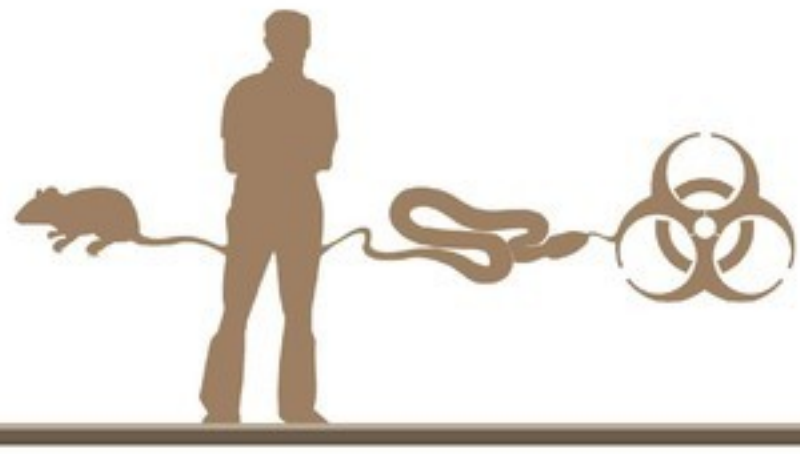
1. Venoms and Envenomations



XIV Reunião Científica Anual do Instituto Butantan
05 a 07 de Dezembro 2012, São Paulo, SP

1.01 Detection of distinct metalloproteinases in the venom of *Bothrops neuwiedi* snake

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Introduction: The SVMPs are responsible for most of the symptoms of *Bothrops* snakes envenoming, acting on different targets of hemostasis. **Objectives:** In this work, our aim is to demonstrate the diversity of SVMPs in the venom of *B. neuwiedi*. **Methods:** Venom was obtained from snakes kept in captivity at Butantan Institute. The venom was fractionated by HPLC (Agilent) in C-18 column or by HiPrep 16/60Sephacryl S-200 followed by Mono-Q 5/5 columns (Äkta, GE). The fractions were analyzed by SDS-PAGE and by the reactivity with specific antibodies anti-SVMPs by dot-blot. **Results and Discussion:** Fractionation of the venom by HPLC showed the existence of at least six different SVMPs. To isolate these SVMPs, the venom fractionated by column Hiprep followed by Mono-Q allowed the identification of at least two distinct fractions with SVMPs class P-III with different hemorrhagic activity, and two fractions with SVMPs class P-I. To demonstrate the activity of the venom on blood coagulation, the crude venom and isolated fractions were tested. Significant decrease of the clotting time was observed after addition of 10 ng of crude venom. Isolated fractions showed distinct activities on blood clotting. Analyzing the data, we could see the high diversity of SVMPs in the venom of *B. neuwiedi,* which presented strong procoagulant activity. We demonstrated the presence of metalloproteinase from classes P-I and P-III. Our data indicate the structural diversity of SVMPs and suggest that different components of coagulation system may be targeted by these toxins, thus accounting for the strong procoagulant activity of *B. neuwiedi* venom.

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XIV Reunião Científica Anual do Instituto Butantan
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1.02 Proteomic analysis of the proteolytic activity of HF3, a hemorrhagic metalloproteinase from the venom of *Bothrops jararaca*, upon human plasma

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Introduction: Snake venom metalloproteinases (SVMPs) are found mainly in viperid venoms and are important players in the local hemorrhage and pro-inflammatory pathogenesis observed upon envenomation. SVMPs are classified in three main classes depending on the organization of their structural domains (P-I, P-II and P-III). The members of the P-III class of SVMPs and the ADAMs/ADAMTSs share homologous disintegrin-like and cysteine-rich domains. This structural similarity has guided a number of functional assays currently employed in toxinology studies. HF3 is a P-III class SVMP, which is extremely hemorrhagic, and shows a minimum hemorrhagic dose of 240 fmol on the rabbit skin. **Objectives:** The aim of this study was to evaluate the substrate repertoire (degradome) of HF3 on plasma *in vitro*, in order to identify plasma proteins that are affected by HF3 in the hemorrhagic process. **Methods:** Human plasma was depleted either of albumin alone using affi-gel blue chromatography or of the 20 most abundant proteins using immunoaffinity chromatography, incubated with HF3 at a 1:100 enzyme-to-substrate ratio, at 37°C for 2h, and submitted to precipitation with acetone. Peptide and protein fractions were analyzed separately by LC-MS/MS using a LTQ Orbitrap Velos mass spectrometer. The peptide fraction was examined in order to find hydrolysis products resulting from the proteolysis by HF3. **Results and Discussion:** Comparing the lists of peptides detected in the control and HF3-treated plasma samples we found a large number of peptides present only in the HF3-treated plasma. The individual analysis of proteins from which these peptides were derived showed that alpha-2-antiplasmin, alpha-2-HS-glycoprotein, alpha-2-macroglobulin, apolipoproteins A-II, A-IV, C-II, C-III, and F, fibrinogen, gelsolin, glutathione peroxidase 3, inter-alpha inhibitor H4 and inter-alpha-trypsin inhibitor H3 were cleaved upon incubation of plasma proteins with HF3. These preliminary results confirm previous studies showing that fibrinogen and alpha-2-macroglobulin were substrates for SVMPs and underscore new targets of HF3 in human plasma, particularly proteinase inhibitors.

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XIV Reunião Científica Anual do Instituto Butantan
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1.03 A new naturally occurring pentapeptide KEILG from *Tityus serrulatus* venom reduces EP24.15 activity *in vitro*

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Introduction: Scorpions are chelicerates that have a medical relevance because they are the major cause of human envenomation by animals in Brazil, whereas the main species is the *Tityus serrulatus*. Most of the functions attributed to *T. serrulatus* venom (TsV) are related to molecules that act upon ion-channels. Moreover, the presence of other biologically active peptides in TsV is poorly explored. Through natural selection, scorpion venoms molecules act upon certain physiological mechanisms which are shared by a great variety of organisms, including the human being. Therefore it is probable that scorpion venom peptides can be prototypes for the development of new drugs. Thimet Oligopeptidase (EP24.15) is a metallopeptidase that was shown *in vivo* to participate in antigen presentation through MHC-I. Actually, EP24.15 has been successfully used to identify new cytosolic peptides in the human HEK29 cell line. **Objectives:** This work aims to find in TsV new bioactive peptides able to modulate EP24.15 activity. **Methods:** The TsV Peptide Pool (PP) was sequentially fractionated through HPLC-C18 and, in parallel, by enzymatic screening using EP24.5, until obtaining the pure peptide. After, the peptide was sequenced by mass spectrometry analysis. **Results and Discussion:** The results of MS/MS analyses revealed a peptide, KEXXG, where X could represent I/L. We observed a similarity between $K^1E^2X^3X^4G^5$ and the propeptides of potassium channel toxins (β-KTx) described in *Tityus* species. All known sequences had a Leu in P4 position, showing to be a conserved residue among species. On the other hand, the residue in the P3 position was reported as Val and also as Ile. Although we found a peptide of expected size, we faced a challenge, as the mass spectrometry technique employed here couldn't determine the correct AA at the indicated positions. In this scenario, we decided to synthesize KELLG and KEILG to observe its performance at RP-HPLC and inhibition screening. We observed that the KEILG fragment is the sequence present in the TsV, but both peptides can inhibit the EP24.15. To our surprise, we observed different mechanisms for each one: KELLG is a competitive inhibitor (Ki = 84 μM) and KEILG acts as a classical uncompetitive inhibitor (Ki = 16 μM). So, although functions attributed to TsV are related to active molecules on ion-channels, we described a new naturally occurring pentapeptide discovered through enzymatic assay selection, using EP24.15. Our results can also indicate a new processing of β-KTX able to produce other biologically active peptides.

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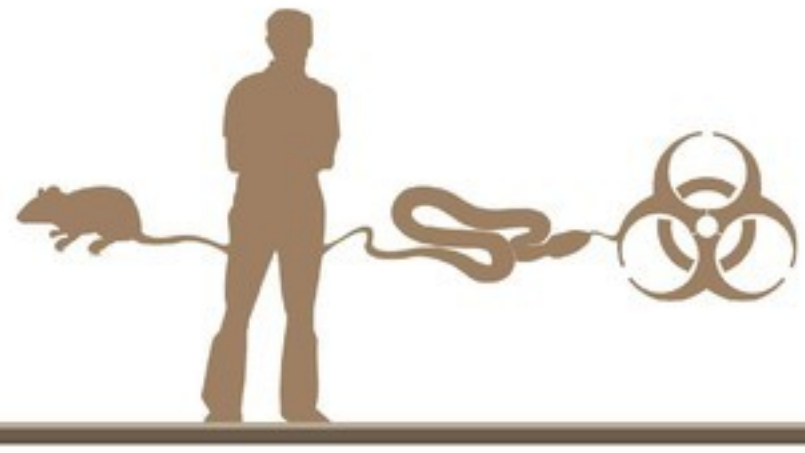

XIV Reunião Científica Anual do Instituto Butantan
05 a 07 de Dezembro 2012, São Paulo, SP

