1. Venoms and Envenomations

1.01 Characterization and validation of a cDNA library from venom glands of *Bothrops jararaca* for the analysis of differential gene expression

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Introduction: The venom gland of the Brazilian venomous snake *Bothrops jararaca* (Crotalinae, Viperidae) is an exocrine gland related to the salivary gland. Its characteristics include a central lumen where the venom produced by secretory cells is stored. When the venom is lost from the gland, the secretory cells are activated and new venom is produced. The production of new venom is triggered by the activation of noradrenaline on both α_1- and β-adrenoceptors in venom gland. Synthetic activity of the secretory cells and mRNA concentration peak at 4-8 days after venom is lost. Afterward, synthetic activity decreases and venom gradually accumulates in the gland lumen. Expressed sequence tags (ESTs) are informative fragments from DNA sequences, obtained from the complementary DNA (cDNA) derived from reverse transcription of messenger RNA (mRNA) of an organism, tissue or even a single cell type. The construction of a cDNA library from the venom glands of snakes provides a collection of specific transcripts and allows inferences about the gene expression pattern of this high specialized tissue. The aim of this work was to construct a cDNA library of the venom gland of *Bothrops jararaca* and validate this library for use in the construction of cDNA nylon arrays. **Methods:** Two adults of *Bothrops jararaca,* one male and one female, were anesthetized and decapitated. The venom gland was removed and frozen in liquid nitrogen. The RNA was extract using TRIZOL ^{R} (Invitrogen). The poly A^+ RNAs were obtained by purification with oligo dT-cellulose. The cDNA library was constructed using *SuperScript™ Plasmid System for cDNA Synthesis and Plasmid Cloning* (Invitrogen). The cDNA library was sequenced, processed and analyzed using specific programs. **Results and Discussion:** We sequenced 384 clones, and after bioinformatics treatments, 297 sequences with high quality were analyzed using BlastX and BlastN, 263 sequences were indentified, 70,72% toxins clones and 29,28% cellular clones (normalized data). Among the toxin clones, we found the major toxin classes (metaloproteinases, 14.52%; PLA2, 11.29%; serine proteinases, 17.2%; C-type lectins, 33.87%), and as expected most of the cellular clones are involved in transcription and translation process (35.06%), which should be important for the further characterization of cell regulation. This library proved to be representative and was used for the construction of the cDNA nylon arrays containing 4500 clones. These arrays are now being probed against messenger RNAs from different times of the venom gland cell cycle in order to define which genes are differentially expressed.

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1.02 Sex-related differences in the antinociceptive effect of crotalphine, a peptide activating κ and δ opioid receptors

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Introduction: Sex differences in pain sensation and in opioid analgesia have been observed in human and rodents. Crotalphine (CRP), a peptide obtained from *Crotalus durissus terrificus* venom, induces analgesia by activation of opioid receptors. Due to its analgesic properties, pre-clinical trials with CRP are now in progress. However, studies have been always developed in male animals, with no information about its effectiveness in female rodents. **Objectives:** In the present study, differences in nociception and in the analgesic effect of CRP between male and female Wistar rats were evaluated. **Methods:** Differences in nociception and in the analgesic effect of CRP were evaluated using acute and chronic experimental pain models. Acute hyperalgesia was induced by intraplantar (i.pl.) injection of prostaglandin E_2 (PGE_2) into one of the hind paws. Neuropathic pain was induced by chronic constriction of the sciatic nerve (CCI) and characterized by the presence of hyperalgesia and allodynia, 14 days after surgery. Mechanical hyperalgesia and allodynia were determined using the rat paw pressure test or von Frey filaments, respectively. CRP (p.o.) was administered immediately before the hyperalgesic agent, or on day 14 after surgery. To determine the influence of the estrous cycle, vaginal smears were examined using 10x and 40x objectives. To determine whether the sex-related differences in nociception and crotalphine-induced antinociception were the result of the effects of gonadal hormones, female rats were submitted to ovariectomy. **Results and Discussion:** Female rats responded to lower hyperalgesic doses of PGE_2 than did males. In PGE_2-induced hyperalgesia, females responded to lower analgesic doses of CRP (p.o.) than did males. In females, the peptide, at 0.008 or 5 μg/kg, suppressed PGE_2-induced hyperalgesia for up to 3 or 6 days, respectively, whereas in males, CRP inhibited hyperalgesia for up to 3 h (0.2 μg/k) or 5 days (5 μg/kg). CRP was also more effective in inhibiting neuropathic pain in females than in males; however, in the CCI model, there were no sex differences in the duration of the analgesic action of the peptide. The antinociceptive action of CRP is mediated, in males and females, by activation of κ- and δ-opioid receptors. To determine the influence of gonadal hormones, females were ovariectomized (OVX) 11 days before the tests. The nociceptive behavior of ovariectomized rats was similar to that of male rats. In contrast, the analgesic effect of CRP was more pronounced in intact females than in the OVX group. Hormonal replacement restored the pain threshold and the effectiveness of CRP in females. The estrous cycle phase did not interfere with pain threshold and with CRP effect. These data indicate that sex differences could be observed between male and female rats in relation to pain threshold. Despite displaying opioid activity, CRP is more effective in females than males. The higher effectiveness of CRP in females is related to the presence of ovarian hormones.

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1.03 Relaxation effects of *Tityus bahiensis* scorpion venom on rat isolated aorta

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Introduction: The study of scorpion venoms of the *Buthidae* family is of interest not only to better understand the pathophysiology of envenomation, but also because these venoms contain neurotoxins, which act mainly on Na^+ and K^+ ion channels, modifying their gating properties. In this way, scorpion venoms have an important role in the study and characterization of ion channels and neurotransmission mechanisms. **Objectives:** We have previously observed that *Tityus bahiensis* scorpion venom induces a relaxation of pre-contracted rat aorta, so the aim of the present study was to better characterize this relaxation. **Methods**: Different concentrations of venom (3; 30; 300; 500 μg/ml) were added to rat aorta rings pre-contracted with noradrenaline (0.3 μM). Venom effect (300 μg/ml) was also analyzed in the absence or presence of different antagonists and blockers [tetrodotoxin (0.3 μM); atropine (1 μM); suramin (0.1 mM); caffeine (0.1 mM); L-NAME (0.1 mM)] and in rings where the endothelium was removed (n=6). **Results and Discussion:** The venom promoted intense relaxation of pre-contracted rings only when higher concentrations of 300 μg/ml (52.66±5.00%) and 500 μg/ml (51.56±7.40%) were tested. The action of 300 μg/ml of the venom was inhibited in the presence of L-NAME and suramin, or in rings without endothelium ($p<0.05$, ANOVA followed by Tukey's test). On the other hand, tetrodotoxin, atropine or caffeine was unable to abolish the relaxation caused by the venom, excluding the participation of tetrodotoxin-sensitive Na^+-channels, acetylcholine or adenosine. These data indicate that *Tityus bahiensis* scorpion venom causes relaxation of rat aorta by an action mediated by endothelium and that ATP and nitric oxide release could be involved in venom actions.

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1.04 Metalloproteinase inhibitors can control damage induced by sphingomyelinases from *Loxosceles laeta* spider venom in human keratinocytes

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Introduction: Sphingomyelinases D (SMases D) are considered the main toxic component present in *Loxosceles* spider venom. The best-known features of the local and systemic reactions induced by *Loxosceles* venoms are local pain, swelling and necrosis, sometimes followed by bleeding from persistent wounds, intravascular hemolysis and renal failure. More specific information about the molecular mechanisms operating during envenomation is needed to develop measures aimed at improving treatment of loxoscelism victms. **Objective:** In this study, the possible association of cell death and matrix metalloproteinase (MMP) expression was investigated. **Methods:** In order to analyze the toxic effects, $2x10^4$ HaCat cells were incubated with increasing concentrations of *L. laeta* venom or recombinant SMase D in the presence or absence of tetracycline and galardin. On the third day, cell viability was analyzed by MTT. Supernatants recovered from HaCat cells, during the period of treatment with venom or SMase D, in the presence or absence inhibitors, were analyzed by gelatin zymography. The action of venom or toxin, on the expression of keratinocyte cell surface molecules was determined after two hours of incubation in the presence of inhibitors, by flow cytometry. **Results and Discussion:** Results show that venom and the recombinant SMase D were able to induce morphologic alterations and loss of viability of human keratinocytes, in a dose-dependent manner. SMase D induces the activation of endogenous metalloproteinases, such as MMP-2, MMP-7 and MMP-9, which were positively associated with reduced cell viability. Besides, induction of EGFR, EPCR, MCP and MHCI cleavage was detected after treatment with the toxins, by action of proteases of the ADAM family. Damage to keratinocytes, induced by SMase D of *L. laeta*, could be controlled by the use of metalloproteinase inhibitors, such as tetracycline and galardin. Our results suggest that endogenous metalloproteinases, from MMP and ADAM families, are induced by the action of SMase D on human keratinocytes; these proteases may play an important role in the development of the dermonecrotic reaction induced by *Loxosceles* venom.

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1.05 Review of accidents caused by snakes of the tribe Tachymenini Bailey, 1967 (Dipsadidae, Xenodontinae) at Hospital Vital Brazil, Instituto Butantan

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Introduction: Snakebites reach 20,000 cases a year in Brazil. Most of these accidents are caused by venomous snakes, represented by: *Bothrops*, *Crotalus*, *Lachesis* and *Micrurus*. However, 40% of all accidents are caused by non-venomous snakes, most of them of Dipsadidae family. Among these non-venomous snakes, some species can cause substantial local effects in humans. We point out the green snake (*Philodryas olfersii*), *Elapomorphus quinquelineatus* (Dipsadidae, Elapomorphini), *Clelia*, *Boiruna*, and some Tachymenini species. Accidents caused by members of the tribe Tachymenini are a neglected subject. **Objectives:** A survey of snakebites caused by Tachymenini snakes that were treated at Hospital Vital Brazil, Instituto Butantan, São Paulo, Brazil. **Methods:** Between 1990 and 2006 48 cases of snakebites were recorded with the following information: sex, age, site of the bite, and symptoms of the victim, and time of the accident. When available, data on snake biology were gathered. **Results and Discussion:** We recorded forty-eight accidents caused by *Tomodon dorsatus* (n= 34), *Thamnodynastes* spp (n= 12), *Ptychophis flavovirgatus* (n= 1), and *Gomesophis brasiliensis* (n= 1). The accidents were more frequent in the spring (n= 18) and summer (n= 14), probably because of intense reproductive and feeding activity during these seasons, although 10 accidents occurred in the autumn and winter seasons. Accidents were prevalent in males, aged from 15 to 40 years, and the regions of the body most affected were feet and hands, including fingers. Usually, these accidents cause pain and local/discrete edema, which regressed in a few hours. However, in one case caused by *Thamnodynastes strigatus* on the index finger of an adult male, there was formation of a hemorrhagic bubble similar to bothropic bite. The species of the genera *Thamnodynastes* and *Tachymenis* can cause substantial local symptomatology in humans, and these accidents deserve not only the attention of health services but also research into the composition and action of its venom.

