or a substrate recognition exosite. Endothelial cells are important targets for venom enzymes like the SVMPs, which play an important role in the inflammatory response that occurs during envenomation. Cell-free protein expression system appeared as a tool for the production of recombinant proteins from crude cell extracts and without the use of living cells. This system is efficient to synthesize toxic proteins that generally in vivo inhibit host cell machinery. Objectives: To obtain two recombinant forms of DC/HF3 protein containing distinct site-directed mutagenesis in the hyper variable region: DC/HF3-Mut Acid, presenting substitutions of acid residues to alanine and DC/HF3-Mut Alkaline, that contains substitutions of basic residues to alanine. To investigate the interaction between HF3 and membrane proteins of blood outgrowth endothelial cells (BOECs). Methods: For a cell-free expression reaction, it was prepared an E. coli extract (S30). To express the proteins DC/HF3-Mut Acid, DC/HF3-Mut Alkaline, and wild-type proteins, a small-scale expression was used to optimize the reaction conditions. BOECS were cultivated, and the interaction of HF3 with BOEC membrane proteins was analyzed by the solid-phase binding assay. Results and Discussion: The mutant proteins DC/HF3-Mut Alkaline and the wild-type protein DC/HF3 were expressed in the soluble form. The proteins were purified, and their identity was confirmed by WB using monoclonal anti-6X His antibody and by mass spectrometry. The solid-phase binding assay showed that native HF3 interacts with BOEC's protein membrane.

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14 BRAZILIAN THREE-FINGER TOXINS: WHAT CAN WE LEARN?FROM MOLECULAR FRAMEWORK TO INSIGHTS IN BIOLOGICAL FUNCTION

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Brazilian Micrurus venoms are mostly predominant in Three-finger toxins (3FTx), which belong to a family of small non-enzymatic proteins constituted by around 58 to 80 amino acid residues. In all members of the family, the protein fold is based on three loops of beta strands that reassemble “fingers" extending to a globular and hydrophobic core, stabilized by four conserved disulfide bonds. Latter studies on 3FTx around the world reaffirmed all the diversity in these proteins. In Brazil, not by far we have accomplished the broad variety in their amino-acid sequence suggesting also diversified structure and function. This work aims to conduct an in silico systematic study on all available Brazilian 3FTx. Using sequence and structural information from homologous proteins, we explored their molecular framework as well as their phylogenetic relationship, rising information about their biological function. In attention to our goal, we elaborated a specific guideline for this toxin family. Since the amino acid sequences are too variable, we grouped them according to their best scored structural homologue as predicted by the HHPred software. For each group, we selected one sequence and created a structural model with the Modeller software, based on the predicted homologue. These models will serve as structural representative of each group. By looking at the conserved functional regions and taking into account the information known about the structural homologue, we managed to suggest biological functions for each group of toxins. The phylogenetic relation of these sequences was estimated by maximum likelihood analyzes and a phylogenetic three was constructed including homologous 3FTx already characterized. Our results highlight an astonishing diversity inside this family of mini-proteins, leading to a proposal of a structure-function classification that guides future studies on this abundant toxin.

15 PHOTOBIOMODULATORY PROPERTIES OF THE ALGAAS LASER DIODE ON LOCAL TOXIC ACTIONS INDUCED BY BOTHROPS LEUCURUS VENOM

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Bothropic venoms present coagulant, hemorrhagic, and proteolytic activities leading to an intense local response. Although antivenom effectively neutralizes circulant toxins preventing death, the local events triggered by the venom are not suppressed by serotherapy. Even when envenomed patients are given antivenom, they experience intense pain, edema, bleeding, and myonecrosis that may demand limb amputation in severe cases. *Bothrops leucurus* is widely spread throughout the northeastern coast of Brazil, where it is the main agent of ophidism. This work aimed to evaluate the photobiomodulatory properties of the AlGaAs laser diode on local effects induced by Bothrops leucurus venom (BLV). The action of laser on myonecrosis, hypernociception, edema, and hemorrhage was evaluated, respectively, by determining the serum levels of creatine kinase (CK), the nociceptive thresholds in the electronic von Frey™ test, the increase in paw volume, and the hemorrhagic area following BLV inoculation. Two laser protocols were tested: (1) 660 nm, 40 mW, 120 s, 4.8 J/cm2 and (2) 780 nm, 70 mW, 60 s, 4.2 J/cm2. The application of protocol (1), but not (2), effectively reduced CK levels when evaluated at 3 h after inoculation. Both protocols reduced BLV-induced hypernociception, but with protocol (2) this action had a quicker start. Although the antiedematogenic effect of laser ceased when it was applied during later stages of envenomation, both protocols reduced edema formation for up to 24 h when performed earlier. Neither of the protocols altered BLV-induced hemorrhage. The harmful local effects promoted by BLV remain an untreatable condition and a cause of high morbidity among bitten patients. Laser photobiomodulation is a promising treatment for the local effects of bothropic envenomation. It is a low-cost technology that can be easily introduced as a complementary therapy for bothropic envenoming.