1.04 Analysis of the individual variation of the venom proteome of *Bothrops jararaca* newborn specimens

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Introduction: The venom of *Bothrops jararaca* is one of the most explored in Brazil due to the large geographical distribution of this snake and the number of envenomation cases. Although a number of toxins have been isolated from this species, the use of powerful tools, like proteomic and transcriptomic techniques, have improved the understanding of this venom as a whole. Previous studies have demonstrated individual, sex-based and ontogenetic variations in *B. jararaca* venom. However, the individual variability among newborn specimens is poorly known. **Objectives:** The aim of this study is to analyze the individual proteomic profile of toxins in the venom of newborn specimens of *B. jararaca*. **Methods:** Venom from 21 newborn specimens of *B. jararaca* born in captivity at the Herpetology Laboratory of Instituto Butantan was milked and stored at -20°C until use. SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was carried out followed by specific staining for total proteins and glycoproteins. Casein, L-BAPNA and citrated human plasma were used to evaluate the proteolytic, amidolytic and coagulation activities, respectively, of the individual venoms. The metalloproteinase subproteome was evaluated by Western blot analysis, using a polyclonal antibody against bothropasin, a P-III class metalloproteinase from *B. jararaca* venom. Differential proteins detected in the SDS-PAGE analysis were submitted to *in-gel* trypsin digestion followed by Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS) and database search for protein assignment. **Results and Discussion:** SDS-PAGE analysis revealed clear distinct profiles among the newborn venoms from siblings and non-siblings specimens. Comparison of eletrophoretic profiles showed a higher proteome complexity in gels run under reducing conditions. *Western blot* analysis showed significant immunoreactivity in all samples, mainly at molecular mass between 25 kDa and 80 kDa. Glycoprotein staining showed a number of glycoprotein bands ranging from 14 to 70 kDa in all venom samples, however, some subtle variations in the venom glycoproteome were detected. The caseinolytic activity did not significantly vary among the venoms; nevertheless, remarkable differences were detected in the venom individual amidolytic activities. High coagulant activity, a striking feature of *B. jararaca* newborn venom, was also observed, with some individual variations. Our partial results on the individual mass spectrometric analyses of these venoms indicate that proteomic variability occurs primarily in toxins of the three most abundant classes of enzymes present *B. jararaca* venom metalloproteinase, serineproteinase and phospholipase A2.

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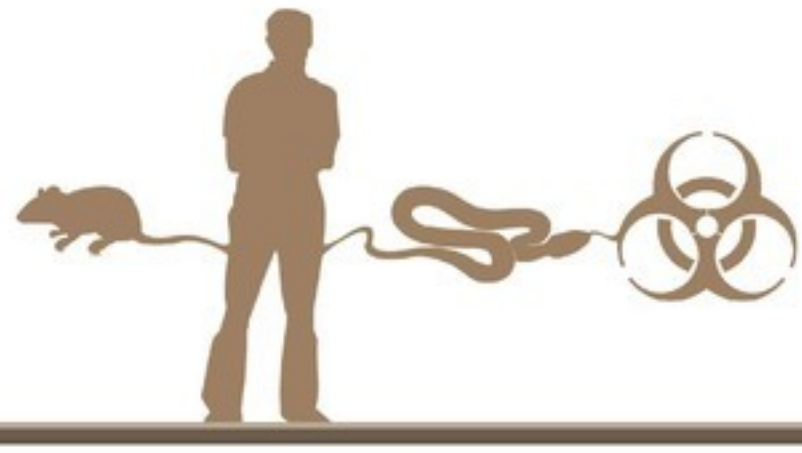

XIV Reunião Científica Anual do Instituto Butantan
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.05 Sphingomyelinase D inhibitors designed by molecular docking: control properties on the *Loxosceles* spider envenomation mechanisms

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Introduction: *Loxosceles* spider venoms consist of a mixture of proteins with enzymatic or toxic activity including sphingomyelinases D (SMases D), considered as the main toxic venom components responsible for the establishment of local and systemic effects. **Objectives:** This study aims to evaluate the potential inhibitory activity of molecules designed by molecular docking on SMases D toxic properties. **Methods:** The action of inhibitors was tested against a recombinant SMase D and the whole venom from *L. laeta* using *in vitro* and *in vivo* models of both cutaneous and systemic loxoscelism. **Results and Discussion:** Fluorometric analysis showed that from the fourteen compounds selected by molecular docking, five (compounds 1, 5, 6, 8 and 10) were able to inhibit the hydrolytic activity of recombinant and native SMases D, present in the venom, on sphingomylein (SM) and lysophosphatidylcholine. Dose-response curves have shown that inhibitor 5 was the most efficient with an IC50 of 45.36 μM. The five inhibitors were able to prevent the SMase D binding to human erythrocytes and the removal of glycophorins from these cells surface, important events for the complement dependent hemolysis induced by *Loxosceles* venoms. Our group has previously shown that SMases D induce death of human keratinocytes, which was associated with an increased expression/secretion of the MMP-2 and 9. Compounds 1, 5 and 6 were able to reduce the binding of SMases D to the membranes of human keratinocytes and cell death. The compounds were able to reduce the expression/secretion of MMP-2 and 9 in the supernatants of keratinocytes cultures, treated with SMase D/venom. Furthermore, four inhibitors were able to prevent the removal of cell surface markers such as MCP, MHCI, β2-microglobulin and EGFR, indirectly induced by SMases D/venom action. In a rabbit model of cutaneous loxoscelism, compounds 5 and 6 were able to reduce approximately 40-60% of the necrotic lesion. Here, we also demonstrated that the venom/SMase were able to reduce the production of NO by keratinocytes over the time, impairing reepithelialization. Inhibitors 1 and 5 were able to restore the levels of NO production. The toxin was also able to induce the activation of MAPK ERK1/2 in keratinocytes and all five compounds were capable of reducing the activation of this cell signaling pathway. These results indicate that SMases D specific inhibitors are able to control local and systemic reactions induced by *Loxosceles* venoms. These are promising tools for function/structure studies and for developing new therapeutic interventions for the loxoscelism.

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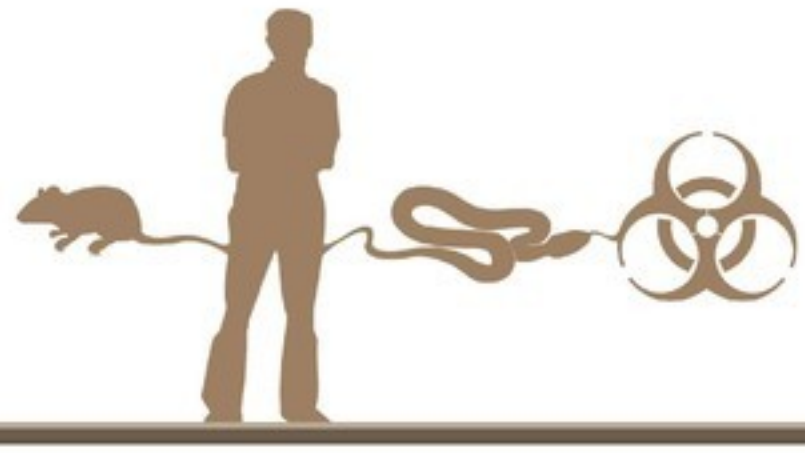

XIV Reunião Científica Anual do Instituto Butantan
05 a 07 de Dezembro 2012, São Paulo, SP

1.06 Purification and identification of two serine peptidases from *Bothrops jararaca* not blocked by the commercial antivenom

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Introduction: Snake venom envenomation is a public health issue for many countries and some studies show that snakebites surpasses several other neglected tropical diseases in occurrence and number of fatalities, such as leishmaniasis, dengue, schistosomiasis, cholera, and Chagas disease. In addition, snake bites only joined the list of neglected tropical diseases recently, in April 2009, showing that it was not seen as an important public health issue by WHO. In Brazil, *Bothrops* spp envenomation is responsible for 90% of snake bites and, these venoms, in particular, are rich sources for proteolytic enzymes (65% of the composition). The treatment recommended for the bothropic poisoning is the administration of the bothropic antivenom that inhibits most, but not all of proteases. **Objectives:** Purify and identify serine peptidases not blocked by the commercial antivenom from Butantan Institute. **Methods:** Two steps of chromatography were used to purify the serine peptidases. The first one was a ion exchange chromatography using a DEAE cellulose column eluted with five different concentrations of NaCl in stepwise (0.03M, 0.1 M, 0.2 M, 0.3 M and 2.0 M). The second step was a reverse phase HPLC using a C4 Shodex column. The fractions were screened through enzymatic assay using a FRET peptide (Abz-RPPGFSPFRQ-EDDnp) as substrate and trying to inhibit the activity of these fractions with the commercial antivenom. The sample purified was submitted to SDS-PAGE and colored by coomassie blue. The bands were excised and submitted to extraction and peptide mass fingerprinting using trypsin to digestion. After, the samples were analyzed by LC/LC-mass spectrometry. **Results and Discussion:** All fractions were submitted to enzymatic and serum neutralization assays and the serine peptidase activity of the fraction eluted in 0.1 M of NaCL was not blocked by the antivenom. Thus, it was submitted to reverse phase chromatography and one peak was selected using the same assays. The sample analyzed by mass spectrometry consisted in two serine peptidases not yet described in the venom of *Bothrops jararaca*. Comparing to *Serpentes* database we found two enzymes that contained all three peptides sequenced by mass spectrometry analyses. The first one is a thrombin-like enzyme from *Bothrops asper* and the second is a serine peptidase from *Bothrops jararaca* obtained from a cDNA library, but never purified before. Biological activity of these enzymes is under investigation.