1.06 Characterization of isolated domains generated by P-I/P-II SVMP precursors

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Introduction: Snake venom metalloproteinases (SVMPs) are zinc-dependent proteolytic enzymes that cause hemostatic alterations in humans, following snake bite accidents. According to their domain structure, SVMPs are classified into three major classes, P-I (metalloproteinase domain only), P-II (metalloproteinase and disintegrin domains) and P-III (metalloproteinase, disintegrin-like and cysteine-rich domains). Proteolytic functions are described mainly for P-I and P-III SVMPs and only a few P-II class metalloproteinases have been studied. We have partially isolated and sequenced a SVMP from *Bothrops neuwiedi* venom, called BnP1, whose partial sequence showed similarity to SVMPs derived from P-II precursors. In this regard, BnP1 could be an interesting model to study the biosynthesis and function(s) of P-II SVMPs. **Objectives:** To purify BnP1 and its corresponding disintegrin, evaluating their capacity in inhibiting platelet aggregation, and to identify the complete cDNA sequence of this putative P-II SVMP by cloning and sequencing cDNAs encoding SVMPs from a *B. neuwiedi* venom gland. **Methods:** BnP1 was purified by FPLC molecular exclusion chromatography (Superdex 75 HR 10/30, pH 7.8) followed by anion exchange chromatography (Mono-Q HR 5/5, pH 7.8). Purification was monitored by relative molecular mass (SDS-PAGE) and fibrinolytic activity (fibrin-agarose plates). Disintegrins were obtained by reverse phase chromatography (Vydac C18 column-HPLC) monitored by inhibition of platelet aggregation. Partial protein sequences were obtained by MS/MS. The inhibition of platelet-aggregation was determined by challenging human platelets with ADP, collagen, thrombin and ristocetin. For cDNA cloning and sequencing, the RNA was extracted from a *B. neuwiedi* snake venom gland. cDNAs were amplified by PCR using primers based on conserved regions of SVMPs, cloned into pGEM–T Easy plasmid. The DH5α transfected colonies were selected by PCR using SVMP-specific primers and the purified plasmids were sequenced. **Results and Discussion:** BnP1 was obtained as a single 25-kDa band, and two new disintegrins were isolated and called D2 and D4, which inhibited platelet aggregation induced by all agonists. BnP1 did not show any significant inhibition of platelet-aggregation. Two distinct cDNAs of each class of SVMP were cloned representing the P-I, P-II and P-III complete sequences. BnP1 partial sequence aligned with high similarity both with P-I (69 out of 73 residues sequenced by MS) and P-II (67 out of 73 residues sequenced by MS) cDNAs. A previous disintegrin sequence identified by MS aligned (100%) with a P-II cDNA, which also encodes BnP1 sequences. Despite sequence similarity with P-II catalytic SVMPs, BnP1 did not show any significant effect on platelet aggregation. Only the isolated disintegrins were able to inhibit platelet aggregation by all tested agonists. The sequence analysis indicated that BnP1 may be coded either by P-I or P-II mRNAs since they encode very similar catalytic domains. This suggests that biosynthesis of low-molecular mass SVMPs is still unclear and may involve rearrangements and processing in different biosynthetic steps rather than the selection of different genes for each SVMP subclass.

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1.07 Antiviral activity of the hemolymph of *Lonomia obliqua* (Lepidoptera: Saturniidae)

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Introduction: Insects are regularly exposed to environmental contaminants. Several studies have detected the presence of peptides, enzymes and metabolites in the hemolymph of insects that curb the proliferation of bacteria and fungi. The immune response of insects to bacterial or fungal infections and to filarial or parasitoid infestations is well documented. However, unlike vertebrates, insects have none of the well-characterized mechanisms for recognizing viruses or virus-infected cells. **Objectives:** The objectives of this work were the identification, isolation, characterization of the hemolymph protein from *L. obliqua* and the evaluation of its antiviral activity against human viruses. **Methods:** The protein responsible for antiviral activity was isolated, purified by gel filtration chromatography using a gel filtration column system (Superdex 75) and further fractionated using a Resource-Q ion exchange column system. Influenza (H1N1), polio (Sabin 1) and measles (Edmonston) viruses were used to determine the antiviral activity of the hemolymph. VERO cells were infected with the measles and polio viruses and MDCK cells with the the influenza virus on the 3rd day post-inoculation at an MOI of 0.1. End-point dilution assays were performed in 96-well microtiter plates to measure virus titers. **Results and Discussion:** Potent antiviral activity against measles, influenza and polio viruses was observed in the hemolymph of *Lonomia obliqua*. Experiments with the purified protein led to a 157-fold reduction (from $3.3\pm1.25\ \mathbf{x}10^{7}$ to $2.1\pm1.5\mathbf{x}10^{5}$ $TCID_{50}$ mL^{-1}) in measles virus production and a 61-fold reduction (from $2.8\pm1.08\ \mathbf{x}10^{9}$ to $4.58\pm1.42\mathbf{x}10^{7}$ mL^{-1}) in polio virus production. Heating and freezing seemed to have no influence on its antiviral activity. The protein did not display virucidal activity and did not act on receptors on the cell membrane. The observations suggest an intracellular mechanism of action and that the protein may act as a constitutive agent that affects the innate antiviral immune response.

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1.08 A rat model of bone cancer pain induced by intrafemoral inoculation of Walker 256 carcinoma cells – analgesic effect of crotalphine

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Introduction: Crotalphine, a peptide first identified and isolated from the South American rattlesnake *Crotalus durissus terrificus*, induces analgesia mediated by the activation of δ- and κ-opioid receptors. **Objective:** The aim of this work was to characterize the analgesic effect of crotalphine in a new rat model of bone cancer pain induced by intrafemoral inoculation of Walker 256 carcinoma cells. **Methods:** Rats were injected with carcinoma cells into the femoral cavity. Bone metabolic alterations were determined by scintigraphy, using ^{99m}Tc-MDP, which is significantly concentrated in areas of osteogenesis. Femoral images were obtained before and 7 and 14 days after tumor cell inoculation. Bone cancer pain was characterized by the presence of hyperalgesia and allodynia, determined using the rat paw pressure test or von Frey filaments, respectively. **Results and Discussion:** Incorporation ^{99m}Tc-MDP was significant 7, 14, 21 days after tumor cell injection, suggesting tumor development in the femoral cavity. Hyperalgesia and allodynia were detected on days 1, 3, 7, 14 and 21 after cell inoculation. Interestingly, we observed that paw withdrawal threshold in von Frey test was reduced not only in the ipsilateral hind paw inoculated with the tumor, but also in the contralateral one, demonstrating the existence of bilateral allodynia (mirror-image pain). Crotalphine administered on day 21, blocked hyperalgesia and allodynia. These results indicate that intrafemoral injection of Walker 256 cells causes bone cancer and pain. Crotalphine induces potent antinociception in this model of cancer pain.

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1.09 Purification and characterization of hyaluronidases from *Otostigmus pradoi* centipede venom

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Introduction: Centipedes are terrestrial arthropods distributed worldwide, except in Antarctica, existing in great abundance in tropical areas. These animals have a long segmented body, which ranges from 4 to 300 mm of length and contains one pair of legs per segment. These arthropods reside in underground galleries, under rocks and tree bark, and also between trash and rubble in urban areas. Accidents occur when centipedes are touched and, as defensive behavior, they inject venom through a pair of forceps - appendages on the first segment behind the head. Centipede envenomation is characterized by pain, erythema and edema. Previous studies showed the presence of hyaluronidases in centipede venoms. **Objectives:** The aim of this work was to purify and characterize hyaluronidases isolated from the venom of the *Otostigmus pradoi* centipede, the species accounting for 27 % of centipede envenomation in the metropolitan area of São Paulo city, Brazil. **Methods:** Animals were captured around the Butantan Institute and kept in captivity. Venom was milked once a month by electric stimulation. Gel filtration (Superdex 75 GL column) and ion exchange (Mono S 5/50 column) chromatography were used to fractionate the venom. SDS-PAGE (10 %) and zymographic analysis, using 170 μg/mL of hyaluronic acid as substrate, were performed to characterize proteins fractionated by chromatography. **Results and Discussion:** Four fractions were eluted after gel filtration chromatography. Hyaluronidase activity was found mainly in fraction 3. This fraction was purified by ion exchange chromatography and eleven peaks were obtained, three of which possessed hyaluronidase activity. The main components with hyaluronidase activity had molecular masses of approximately 53-41 and 32 kDa, which were eluted with 0.14 to 0.27 M NaCl. This result indicates the presence of at least two different enzymes able to hydrolyze hyaluronic acid in *O. pradoi* centipede venom. Hyaluronidases are enzymes that have a biotechnological potential due to their ability to spread substances under the skin. Herein, two hyaluronidases were partially purified from *O. pradoi* centipede venom. Further studies are in progress to determine the optimal conditions of these enzymes, such as pH and temperature.

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1.10 Effect of insularin, a disintegrin from *Bothrops insularis* venom, on endothelial cells

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Introduction: Endothelial integrins play an essential role in angiogenesis and cell survival. Disintegrins are a family of low-molecular-weight and cysteine-rich proteins derived from viper venom. They bind to Arg-Gly-Asp (RGD)-responsive integrins with high affinity and block integrin function. We isolated insularin, a RGD-disintegrin from *Bothrops insularis* snake venom, which inhibits platelet aggregation by glycoprotein (GP) IIb-IIa with an IC_{50} of 0.80 μM. **Objectives**: The aim of this study was to evaluate the effects of insularin on human umbilical vein endothelial cell (HUVEC) adhesion and the inhibition of capillary-like tube formation by the Matrigel assay. **Methods:** The adhesion assay was performed after preincubation of insularin with HUVECs (10^6 cells/mL), for 1 h at 37° C. The cells were added to fibrinogen-coated plates, and after 1 h of incubation, adhesion was determined using the MTT method. For HUVEC tube formation assay, Matrigel was added to the *chamber slide system* in a volume of 50 μL in each well. After gel formation (30 min at 37° C), HUVECs, in the presence of various concentrations of insularin or PBS (control samples), were applied to each well and incubated at 37° C for 24 h in 5% CO_2. **Results and Discussion:** Insularin inhibits the HUVEC adhesion to immobilized fibrinogen with an IC_{50} of 36 nM in a dose-dependent manner and significantly inhibits (1μM) tube formation on Matrigel. Several lines of experimental evidence suggest the potential use of RGD-disintegrins for the development of therapeutic anti-angiogenic and/or anti-tumorigenic agents. These results provide further evidence that insularin may act as an anti-angiogenic agent, in *in vitro* assays, by interaction with integrin αvβ3 present in the endothelial cells. Furthermore, the antiangiogenic activity of insularin could be confirmed by *in vivo* assays.