16 USE OF ALTERNATIVE ADJUVANT SYSTEMS TO PRODUCE NEUTRALIZING IGY ANTIBODIES AGAINST BOTHROPS ALTERNATUS VENOM

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The use of adjuvant is a key point in the production of antivenoms and currently immunization schemes mostly consider Freund adjuvant. Anyhow, there are other adjuvants that may have a better performance, particularly when the antivenoms are produced on other platforms different from horses, such as egg yolk antibodies (IgY). When choosing an immunomodulator, also tissue damage in the site of injection or economical costs should be considered. The aim of this study was to evaluate the performance of a commercial Montanide™ adjuvant to produce an IgY-based antivenom against *Bothrops alternatus*. Groups of laying hens (n=2) were immunized via i.m. with increasing doses of the venom (40, 80, 120 µg) at days 0, 14 and 28. Hens from Group I received the venom in saline solution (all injections); hens from Group II received venom emulsified only with Montanide™ (all injections); and Group III were immunized with the venom emulsified with the adjuvant added with inactivated Salmonella (1st injection) or only the adjuvant (boosters). Serum samples were taken 7 days after each immunization and IgY-based antivenoms were obtained by ammonium sulphate precipitation. To evaluate the seroconversion levels in each Group, titers against the venom were analyzed by ELISA. Median effective dose (ED50) of the antivenoms was assessed in mice according to WHO guidelines. Increasing level of antibodies were observed in the hens from Group III after subsequent immunizations, while no seroconversion was observed in hens from Groups I or II. Regarding the potency of the antivenom obtained from hens of Group III, 1 ml IgY antivenom was able to neutralize 500 µg of the venom. In conclusion, Montanide™ commercial adjuvant could be used to produce IgY based-antivenoms against *B. alternatus* but an immune-stimulant component such as inactivated Salmonella is needed to elicit the response.

17 EVALUATION OF AN IGY-BASED ANTIVENOM AGAINST APITOXIN FROM HONEYBEES

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Severity of the envenoming by apitoxin (the venom from honeybees, composed by several peptides) depends on the individual's sensitivity and also on the number of stings. In hypersensitive patients it can even lead to death when no proper treatment is administered. No specific therapy is currently available and then a safe and effective treatment, such as antivenoms, are urgently required. An alternative to mammal polyclonal sera is the use of egg yolk antibodies due to its advantages regarding animal welfare and lower costs of production. In this work, we evaluated the efficacy of an IgY-based antivenom against apitoxin obtained from honeybees (*Apis mellifera*). Laying hens were immunized via i.m. with 100 µg of apitoxin (LD50 9 mg/kg) emulsified with Freund´s complete adjuvant (first inoculation) and Freund´s incomplete adjuvant (3 boosters). Eggs were collected during 10 days after the last immunization and IgY antivenoms were produced by ammonium sulphate double precipitation method and preserved using 0.01 % (w/v) thimerosal. Immunochemical analysis was done by SDS-PAGE and Western Blot. Seroconversion after the immunizations was analyzed in serum by ELISA and the Median Effective Dose (ED50) was determined on a mouse model by mixing 3 LD50 of the apitoxin with increasing volumes of IgY antivenom, according to WHO guidelines. Titers of specific IgY were increased after the subsequent immunizations and IgY antibodies detected the main components of the apitoxin (mellitin, phospholipase A). The ED50 of the antivenom was 20 µg apitoxin / mg IgY. Thus, these results show the feasibility to develop antivenoms based on egg yolk antibodies for the treatment of hypersensitive patients to bee venom.

18 BETA-CARDIOTOXIN, A NOVEL COMPOUND FROM KING COBRA VENOM, SUPPRESSES CARDIAC FUNCTION VIA NON-BETA-ADRENERGIC PATHWAY

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Beta-cardiotoxin (β-CTX), a compound isolated from king cobra (*Ophiophagus hannah*) venom, is a proposed beta-blocking agent (Rajagopalan et al., 2007). In our previous report, we found that β-CTX suppressed cardiomyocyte shortening without changing the calcium transient. The compound also induced this depression in myofilament dynamics without affecting cross bridge cycling kinetics. In order to further elucidate the mechanism of the compound, we tested the effects of β-CTX on isolated cardiomyocytes in the presence of isoproterenol (ISO), the standard beta-agonist. Moreover, post-translational modifications of the proteins involved in beta-adrenergic signaling were also investigated. Findings demonstrated that the pre-incubating β-CTX attenuated the ISO stimulatory effects on both myocyte shortening and calcium transient which were comparable to propranolol. To understand the mechanisms of these effects, we first determined phosphorylation of cardiac myosin binding protein C, cardiac troponin I and phospholamban. Cardiomyocytes were pre-treated with either propranolol or β-CTX, in two conditions, with or without ISO incubation. Before treatment with ISO, no changes in levels of the measured phosphorylation sites were detected between myocytes from controls and myocytes treated with β-CTX. With ISO stimulation, cells pre-treated with β-CTX did not exhibit a reduction in protein phosphorylation induced by ISO. On the other hand, cells pre-incubated with propranolol showed inhibition of ISO effects in all interested phosphorylation sites. We interpret these findings to indicate that the suppression of ISO effects on cardiomyocyte function and calcium transient by β-CTX occur independently of changes in the major phosphorylation sites associated with beta-adrenergic stimulation. Further investigations are required to determine the possibility of other mechanisms involving novel ion-channel or receptor-mediated pathways.