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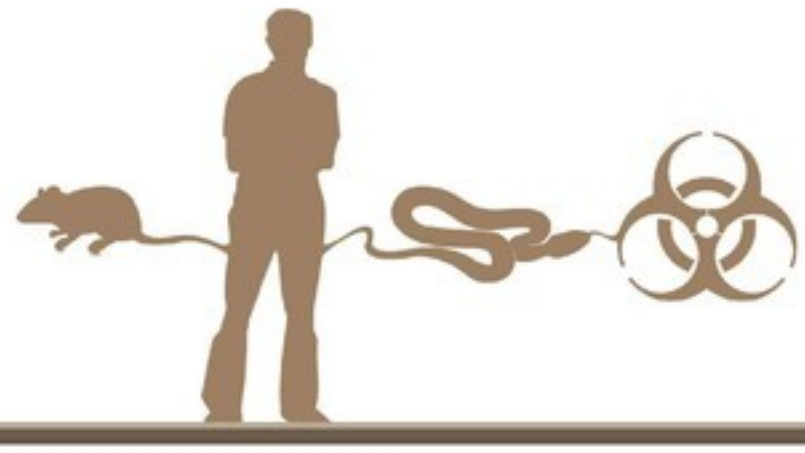

XIV Reunião Científica Anual do Instituto Butantan
05 a 07 de Dezembro 2012, São Paulo, SP

1.07 Cloning and expression of a new metaloproteinase, BnMPIIx, of *Bothrops neuwiedi* snake venom

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Introduction: Snake Venom Metaloproteinases (SVMPs) are toxins responsible for most of the symptoms of envenoming, acting on hemorrhage, activation or hydrolysis of components of the coagulation cascade and inhibition of platelet aggregation. They are divided into PI, PII and PIII classes. BnMPIIx is an atypical SVMP because it has a catalytic domain of class PIII and a disintegrin domain of class PII SVMPs, found in a cDNA library of *Bothrops neuwiedi*. **Objectives:** In this study, our objective is to clone and express BnMPIIx in bacterial system in order to access the biological activity of this new protein and its importance in the envenoming. **Methods:** The cDNA of complete BnMPIIx (catalytic and disintegrin domains) or its disintegrin domain were amplified by PCR, digested by specific endonucleases and cloned into pET20 vector. The disintegrin domain was also cloned into pET32 vector. The sequencing of both inserts did not show mutations during the cloning procedures. These vectors were transformed into *E.coli* BL21(DE3) or C43(DE3) strains and expression was evaluated in two temperatures (30ºC and 37ºC). **Results and Discussion:** The SDS-PAGE analysis showed that recombinant BnMPIIx as well as its disintegrin domain were expressed in *E.coli* C43 (DE3) in both temperatures (30° C and 37° C), but were insoluble when using pET20 vector generating inclusion bodies. When using pET32, the recombinant disintegrin domain was successfully expressed in a soluble form. At present, refolding procedures are being conducted in order to obtain the pET20 proteins in active state. Also, the purification of pET32 disintegrin domain is been optimized in order to analyze the biological activity of these protein.

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XIV Reunião Científica Anual do Instituto Butantan
05 a 07 de Dezembro 2012, São Paulo, SP

1.08 Sympathetic outflow and the production of different toxins in the snake venom gland

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Introduction: Noradrenaline, released just after venom removal, regulates the proteins synthesis of the gland which is important to activate it for venom production. In previous studies, we identified a set of proteins that had their expression regulated during venom production cycle by using MALDITOF/TOF mass sprectrometry (MS). Besides, sympathetic outflow also regulated the expression of some toxins. **Objectives:** The aim of this study is to continue the identification of other set of proteins of venom gland that had their expression regulated during venom production cycle or by sympathetic outflow **Methods:** Venom glands were obtained from female *Bothrops jararaca* snake in quiescent stage and in activated stages (4 and 7 days after milking). Some snakes were treated with reserpine or reserpine plus phenylephrine and isoprenaline just after milking (N=3 for each group). Specific spots from each group that was not identified previously were reanalyzed by ESILTQ XL/Orbitrap MS. All MS data were analyzed using PEAKS Studio 5.3 and searches were made using NCBInr and snake venom BSI databases. All information about proteins identified is collected on UniProt. The expression of some proteins was further confirmed by Western blot (WB). **Results and Discussion:** The expression of actin and protein disulfide isomerase (PDI), a cytoskeleton and an endoplasmic reticulum protein, respectively, were up-regulated in activated venom gland. The total expression of secreted proteins decreased during venom production cycle, but it is interesting to note that glycoprotein IB-binding protein (GPIb-BP) and thrombin-like enzyme is only present in quiescent venom gland. PIII metalloproteinase (SVMP) is present in both stage of the venom gland, but was up-regulated in 7 days activated stage. After treatment with reserpine, detection of GPIb-BP and PIII SVMP was similar to the quiescent gland and after administration of α and β-adrenoceptors agonists the detection of these toxins is similar to the activated gland. Expression of actin, PDI and PIII SVMP was confirmed by WB. In conclusion, these results suggest that the higher expression of actin and PDI in activated than in quiescent stage are essential to regulate the venom production and secretion. Moreover, the stimulation noradrenergic innervation is probably involved in the secretion of GPIb-BP toxin and also in up-regulating the PIII SVMP. We also showed for the first time that the synthesis of toxins occurs not only in activated stage, but also in quiescent stage. The identification of these proteins will give us new insides to understand the mechanism of venom gland activation and venom production.

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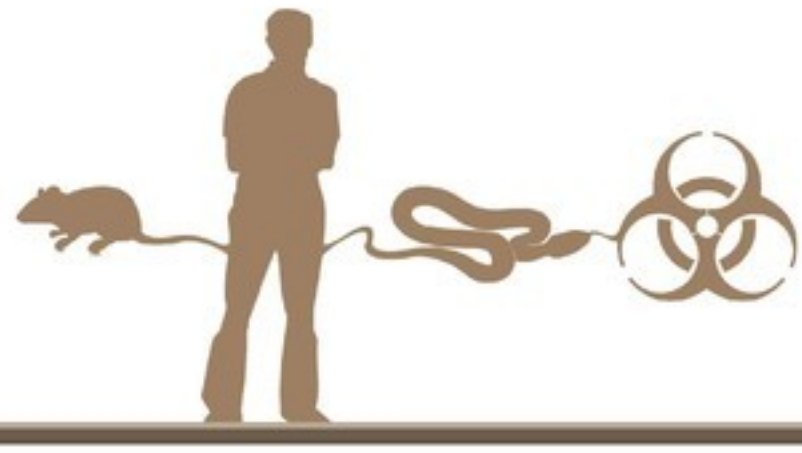

XIV Reunião Científica Anual do Instituto Butantan
05 a 07 de Dezembro 2012, São Paulo, SP

1.09 Histological evaluation of brain in adult offspring of mothers treated with *Tityus bahiensis* scorpion venom during the lactation

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Introduction: Previous studies with *Tityus bahiensis* scorpion venom demonstrated that when injected into rats during pregnancy it causes behavioral changes and neuronal loss in pups at the adulthood. This venom also causes behavioral and reflexological changes in pups whose mothers were treated during lactation. **Objectives:** Because we do not know how harmful is the effect of inoculation of this venom during lactation period to neuronal cells in this study we evaluated the possible alterations that may compromise the development of the hippocampus in relationship to cell proliferation. **Methods:** Pregnant females were injected with saline or venom (2.5 mg / kg sc) on the 10^{th} postnatal day. Pups with 22 (n = 6) or 60 days of life (n = 6) were deeply anesthetized with CO2 and perfused using saline injected directly into the left ventricle of the rat, followed by injection of formalin to fix the tissue. These animals were decapitated and had their brains removed and stored in formalin solution until embedded in Paraplast. Coronal brain sections of 10 μm were cut and the slides were stained with cresyl violet solution and analyzed in an optical microscope at 40x magnification. For statistical analysis Student "t" test were used. **Results and Discussion:** CA1, CA3 and CA4 regions of hippocampus were selected for cell counts. Number of cells from 22 days old offspring of mothers treated on the 10^{th} day of lactation was 74.4 ± 5.0 in control group (C), while the experimental group (E) averaged 87.0 ± 3.2 in the CA1 region. CA3 region showed 45.4 ± 2.4 in C group and 47.5 ± 4.2 cells in E group, and in CA4 area of C group was counted 34.7 ± 2.4 cells and in E group 39.7 ± 1.5. In the same way, animals with 60 days old were count 62.1 ± 4.9 cells in CA1 in the control group and 74.4 ± 5.0 cells in the experimental group. CA3 region in group C showed 39.4 ± 5.1 and E group 52.4 ± 5.1 cells, and the area CA4 cells showed 36.5 ± 3.3 in group C and 33.3 ± 3.8 in group E. Although in our previous experiments we found behavioral and reflexological changes induced by *Tityus bahiensis* scorpion venom inoculation on 10^{th} day of lactation the venom does not cause cell damage in the hippocampus.