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1.11 Effects of PA-BJ and gyroxin, two serine proteinases isolated from snake venom, on endothelial cells *in vitro*

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Introduction: The disturbances in blood clotting are among the most dramatic effects of envenomation by viperid snakes. Venom serine proteinases are involved in proteolytic events, causing disturbances in hemostasis observed upon envenomation. However, their effects on endothelium are still unknown. PA-BJ and gyroxin are serine proteinases isolated from *Bothrops jararaca* and *Crotalus durissus terrificus* snake venom, respectively. PA-BJ induces platelet aggregation mediated by proteinase activated receptors -1 and -4 (PAR -1 and -4), which are also activated by thrombin. Gyroxin, in turn, acts on the central nervous system, causing temporary episodes in mice, characterized by opisthotonos and rotation around the long axis of the animals, and produces clotting on fibrinogen. **Objective:** To investigate the effects of PA-BJ and gyroxin on endothelial cells in culture, evaluating: a) viability and integrity of endothelial cells. b) release of prostacyclin (PGI_2) and c) protein expression of cyclooxygenase-1 and -2 (COX-1 and -2). **Methods:** Endothelial cells (ECs) from the murine endothelioma cell line (tEnd) were grown in RPMI-1640 medium with 10% FBS and seeded on 96 well microplates for formation of monolayers. After reaching confluence (48 h), ECs monolayers were incubated with PA-BJ (0.01, 0.1, 0.5 and 1 μM) or gyroxin (1 μM) or RPMI (control) or Triton X-100 0.1% for selected periods of incubation (3 or 24 h). ECs viability was evaluated by determination of lactate dehydrogenase (LDH) activity and tetrazolium salt reduction assay (MTT). The integrity of monolayers was determined by crystal violet staining. Prostacyclin production was evaluated by enzyme immunoassay and COX-1 and -2 protein expression by Western blotting. **Results and Discussion:** Incubation of ECs monolayers with PA-BJ and gyroxin neither affected the integrity of monolayers nor modified ECs viability at all concentrations and periods of incubation tested. At the highest concentration, these serine proteinases induced release of PGI_2 after 24 h of incubation, but did not affect basal levels of COX expression. The data show the ability of PA-BJ and gyroxin to stimulate the production of prostacyclin by the endothelium in culture. This effect is not related to increased protein expression of COX-2. Since PGI_2 is a potent vasodilator, this mediator may contribute to interference in hemostasis induced by both PA-BJ and gyroxin.

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1.12 Effect of *Crotalus durissus terrificus* snake venom and crotoxin on neutrophil functions *in vitro*

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Introduction: Previous works showed that *Crotalus durissus terrificus* snake venom (CdtV) modulates macrophage function, inhibiting spreading and phagocytic activity but increasing the oxidative burst of these cells. In addition, crotoxin (CTX), the main component of the venom, was reported to inhibit this phagocytic activity. Recently, CdtV was shown to inhibit carrageenan-induced inflammatory response and phagocytosis by neutrophils. Despite these findings, the component of CdtV responsible for the inhibition of phagocytosis is still unknown. Moreover, the effect of crude CdtV in other important functions of neutrophils, such as reactive oxygen species production, has not yet been investigated. **Objectives:** The aim of this study was to investigate the effect of CTX on phagocytosis activity, via the C3b receptor, and the effect of crude CdtV on hydrogen peroxide (H_2O_2) production, both by neutrophils obtained by carrageenan-induced peritonitis. **Methods:** Neutrophils were obtained from the peritoneal cavity of male Wistar rats (170g) (Institutional Animal Care Committee at Butantan Institute, protocol number 407/07) 4 h after the intraperitoneal (i.p.) administration of carrageenan (cg) (4.5 mg/kg). Phagocytosis of opsonized zymosan was evaluated after in vitro treatment with CTX. For this treatment, cells ($1.2x10^6$ cells/mL) were incubated (1 h) with RPMI 1640 medium (control) or with RPMI 1640 medium containing CTX (0.02, 0.04, 0.08, 0.16 and 0.32 μg/mL). Reactive oxygen species production was determined by H_2O_2 production. For this assay, cells ($4x10^5$ cells/mL) were incubated (1 h) with RPMI 1640 medium (control) or with RPMI 1640 medium containing CdtV (0.25, 0.5 and 1.0 μg/mL), and H_2O_2 production was evaluated by the phenol red oxidation method. **Results and Discussion:** In vitro, CTX significantly reduced the phagocytic activity of neutrophils at the following concentrations: 0.02 μg/mL: 24% (cg+CTX: 86.6±7.7; $p<0.05$), 0.04 μg/mL: 26% (83.3±11.7; $p<0.01$), 0.08 μg/mL: 27% (87.2±8.4; $p<0.05$). However, CTX at 0.16 and 0.32 μg/mL did not alter the phagocytic activity of neutrophils. For reactive oxygen species production, crude CdtV, at all concentrations, did not alter H_2O_2 production by neutrophils. These results show that CTX inhibits phagocytosis in neutrophils, as has been described for macrophages. However, CdtV did not stimulate H_2O_2 production in neutrophils, unlike in macrophages. Considering the important role of neutrophils in inflammation, the data presented herein contribute to the characterization of the anti-inflammatory effect of the CdtV, particularly of the CTX, recently described. These data reinforce the role of CTX as a new approach to control inflammatory diseases.

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1.13 Seroneutralization and pharmacological inhibition of the main toxic activities induced by venom of the freshwater stingray *Potamotrygon falkneri*

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Introduction: Pain and edema are the most conspicuous symptoms observed in patients wounded by stingrays. Moreover, skin necrosis is commonly observed in injuries caused by freshwater stingrays. There is no commercially available antivenom, and the treatment of patients is exclusively symptomatic, but not entirely satisfactory. **Objectives:** The objective of this work was to evaluate the effectiveness of anti-*P. falkneri* serum as well as anti-inflammatory drugs (cyclooxygenase inhibitors) to block toxic activities induced by *P. falkneri* venom. **Methods:** IgG was isolated by affinity chromatography (Protein A-Sepharose) from sera of rabbits immunized against *P. falkneri* venom. ELISA and Western blotting were used to verify the immunogenicity of the venom. Three doses of venom (16, 32 and 64 μg) were administrated to the hind paw of mice for evaluation of edema (measured at 30 min and 1, 2, 24 and 48 h) and nociception. Local tissue injury – characterized by necrosis, ecchymosis and ischemia – was observed 72 h after venom injection (200, 400 and 600 μg). Myotoxicity was evaluated by measuring the levels of creatine kinase (CK) in serum of mice injected i.m. with venom (50, 100 and 200 μg). In order to investigate the ability of specific antibodies to neutralize *P. falkneri* venom activities, groups of mice were inoculated with a mixture of different doses of venom that had been preincubated with a fixed volume of antibodies (30 min, 37° C). To verify the action of anti-inflammatory drugs, indomethacin (4 mg/kg), dipyrone (200 mg/kg) or etoricoxib (10 mg/kg) was administered (i.p.) 30 min before *P. falkneri* venom injection, and the edematogenic and nociceptive activities were evaluated. **Results and Discussion:** By ELISA, *P. falkneri* venom was found to induce high levels of antibodies (titer 2,048,000) in rabbits. Many venom components, mainly above 20 kDa, were recognized by antibodies using Western blotting. Antibodies were effective in neutralizing necrosis (above 68%) and nociception (above 56%), and partially neutralized the edema from 30 min to 24 h. However, antibodies failed to neutralize the myotoxicity elicited by venom. The results demonstrate the efficacy of specific anti-*P. falkneri* antibodies in neutralizing the main toxic activities induced by crude venom, except for myotoxicity. Dipyrone and etoricoxib were effective in reducing nociception in 85% and 59%, respectively. In contrast, dipyrone, but not etoricoxib, was effective in abrogating nociception. However, indomethacin failed to inhibit this venom toxicity. All drugs partially reversed venom-induced edema. Our results demonstrate that eicosanoids are involved in both nociception and edema induced by *P. falkneri* venom, and that anti-inflammatory drugs may be useful to treat pain in patients wounded by this stingray. However, more studies are necessary to demonstrate that the specific antivenom as well as cyclooxygenase inhibitors may be effective in treating patients wounded by *P. falkneri*.

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1.14 *Tityus serrulatus* venom downregulates ENaC and AQP5, as well as upregulating NKCC1, in rat lungs

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Introduction: *Tityus serrulatus* envenomation can cause pulmonary edema of cardiogenic or noncardiogenic origin. Pulmonary edema clearance is largely related to active Na+ transport out of the alveoli, rather than the reversal of Starling forces. Pulmonary edema is resolved through active Na transport across the alveolar epithelium via apical amiloride-sensitive Na channels and via basolateral alpha-Na-K-ATPase. This active vectorial sodium flow produces a transepithelial osmotic gradient that results in passive movement of water from the air spaces into the alveolar interstitium. Found in virtually all cells, NKCC1 mediates the Na-/K-/Cl-coupled influx and regulates cell volume. In the lung, AQP5 provides the principal route for osmotically driven water transport. **Objectives:** The aim of the present study was to determine the effects that *T. serrulatus* envenomation has on the mechanisms of pulmonary edema. **Methods:** Rats were inoculated intraperitoneally with *T. serrulatus* venom (3.8 mg/kg BW; V group, n = 13). Control rats were injected with saline (C group, n = 9). Serum urea, creatinine, Na, K, creatine kinase (CK), lactate dehydrogenase (LDH), troponin and amylase were measured one hour after inoculation. In lung tissue, protein expression of the epithelial Na channel, alpha subunit (α-ENaC), Na-K-ATPase, NKCC1 and AQP-5 was determined by Western blotting. Data are expressed as mean ± SEM. **Results and Discussion:** In the V group, 9 rats developed clinical signs of severe envenomation, and serum urea was higher than in the controls (39.4 ± 2.4 vs. 51.8 ± 3.5, $P < 0.036$). There were no differences between the groups in terms of the serum levels of Na, K and creatinine, amylase, CK or LDH. However, envenomation led to marked downregulation of pulmonary expression of α-ENaC (100.7 ± 6.5 vs. 48.8 ± 5.4, $P < 0.0061$) and AQP-5 (98.7 ± 2.2 vs. 61 ± 7.3, $P < 0.012$), whereas NKCC1 protein expression increased (102.1 ± 6.2 vs. 141.2 ± 2.0, $P < 0.0061$) and Na-K-ATPase protein expression was unaffected. We hypothesize that *T. serrulatus* venom decreases α-ENaC protein abundance, reducing the transport of Na from the lumen to the interstitium, as well as decreasing the movement of water from the lumen to the interstitium, thereby lowering the osmotic gradient and impairing alveolar clearance. The resulting cell shrinkage can stimulate basolateral NKCC1. The decreased influx of Na from the lumen into the cells (induced by the lower α-ENaC levels), together with the increased influx of Na from the interstitium into the cells (induced by the higher NKCC levels), can block the net influx of Na and water from the alveoli. Our data show that *T. serrulatus* venom has a profound effect on the Na+ and water transport capacity of alveolar epithelial cells. Inadequate pulmonary fluid handling can impair lung function and increase susceptibility to lung injury.