19 PEGYLATING TOXINS: A NEW TREND IN TOXINOLOGY? A SUCCESSFUL EXAMPLE OF A PEGYLATED SNAKE VENOM SERINE PROTEASE

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PEGylation has been used for more than 20 years as a strategy to decrease immunogenicity and improve pharmacokinetic properties of biopharmaceuticals. However, it remains poorly employed in toxinology, even though it may be a promising strategy to empower molecule candidates in therapeutics. rCollinein-1 is a recombinant snake venom serine protease (SVSP) derived from *Crotalus durissus collilineatus* venom, with effects on fibrinogen consumption and inhibitory activity on hEAG channels. This work compared the functional, structural and immunogenic properties of the non-PEGylated (rCollinein-1) and the PEGylated form (PEG-rCollinein-1) of this SVSP. PEG-rCollinein-1 shares similar kinetic parameters with rCollinein-1, presenting comparable Km, kcat and Km/kcat values. PEGylation also maintained its capability of degrading fibrinogen, but strongly reduced its blocking activity on hEAG channels. Both PEGylated and non-PEGylated enzymes showed to be non-toxic to peripheral blood mononuclear cells (PBMC), even in high concentrations, with cell viability higher than 85%. Structurally, circular dichroism (CD) analysis revealed the maintenance of protein conformation after PEGylation, sharing similar content of secondary elements with the non-PEGylated form. Regarding their immunogenicity, in silico analysis indicated four putative amino acid epitopes, located in the surface of the protein structure, near to possible sites of PEGylation. Interestingly, immune response on mice showed PEG-rCollinein-1 was devoid of immunogenicity, even after four sensibilizations, whereas the non-PEGylated enzyme showed low immunogenicity. Consequently, besides reducing the immunogenicity of this SVSP, PEGylation also directed its activity towards hemostasis control, broadening its possibilities to be employed as a defibrinogenant agent in conditions related to imbalances in this system, such as stroke, thrombosis, and pulmonary embolism.

20 EXTRACELLULAR VESICLES FROM BOTHROPS JARARACA VENOM: COMPOSITION AND INITIAL ASSESSMENT OF BIOLOGICAL FUNCTIONS

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Venoms are mainly composed by proteins originally believed to be secreted by the conventional protein secretion pathway. This concept has been questioned based on evidences of extracellular vesicles (EVs) occurrence in venom-producing glands and fresh venom. EVs are membrane-enclosed particles secreted by cells for cellular communication. Their specific cargoes modulate the recipient cell's function and physiology. Although the existence of EVs in snake venoms is unveiled, their roles in cross-organism communication are completely unknown. To advance in the comprehension of their biological function we firstly isolated and characterized EVs present in *Bothrops jararaca* venom. Fresh *B. jararaca* venom pools were submitted to standard EVs isolation procedures by sequential centrifugation, resulting in two populations of vesicles. Electron microscopy and Nanoparticle Tracking Analysis revealed typical EVs' morphology and size range, mostly at 100 nm. The EVs-associated proteins were analyzed using shotgun proteomics, which allowed the identification of proteins, including actin, flotilins, annexins and syntenin considered EVs markers in other organisms. Among the most abundant proteins are those shown to play key roles in several biological processes, such as cancer malignancy and host-parasite interactions. In parallel, the uptake of venom EVs by muscle cells and macrophages were observed by Dil-labeled particles using fluorescence microscopy and 3D deconvolution. Currently, the investigation of the biological relevance of some identified proteins and the EVs themselves is in progress. The ontologies of *B. jararaca* EVs proteins and results of this study are crucial to comprehension of the potential EVs-mediated cross-talk between snakes and other organisms and also the involvement of EVs in venom processing and production.

21 BOTHROPS MOOJENI WHOLE VENOM ACTIVATES PREADIPOCYTES TO RELEASE PROINFLAMMATORY MEDIATORS

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Bothrops moojeni snakes cause most of ophidic accidents in the center-western and southeastern areas of Brazil and trigger severe local inflammatory reaction in their victims. However, the cellular elements involved in this venom-induced effect are not completely clarified. The white adipose tissue (WAT) is an endocrine organ capable of releasing a range of inflammatory mediators and immunomodulatory molecules, such as adipocytokines. Preadipocytes constitute the major cell type in adipose tissue and are directly related to biosynthesis of proinflammatory mediators. However, the effect of snake venoms on these cells is still unknown. In this study, we investigated the effects of B. moojeni snake venom (Bmv) on preadipocytes, with focus on: (1) release of PGE2 and IL-6 and (2) mechanisms involved in production of PGE2. 3T3-L1 murine preadipocytes were used. These cells were cultivated in DMEM (10% BFS) until confluence and then incubated with Bmv (1 µg/mL) or DMEM supplemented with BSA 0.2% (negative control) for selected time intervals. Release of IL-6 and PGE2 was determined by EIA. Participation of COX-1 and -2 in venom-induced release of PGE2 was evaluated by pharmacological interventions. Stimulation of cells with Bmv induced a significant release of PGE2 after 12 and 24 h and IL-6 at 24 h. Pre-treatment of cells with SC-560 (1 µM), a COX-1 inhibitor, abrogated PGE2 release induced by Bmv whereas pretreatment with the COX-2 inhibitor, NS-398 (1 µM), significantly reduced PGE2 release. In conclusion, obtained results indicate the ability of Bmv to directly activate preadipocytes and induce biosynthesis of important inflammatory mediators (PGE2 and IL-6) by these cells. Moreover, production of PGE2 occurred via COX-1 and COX-2 pathways. Altogether, these data demonstrate for the first time that preadipocytes are cellular targets for snake venoms and potential sources of inflammatory mediators during envenomation by *Bothrops spp* snakes. Support: CAPES, FAPESP and CNPq