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XIV Reunião Científica Anual do Instituto Butantan
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1.10 Expression of perilipin 2 (PLIN2) and formation of lipid bodies in leukocytes recruited by venoms of distinct species of *Bothrops* snakes

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Introduction: Lipid bodies (LBs) are inducible lipid-rich cytosolic inclusions abundant in inflammatory cells and involved in the synthesis of inflammatory lipid mediators. LBs formation is dependent on the scaffold protein perilipin 2 (PLIN2). **Objectives:** To study the effects of crude venoms of *B. jararaca (*BjV), *B. insularis* (BiV), *B. moojeni* (BmV) and *B.atrox* (BatxV) in peritoneal leukocytes of mice, evaluating: *i*) LBs formation and *ii)* protein expression and localization of PLIN2. **Methods:** Swiss male mice were used (Butantan Institute Ethical Committee 729/10). These animals received intraperitoneal injection of an inflammatory dose of each venom species (0.250 mg/g) or saline (control). After 6 h, inflammatory exudates were harvested to determine: a) total number of leukocytes in Neubauer chamber, b) leukocyte subtypes in Hema3 stained cell smears and c) LBs formation in leukocytes stained with osmium tetroxide (1%) followed by counting under phase contrast microscopy. PLIN2 expression was evaluated by Western blotting. **Results and Discussion:** Intraperitoneal injection of BjV or BiV or BmV or BatxV significantly increased LB numbers in leukocytes collected 6 h after their injections in comparison with controls, without statistical difference among venoms. Moreover, BiV, BmV and BatxV, significantly increased PLIN2 protein expression at 6 h and BjV at 12 h after i.p. injection as compared with controls. These data indicate the ability of distinct species of *Bothrops* snake venoms to induce formation of LBs and expression of PLIN2 in leukocytes. Expression of PLIN2 may be relevant for venom-induced LB formation. LBs may have a role in the inflammatory reaction induced by *Bothrops* snake venoms.

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1.11 Cloning, expression and characterization of human anti-crotoxin scFv mutants suggested by *in silico* studies

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Introduction: Crotoxin is the main toxic component of *Crotalus durissus terrificus*. It is a heterodimeric β-neurotoxin that consists of a weakly toxic basic phospholipase A2 and a non-enzymatic, non-toxic acidic component (crotapotin). The human neutralizing recombinant anticrotoxin scFv6 was isolated by phage display technology from a naive library of more than 10^{10} single-chain Fv (scFv). Single-chain variable fragments (scFv) consist of VH and VL domains joined by short flexible linker and may be useful as auxiliary therapy to envenoming by snake bite. In order to clarify the mechanisms of neutralization, docking and energy minimization calculations of the antibody-CTX were also conducted. From these simulations, three single changes were chosen to be mutated. Mutants (S30A) and (Y31F) have a mutation in CDR H1 and (R103H) in CDR H3. **Objectives:** To produce scFvs antibodies with improved affinity viewing a possible therapeutic alternative for the local effects caused by envenoming. **Methods:** The first mutant (S30A) was obtained by site-directed mutagenesis, while the others (Y31F and R103H) were obtained from synthetic genes with codons optimized for bacteria expression. ScFv original and mutants were cloned into pET20b+ vector and the constructions were used to transform C43 bacteria. The production of scFvs was accomplished by induction with IPTG. The mutated proteins and the original scFv were all expressed in soluble form. Periplasmic fractions were isolated through osmotic shock and further purified by Ni(2+)-immobilized metal affinity chromatography and the purity of scFvs was analysed by SDS-PAGE. Circular dichroism was performed to analyse the secondary structure of original scFv. **Results and Discussion:** Sequencing confirmed the desired mutations. Preliminary results show that all scFvs mutants presented similar expression levels. The circular dichroism of scFv revealed preserved secondary structure. ScFvs will be now analysed regarding their affinity to CTX by surface plasmon resonance (Biacore) assay.

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XIV Reunião Científica Anual do Instituto Butantan
05 a 07 de Dezembro 2012, São Paulo, SP

1.12 Comparative trancriptome analysis of the venom gland from *Tityus serrulatus* and *T. bahiensis*

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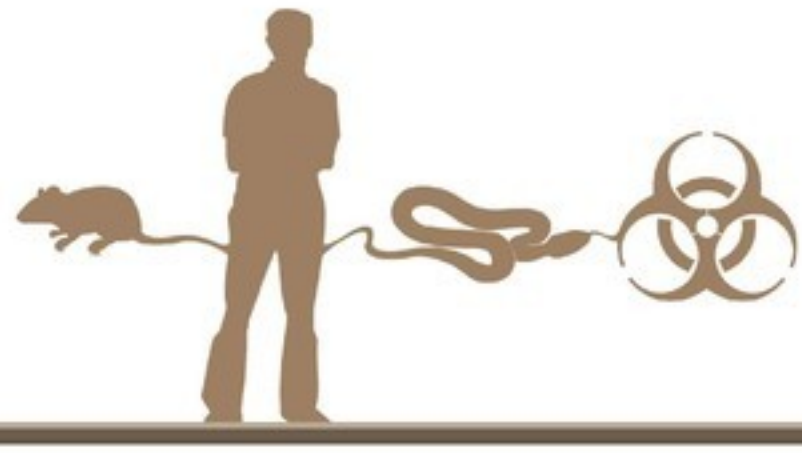
Introduction: *Tityus serrulatus* and *T. bahiensis* are scorpions widely distributed in Brazil with the exception of North region. Scorpion venom is a mixture of toxins, neurotoxins, as well as ions channels blockers which are responsible for the symptoms of clinical manifestation. Other known components are antimicrobial peptides, bradykinin-potentiating peptides, anionic peptides, metalloproteases and phospholipases. **Objectives:** Here we sequenced and analyzed the transcripts from the venom glands of these two species, aiming at identifying, annotating and comparing the venom expressed genes. **Methods:** Telsons of *T. serrulatus* and *T. bahiensis* were removed 48 hours after being milked by electrical stimulation. For total RNA isolation the telsons were ground into powder in liquid nitrogen and homogenized in Polytron® Tissue Homogenizer. The extraction was done using TRIZOL Reagent and mRNA isolation was carried out with oligo dT magnetic beads. mRNA was quantified by Quant-iT™ RiboGreen® RNA reagent and Kit. A cDNA library from each species was constructed using 500 ng of mRNA and cDNA Synthesis System. Sequencing was performed in a GS Junior 454 Sequencing System following the manufacturer protocols. Sequences were assembled with Newbler Software. Parameters were set as default with the exception of a minimum overlap length of 50 bp and a minimum overlap identity of 95%. Sequences were subjected to a BLAST search (at NCBI and UniProt databases) and an Automatic annotation using Blast2Go. **Results and Discussion:** A total of 138,855 and 116,027 reads were generated by pyrosequencing from *T. serrulatus* and *T. bahiensis*, respectively, these reads were assembled, respectively, in 5,984 and 5,615 contigs. Annotation identified transcripts with high degree of similarity to known scorpions toxins and also to products involved in cellular process. Among toxin transcripts, it was possible to identify potassium and sodium channel toxins, antimicrobial peptides, anionic peptides, and bradykinin-potentiating peptides. Regarding non-toxin transcripts, several sequences code for actin, tubulin, cysteine peptidase cathepsin, ATPases, splicing factor, transposases and mitochondrial proteins (cytochrome b and cytochrome oxidase I), among other cellular proteins. There were also many transcripts with hypothetic conserved functions and other ones with unidentified sequences. The initial gene profile provided here represents the first attempt to massively identify the components of these two species and one of the few transcriptomic efforts on the genus *Tityus*.

Supported by: CAPES and FAPESP



1.13 Inhibition of KV channels by Crotamine

Peigneur S[1], Orts DJ[2], Prieto da Silva ARB[3], Oguiura N[4], Boni-Mitake M[5], de Oliveira EB[6], Zaharenko AJ[3], de Freitas JC[2] & Tytgat J[1]
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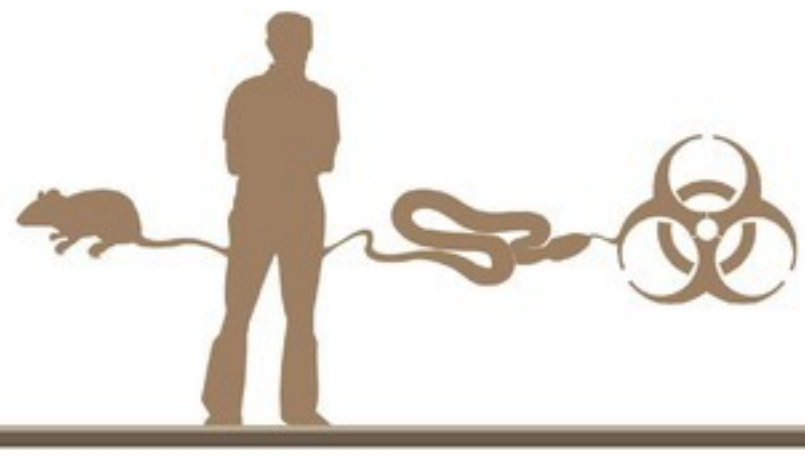
Introduction: Crotamine, a myotoxin from *Crotalus durissus terrificus* venom, possess exceptional biological versatility. As well as, its cell penetrating ability, which allows for crotamine to translocate cell membranes and to accumulate in the nucleus; its property of intracellular vesicle tracking and cell cycle marker; its capability of delivering DNA into replicating mammalian cells *in vitro* and *in vivo*. And more recently, its selective toxicity against aggressive types of cancer, its potential application as a selective metastatic cell marker and target mediated drug delivery into cell. Several early studies have attempted to elucidate the exact molecular target responsible for the crotamine-induced skeletal muscle spasm without success. **Objectives:** The aim of this study was to investigate whether crotamine affects voltage-gated potassium (KV) channels in an effort to explain its in vivo effects. **Methods:** We investigate whether crotamine affects voltage-gated potassium (KV) channels in an effort to explain its *in vivo* effects. To achieve that aim Crotamine was studied on ion channel function using the two-electrode voltage clamp technique on 16 cloned ion channels (12 KV channels and 4 NaV channels), expressed in *Xenopus laevis* oocytes. **Results and Discussion:** Crotamine selectively inhibits KV channels with IC50 values yielded 369 ± 56 nM for KV1.1, 386 ± 11 nM for KV1.2 and 287 ± 92 nM, to KV1.3 as 90% of inhibition. Moreover, several amino acid residues have been suggested to play a functional and critical role in the KV channel inhibition of this toxin by tridimensional homology model evaluation with other toxins. Our results demonstrate for the first time that the typical crotamine syndrome symptoms may result from the inhibition of KV channels. The ability of crotamine to inhibit the potassium current through KV channels unravels it as the first snake peptide with the unique multi-functionality such as cell penetrating, anti-tumoral activity and KV channel inhibiting properties. The potent and selective KV channel inhibiting properties, as demonstrated in this work, can be an advantage for the use of crotamine or its derivatives as antitumor drug and may perhaps explain its interference in cell cycle progression mediated by KV channels.