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1.15 Melittin-S: a novel melittin isoform from *Apis mellifera* venom

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Introduction: Melittin, the main component of *Apis mellifera* venom (~50% by dry weight), is a peptide with 26 residues and a characteristic structure of membrane bound cytolytic, channel-forming peptides and trans-membrane protein helices. This peptide shows hemolytic activity, other than being the primary allergen in bee venom. **Objectives:** To analyze the composition of *A. mellifera* venom over one year, in one specific hive, and to identify the main components. **Methods:** Venom from Africanized honeybee workers was obtained by electric stimulation of the animals. The venom of each month was analyzed and purified by RP-HPLC (C8 column), and the major peaks were analyzed by mass spectrometry and/or Edman degradation. A hemolytic assay was carried out as well. **Results and Discussion:** It was possible to perceive a seasonal variation in the venom contents of melittin. Moreover, we have been able to identify a novel melittin isoform, named melittin-S, produced during the southern winter. Melittin-S levels are 1-2% throughout the year, but abruptly rise to 10% during the southern winter months, thus becoming a 'new' major component. This isoform has the same amino acid sequence of melittin, but has a Ser residue instead of Thr at the tenth position. The hemolytic activity level of melittin-S is equivalent to melittin. Although, a melittin from *A. dorsata* has already been described showing a Ser at this tenth position, it was one amino acid shorter than melittin and melittin-S described here. For *A. mellifera*, this is the first description of a melittin isoform. Moreover, it was observed that the total melittin content remains rather constant throughout the year, indicating that the bee responds to some environmental change by synthesizing melittin-S. The hemolytic activity present in melittin-S indicates that the amino acid modification should retain the other physiological roles of melittin.

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1.16 Mechanisms involved in prostacyclin production induced by CB, a crotoxin subunit with phospholipase A_2 activty, isolated from *Crotalus durissus terrificus* snake venom

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Introduction: Crotoxin (CTX), the major component of *Crotalus durissus terrificus* snake venom (CdtV) contains two subunits: crotapotin (CA) and phospholipase A_2 (CB). The CB subunit exerts neurotoxic and myotoxic effects and inhibits macrophage functions. The phospholipase A_2 (PLA_2) enzymes catalyze the cleavage of arachidonic acid (AA) from the *sn*-2 position of phospholipids with subsequent conversion of free AA into prostaglandin H_2 by two distinct isoforms of cyclooxygenases (COX-1 and COX-2); newly formed PGH_2 is then converted into distinct prostaglandins by terminal synthases. Prostacyclin (PGI_2), a potent vasodilator and inhibitor of platelet aggregation, is the predominant prostaglandin synthesized by vascular endothelial cells (ECs). Recent data from our laboratory demonstrated the ability of CB to release PGI_2 from endothelial cells *in vitro*. **Objective:** In this study, the role of COX-1 and -2 enzyme systems on CB-induced prostacyclin production by endothelial cells was evaluated. **Methods:** ECs from a murine endothelioma cell line (tEnd) were grown in RPMI-1640 medium with 10% FBS and seeded on 96 well microplates for formation of monolayers. After reaching confluence, ECs monolayers were incubated with CB (0.4 μM) or RPMI-1640 (control) for selected periods of incubation. Inhibition of COX activities was performed by the pre-incubation of ECs with indomethacin (non-selective COX inhibitor) or valeryl salicylate (selective COX-1 inhibitor) or etoricoxib (selective COX-2 inhibitor). Production of PGI_2 was measured using enzyme immunoassay (EIA) and COX protein expression evaluated by *Western blotting* analysis. **Results and Discussion:** Pre-incubation of ECs with indomethacin or valeryl salicylate or etoricoxib significantly decreased the prostacyclin production induced by CB subunit (78%, 42% and 61%, respectively) ($p \leq 0.05$). This toxin also up-regulated COX-2 protein expression (53%), whereas 4-bromophenacyl bromide (BPB), an inhibitor of phospholipase A_2 enzyme activity, abrogated this effect. However, the COX-1 pattern of protein expression was not modified by CB. These data indicate that COX-1 and COX-2 activities are involved in CB-induced prostacyclin production from endothelial cells. In addition, up-regulation of COX-2 protein expression is important for the effect induced by CB. Moreover, the catalytic activity of CB is essential for the stimulatory effect of this phospholipase A_2 on the biosynthesis of PGI_2. Finally, these findings indicate novel regulatory mechanisms for venom secretory PLA_2 in endothelial cells.

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1.17 *Loxosceles intermedia* sphingomyelinase D induces the activation of proteases on the human erythrocyte membrane, able to hydrolyze fluorescence substrates

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Introduction: Spiders of the *Loxosceles* genus, also known as "brown spiders," are distributed worldwide. Accidents caused by these spiders are associated with severe clinical symptoms, including dermonecrotic lesions, thrombosis, vascular leakage and, eventually, intravascular hemolysis and renal failure, which can be lethal. In Brazil, three major species, implicated with human envenoming, are responsible for more than 6000 cases of envenomation each year. The main toxic component present in the venom is a sphingomyelinase D (SMase D). *Loxosceles* SMase D promotes the activation of membrane bound proteinases on erythrocytes and nucleated cells. In the case of erythrocytes, this leads to an increased susceptibility to activation of complement (C), *via* the alternative pathway, because of proteinase-induced cleavage of glycophorins, which are important regulators of C-activation. **Objectives:** The aim of the present study was to further investigate the activation mechanism of the membrane-bound proteinase(s), responsible for the cleavage of the erythrocyte glycophorins, induced by SMases D. **Methods:** We used the fluorescence resonance energy transfer (FRET) substrate Abz-FRSSRQ- EDDnp as a tool to explore the proteolytic activity of human erythrocyte membranes. Membranes were incubated with 5 mM substrate, in the presence or absence of a recombinant SMase D from *L. intermedia* (referred to as P2 - 5 μg) at 37°C, in a 96-well plate. The hydrolysis reaction, of the fluorogenic peptide, was monitored during 40 min in a spectrofluorimeter. In addition, inhibition assays were performed in order to determine the class of the activated proteinase(s). **Results and Discussion:** Data obtained show that the erythrocyte membrane preparation have a basal proteolytic activity on the fluorogenic peptide. However, treatment with SMase P2 induces an increase of this enzymatic activity by approximately 20%. Moreover, the use of an elastase inhibitor was able to reduce the membrane proteolytic activity, induced by treatment with P2, by about 40%. Our results suggest that SMase P2 is able to activate proteinase(s) on erythrocyte membrane preparations and that elastase may be involved in the process.

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1.18 Purification and partial characterization of blood coagulation Factor X activator from *Bothrops jararaca* snake venom

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Introduction: Snake venoms of the viperidae family contain several proteins that cause hemostatic disturbances. Envenomation by this family is characterized by hemorrhage, edema, local tissue damage, myonecrosis, and fibrinolytic and kinin releasing activities. *Bothrops jararaca* envenomation causes local and systemic hemorrhage, together with blood clotting disorders, mainly because of the presence of thrombin-like, factor X and prothrombin-activating enzymes. Although thrombin-like and prothrombin activators have already been isolated from this venom, factor X activators have not yet been purified. Considering the importance of these proteases in the coagulation process, these enzymes have been largely studied as drugs in the clinical therapeutic area. Thus, this work shows the isolation of the factor X activator from *Bothrops jararaca* snake. **Objectives:** Purification and partial characterization of blood coagulation factor X activator from the venom of *Bothrops jararaca*. **Methods:** An amount of 100 mg venom was dissolved in 1 mL 20 mM Tris-HCl (pH 7.5) containing 5 mM benzamidine and 200 mM NaCl, and centrifuged at 5400 g for 10 min. The clear supernatant was filtered through a 0.22 μM membrane and applied to a Superdex 75 column, followed by a benzamidine Sepharose column and finally a SP Hitrap XL column. Along all the purification steps, protein concentration was determined by absorbance at A_{280}. Protein activity was measured using a specific chromogenic substrate. The fractions were analyzed by SDS-PAGE (10%). **Results and Discussion:** The factor X activator was isolated from the *Bothrops jararaca* snake venom using three chromatography steps: gel filtration, affinity and cation-exchange columns. The purified protein was analyzed by SDS-PAGE showing a molecular mass of 67 kDa. This is the first report on the isolation and partial characterization of a factor X activator from *Bothrops jararaca* snake venom. The perspectives for this work are to obtain a larger amount of protein for biological and biochemical characterization.

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1.19 Identification of novel compounds from solitary wasp venom

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Introduction: Bradykinin (BK) is one of the most important compounds generated following tissue injury. This nonapeptide is released by the action of endogenous or exogenous kininogenases on plasma kininogen, and is a potent algesic, hypotensive, and proinflammatory endogenous substance. It is rapidly hydrolyzed by the action of angiotensin-converting enzyme (ACE). BK and BK-related peptides (BRPs) are also widely distributed in venomous animals, including frogs and insects such as wasps. In a survey of the solitary wasp *Cyphononyx fulvognathus* venom, three novel BRPs, besides the well-known Thr^6-BK, were identified. Herein, we report the chemical, biochemical and pharmacological characterization of Thr^6-BK and of these novel BRPs, named *Cf*-32, *Cf*-46, and *Cf*-146. **Objective:** Aiming for a better understanding of the wasp envenomation process, the extract of the solitary wasp *Cyphononyx fulvognathus* venom was analyzed. The identified compounds were both biochemically and pharmacologically characterized. **Methods:** The venom extracts of *C. fulvognathus* were subjected to reverse-phase HPLC, and the purity and complexity of each fraction was examined by MALDI-TOF MS. The primary sequence of the observed peptides was determined by Edman degradation and ladder sequencing. Enzyme activity assay and determination of kinetic parameters of ACE for these wasp peptides were performed using the synthetic analogs obtained by synthesis on an automated PSSM-8 peptide synthesizer. These same analogs were employed for the BK-potentiation assays on isolated guinea pig ileum and pain threshold evaluations (CEUAIB no. 532/2008). **Results and Discussion:** Interestingly, we found that these peptides showing the highest structural similarity to BK, which was the case with Thr^6-BK and *Cf*-46, were shown to be able to contract smooth muscle preparation, while the other two peptides studied herein, namely *Cf*-32 and *Cf*-146, could not. On the other hand, all these peptides were able to inhibit ACE as well as to induce the hyperalgesic effect in living rats after intraplantar injection. The use of specific BK-receptors antagonists also allowed the identification of BK-receptors as the target of these wasp peptides. We believe that this knowledge will contribute to a better understanding of the wasp envenomation process, which could be a good support in proposing the inclusion of novel elements to be considered for the treatment of current wasp accident symptoms.