22 EXPOSURE OF LACTATING WISTAR RATS TO TITYUS BAHIENSIS SCORPION VENOM: EFFECTS ON CYTOKINE AND GROWTH FACTOR (BDNF) LEVELS OF OFFSPRING.

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Scorpionism is a serious public health problem in Brazil and in the world. According to the Brazilian Ministry of Health, accidents involving women during the fertile time of their lives accounted for more than 40% of the cases between 2007 and 2012. Thus, the possibility of a breastfeeding woman being stung by a scorpion is great and the study of possible consequences for the newborn becomes extremely important. Previously we observed that the administration of *Tityus bahiensis* scorpion venom in lactating female rats affects the physical, reflexological and behavioral development of offspring both in the perinatal and adult phases. On the other hand, the mechanisms responsible for these effects are not yet established. Cytokines and growth factors are fundamental for an adequate development of the individual. In order to better understand the issue, we evaluated the levels of cytokines and growth factor BDNF in the blood and brain of offspring from female rats injected with *T. bahiensis* venom on the 2nd (PND2), 10th (PND10) or 16th (PND16) day of lactation. BDNF levels were not altered in the offspring blood, but they were decreased in brain structures of the PND2 group and increased in the PND10. The most changes in cytokine levels occurred in the brains of the PND2 group, with decreased levels of IL-1α, IL-1β, INF-γ and IL-6 in various structures. The PND10 group had an increase in the levels of INF-γ and IL-6 in the cerebellum. The PND16 group had a decrease in IL-1β, INF-γ and IL-6 levels in the hippocampus and cerebellum. Our findings show that maternal envenomation on a single day of lactation promotes changes in the biochemical parameters of the offspring, which can be deleterious, especially in the early postnatal phase, when the neuroimmunoendocrine system is more immature and susceptible to environmental interference.

23 ANTI-TUMORAL POTENTIAL OF A NOVEL DISINTEGRIN ISOLATED FROM PORTHIDIUM LANSBERGII LANSBERGII VENOM ON BREAST CANCER CELLS

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Disintegrins from snake venoms recognize and bind to cell surface integrins with high specificity. Some of these receptors are differentially expressed by cells in the process of cancer development and progression. In this study, we describe for the first time the sequencing, modeling and the antitumoral effects of a novel disintegrin isolated from *Porthidium lansbergii lansbergii* venom on breast cancer cells. This RGD-containing disintegrin has a low molecular weight and showed potent inhibition of platelet aggregation on ADP and collagen-induced human plasma and also displayed inhibitory effects on the adhesion and migration of breast cancer cell lines MCF7 and MDA-MB 231, without affecting non-tumorigenic breast MCF-10A. The disintegrin also prevented MCF7 cells to adhere to fibronectin and collagen by interfering with α2 and/or β1-containing integrins. This result was verified experimentally by flow cytometry and confocal microscopy. In addition, the protein also inhibited in vitro angiogenesis on human endothelial cells-HUVEC. Our results display the first report on the characterization of a disintegrin isolated from Porthidium snake venom and introduce it as an attractive model for elucidating the antitumor effects of disintegrins against breast cancer development.

24 DISCOVERY AND CHARACTERISATION OF NOVEL PEPTIDES FROM AMAZONIAN STINGING ANT VENOMS WITH ANTIPARASITIC ACTIVITY

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Parasites remain a serious challenge for veterinary medicine and livestock production worldwide. The Australian sheep industry is severely threatened by gastrointestinal nematodes, with control costs exceeding $430 million AUD annually. Control of blowflies, which cause myiasis, cost a further $222 million. Widespread drug resistance necessitates the development of new therapies to control these infections. Venoms have evolved over millions of years to become cocktails of selective and potent bioactive molecules, but their potential as sources of novel antiparasitic compounds has been under explored. We screened over 250 crude venoms from a diverse panel of spiders, scorpions, assassin bugs, caterpillars, marine snails, ants and wasps for anthelmintic activity against the blood-feeding small ruminant nematode *Haemonchus contortus* in a larval development assay. At 0.2 g/l crude venom the hit rate for the screen was 21%, with hits dominated by arthropod venoms, particularly tarantulas and ants. Candidate venoms were characterised using bioassay-guided fractionation to identify the active compounds. Five novel small linear peptides were identified from Amazonian stinging ant venoms. Peptides were synthesised and found to have low micromolar activity against *H. contortus* larvae (2–30 μM). The peptides were also injected into adult *Lucilia cuprina* sheep blowflies, resulting in paralysis within 1 hour of injection at moderate doses (PD50 = 0.5–38 nmol/g) and lethality at high doses. The peptides were counter-screened for cytotoxicity, haemolysis and activation of sensory neurons (as a proxy for pain). One peptide showed >10-fold selectivity for *H. contortus* over mammalian cell lines and no sensory neuronal activation. We subsequently generated mutant peptides to improve potency and selectivity against *H. contortus* and *L. cuprina*, but found that these activities were closely correlated with their cytotoxic and haemolytic activities. Overall, our data indicate that arthropod venoms may be a useful source for antiparasitic drug discovery.