XIV Reunião Científica Anual do Instituto Butantan
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1.14 A β-adrenergic antagonist molecule isolated from *Pipa carvalhoi* skin secretion

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Introduction: *Pipa carvalhoi* is an aquatic anuran of the Pipidae family, exclusive to South America, which mainly inhabits Amazon and the Northeast of Brazil. Previous histological studies on the skin demonstrated the presence of scarce granular cells. Nevertheless, the skin secretion of this animal has never been biochemically studied. **Objectives:** Obtain a skin secretion solution of *P. carvalhoi* and biochemically characterize its major components. **Methods:** Specimens of *P. carvalhoi* were maintained in 200 L stainless steel tanks. A skin secretion solution of male and female (without eggs) animals was obtained after submerging the subcutaneously noradrenaline injected (100 μM) animals in 100 mM ammonium acetate (pH 7.4). The skin secretion solution was concentrated, filtered and was fractionated by C18-RP-HPLC, and the major peak was collected and characterized by mass spectrometry (MS), nuclear magnetic resonance (NMR) and infrared (IR). Possible antagonism on α and β receptors was tested in anaesthetized rats by measuring mean arterial pressure (MAP) and heart rate (HR) responses elicited by isoprenaline (0.25 mg/kg) or adrenaline (0.1 mg/kg) (β-adrenergic and α-adrenergic agonists, respectively) before and after intravenous injection of the major peak (10^{-3} M). **Results and Discussion:** The major component of *P. carvalhoi* secretion is currently under structural elucidation, but preliminary MS/NMR/IR data made it possible to correlate it to Propranolol. According to the initial tests, this molecule, when injected in rats does not possess any deleterious effect nor does it elicit any effect MAP or HR of anaesthetized rats, but interfere with the hypotensive response elicited by isoprenaline, indicating a β-antagonist effect. In this work we present the first toxinological approach to the skin secretion of *P. carvalhoi* and describe the presence of one propranolol-like molecule, which antagonizes the β-adrenergic receptors, thus decreasing the rat blood pressure. However, more experiments are necessary to confirm these effects which are currently being performed.

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XIV Reunião Científica Anual do Instituto Butantan
05 a 07 de Dezembro 2012, São Paulo, SP

1.15 Venom of the *Tityus obscurus* scorpion induces changes on the general activity of Wistar rats

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Introduction: In the Brazilian Amazonia, the reported scorpion stings are related mainly to the species *Tityus obscurus*, *Tityus silvestris* and *Tityus metuendus* and some reports related to *Brotheas amazonicus*, the first being a species of importance in health and related to the most important cases of envenomation. In the west Pará the envenomation caused by this species shows clinical picture characterized by local effects such as pain and parenthesis, however, is distinct from that described in other areas of the Amazonia and other regions of the country which includes myoclonus, patients refer as an"electric shock in the body", ambulation difficult and muscular contraction. **Objectives:** This work aimed to verify if *Tityus obscurus* scorpion venom induces changes on the general activity as observed to others *Tityus* species. **Methods:** The dose of 10 mg/kg diluted in 1.46% NaCl was used. Male Wistar rats (230-260 g) were injected with 1.46% NaCl (control) or scorpion venom (experimental) intraperitoneally. Then, one experimental group and one control group were evaluated five minutes after the venom injection in activity box, and the other 2 groups were underwent the same test 1 hour after injection of the venom. Each animal was tested individually only once by 10 minutes. The activity box measures separately the locomotor activity and the general activity (horizontal locomotion plus other movements) of the animal. **Results and Discussion:** The venom injected 5 minutes before the test does not changed the general and locomotor activity of the animals. However, animals evaluated 1 hour after injection of the venom showed decreased general and locomotor activity compared to the control group. The time of 5 minutes after injection may be not sufficient to induce changes on the exploratory activity of these animals. The decrease in general activity of the animals evaluated 1 hour after the venom injection can be explained by the effects of the envenomation or changes in the dopaminergic neurotransmitters pathways, since the dopaminergic system regulates motor activity of rats. It is necessary more detailed studies in order to find conclusive answers about the effect of this venom on motor activity of rats.

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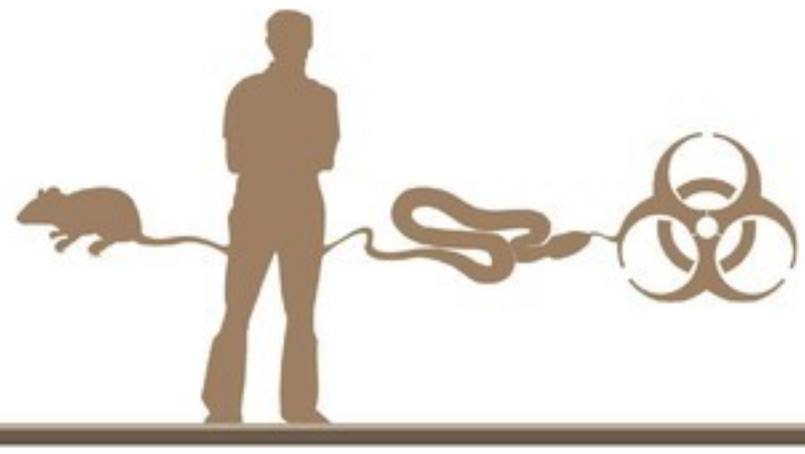

1.16 Insularin (GST-INS), a recombinant disintegrin from *Bothrops insularis* venom, inhibit adhesion of endothelial and melanoma cells to platelets *in vitro*

Silva-Mendonça R[1], Clissa PB[1], Lima IC[1], Mendes VA[2], Vassão RC[2], Della-Casa MS[1]
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Introduction: The adhesion of tumor cells with platelets is important in the process of tumor metastasis. *In vivo* tumor cells interact with a variety of host cells such as endothelial cells and platelets, and these interactions are mediated by integrins GPIIbIIIa (αIIbβ3) and αvβ3. Agents that block multiple integrin receptors may be more effective at inhibiting tumor growth and angiogenesis. Insularin (GST-INS) is a recombinant disintegrin from *Bothrops insularis* venom that inhibits the platelet aggregation by ADP and inhibits the endothelial adhesion to a fibrinogen by block αIIbβ3 and αvβ3 integrins, respectively. **Objectives:** The aim of this study was to investigate the potential roles of GST-INS on inhibition of endothelial and melanoma cells adhesion to platelets *in vitro* study. **Methods:** Human whole blood from healthy donors and volunteers were collected in 3.8% citrate buffer and centrifuged at 800 rpm for 20 min to obtain platelet-rich plasma (PRP). 400µl PRP were incubated with PBS or GST-INS for 3min and then the platelets were challenged by ADP (10µM). The platelet aggregation was monitored by aggregometer (CHRONO-LOG). For static adhesion, 96-well polystyrene plates were coated with platelets ($1x10^8$/mL) for 2hr, washed with Tyrode´s buffer to remove nonadherent platelets, and blocked for 30 min with 1% BSA in Tyrode´s Buffer. HUVECs (Human Umbilical Vein Endothelial Cell) or Murine B16F10 melanoma cells ($1x10^6$/mL) were preincubated for 30min with GST-INS or Agrastat (a potent inhibitor of platelet aggregation and selective to αIIbβ3 integrin). Subsequently, HUVECs or B16F10 were added and allowed to adhere for 40 min under cell culture conditions. After careful washing with PBS, adherent cells were analyzed per visual field by microscopy. **Results and Discussion:** The GST-INS was successfully expressed, resulting in 1mg/mL of protein. Furthermore, this protein inhibited platelet aggregation confirming its biological activity. The GST-INS inhibited both HUVECs and B16F10 cell adhesion to platelets at concentration as low as 0.32µM. This inhibition was dose dependent and significantly higher than the inhibition induced by Agrastat when used at the same dose. These results suggest that combined inhibition of platelet/endothelial cell and platelet/melanoma by GST-INS may be an important approach to inhibiting tumor growth, angiogenesis, and metastasis. Further studies *in vivo* are important for the confirmation of this approach.