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1.20 Human anti-crotoxin single-chain variable-fragment (scFv) expressed in bacteria and refolded

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Introduction: Nowadays, single chain variable fragments, scFv, are becoming therapeutic alternatives to whole monoclonal antibodies (AcMos), since these are smaller, have different advantages in certain medical applications and are easily genetically manipulated. Recently, an antibody isolated by phage display technique has just been approved for therapeutic use in humans (adalimumab, anti-TNF, form Humira; Abbott Laboratories). At least 14 additional antibodies derived from recombinant libraries are in clinical trial. ScFv contains the variable domain of heavy (VH) and light (VL) chains linked by a flexible polypeptide $(G_4S)_3$ and may be useful as auxiliary therapy to envenoming by snake bite. The South American rattlesnake *Crotalus durissus terrificus* is responsible for about 8% of envenoming in Brazil. Its venom contains many toxins, such as gyroxin, convulxin, crotamin and crotoxin (CTX). CTX is the main toxic component of the venom and responsible for most of its toxic effects, such as neurological disorders, myotoxicity, and renal failure. It is composed of two subunits, an acidic, nontoxic, and nonenzymatic component (crotoxin A, CA, or crotapotin) and the toxic molecule, a basic Asp49-PLA2 (crotoxin B, CB, or Cdt PLA2). ScFv6 anti-crotoxin was isolated by phage display technology from a naive library of more than 10^{10} scFv clones with *in vivo* CTX and crude venom neutralizing activities. Many attempts have been tried to improve its expression in *E. coli* system such as the use of a synthetic scFv6 gene devoid of rare codons with a MBP-linker. However, this strategy resulted in the expected complete protein, in which MBP could not be removed. **Objective:** To express the scFv6 in the cytoplasm of bacteria and optimize refolding conditions to obtain the molecule in its soluble and functional form. **Methods:** ScFv6 coding sequence was cloned into a pAE vector that contains His-tag and used to transform BL21 (DE3) bacteria. The production of scFv was accomplished using 0.5 mM IPTG and growth condition at 37°C for 4 h. The insoluble ScFv was extensively washed and incubated in the reducing buffer (8 M urea solution). Renaturation of reduced ScFV was achieved by dialysis with buffer containing urea. The urea concentration of the dialyzing bottle was gradually diluted with buffer without urea at a flow rate of 0.1 ml/min by high-pressure pump. The purity of the sample was evaluated by SDS-PAGE and Western blotting using mouse anti-His antibody followed by anti-mouse-peroxidase. **Results and Discussion:** The construct expressed a 30 kDa expected protein and the refolding resulted in a 40 μg/mL yield. DNA sequencing of scFv6 revealed identity to the reported sequence (Genbank: AJ132608). The refolding system used had some drawbacks and to solve these problems we are now trying to refold using hydrostatic pressure. Reproducible refolded scFv will be biochemically characterized regarding its affinity and ability to neutralize CTX and venom toxic activities. Satisfactory results will lead us to the next step: improvement of the affinity and specificity of scFv6 using suggestions of the Antibody Evolution (AbEvo) software that predicts advantageous mutations.

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1.21 Structure-activity studies of different peptide-like BPP-10c. Evaluation of cardiovascular effects in SHR

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Introduction: The bradykinin-potentiating peptides (BPPs) from *Bothrops jararaca* snake venom were the first angiotensin I converting enzyme (ACE) inhibitors described, providing the basis for the development of captopril. *In vivo* studies showed that the BPP-10c can cause a potent and long-lasting decrease in mean arterial pressure (MAP) and heart rate (HR) of spontaneously hypertensive rats (SHR) independent of ACE activity. Recently, argininosuccinate synthetase (AsS) was identified as a novel target for BPP-10c, suggesting that AsS could be a new mechanism for the antihypertensive effect of the decapeptide in SHR. **Objectives:** To evaluate the cardiovascular effects of peptide-like BPP-10c in SHR and to identify the responsible amino acid(s) for the BPP-10c effects. **Methods:** Twenty hours before the experiment, polyethylene catheters were introduced into abdominal aorta and femoral vein for measurements of cardiovascular parameters and intravenous (i.v.) injection, respectively. Before drug administration, the cardiovascular parameters were monitored for 30 min (baseline period). Following i.v. injection of the BPP-10c, peptide-like BPP-10c (71 nmol/kg) or vehicle (0.9% NaCl) was given in a total volume of 0.5 ml. The cardiovascular parameters were monitored for 6 h after drug administration. Six peptide-like BPP-10c were evaluated: BPP-10c des<ENW, BPP-10c desPro^{10}, BPP-10c E^{1}, BPP-10c Scrambled, BPP-10c Ala^{5} and BPP-10c Ala^{3}. Data were compared by Student's *t* test or ANOVA followed by Newman-Keuls post-test when appropriate. **Results and Discussion:** The BPP-10c des<ENW, BPP-10c E^{1}, BPP-10c Scrambled and BPP-10c Ala^{5} were able to cause a significant decrease in MAP. The maximal change of MAP ranged from -21±3 mmHg to -25±4 mmHg *vs* -11± 2 mmHg (control group), $p<0.05$. The MAP changes caused by the modified peptides were smaller than that described for BPP-10c (-36±3 mmHg, $p<0.001$). The four peptide-like BPP-10c caused maximal MAP changes from 58 to 70% of the BPP-10c maximal effect. Similar to BPP-10c, bradycardia was observed after BPP-10c des <ENW, BPP-10c desPro^{1}, BPP-10c Ala^{5} and BPP-10c Ala^{3} injection. BPP-10c Scrambled and BPP-10c E^{1} did not cause changes in HR. The BPP-10c des<ENW, BPP-10c E^{1} and BPP-10c Scrambled and BPP-10c Ala^{5} were able to decrease blood pressure in SHR. The absence of tripeptide (<ENW) at N-terminal portion, such as the change in the pyroglutamic acid for glutamic acid at the N-terminal position or replacement of the amino acids at positions 2, 5 and 9 or change of the amino acid at position 6 (proline for alanine) did not make BPP-10c inactive. On the other hand, the BPP-10c desPro^{10} was not able to decrease arterial pressure. It suggests that the presence of dipeptide (proline-proline) at C-terminal position is required to evoke the antihypertensive effect in SHR. Further studies are needed to reveal which amino acid(s) is/are essential(s) to evoke the antihypertensive effect. This approach is important to develop stable molecules to be used to treat hypertension and other cardiovascular diseases.

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1.22 Snake venoms of the *Bothrops* genus activate complement by cleaving C1-inhibitor, C3 and C4

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Introduction: Snake venoms are a complex mixture of components, which have a wide range of actions both on prey and human victims. Many of these components are biologically active proteins that function to kill or immobilize the prey as well as assist in the digestion process. The genus *Bothrops* inflicts the vast majority of snakebites in Central and South America, being responsible for 90% of snake envenomations in Brazil. Envenomations are characterized by prominent local effects, including edema, hemorrhage and necrosis, which can lead to permanent disability. Systemic manifestations such as hemorrhage, coagulopathy, shock and acute renal failure may also occur. **Objective:** In the present study, we investigated the action of venoms from 19 species of snakes from the genus *Bothrops* occurring in Brazil on the complement system in *in vitro* studies. **Results and Discussion:** All venoms were able to activate the classical complement pathway, in the absence of sensitizing antibody, in a dose dependent manner. This activation was in part associated with the cleavage of C1-inhibitor by proteases present in these venoms, which disrupt complement activation control. No modification of the membrane bound complement regulators, such as DAF, CR1 and CD59 was detected, after treatment of human erythrocytes with the snake venoms. Moreover, some of the *Bothrops* venoms were also able to activate alternative and lectin pathways, as measured in hemolytic and ELISA assays. C3a, C4a and C5a were generated in sera treated with the venoms, not only by C-activation, but also by the direct cleavage of complement components, as determined using purified C3 and C4. Metalloproteinase and/or serineprotease inhibitors prevented cleavage of C3 and C4. These results suggest that *Bothrops* venoms can activate the complement system, generating a large amount of anaphylatoxins, which may play an important role in the inflammatory processes shown in humans after snake envenomations.

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1.23 Channel formation in lipid bilayers induced by toxins of the marine sponge *Amphimedon viridis*

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Introduction: Recently, membrane pore-forming antimicrobial peptides have attracted attention as a new type of antibiotic, mainly because their antimicrobial activity is induced through interaction with the cell membrane and thus drug resistance would not be developed. **Objective:** The aim of this work was to investigate channel incorporation in artificial lipid bilayers (LBs) in the presence of the methanol-water extract of the marine sponge *Amphimedon viridis* (AvM) as a possible mechanism of action of the antimicrobial and hemolytic properties of the extract. **Methods:** AvM was prepared from *A. viridis* specimens collected (in Maceio, Alagoas, Brazil) and identified by Profs. Drs. Monica D. Correia and Hilda H. Sovierzoski, of the Biomedical Sciences and Health Institute of Federal University of Alagoas. The LBs were formed by giant unilamellar vesicles (GUVs). The experiments were performed with the patch clamp setup "Port-a-Patch" (Nanion Technologies), using borosilicate glass chips NPC-1. Current signals were recorded and amplified (Heka EPC-10, A/D interface ITC-1600, Patch Control™ software). **Results and Discussion:** AvM (~0.1 to 0.2 μg/ml) induced channel formation in DPhPC/cholesterol (80:20) and asolectin LBs, using both positive and negative holding potentials, and with two electrolyte solutions (150 mM KCl and 100 mM HCl). Conductance varied from 16 to 600 pS (V_{hold} = -100mV, asolectin), and from 10 to 734 (V_{hold} = -100mV, DPhPC/cholesterol). Within a few minutes, the great number of incorporated channels induced the LB break. Channel opening showed varied conductance, which may be due to the presence of more than one pore-forming substance in the extract, or by the polymerization of the substance during the experiment. Previous studies with sponges of the same genus/family demonstrated that alkylpyridinium polymers, which can assemble forming pores in biological and artificial membranes, are responsible for the biological activities of the extracts. Chemical fractionation and isolation of the active compounds are under investigation and will corroborate (or not) the presence of this type of polymer in AvM.