25 INVESTIGATING THE NEUROMODULATORY EFFECTS OF CORAZONIN IN EMERALD JEWEL WASP VENOM

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The parasitoid jewel wasp, *Ampulex compressa*, induces hypokinesia (a sleep-like state) and reduced fecundity in its host, the American cockroach *Periplaneta americana*, through direct envenomation of its central nervous system. A proteomic screening of the venom identified over 250 protein components in the venom, with many not being observed before in arthropod venoms nor found to play a role in modulating insect locomotion. Of the multitude of toxins identified, the presence and function of corazonin was investigated as it was able to bind to *Rhodnius proxilus* corazonin receptors. Corazonin is a highly conserved peptidergic neurohormone found within all insect orders except Coleoptera (beetles). Despite its conserved sequence, its function between insect genera varies greatly. A recent study revealing the involvement of corazonin in the behavior switching of ponerine gamergates to infertile workers associated Ampulex corazonin's involvement in suppressing fecundity. These findings led us to investigate the function of corazonin in the venom via injecting Ampulex corazonin into the brains of virgin females. Following treatment, changes in fecundity was monitored by measuring relative gene expression of vitellogenin yolk proteins, average ovariole protein content, and average ovariole volume size in stung and corazonin-injected females. Changes in cockroach fecundity was compared to non-treated and saline-injected controls. Both the sting and corazonin injections resulted in significant decreases in vitellogenin gene expression as well as a reduction in average ovariole protein content and ovariole size in virgin female cockroaches. Thus, it is suggested that the role of corazonin in the venom may be to suppress ovary development and preserve energy within females. Our findings elucidate alternate mechanisms of how venoms can inhibit reproductive abilities as well as provide further insight on the effects corazonin in the central nervous system of arthropods.

26 POLYVALENT ANTINEUROTOXIC ARACHNIDIC ANTIVENOM. PRELIMINARY REPORT.

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In Argentina sting or bite of three different arachnids may produce human neurotoxic envenomations that can lead to death. These are spiders of Latrodectus and Phoneutria genera and scorpions of Tityus genus. Although physiopathology of the envenomations and the toxins of these venoms are very different and death, if it occurs, is mainly due to cardiac failure and pulmonary edema or respiratory stress in the case of scorpions, the clinical signs can be very similar and difficult to differentiate, especially for non-experienced physicians. Anamnestic data are not always reliable or absent, especially in pediatric cases. This poses a serious problem for therapeutic success, as the fast instauration of a specific treatment is vital, and this demands the rapid application of antivenom. In those cases lacking proper identification of the arachnid, the use of a polyvalent antivenom could help to avoid binding of the majority of the different toxins to their target sites and thus enhance the success of the treatment. We produced an equine experimental anti Phoneutria-Latrodectus-Tityus antivenom, applying the classical procedures. The hyperimmune serum or its purified IgG were able to neutralize the different venoms. Mice (CF-1, 18-22g) were fully protected against a challenge dose of 3.0LD50s of Latrodectus spp. venom incubated with 100µl of crude serum. One ml of crude serum, protected guinea pigs (Hartley) against 1.5 MMD of *Phoneutria nigriventer*. The purified IgG of the antivenom protected 100% of mice challenged with 3.0LD50s of *Tityus trivittatus* venom, in a dose of 250µl. Considering the neutralizing potency of the specific antivenoms currently used in Argentina for the treatment of these envenomations, the antivenom produced could be a valuable therapeutic option, since a 5.0ml vial would have a similar or higher neutralizing potency than the one required by the sanitary authorities for this type of product.