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XIV Reunião Científica Anual do Instituto Butantan
05 a 07 de Dezembro 2012, São Paulo, SP

1.17 Comparative analysis of venoms from *Bothrops*, *Bothropoides* and *Rhinocerophis*, snakes: Protein composition and reactivity with antivenom

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Introduction: It is currently accepted that snake phylogeny may be represented in venom composition with implications in the production of antivenoms and treatment of envenoming. **Objectives:** Analyze the chromatographic and electrophoretic profiles of venoms of snakes from three different genera and reactivity of each fraction with Bothrops antivenom (SAB) produced in Butantan Institute and with anti-SVMP monoclonal antibody. **Methods:** Samples of 5mg of venoms of *Bothropoides jararaca*, *Bothropoides neuwiedi*, *Bothrops atrox*, *Bothrops jararacussu*, *Rhinocerophis cotiara* and *Rhinocerophis alternatus*, were submitted to reverse-phase chromatography in HPLC and the reactivity of the venoms and their fractions with the antivenom were performed by ELISA and western blotting. **Results and Discussion:** The electrophoretic profile of venoms by SDS-PAGE (12.5%) showed that the majority components presents are localized between 20 and 66 kDa. In the venoms of *Bothrops atrox* and *Rhinocerophis cotiara* predominated bands of molecular masses correspondent to Snake Venom Metalloproteinase (SVMPs), while the venom of *Bothrops jararacussu* had a predominance of bands corresponding to phospholipases. The components of molecular masses around 20 and 66 kDa, which corresponds mostly to molecular masses of SVMPs class P-III and P-I, respectively, were better recognized by SAB, in the western blotting, than the components with low molecular masses. Each venom showed a distinct chromatographic profile, without apparent correlation to phylogeny. *Bothropoides jararaca/neuwiedi* venoms had a diverse elution profile as well as *Bothrops atrox/jararacussu* venoms. *Bothrops atrox* showed the greatest abundance of SVMPs, while phospholipases were more abundant in the venom of *Bothrops jararacussu*. *Bothropoides neuwiedi* presented the highest diversity of SVMP isoforms. Only venoms of the genus *Rhinocerophis* (*alternatus* and *cotiara*) showed relatively similar profiles. The antivenom recognized venoms of different genera with similar antibody titers (640,000). For all venoms tested, antivenom equally recognized fractions that correspond to SVMPs. There was no apparent correlation between the phylogeny of snakes and the venom composition, based on their chromatographic and electrophoretic profiles. Moreover, the antivenom recognized similarly the venoms of all genera, with predominance of SVMPs, which are key toxins for venom induced pathology in cases of snake bite.

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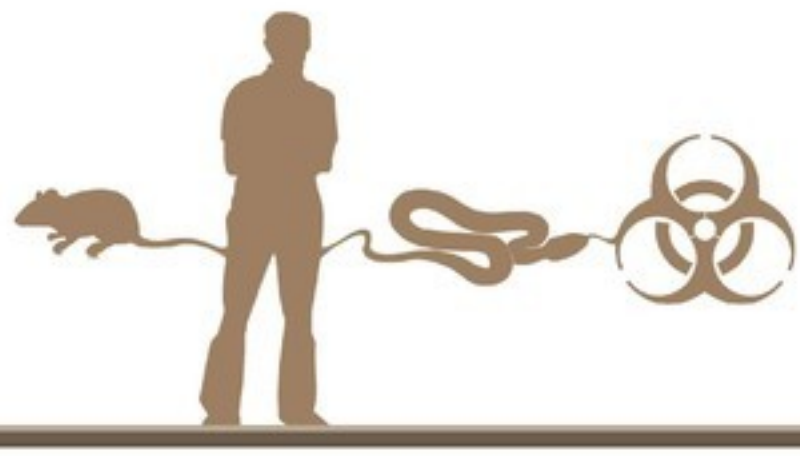

XIV Reunião Científica Anual do Instituto Butantan
05 a 07 de Dezembro 2012, São Paulo, SP

1.18 Identification of proteins in the microvesicles present in *Crotalus durissus terrificus* venom

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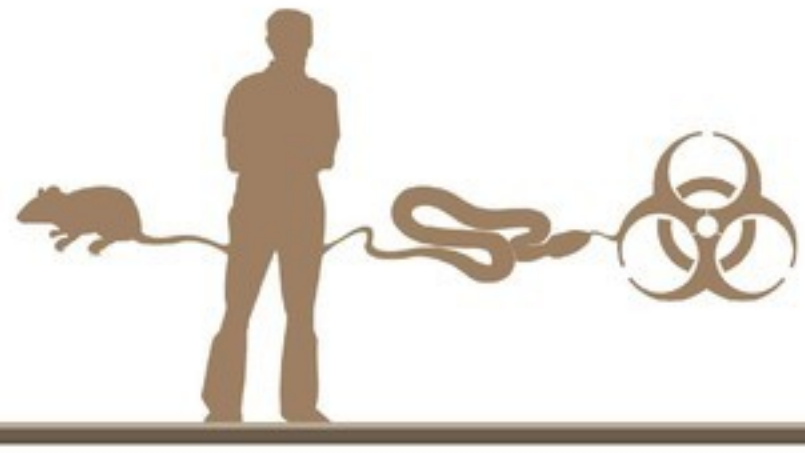
Introduction: *Crotalus durissus terrificus* (Cdt) venom is not only constituted by toxins that are responsible for the clinical complications caused by envenoming. Numerous electron-dense microvesicles (40 – 80 nm in diameter) are observed on the luminal face of secretory cells of venom gland and in the venom of the *Cdt*. These microvesicles originate from microvilli by fragmentation or membrane budding and have intramembranous particles on the cytoplasmic leaflet, suggesting the presence of transmembrane proteins. We have detected specific proteins in the microvesicles present in *Cdt* venom. The molecular weights of these proteins are approximately 72, 148, 176, 272 and 323 kDa. The *Crotalus* antivenom recognized only some of these proteins **Objectives:** The aim of this study is to identify the specific proteins in the microvesicles from venom of the *Cdt*. **Methods:** The venom used was manually extracted from *Cdt* maintained in the Laboratory of Herpetology at Instituto Butantan. The microvesicles were isolated by two ultracentrifugations. Supernatant (S2) from the first ultracentrifugation, venom without microvesicles, and crude venom were used as a control. The resulting pellet was processed for one-dimensional electrophoresis (4-20% gradient gel). Bands of proteins were excised from the gels, digested with trypsin and analyzed by Ion Trap/time of flight (LCMS-IT-TOF) or LC-MS/MS (nano-HPLC ESI-Q-TOF) mass spectrometry. All mass spectrometry data were analyzed using NCBI non-redundant database. Information about the proteins identified is in accordance to data collected on UniProt. **Results and Discussion:** The identification of proteins by mass spectrometry showed the presence of cellular membrane and intracellular proteins. Cellular membrane proteins identified were ecto-5´nucleotidase, aminopeptidase N and angiotensin-converting enzyme-like. The intracellular proteins indentified were ankyrin repeat domain-containing protein 62, N-acylglucosamine 2-epimerase, leucine-rich repeat immunoglobulin-like domain and transmembrane domain-containing protein 3, DNA fragmentation factor subunit alpha isoform 1, protein phosphatase 1 regulatory subunit 26. In conclusion, we identified specific proteins in the microvesicle present in the *Cdt* venom and these proteins could have a role in metabolic pathways that regulates the integrity of the secretory cells. Besides, the cellular membrane proteins could participate of the pathophysiology process of the envenoming.

Supported by: FAPESP, CNPq, CAPES



XIV Reunião Científica Anual do Instituto Butantan
05 a 07 de Dezembro 2012, São Paulo, SP