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1.24 Participation of mast cells and histamine receptors in leukocyte recruitment induced by *Bothrops moojeni* venom (BmV)

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Introduction: *Bothrops moojeni* snake bite causes a marked local inflammatory reaction, but the events involved in this reaction are still unclear. Mast cells (MC) are central elements of innate immune responses releasing a vast array of inflammatory mediators. However, their role in *B. moojeni*-induced envenomation is unknown. **Objectives:** To investigate the inflammatory response to BmV analyzing: a) leukocyte (LK) infiltration and release of chemotactic mediators into mouse peritoneum, b) the number of LKs in peripheral blood, c) the role of MC and histamine receptors in LK infiltration and d) the ability of BmV to induce MC degranulation both *in vivo* and *in vitro*. **Methods:** Male Swiss mice were used. LK numbers were evaluated in both blood (24 h before and at 1-6 h) and in peritoneal washes (1-48 h) after intraperitoneal (ip) injection of BmV (0.25 μg/g) or saline (control). Total and differential LK counts were determined in a Neubauer chamber after dilution in Turk solution and in Hema[3] stained smears, respectively. Groups of mice were treated with an inhibitor of MC degranulation or distinct histamine receptor antagonists or their vehicles before BmV ip injection, and LK influx was evaluated. Levels of chemotactic mediators (LTB_4, TXA_2, MCP-1 and KC) were determined by enzyme immunoassay (EIA). MC degranulation was evaluated by light microscopy after histological processing of mesentery of mice from 5 up to 30 min after BmV (0.025 – 0.5 μg/g, ip) or saline (control) injection. MC activation was evaluated by measuring PGD_2 release by EIA at 5-60 min after injection of either BmV (0.25 μg/g) or saline. *In vitro* MC degranulation was determined by measuring β-hexosaminidase release from MC of cultured PT18 line incubated with BmV (1 – 10 μg/mL) or Tyrode buffer (control). **Results and Discussion:** BmV ip injection markedly increased the peritoneal number of total LK from 3 up to 24 h with polymorphonuclear cells at 3 - 6 h and mononuclear cells from 3 up to 24 h. The number of blood neutrophils were increased at 3 h. Inhibition of MC degranulation by cromoglycate abolished infiltration of LK, whereas the histamine antagonists, diphenhydramine, ranitidine or thioperamide reduced PMN influx by 54, 47 and 78%, respectively. Moreover, BmV significantly increased peritoneal levels of TXA_2 at 1 h, LTB_4 at 6 h, MCP-1 at 3 h and KC at 30 min, and caused a dose-related increase in the number of degranulated MCs at 10 min, in comparison with controls. In addition, BmV caused a significant increase in PGD_2 levels from 5 up to 30 min, indicating activation of MCs. Incubation of PT18 MCs with non-cytotoxic concentrations of BmV increased β-hexosaminidase release compared with controls. BmV was able to recruit leukocytes into the site of its injection, which was dependent on MC degranulation and activation of H_1, H_2 and H_4 histamine receptors. BmV-induced both MCs activation and degranulation in mice may be due at least in part to a direct action of venom on these cells. Moreover, the ability of BmV to release chemotactic mediators such as LTB_4, TXA_2, MCP-1 and KC, and mobilize neutrophils from bone marrow compartments is important for the recruitment of leukocytes to the site of its injection.

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1.25 Autolysis of patagonfibrase, a metalloproteinase isolated from *Philodryas patagoniensis* venom (Serpentes: Colubridae)

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Introduction: Patagonfibrase is a 57.5-kDa hemorrhagic metalloproteinase isolated from the venom of the South American colubrid snake *Philodryas patagoniensis*. Metalloproteinases isolated from *Bothrops* venoms with molecular mass similar to patagonfibrase are known to undergo autolysis under different conditions, giving rise mainly to one fragment lacking the metalloproteinase domain, that is, containing only disintegrin-like/cysteine-rich domains. **Objectives:** Taking into consideration that patagonfibrase is the only metalloproteinase isolated to date in native form from a colubrid snake venom that inhibits platelet aggregation, and that this enzyme exhibits various biological activities similar to those exhibited by *Bothrops* venom metalloproteinases with similar molecular mass, in this study we aimed to evaluate the autoproteolytic activity of patagonfibrase. **Methods:** Patagonfibrase was incubated at 37°C at a concentration of 0.5 mg/mL in 50 mM Tris-HCl buffer, pH 7.4, for different time intervals (0, 5, 15, 30, 60 and 120 min; 18 h), in the presence and absence of 1 mM $CaCl_2$. Immediately after incubation, autolysis was interrupted by addition of SDS-PAGE sample buffer containing β-mercaptoethanol. Autolysis was visualized by SDS-PAGE (12% running gel) and silver staining. Residual proteolytic activity of patagonfibrase (30 µg/mL), was also evaluated using azocasein as substrate. **Results and Discussion:** Patagonfibrase was able to undergo autolysis at 37°C. The analysis of electrophoretic migration pattern showed a progressive fainting of the proteinase band and an increase in the staining density of bands below 45 kDa over incubation up to 18 h. In this period of incubation, the enzyme was almost totally autolysed, and thus it failed to hydrolyze azocasein. However, in the presence of 1 mM $CaCl_2$, patagonfibrase was only partially autolysed, even after 18 h of incubation, giving rise mainly to one fragment of 52.2 kDa. In addition, preincubation of patagonfibrase at 37°C for 18 h in the presence of 1 mM $CaCl_2$ caused an increase in its azocaseinolytic activity by about 50%. Taken together, these results imply that the calcium ions are important for the structural stabilization of patagonfibrase, as demonstrated for other snake venom metalloproteinases found in different ophidian taxa. Moreover, this work demonstrates for the first time the autoproteolytic processing of a colubrid snake venom metalloproteinase, which will contribute to a better understanding of the structural and mechanistic basis of this type of proteins that are widely distributed among snake venoms.

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1.26 The peristomial coelomic fluid of the sea urchin *Echinometra lucunter* contains antibiotic molecules

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Introduction: The immune function of the sea urchins has already been described and it is carried out by coelomocytes, the cells present in the coelomic fluid, which are able to neutralize bacteria. However, few molecules in sea urchin coelomic fluid have been described, so far, that are active against these pathogens. **Objectives:** To identify and characterize antibacterial molecules from the peristomial coeloma of *E. lucunter* sea urchin, a common species found in the Brazilian coastal waters. **Methods:** Peristomial coelomic fluid was collected by puncturing the *E. lucunter* sea urchin. The fluid was centrifuged and the supernatant was processed by solid phase extraction (C18 cartridges) with step gradients of 25, 50, 75, 100 % acetonitrile containing 0.1% TFA. The fractions were assessed on bacterial cultures (*E. coli*, *S. aureus*, *P. aeruginosa* and *M. luteus*), in order to determine possible growth inhibition effects. The active fractions were then purified by RP-HPLC (C18 column) and the peaks obtained were re-tested and analyzed by mass spectrometry (MALDI-TOF/MS and GC-MS). **Results and Discussion:** SPE 50% was active in the growth inhibition of *E. coli*. This fraction, under RP-HPLC-C18 chromatography, yielded 8 peaks, where one of them was active. This peak was able to cause 100% growth inhibition of *E. coli*; furthermore, this peak was not active against other bacteria. MALDI-TOF/MS analyses of selected peaks showed molecular masses of 582,029 and 657,055 Da. Moreover, GC-MS analyses confirmed the same masses. Although theses mass spectrometric analyses indicate that the sample is not yet 100% pure, it was possible to correlate the biological activity to the 657,055 Da moiety. This work reports the identification of one specific antibiotic isolated from sea urchin. Although its molecular structure is not unveiled, several pieces of information sum up to the understanding of this molecule's physicochemical characteristics: it is a non-polar (small) organic molecule (higher ACN% in SPE, low RT in CG) that contains one bromine atom, as indicated by GC-MS. Altogether, these data indicate that the peristomial coelomic fluid of *Echinometra lucunter* sea urchin contains biological active molecules, one of them being active against bacterial growth.

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1.27 Involvement of factor II and X activators of *Bothrops jararaca* venom in the pathogenesis of hemostatic disturbances and intravascular hemolysis during envenomation

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Introduction: Vitamin-K dependent coagulation factors (VKDCF, factors II, VII, IX, X, and proteins C and S) are essential to maintain hemostasis. To attain fully functional biological activity, they are γ-carboxylated prior to secretion from hepatocytes, and this process is dependent on vitamin K. The anticoagulant warfarin limits the γ-carboxylation of VKDCF. *Bothrops jararaca* (Bj) snake venom possesses several activators and inhibitors of coagulation, especially procoagulating enzymes (factor II and X activators) and thrombin-like enzymes. **Objective:** To clarify the participation of factor II and X activators in evoking hemostatic disturbances and intravascular hemolysis during experimental Bj envenomation. **Methods:** Male Wistar rats, weighing 180 to 200 g, were administered warfarin (5 mg/kg, i.v.) or saline for 3 days. To evaluate the effect of *in vivo* VKDCF depletion on the severity of hemostatic disturbances induced by Bj venom, rats were envenomed (1.6 mg/kg, s.c) after the aforementioned treatment with warfarin (warfarin group, WG, n= 6). The positive control group (PCG, n=6) received saline i.v., instead of warfarin, prior to the administration of Bj venom s.c., and the negative control group (NCG, n= 6) received only saline. Blood samples were used for performing complete blood cell counts and differential blood counts and for determining whole blood platelet aggregation induced by collagen; fibrinogen, factors II and X, and plasma hemoglobin were determined in citrated plasma. **Results and Discussion:** The platelet counts of the PCG and WG groups were respectively 14 and 5 times lower than that of NCG (1156 ± 80.0 x 10^9/L), indicating that platelet consumption is partially due to intravascular thrombin generation during Bj envenomation. Platelet aggregation in NCG (15.6 ± 3.8 Ω) was normal, while in the PCG and WG groups it was completely inhibited (0.0 ± 0.0 Ω, and 1.5 ± 1.4 Ω, respectively). No statistical difference was noted between the three groups regarding the red blood cell and white blood cell counts. Plasma fibrinogen levels dropped abruptly after venom administration in PCG (34.5 mg/dL ± 2.0) in relation to NCG (244.0 mg/dL ± 7.0), and fibrinogen consumption was partially reversed by previous warfarin treatment in WG (169.0 mg/dL ± 20.0). Plasma levels of factor II and X decreased in PCG (64.0 ± 3.0%, and 36.0 ± 2.0%, respectively) and WG (2.8 ± 1.5%, and 0.2 ± 0.2%, respectively) in comparison with NCG (107.0 ± 7.0%, and 66.0 ± 6.0%, respectively). Schistocytes and microcytes were observed in blood smears of the PCG group, likely due to intravascular thrombin generation, which forms cross-linked fibrin and causes microangiopathic anemia; these cells were absent in the blood of NCG and WG animals. In agreement with previous observations, there was a five-fold increase in plasma hemoglobin levels in PCG group in comparison with the NCG and WG groups, demonstrating that VKDCF depletion abrogated the lysis of red blood cells in the blood stream. These data indicate that thrombin production is of major importance to in provoking coagulopathy and hemolysis during *B. jararaca* envenomation in rats, and that the activation of factors II and X by procoagulating enzymes of Bj venom has been underestimated so far.