27 SOME MOLECULAR PROPERTIES OF AN ACID PHOSPHOLIPASE A2 FROM THE VENOM OF BOTHROPS ATROX SNAKE AND ITS RELATIONSHIP WITH MYOTOXICITY

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Phospholipases A2 (PLA2) from snake venoms are enzymes with highly variable biological effects due to different isoforms found that are directly involved in envenomation. On the other hand, *Bothrops atrox* is responsible of the vast majority (~87.6%) of reported snakebites in Peru, which are characterized by severe myotoxic effects. For this reason, this research aimed to purify an acidic isoform of PLA2 from B. atrox venom in order to characterize at molecular level and evaluate its role on myotoxicity. For purification, three chromatographic steps in DEAE-Sephadex A50, Sephadex G75 and anion exchange-medium pressure chromatography system were used. From 120 mg of venom, it was purified 0.275 mg of BaPLA2a with a specific activity of 34.1 U/min/mg and a MW of 14.5kDa by SDS-PAGE. Also, myotoxicity assays on BaPLA2a show no activity, however, when BaPLA2a and myotoxic basic PLA2 were combined, an increase of 21.58% in activity was obtained. For molecular analysis, 40 mg of venom sample were used to obtain 77ng/µL of total RNA and after reverse transcription; 541ng/µL of cDNA was obtained while an amplicon amplified by PCR was around 480 bp. In addition, based on sequence of cDNA, the primary structure of BaPLA2a is formed by 124 amino acids with conserved domains, the Asp 49 residue was identified join to His48 in the catalytic center and the isoelectric point deduced correspond to 4.41. This study will allow cloning the cDNA and expressing it in eukaryotic system for its characterization.

28 METALLOSERRULASES 3 AND 4 FROM THE TITYUS SERRULATUS SCORPION VENOM AND ITS INFLAMMATORY PROPERTIES.

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Scorpionism has grown alarmingly in Brazil in recent years. The occurrence of accidents is associated with the adaptation and proliferation of scorpions in urban areas, where they find shelter, food and absence of natural predators. Along with this, the *Tityus serrulatus*, the species responsible for the greatest number of serious accidents, reproduces by parthenogenesis. The *T. serrulatus* venom is a complex mixture of components which can affect the majority of physiologic human systems. Recent transcriptomics studies showed that metalloproteases make up around 30% of the venom's components. However, there is little information about this class of toxins and its possible role in the envenomation. Metalloserrulase 3 and 4 (TsMS 3 and TsMS 4) were recently purified and characterized as possible neuropeptidases, since both cleave human neuropeptides such as dynorphin and peptides from the neuropeptide Y family, in in vitro studies. In this work, we aimed to analyze the effects of TsMS 3 and 4 on pro- and anti-inflammatory cytokines levels using murine peritoneal macrophages (BALB / c mice). For this, both proteases were purified after two chromatographics steps, and identified by mass spectrometry analyzes. Our results indicate that TsMS 3 and TsMS 4 lack cytotoxicity at the concentrations tested (1 µg/mL and 5 µg/mL), and can induce increased production of proinflammatory cytokines, IL-6, MCP-1 and TNF-α in murine macrophages. Especially, we have observed that TsMS 3 is capable of increasing the level of TNF-α in a manner comparable to LPS (used as a positive control). In addition, TsMS 3 cleaves a fluorescent substrate analogous to the pro-TNF-α, suggesting a possible TACE-like activity. These results indicate that metalloserrulases, particularly TsMS 3, may play a role in the inflammatory process present in *Tityus serrulatus* envenomation, and we are looking for this confirmation.

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29 ACTIVITY IN TWO KEY TOXIN GROUPS IN AUSTRALIAN ELAPIDS SHOW A STRONG CORRELATION TO PHYLOGENY BUT NOT TO DIET

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We measured the activity of two enzyme groups – phospholipase A2 (PLA2), and L-amino acid oxidase (LAAO) – in the venom of 39 species of Australian elapids (40% of terrestrial species diversity). PLA2 activity ranged from 0 to 481 nmoles of chromophore/min/mg of venom, and LAAO activity ranged from 0 to 351 nmoles of H2O2/min/mg of venom. Phylogenetic comparative methods, implemented in BayesTrait showed that venom activity was strongly correlated to phylogeny but not to diet. Some species/individuals lacked activity in one protein family suggesting that snake venoms are redundant systems and that lack of activity of one protein family may not incur a significant fitness cost. Testing for accelerated evolution of these enzyme groups using phylogenetic comparative methods showed strong evidence for faster initial rates of change for LAAO (delta parameter mean 0.2) but no such pattern of evolution in PLA2 (delta parameter mean 0.9), suggesting that PLA2 may confer a higher ongoing fitness benefit. *Notechis scutatus* showed remarkable intra-specific variation in LAAO activity within populations that was not correlated to geographical distribution. LAAO activity was absent in both Vermicella and the Pseudonaja/Oxyuranus clade supporting the proposed relationships among these disparate taxa. We found two examples of venom activity differences in sister-species with similar diets that cannot be convincingly explained by positive selection, suggesting that genetic drift (founder effect), may in some instances play an important role in shaping venom composition.

30 THREE-FINGER TOXINS AND AUSTRALIAN ELAPID VENOMS: ARE THEY IMPORTANT?

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Three-finger toxins (3FTxs) are the dominant toxin family in elapid venoms, assumed to be important in prey capture and possibly post-synaptic neuroxicity in humans. We investigated the composition of Australian elapid venoms focusing on the presence and importance of 3FTxs.