1.19 Proteomic and peptidomic characterization of venoms from the spider *Acanthoscurria gomesiana*

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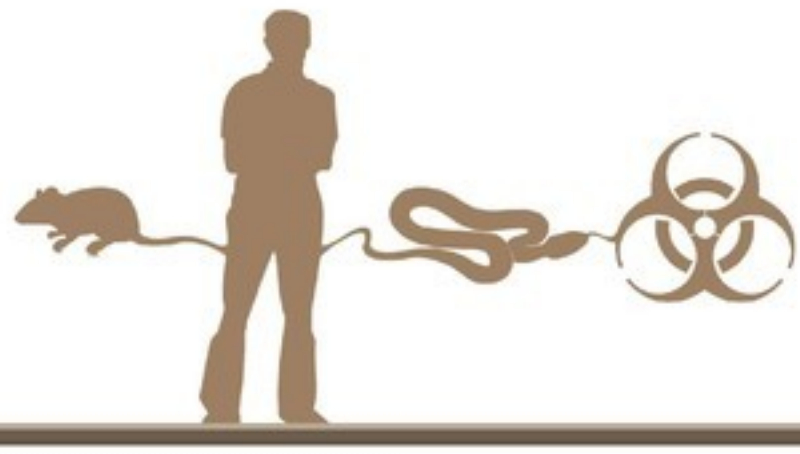
Introduction: The order of spiders (Araneae) is considered one of the most diverse in number of species, and also one of the most successful among poisonous animals on Earth. One of the key factors to the success of spiders is the production of a highly toxic venom from their glands, used to subdue prey and for protection against potential predators. These components are primarily proteins and peptides which act mainly as neurotoxins, cytotoxins, and as ion channel modulators. However, despite several advances, the number of proteins and peptides isolated and characterized from spider venoms is estimated to be only 0.01% of all possible sequences. **Objectives:** Considering the biological potential of spider venom toxins and the lack of studies from Brazilian spiders, the objective of this work is to characterize the protein and peptide composition of the venom from *Acanthoscurria gomesiana* by mass spectrometry, prospect molecules with biological activity and identify sex-based differences. **Methods:** The venoms were obtained from adult male and female specimens separately. Venoms were fractionated by RP-HPLC and the fractions were used in antimicrobial and platelet aggregation assays. The fractions were submitted to SDS-PAGE, mass spectrometric analysis and database search. **Results and Discussion:** The venoms of male and female specimens of *Acanthoscurria gomesiana* present differences in composition. In males, the average protein concentration was 240 mg/mL, while in females it was 129 mg/mL. In addition, RP-HPLC and SDS-PAGE analysis indicate differential expression of peptides and proteins. The crude venoms did not inhibit platelet aggregation, but some fractions presented antimicrobial activity. Due to the lack of sequences in public databanks, automated database search of mass spectra did not retrieve any known sequence, which indicates that de novo sequencing will be required in this work.



XIV Reunião Científica Anual do Instituto Butantan
05 a 07 de Dezembro 2012, São Paulo, SP

1.20 Antimicrobial activity of the toxin VdTX-I and fractions present in the venom of the tarantula spider *Vitalius dubius*

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Introduction: Antimicrobial molecules are widely distributed in organisms. The widespread occurrence of these substances suggests that they play. The spiders occupy ecological niches generally hot and humid. Under these conditions, are prone to pathogens, the use of antimicrobial molecules can be explained by the constant contact throughout the evolution of species with such pathogens to prevent infections. **Objectives:** To investigate the antimicrobial action of VdTX-I, an acylpoliamin, purified from the venom of the spider crab *Vitalius dubius*. **Methods:** Antimicrobial assays were performed in cultures made in 96-well micro plates against 14 microorganisms (fungi, yeasts and bacteria), which was tested several VdTX-I concentrations of 0.19 μM to 100μM, and evaluated whether there was antimicrobial activity, and this qualitative analysis was also performed a dose-response curve for two microorganisms (*Candida albicans* and *Candida guillermondii*). **Results and Discussion:** The antimicrobial activity of the toxin VdTX-I was observed in 12 tested microorganisms whose activity is present in concentrations of 100, 50, 25 and 12.5 μM, the same could be observed in the dose-response curve for both tested microorganisms, where the maximum activity occurs at 100μM concentration decreased up to 0.76 μM. The toxin VdTX-I was shown to be active against most of the microorganisms tested, inhibiting the growth at relatively high concentrations, and within this concentration range, the VdTX-I inhibits growth in a dose-dependent manner. The toxin VdTX-I do not have hemolytic activity in any of the tested concentrations, as well as their cytotoxic activity.

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XIV Reunião Científica Anual do Instituto Butantan
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1.21 Histological analysis of local reaction induced by *Scolopendra viridicornis* centipede venom

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Introduction: Envenomation caused by centipede bites are generally mild, and human victims usually manifest burning pain, paresthesia and edema, which may develop into superficial necrosis. Despite the abundance of these animals and frequency worldwide, centipede venoms have been poorly characterized in literature. **Objectives:** To evaluate microscopically the local reaction induced by *S. viridicornis* venom in mice. **Methods:** *S. viridicornis* venom (3.8, 15 or 60 μg) or PBS (negative control) were injected into the right hind paw of mice. At 0.5, 1, 4, 6, 24 and 48 h after injection, the injected paws were removed and processed for histological analysis. The samples were fixed in Bouin fixative and embedded in paraffin. The sections were stained with HE and photographed using a digital camera. **Results and Discussion:** The intraplantar injection of *S. viridicornis* venom induced the formation of a rapid and persistent edema, which is dependent of dose. The highest edematogenic activity was noticed within 30 min although edema was still observed up to 6 h in all doses tested. After 24 h, edema was detected in animals that received 15 or 60 μg of venom, and after 48 h only in animals that were injected with the highest dose (60 μg) of venom. Histological analysis showed that at 0.5 h after venom injection the footpads injected with venom, when compared with the PBS-injected mice (control), showed an intense edema and only a few inflammatory cells. At 4, 6 and 24 h after injection a great inflammatory influx was detected, together with disruption of the epidermal cells that culminates at 6h after injection. After 48 h of venom injection, only a mild amount of leukocytes was detected and epidermis is practically recovered, even when the highest dose (60 μg) of venom was injected. In conclusion, the histological analysis showed that *S. viridicornis* venom induces edema formation and cell influx in footpad of mice, demonstrating that centipede venoms induce a rapid and substantial inflammatory response and skin necrosis.

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XIV Reunião Científica Anual do Instituto Butantan
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1.22 *Premolis semirufa* envenomation: toxic properties and immune response

Villas-Boas IM[1], Gonçalves-de-Andrade RM[1], Pidde-Queiroz G[1], Squaiella CC[1], Portaro FCV[1], Sant'Anna OA[1], van den Berg CW[2], Tambourgi DV[1]
[1]Immunochemistry Laboratory, Butantan Institute, Brazil; [2]Cardiff School of Medicine, UK

Introduction: The Brazilian caterpillar of *Premolis semirufa*, usually called as Pararama, belongs to the Arctiidae family and inhabits rubber plantations in the Amazon region. The contact with the bristles, after the first accident, causes an acute inflammation. However, after multiple contacts, a chronic inflammatory reaction can occur with the immobilization of the joints. Specific treatment for this disease does not exist, but corticosteroids are frequently administered. **Objectives:** The present study was carried out to evaluate the toxic properties of the caterpillar's bristles extract, as well as the immune response induced by the pararama venom components in a murine model. **Methods:** For this purpose, the proteolytic, phospholipase, hyaluronidase activities and the Complement system-activating property of the bristles extract were analyzed *in vitro*. In addition, BALB/c mice were injected with the bristles extract or saline (control) into the footpad for seven times, every two weeks. The presence of edema and the production of specific antibodies and autoantiboides were measured during the inoculation process. Cytokines measurements in the footpads and popliteal lymph nodes cells immunophenotyping were performed after 24 h and 48 h of the 1st, 4th and 7th extract inoculations. Immunohistochemistry analyses were carried out with mice footpads collected after 48 h of the 1st, 3rd, 5th and 7th inoculations. **Results and Discussion:** The results showed the presence of significant proteolytic and hyaluronidase activities in the extract. The bristles extract was capable to induce alternative and lectin complement (C) pathways activation, with the generation of large amounts of the potent pro-inflammatory C-factors, *i.e.*, the anaphylatoxins C3a, C4a and C5a. *In vivo*, a significant edematogenic response and an intense infiltration of inflammatory cells to the envenomation site were observed. Immunohistochemical analysis has shown the presence of a large number of neutrophils and macrophages in the paw tissues of the envenomated mice. The extract was also able to induce high specific antibody titers, but not autoantibodies (anti-DNA or anti-collagen type II), and the proliferation/migration and activation of T and B lymphocytes in the lymph nodes. Cytokines evaluations demonstrated elevated levels of IL-6, IL-10, IL-12 and IL-17 after the 7th bristle extract inoculation. These data show that the intense bristles proteolytic activity and the high cellular and humoral immune responses induced by pararama venom toxic components may play an important role in the establishment of the pararamose.

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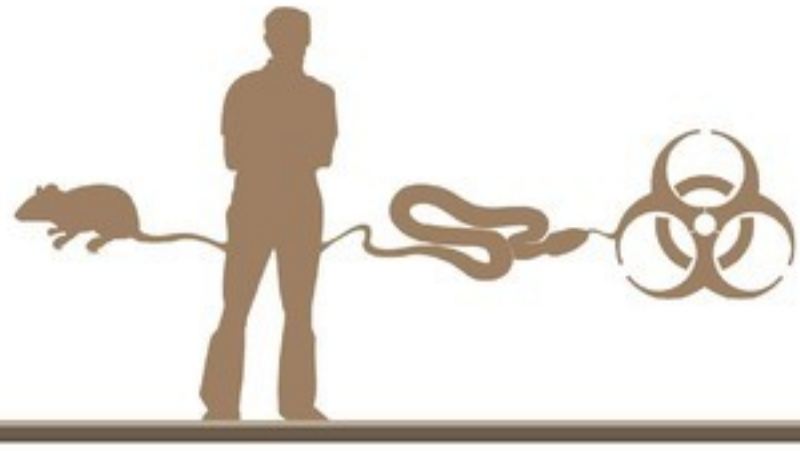

XIV Reunião Científica Anual do Instituto Butantan
05 a 07 de Dezembro 2012, São Paulo, SP