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1.28 Leukocyte accumulation and release of inflammatory mediators at the site of *Bothrops insularis* venom injection

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Introduction: *Bothrops insularis* snake is an endemic species restricted to the island of Queimada Grande, coast of the State of São Paulo, Brazil. Previous studies showed the ability of *B. insularis* venom (BiV) to induce local edema. However, the subsequent events involved in the local inflammatory response to this snake venom are still unclear. **Objectives:** To investigate the effect of BiV on the number of leukocytes in the blood and release of TXB_2, LTB_4, MCP-1 and KC into the peritoneal cavity. In addition, the effect of venom on the number of peritoneal leukocytes was evaluated. **Methods:** Male Swiss mice (18-20 g) (protocol no. 463/08) were injected either with BiV (0.05 μg/g) or saline (control) by the intraperitoneal (i.p.) route. At selected time intervals after these injections, peritoneal exudates were harvested and centrifuged. Supernatant concentrations of mediators were measured by a specific enzyme immunoassay. Total and differential blood leukocyte numbers were evaluated at 24 h before or 1, 3 or 6 h after injection of BiV or saline and peritoneal leukocytes at 1 h up to 72 h after these injections. Total cell counts were determined in a Neubauer chamber after dilution in Turk solution (1:20 v/v) and differential cell counts performed in HEMA[3] stained smears. **Results and Discussion:** BiV significantly increased the number of total leukocytes from 3 h up to 72 h in the peritoneal cavity of animals (mean BiV= 20.8 ± 2.1 cells/mL; control= 9.4 ± 1 cells/mL). Moreover, BiV significantly increased the peritoneal levels of TXB_2 (2.9 ± 0.3 ng/mL) and LTB_4 (2.6 ± 0.5 ng/mL) at 6 h after injection, compared with respective controls (1.7 ± 0.2 and 0.5 ± 0.2 ng/mL). Levels of MCP-1 were significantly increased from 0.5 up to 48 h (mean: 9.2 ± 0.2 ng/mL; control: 3.3 ± 0.2 ng/mL) whereas levels of KC were not modified by venom injection. In addition, BiV increased total leukocyte (146 ± 16.4 $x10^5$ cells/mL), MN (102.5 ± 11.05 $x10^5$ MN/mL) and PMN (43.5 ± 7 $x10^5$ PMN/mL) numbers in the blood at 3 h after injection, compared to control (64.6 ± 5.4, 54.25 ± 5 and 10.4 ± 0.6$x10^5$ cells/mL, respectively). BiV induces a marked leukocyte infiltration at the site of its injection. The ability of BiV to release chemotactic mediators such as LTB_4, TXA_2 and MCP-1 but not KC, as well as to mobilize leukocytes from bone marrow compartments is important for the recruitment of leukocytes to the site of its injection.

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1.29 Toxic properties of venoms from Micrurus genus: neutralization potential of Brazilian anti-elapidic serum

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Introduction: *Micrurus* bites can cause death by muscle paralysis and further respiratory arrest few hours after envenomation. The specific treatment for *Micrurus* envenomation is the application of heterologous antivenoms. **Objectives:** The aim of this study was to compare the toxic potential of venoms from nine species of coral snakes (*M. corallinus, M. frontalis, M. fulvius, M. surinamensis, M. lemniscatus, M. altirostris, M. spixii, M. hemprichii and M. ibiboboca*) and analyze the ability of the Brazilian anti-elapidic serum to neutralize their main toxic enzymes, *i.e.*, phospholipases and hyaluronidases. **Methods:** The lethal potential of the *Micrurus* spp venoms was assessed in outbred mice by intraperitoneal injection of different amounts of venoms. Phospholipase A_2 activity was determined venom samples incubated with a mixture containing: 5 mM Triton X-100, 5 mM phosphatidylcholine (Sigma), 2 mM HEPES, 10 mM calcium chloride and 0.124% (wt/vol) bromothymol blue. The hyaluronidase activity of *Micrurus* venoms was measured with the substrate hyaluronic acid for 15 min at 37°C. The ability of the anti-elapidic serum to neutralize venom phospholipase A_2 and hyaluronidase was estimated by incubating *Micrurus* spp venoms with the antivenom. **Results and Discussion:** The LD_{50} values, calculated by probit analysis at 95% confidence, were variable among *Micrurus* venoms, with the more lethal being the ones from *M. corallinus*, *M. spixii, M. altirostris* and *M. lemniscatus*. High phospholipase activity was detected in *M. hemprichii*, *M. frontalis*, *M. fulvius*, *M. lemniscatus*, *M. altirostris*, *M. ibiboboca*, and *M. spixii* venoms. On the other hand, *M. lemniscatus*, *M. corallinus, M. hemprichii* showed higher hyaluronidase activity when compared with the other venoms. In contrast to the high phospholipase and hyaluronidase activities, these *Micrurus* venoms exhibited low proteolytic activity. Neutralization studies showed that the Brazilian anti-elapidc serum was unable to fully block the phospholipase activity of *M. spixii*, *M. frontalis* and *M. fulvius* venoms and the hyaluronidase action of *M. lemniscatus* and *M. hemprichii* poisons. These results suggest that other coral snakes venoms should be included in the immunization pool, in order to produce a fully neutralizing antiserum for the Brazilian coral snake venoms.

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1.30 Comparative peptidomics of *Bothrops cotiara*, *Bothrops fonsecai* and *Bothrops jararaca* snake venoms

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Introduction: Snake venoms are rich sources of bioactive proteins and peptides of interest in clinical, biochemical and drug discovery research. In this work we analyzed the venom peptidomes of three Bothrops species by mass spectrometric techniques. We found new peptides from the bradykinin-potentiating peptide/C-type natriuretic peptide precursor (BPP/CNP) in the venom of *B. jararaca* and, interestingly, fragments of L-amino acid oxidases (LAAO) in the venoms of the three snake species. **Objective:** The objective of this work was to compare the venom peptidomes of three Bothrops species. **Methods:** The venom peptidomes were analyzed by LC-MS/MS and peptide sequences were determined by de novo sequencing or by automated database searches using the software MASCOT. **Results and Discussion:** The LAAO peptides identified in the venoms provided sequence coverages of up to 56%, 52% and 48% and MOWSE scores of 3914, 1991 and 488 for the protein in the venoms of *B. cotiara*, *B. fonsecai* and *B. jararaca*, respectively. As expected, most of the known BPPs were identified in the venom of *B. jararaca* along with a new glycine-rich peptide derived from the BPP/CNP precursor: HHDHHAAVGGGGGGGGGGA. A similar peptide was also found in the venoms of *B. cotiara* and *B. fonsecai* containing nine glycine residues instead of ten. None of the known *B. jararaca* BPPs was identified in the venoms of *B. cotiara* and *B. fonsecai*; however, new BPP sequences were found in these venoms.

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1.31 Maintenance of blood-brain barrier integrity and decreased severity of experimental multiple sclerosis in mice treated with tempol

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Introduction: Multiple sclerosis (MS) is a prevalent disease of the central nervous system (CNS). Nitric oxide-derived oxidants have been implicated in the pathogenesis of MS. **Objective:** Here, we studied the effect of tempol, a stable nitroxyl antioxidant in the C57BL/6. strain of mice intracerebrally inoculated with A59-strain coronavirus mouse hepatitis virus. **Methods**: Tempol was administered by i.p. injection (24 mg/Kg, 9 doses and in drinking water, 2 mM, ad libitum). Mice were assayed for: clinical score; spinal cord viral titration by plaque assay; spinal cord BBB permeability using sodium fluorescein as a tracer molecule; and analysis of spinal cord protein nitration by immunoblot. **Results and Discussion:** Our results showed that the effect of tempol in 60% of immunized mice (6-7d.p.i) is associated with the maintenance of spinal cord tissue BBB integrity by a mechanism that inhibits protein nitration. The permeability in tempol-treated mice was about 2 times lower than in vehicle-treated mice. Tempol treatment significantly inhibited spinal cord protein nitration from apparently healthy or mildly sick mice. In contrast, high levels of 3-nitrotyrosine residues were detected in spinal cord proteins from severely sick mice We also showed inhibition of virus proliferation and a survival advantage in tempol-treated mice that extended beyond 60 days in about 60 % of immunized mice. Using this model of MS, we also observed that females are more resistant than male mice. In addition, we performed a pharmacokinetics assay of tempol detection by paramagnetic resonance spectroscopy of mouse brains and demonstrated that the CNS is highly permeable to this drug, reaching the maximal concentration early (5-10 min.) after ip treatment. In conclusion, our results indicate that tempol readily penetrates de blood brain-barrier and can, at least partially, preserve BBB integrity by a mechanism that inhibits protein nitration, indirectly inhibits virus proliferation and prevents and/or delays the onset of multiple sclerosis clinical signs.

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1.32 Toxin cross-reactivity after oral tolerance induction with *Bothrops jararaca* venom

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Introduction: Oral tolerance is classically defined as specific suppression of cellular and humoral immune responses to a determined antigen with previous oral administration of antigen. It has a unique immunological importance because it is a natural and continuous event driven by external antigen. It is characterized by low IgG levels in the serum of animals after immunization with the antigen. Some groups reported low IgE levels in the serum of animals with induction of tolerance. As for the main immunobiological characters, genetic and environmental factors are involved in the immunological tolerance by oral administration, demonstrating that this trait is a process under the influence of multiple factors. Autoimmune processes are an example of this phenomenon. There is no report of oral tolerance induction to serpent venoms. We propose a methodology for knowledge of the major common tolerogenic epitopes and the correlation analysis with the main toxic components among snake venoms pertaining to a given genus. **Objectives:** To induce oral tolerance to *B. jararaca* venom in mice and to evaluate specific tolerance and the cross reactivity to other *Bothrops* species toxins. **Methods:** BALB/c mice received oral administration of *B. jararaca* venom [Group 1: high concentration; Group 2: low concentration on alternate days]. After 7 days, the animals were immunized with *B. jararaca* venom adsorbed/encapsulated into nanostructured SBA-15 silica. Two other groups that received low concentration of venom orally were immunized with *B. atrox* or *B. jararacussu* venom. As a control, a group of animals was only immunized with *B. jararaca*. The antibody titers were determined by ELISA. **Results and Discussion:** Animals orally receiving high concentration of *B. jararaca* venom were responders, showing antibody titers similar to those of immunized animals. On the other hand, mice orally tolerized with low concentration of venom showed low antibody titers after immunization with *B. jararaca* venom. In animals that received low concentration of *B. jararaca* and then immunized with *B. atrox* venom, tolerance was not observed. At least in part, these preliminary results demonstrate that the tolerance to toxins seems specific for the majority of the venom constituents. Immunoblotting analysis of distinct *Bothrops* venom species will provide details about the main tolerogenic epitopes.