We included venoms from 35 species (17 genera) of Australian elapids representing 8 of the 10 major terrestrial clades. Each venom was subjected to reverse-phase high-performance liquid chromatography (RP-HPLC). HPLC profiles were examined visually, confirmed for the presence of 3FTxs and area under the curve (AUC) estimated for identifiable peaks. The 40 minute retention peak on the RP-HPLC was identified as containing 3FTxs based on previous published HPLC profiles of Australian elapids, and confirmed by mass spectrometry for selected venoms. Based on the AUC of this 40 minute first peak, Australian elapids contained a median proportion of 5.4% of 3FTxs (interquartile range: 1.2 – 9.5; range: 0 to 42%). Some species (and possibly entire genera) in the Rhinoplocephalus/Suta clade have effectively lost the 3FTx protein family. Two species' venom contained a large proportion of 3FTx, 33% and 42% of total venom. There appeared to be only a weak phylogenetic relationship at genus level, but no dietary association with levels of 3FTxs.

Discussion

Australian elapid venoms appear to be contain much smaller amounts of 3FTxs compared to other elapids worldwide which commonly contain greater than 50% 3FTxs. The weak phylogenetic relationship and lack of correlation to diet and foraging strategy could suggest that genetic drift may be more influential than positive selection in determining the abundance of this protein family in Australian elapids.

31 SYSTEMIC AND VASCULAR ACTIONS OF LEPTODEIRA ANNULATA (BANDED CAT-EYED SNAKE; DIPSADIDAE) VENOM

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The venom composition and activities of most rear-fanged snakes are largely unknown. In this work, we investigated the action of *Leptodeira annulata* venom in anesthetized rats and in vascular tissue in vitro. Rat aortic, mesenteric and pulmonary artery rings were mounted in organ baths containing aerated Krebs solution. After stabilization, the rings were contracted with KCl (40 mM) and then pre-contracted with phenylephrine (PE; 1 µM) followed by addition of acetylcholine (1 µM) to assess endothelial function. The rings were pre-contracted again with PE and venom (10-30 mg/ml) was added. Some experiments were done in aortic and mesenteric rings with or without endothelium and in the presence of EDTA (1 mM; metalloprotease inhibitor), varespladib (1 mM; PLA2 inhibitor), indomethacin (10 µM; cyclooxygenase/COX inhibitor), L-NAME (100 µM; nitric oxide synthase inhibitor), ODQ (10 µM; soluble guanylate cyclase inhibitor), KT5720 (100 µM; protein kinase A inhibitor), 4-aminopyridine (1 mM) or tetraethylammonium (10 µM) (both voltage-gated K+ channel blockers). The hemodynamic responses were studied in isofluorane-anesthetized rats cannulated for measurement of arterial blood pressure, heart rate, ECG and respiratory rate. Samples of lung, liver, kidney and heart were collected for histopathological analysis. The venom relaxed the three types of vessels, with aortic rings being the most sensitive. Endothelium removal partially attenuated the aortic relaxation but enhanced it in mesenteric rings. Pre-incubation of aortic rings with indomethacin, ODQ, L-NAME, EDTA and varespladib attenuated the relaxation; the other inhibitors had no effect. The venom did not alter the hemodynamic parameters, although hemorrhage, inflammation and extensive thrombus formation occurred in the lungs, with no damage to other organs. These results show that *L. annulata* venom relaxes primarily aortic rings in vitro via endothelium-dependent mechanisms involving NO/GC/GMPc and COX pathways mediated by venom metalloproteinases and PLA2. The lack of hemodynamic alterations despite pulmonary thrombosis requires further investigation.

32 BIOCHEMICAL CHARACTERIZATION OF THE MAJOR TITYUS BAHIENSES VENOM TOXINS

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Scorpion toxins have long been studied from different perspectives, such as the clinical, medical and biotechnological approaches. The peptide neurotoxins are the major components of the venom; therefore, they have been considered as potential tools for the structural and functional characterization of the ion channels due to their neurophysiological effects, including the pharmaceutical and pesticides industries.

Nevertheless, Tityus venoms, particularly *Tityus bahiensis* venom remains poorly biochemically characterizes. In spite toxins acting in ionic channels, there are still other described venom peptides presenting diverse biological activities, such as antimicrobial, hypotensive, anti-inflammatory and enzyme inhibition, for example. Biotechnological uses of these peptides would include assessing neurological diseases, such as apoplexy, epilepsy and cerebral palsy. Taking into account the number of bioactive molecules in the venom of T. bahiensis, our proposal was to decomplex and characterize the venom through chromatographic, spectrometry (MALDI-TOF) and proteomic techniques (LC-MS/MS).

We were able to optimize an C18-RP-HPLC chromatography separation methodology and obtained 27 fractions, in which the mass spectrometric and proteomic analyses revealed 116 proteins, being 33 (19,87%) new proteins not previously characterized for this venom and 94 (81,03%) homologous proteins to other arachnids. Such proteins may be isoform, presenting diverse mechanisms of actions. Our results also include proteins with potential antimicrobial applications, like microporins and scorpine-like peptides, besides putative enzyme inhibitors.

These preliminary results clearly indicate that there are still a myriad of proteins to be identified and characterized in the venom. Additionally, biological tests will still necessary in order to fully characterize their biological roles.