1.23 Pathogenesis of systemic hemostatic disturbances in *Bothrops jararaca* snake envenomation in rats

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Introduction: Bites by *Bothrops jararaca* (Bj) snakes evoke hemostatic disturbances in patients and thrombocytopenia and platelet dysfunction are the major manifestations during envenomation. The pathophysiology of such hemostatic disturbances in *Bothrops* envenomation is complex. Metalloproteinases and serine proteinases found in Bj venom with anti-hemostatic activity may account for engendering hemostatic disorders in patients. Moreover, local injury induced at the site of venom inoculation might also stimulate both the release of tissue factor (TF) into bloodstream, favoring the coagulopathy. **Objectives:** To investigate the contribution of Bj venom toxins and TF expression to the genesis of hemostatic disturbances, especially thrombocytopenia, in rats. **Methods:** Crude Bj venom was previously incubated with 269 mM Na2-EDTA or 200 mM AEBSF to inhibit metalloproteinases and serine proteinases, respectively, administered s.c. or i.v. into rats, and hemostatic parameters were evaluated 3 and 6 h later. **Results and Discussion:** Platelet counts showed a marked decrease in all groups administered with Bj venom in comparison with saline-treated rats; animals administered i.v. showed more intense fall in circulating platelets. The pre-treatment of the venom with AEBSF failed to block the fall in platelets count, and only Na2-EDTA minimally reversed thrombocytopenia. Similar, Bj venom caused plasma fibrinogen and vWF consumption (in s.c. groups). In addition, envenomed animals showed a marked local hemorrhage at the local of Bj venom inoculation. However, Na2-EDTA completely blocked plasma fibrinogen consumption and local hemorrhage in animals. Envenomed rats showed a marked elevation in plasma tissue factor levels after 3 h s.c., whose increase was also blocked by Na2-EDTA. These findings demonstrate that metalloproteinases are essential venom components involved in the coagulopathy resulting from Bj envenomation. Nonetheless, serine proteases have no involvement in the genesis of thrombocytopenia and other venom components/mechanisms seem to be associated therein. High levels of TF in plasma may occur during snake envenomation, so that the etiopathogenesis of the coagulopathy in snake envenomation resembles that of true disseminated intravascular coagulation syndrome.

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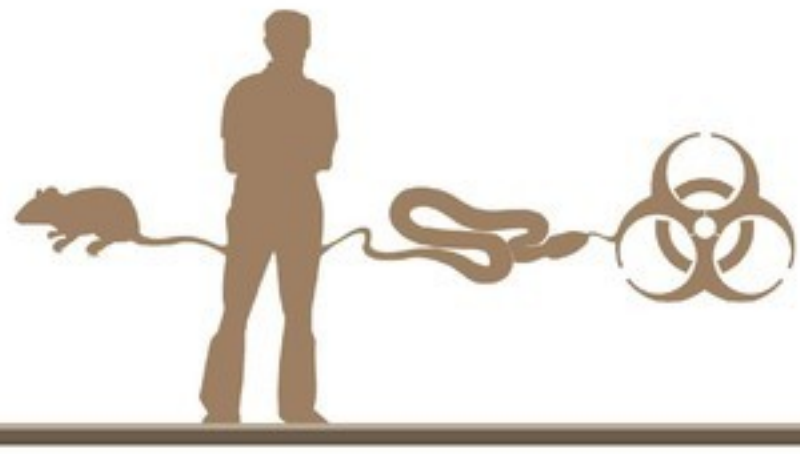

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1.24 Experimental envenoming induced by *Bothrops jararacussu* venom (Viperidae, Crotalinae): efficacy of Botropic, Botropic/Crotalic and Crotalic antivenin treatment

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Introduction: The snakebites are a public health problem in Brazil and twenty thousand bites by venomous snakes are reported each year, and approximately 90% of them are inflicted by the genus *Bothrops*. The most effective treatment for snakebites is serum therapy and the effectiveness of antibotropic serum for the neutralization of *Bothrops jararacussu* venom (Bjuv) has been discussed by many groups. **Objectives:** The aim of this study was to compare the efficacy of different antivenin treatments in the experimental envenomation induced by Bjuv. **Methods:** To evaluate the neutralization of the coagulant activity, the animals were injected *i.v.* with 0.50 mg/Kg of de Bjuv or saline and treated *i.v.* after 1 h, with Botropic antivenom (BAV), Crotalic Antivenom (CAV), Botropic/Crotalic Antivenom (BCAV) or saline. Blood samples were collected 3 and 6hs after the treatment to dose plasmatic fibrinogen and for thromboelastography analysis (TE) by ROTEM®. To evaluate the myotoxic activity, the animals were injected *i.m.* with 0.75 mg/Kg of Bjuv or saline, and also treated *i.v.* after 1 h. Blood samples were collected at 3, 6 and 12 h after treatment to quantify plasmatic creatine phosphokinase (CK). To evaluate the muscle regeneration, the animals were treated with antivenins or saline 15 min after Bjuv injection. The injected muscle was removed to residual CK and histological analysis. **Results and Discussion:** The results show that in 3 h after the treatment, the fibrinogen is not yet in hemostatic levels. At 6 h, the fibrinogen levels and the TE analysis show that the BCAV were more effective than BAV and CAV. Regarding the myotoxic activity, there were not significant differences between the treated or saline animals at 3, 6 or 12 hours. Similarly, with regards to the muscle regeneration, at 24 h after the treatment it was not detect differences among the groups studied. On the 7th day after treatment, it was noticed that all the antivenins were significantly efficient to recover muscle tissue, as show residual CK and the histological analysis. There seems to be a trend for better recovery in BCAV-treated animals, in comparison with BAV or CAV ($p<0,055$). However, the histological analysis was not conclusive, since morphometric analysis was not done yet. On 14th and 30th days after treatment, there was not difference among the envenomed and treated animals. In conclusion, the results show that the BCAV is more efficient than the BAV and CAV to restore coagulopathy, but this effect was not so evident regarding the myotoxic activity.

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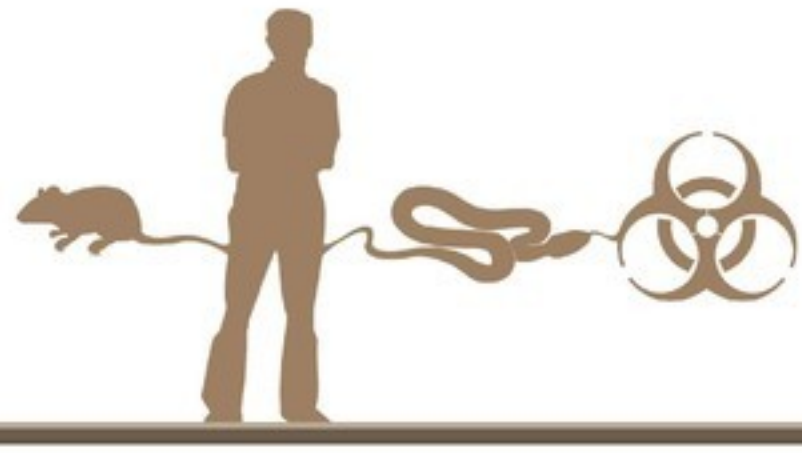

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1.25 Role of different domains of *Bothrops* metalloproteases on the alterations in leukocyte-endothelium interaction of murine cremaster microcirculation

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Introduction: Snake venom metalloproteases (SVMP) are classified as PI-PIII according to their composition domains and are involved in local inflammatory reactions observed in envenoming. **Objectives:** We evaluated the participation of different domains on the leukocyte-endothelial interaction (LEI) alterations, after exposure of mice cremaster microcirculation to toxins. **Methods:** We used jararhagin (Jar), a SVMP-PIII with a strong hemorrhagic activity that has catalytic, ECDdisintegrin and cysteine-rich domains; JAR-C a degraded form of JAR lacking of catalytic domain and hemorrhagic activity, and BnP1 a SVMP-PI weakly hemorrhagic that has only the catalytic domain. Toxins (0.5μg) or PBS (100μL) were injected into the mice scrotum bag. Adhered and migrated leukocytes were counted in post-capillary venules by intravital microscopy 2 or 24h after injections. **Results and Discussion:** An increase of adhered and migrated leukocytes was observed after toxins injection. We studied the protein and gene expression of the adhesion molecules in vascular endothelium, ICAM-1 and PECAM-1 involved in cell adhesion and migration, respectively and integrins CD11a and CD11b expressed on leukocytes. There was an increase of ICAM-1, CD11a and CD11b at the initial time and an increase of PECAM-1 at later times after injection with toxins. Inhibiting the catalytic activity of Jar and BnP1 by treatment with 1,10-phenanthroline (oPhe) a complete inhibition on the alteration in LEI was observed when compared to toxins without inhibition. Since Jar has also ECD disintegrin and cysteine-rich domains and Jar-C results showed that those domains can also induce changes in LEI, we analyzed by circular dichroism if oPhe treatment had caused a conformational change in Jar. The structure of Jar changed, showing that the chelant promoted reduction of α-helical structures. Yet, to identify if changes on oPhe-Jar were restricted to the catalytic domain or if they also affected ECD-disintegrin domain, an adhesion assay was performed to collagen, showing no differences among Jar or oPhe-Jar. Our data suggests that the catalytic domain of SVMP is the major domain for induction of changes in LEI, and apparently ECD-disintegrin and cysteine-rich domains needs to be separated from catalytic domain to induce these alterations. Despite the differences in hemorrhagic activities and domain compositions, the three toxins used induced changes in LEI by expression of adhesion molecules such as ICAM-1, CD11a, CD11b and PECAM-1.

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