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1.33 Production of native Fab from an anti-jararhagin monoclonal antibody - MAJar3 - and sequencing of immunoglobulin variable regions for structural studies

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Introduction: Snake venom metalloproteinases (SVMPs) are zinc-dependent proteolytic enzymes that play an important role in hemostatic disturbances and local lesions in bothropic envenomings. Jararhagin is a hemorrhagic PIII metalloproteinase isolated from *Bothrops jararaca* venom, comprised of metalloproteinase, disintegrin-like and cysteine-rich domains. The monoclonal antibody MAJar3 neutralizes the hemorrhagic effect of *Bothrops* venoms and the binding of jararhagin to collagen, suggesting that collagen-binding is a key factor for SVMPs hemorrhagic activity. The binding site of jararhagin to collagen was indirectly analyzed through the molecule region that was recognized by MaJar3. Studies of molecular modeling revealed that it was located in the desintegrin-like domain, and further crystallographic studies are required to confirm the exact interaction between collagen and jararhagin. **Objectives:** To isolate the Fab fragment from MAJar3 and to sequence the variable regions of its heavy and light chains in order to obtain the jararhagin-FabMAJar3 immunocomplex in appropriate conditions for structural studies. **Methods:** MAJar3 was purified from the hybridoma culture supernatant by affinity chromatography using protein-G Sepharose. Purified IgG was digested with 1mg/mL immobilized papain and the Fab was purified by affinity chromatography (HiTrap protein A HP/1mL) in FPLC system. The immunocomplex FabMAJar3/Jararhagin was assembled by incubation at 37°C for 30 minutes, tested for neutralization of hemorrhagic activity on mouse skin and isolated from free antigen or antibody by size exclusion chromatography. To sequence MAJar3 variable regions, total mRNA was isolated from $5x10^6$ mouse hybridoma cells and purified on oligo (dT)-cellulose columns (QuickPrep *Micro* mRNA Purification Kit, Pharmacia Biotech.). The purified mRNA was transcribed into cDNA using the reverse transcriptase (Superscript III) and the amplification of variable light (V_L) and heavy (V_H) chains of the antibody was carried out using the light and heavy chain primers from Amersham Biosciences. These cDNAs were cloned into pGEM-T Easy vector and sequenced. **Results and Discussion:** The purification of MAJar3 from hybridoma culture supernatant had a yield of 25 mg IgG/L of supernatant. After the digestion with papain, the antibody was submitted to a protein A chromatography which effectively retained the Fc fragments and undigested IgG, being the Fab portion obtained in the flow-through fraction, resulting in 1.9 mg Fab/L of supernatant. The SDS-PAGE showed a double band of mol. mass ~25 kDa. The preliminar assay of hemorrhagic activity using the FabMAJar3/Jararhagin immunocomplex revealed a neutralization in mouse skin tests. Size exclusion in HPLC system demonstrated the retention time of Fab, jararhagin and immunocomplex were 32.1, 29.0, 28.0, respectively. The sequence of variable light and heavy chain were obtained and confirm an identity with mouse immunoglobulins. We are currently improving the protocols of Fab purification in order to get enough yields of jararhagin-FabMajar3 immunocomplexes, in the appropriate conditions for crystallization assays with the objective to elucidate the preliminary results for characterization of jararaghin epitope.

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1.34 Venom of the caterpillar *Premolis semirufa* induces the generation of anaphylatoxins by activating the complement system in human serum

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Introduction: The Brazilian caterpillar of *Premolis semirufa*, commonly called *pararama*, belongs to the *Arctiidae* family of the order *Lepidoptera*. This caterpillar is native to the Amazonian rubber tree plantations and the accidental contact with its bristles causes local symptoms such as edema, intense itching and pain, lasting three to seven days, where after multiple accidents it can lead to joint-space narrowing and bone alteration, as well as degeneration of the articular cartilage and immobilization of the affected articulations. Specific treatment for this disease does not exist, but corticosteroids are frequently administered. Despite the public health hazard of *Premolis semirufa* poisoning, little is known about the pathological alterations of poisoned victims and the biochemical and biological properties of the extract of the caterpillar bristles are poorly understood. **Objectives:** Considering that the complement system is an important component of innate immunity and that it may be involved in the pathogenesis of the disease, the present study was carried out to evaluate the *in vitro* effects of the crude extract of the caterpillar's bristles on the complement system. **Methods:** For this purpose, normal human serum was incubated with increasing concentrations of bristles crude extract and the remained complement activity assessed in hemolytic assays, using conditions to develop alternative and classical pathways. Furthermore, activation of the lectin pathway was also determined in microtiter plates coated with mannan (10 μg/mL) and the production of anaphylatoxins C3a/C3a desArg, C4a/C4a desArg, and C5a/C5a desArg was measured using the Cytometric Bead Array (BD Biosciences PharMingen, USA). In addition, direct proteolytic activity of the bristles crude extract on components of the complement system, such as C3, C4 and C1esterase inhibitor (C1inh) was also evaluated. **Results and Discussion:** Results show that the bristles extract was able to induce a dose-dependent inhibition of the alternative complement pathway and a low inhibition of the lectin pathway; however, the extract had no influence on the classical pathway. Moreover, the caterpillar extract induced anaphylatoxin production and caused cleavage of C3, C4 and C1inh components. These results suggest that *Premolis semirufa* venom can activate the complement system, generating a large amount of anaphylatoxins, which may play an important role in the inflammatory processes seen in humans after envenomation.

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1.35 Peripheral opioid receptor expression is distinctly regulated by the presence of acute or chronic tissue injury

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Introduction: The main challenge in the therapy of pain is providing relief without causing adverse effects. While opioids efficiently alleviate pain, they induce serious side effects. Besides their central mechanisms of action, opioids also exert analgesia through peripheral mechanisms. This alternative mechanism allows for antinociception after the application of systemically inactive doses of opioids directly into injured peripheral tissue, minimizing adverse central actions. Interestingly, clinical and experimental data have shown that the local efficacy of opioid drugs is enhanced in the presence of tissue injury/inflammation, but the mechanisms involved in this phenomenon are not well characterized. Previous data of our group demonstrated that, in rats, prostaglandin E_2 (PGE_2, intraplantar/i.pl.) and chronic constriction injury (CCI) of the sciatic nerve increase the peripheral analgesic efficacy of opioid agonists and of crotalphine (CRP), a peptide obtained from *C. d. terrificus* snake venom. CRP has a local antinociceptive effect mediated by the activation of κ-opioid receptor in PGE_2-induced hyperalgesia model or κ- and δ-opioid receptor in the CCI model. **Objectives:** The aim of this study was to characterize some of the mechanisms involved in the increase of the analgesic efficacy of opioids caused by inflammation and tissue injury. For this purpose the effect of PGE_2-induced hyperalgesia and CCI on opioid receptor expression in the dorsal root ganglia (DRG) and nerve paw (NP) was evaluated. **Methods:** The expression of μ–, κ– and δ-opioid receptors was evaluated by immunoblotting, in DRG or NP (ipsilateral and contralateral to injury), of male Wistar rats, 3 h after i.pl. injection of PGE2 (100 ng/paw) or 14 days after CCI. **Results and Discussion:** PGE_2 increases the expression of μ- and κ-opioid receptors in NP (43% and 71%, respectively) and decreases (30%) the expression of δ-opioid receptors, when compared to naïve rats. μ-Opioid receptor expression is also increased in the ipsilateral and contralateral DRG (79 and 27%, respectively), while κ-opioid receptor expression is increased only in the ipsilateral DRG (168%), when compared to naïve rats. CCI up-regulates m-opioid receptors in NP (27%) and DRG (ipsilaterally and contralaterally, 49 and 20%, respectively) and δ-opioid receptors in the ipsilateral DRG (35%). On the other hand, κ-opioid receptors are down-regulated by CCI in both NP (51%) and DRG (21%), when compared to naïve rats. Thus, the results obtained indicate that peripheral opioid receptor expression is distinctly regulated by the presence of acute or chronic tissue injury and provide evidence regarding the effectiveness of peripheral opioids in both acute and chronic pain. The different expression patterns of κ- and δ-opioid receptors caused by acute and chronic injury may contribute to the comprehension of the mechanisms involved in the activation of opioid receptors by CRF in PGE_2-induced hyperalgesia and CCI models. In addition, these data also point out that drugs that activate peripheral opioid receptors, including substances derived from animal toxins, could have therapeutic potential as peripherally active analgesics.

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1.36 Proteomics profiling of age- and sex-based variability in *Bothrops jararaca* snake venom

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Introduction: Snake venom composition is a result of multiple factors and its inherent variability is often related to environmental and ecological traits, which can change from species to species. **Objectives:** The aim of this work was to explore the age- and sex-based variability of the *B. jararaca* venom proteome. **Methods:** Venoms from 694 two-week-old newborns and 110 adults (49 males and 61 females, older than 3 years) from São Paulo state were milked and the venom lyophilized. Venoms were analyzed by two-dimensional electrophoresis (2D-PAGE), and spot identification was performed by in-gel trypsin digestion followed by liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) analysis. **Results and Discussion:** 2D-PAGE showed striking differences among the samples analyzed. Newborn venom showed a complex profile of high-molecular-weight acidic proteins, identified as P-III class snake venom metalloproteinases (SVMPs). Conversely, adult venom showed several spots identified as P-I class SVMPs, almost absent in newborn venom. L-Amino acid oxidase (LAAO) and nerve growth factor (NGF) are among the highly abundant spots identified in adult male venoms. On the other hand, adult female venom showed several isoforms of C-type lectins (CTLs) and abundant spots identified as P-III class SVMPs. *N*- and *O*-glycosylation showed distinct profiles among the samples, being markedly different between newborn and adult venoms. For a quantitative comparative analysis, we employed isobaric tag peptide labeling (iTRAQ) coupled to 2D LC-MS/MS. Analysis by the iTRAQ approach identified over 70 proteins in the venoms. The major quantitative differences were detected in newborn and adult venoms, where SVMPs, serine proteinases, CTLs, phospholipase A2 and NGF were found in higher abundance in adult venom. However, the presence of unknown toxins in newborn venom may have influenced these results, since identification and quantification are dependent on the availability of the protein sequence in databanks.

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