33 CHARACTERIZATION OF INSECTICIDAL PEPTIDES FROM THE VENOM OF THE NORTH AFRICAN SCORPION, BUTHACUS LEPTOCHELYS

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Scorpions use venom to capture prey and protect themselves from predators. Scorpion venom is a complex mixture of bioactive peptides, which show various biological activities such as insecticidal, antimicrobial or hemolytic effects. Many peptides have been identified from various scorpion species, but venoms of minor species remain largely unstudied. In this study, we chemically and biologically characterized components of the venom of the North African scorpion *Buthacus leptochelys*.

The presence of insecticidal components was confirmed by the symptom observed after the injection of the crude venom into abdominal cavity of crickets, and the LD50 value was 30 ng/mg body weight. Mass spectrometric analyses revealed that the venom contained 148 components, and bioassay-guided fractionation using RP-HPLC led to the isolation of 4 insecticidal peptides, Bl-1~4. Of these, Bl-1 showed the strongest insecticidal activity, whose structure was determined by a combination of Edman degradation and MS/MS de novo sequencing analyses. Bl-1 consists of 67 amino acid residues, having 4-intramonecular disulfide bridges. A BLAST search revealed that Bl-1 shared high sequence similarity with scorpion α-like toxins that act on sodium channels of insects and mammals.

34 COMPARISON OF THE EFFICACY OF TWO MONOVALENT EXPERIMENTAL ANTIVENOMS FOR BOTHROPS ASPER FROM COLOMBIA

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The antivenom is the only specific therapy for treating snakebite. Our objective was to test the efficacy of two experimental monovalent antivenoms developed with *Bothrops asper* venom from the Andina (ANDI) ecoregion of Colombia. The project was approved by the ethics committee of the Pontificia Universidad Javeriana, the Institutional Committee for the Use of Laboratory Animals and the Uniagraria Foundation. The lethal dose 50 and effective dose 50 were carried out in mice Balb-c, between 18-20g, of the Comparative Biology Unit (UBC) of the Javeriana University. A 4-month immunization schedule was performed in a creole equine using *Bothrops asper* ANDI venom. Both antivenoms were manufactured from the same pool of hyperimmune plasma with the Bothrops asper venom from Atlántica (ATL) ecoregion. The whole immunoglobulins IgG antivenom was produced by caprylic acid precipitation, while the F(ab)2 was generated by pepsin digestion and caprylic acid precipitation. Electrophoresis was performed to check the antivenom´s weight. The data was analyzed using the Prisma-statMate combined software, San Diego, CA. The venom potency of *Bothrops asper* ANDI was 57.5 mcg/mouse, 2.8 +/- 0.3 mg/kg and for *Bothrops asper* ATL at 45.7 mcg/mouse, for 2.3 +/- 0,2mg/kg. The quantification of total proteins for the whole immunoglobulins was 9.8 +/- 1.2 mg / ml, while for the F(ab)2 it was 11.2 +/- 0.8 mg / ml. The neutralizing capacity was calculated at 1.4 mg/ml for whole immunoglobulins and at 1.75 mg/ml for F(ab)2. We found both antivenoms were efficient to neutralize the venom of *Bothrops asper* ATL, without statistically significant differences, which is justified by sharing some proteins in their venom, even though they are of different ecoregion, but also suggests that part of the neutralization is by antivenom cross-reactivity.

35 CLINICAL MANIFESTATIONS IN OPHIDIAN ACCIDENTS BY SERPENTS OF THE BOTHROPS GENUS IN THE STATE OF AMAZONAS: DIFFERENCES BETWEEN THE SOLIMÕES AND JURUÁ RIVERS.

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In the state of Amazonas, more than 80% of ophidian accidents are caused by snakes of the genus Bothrops. Environmental characteristics facilitate the diversity and maintenance of the genus in the region and in addition, the approach of the man of these environments promotes the increase of the risk of accidents. In order to characterize and identify the post-accident clinical manifestations caused by Bothrops, our study used field interviews in different communities along two regions of the state on the banks of the Solimões and Juruá rivers from January to April 2019. In this period, 157 victims were interviewed. Initially, we highlight the victims who reported having suffered 2 (n=25, 15.92%), 3 (n=24, 15.92%) or more times (n=9, 5.73%). Of the local manifestations present, pain (97.45%) and edema (92.99%) are commonly reported and necrosis (40.13%) was reported as the most frequent local complication for both localities. Interestingly, there were differences between the river banks in regard to systemic manifestations. While the respondents of the Solimões River reported that the most present systemic manifestations after the accident were hemorrhagic (55.56%) followed by myolitic/hemolytic (47.22%); the interviewees of the Juruá River reported the highest presence of myolitic/hemolytic manifestations (50.57%) followed by neuroparalytic (18 cases, 45.00%). Vagal manifestations (31.21%) and renal (27.39%) were also reported by patients. Of the total number of interviewees, 88 of these (56.05%) did not seek health care, and 24 patients (37.68%) had some type of sequelae. The presence of scars (96.17%), loss of movement (7.85%), amputation (3.14%) were also reported. Several studies carried out in the region aimed at evaluating the venom properties of snakes of the genus Bothrops show that there may be differences in the toxicity of venom from the same snake species, thus causing different clinical manifestations.