1. Venoms and Envenomations

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

1.01 Comparison of Bothropoides jararaca bites with and without envenoming treated at Hospital Vital Brazil of Instituto Butantan, São Paulo, Brazil

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Introduction: In Brazil, 90% of the venomous snake bites are caused by *Bothrops, Bothropoides, Bothriopsis, Bothrocophias* and *Rhinocerophis*, predominantly in hot and rainy months. *Bothropoides jararaca* is widespread in south, southeastern and part of northeastern Brazil. Due to the fact they have a great adaptive capacity, it is the predominate species in São Paulo City and neighborhood. **Objectives:** The objective of this study was to examine the cases of all bites (including dry bites) caused by Bothropoides jararaca treated at the Hospital Vital Brazil do Instituto Butantan, São Paulo, Brazil (HVB), only when the snake was brought by the victim, examining epidemiological, clinical and laboratory aspects. **Methods:** A retrospective study was made in patients bitten by *Bothropoides jararaca* (n= 792) between 1990 to 2004 in Hospital Vital Brazil, São Paulo, Brazil. The data were obtained from medical records. **Results and Discussion:** The majority of the cases in this study were caused by female and juvenile snakes. This could be explained by the fact that *Bothropoides jararaca* females grow to greater lengths than males. Due to this fact as well as to pregnancy, females have higher food requirements and forage more actively, which demands more thermal control, and consequently, they become more susceptible to human encounters. Juvenile snakes probably also cause more bites because visualization of the snake is more difficult. No stomach contents were found in 93.4% of the snake specimens. There was no statistical difference between the occurrence of dry bites and maturity or sex of the snake. We observed that necrosis was more common in the digits of the feet and hands (4.8%) compared to the other body regions (1.8%). It is possible that there is a likelihood of developing compartment syndrome of the lower limbs is greater than in other regions of the limbs. A significant difference was found between severity and time interval greater than 6 hours between the bite and hospital admission. We found a significant association between gingival bleeding and abnormal blood coagulability. It was observed that in accidents caused by adult snakes, necrosis was more frequent (7.2%) when compared to accidents caused by juvenile snakes (1%). In this work, we highlight the association between some epidemiological data and biological parameters evolved in the clinical picture of *Bothrops*-like accidents, contributing to the improvement of snake bite care.

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

1.02 Unusual bites by Bothrops-like species treated at Santarém Municipal Hospital, Pará, northern Brazil

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Introduction: Ninety percent of venomous snake bites in Brazil are caused by *Bothrops, Bothropoides, Bothriopsis, Bothrocophias* and *Rhinocerophis*, mainly in warm and rainy months. In 2008, there were reports of 26,654 snakebites with a fatality rate of 0.44%. The *Bothrops*-like snakes were responsible for 86.9%. The venom has local acute inflammatory, coagulant and hemorrhagic effects. The most common signs and symptoms include pain, swelling and bleeding, occurring in the first six hours after envenomation. The municipality of Santarém, State of Pará, is characterized by a significant number of accidents by venomous animals, mainly *Bothrops*-like snakes. **Objectives:** Retrospective study of data obtained from medical records. Case 1: A 43-year-old man was bitten on the forehead, by a green snake that was on a tree, when he was fishing. He denied any symptoms. Physical examination revealed only one perforating mark. Clotting time (CT), hematologic parameters and renal function were normal. The accident was considered a dry bite. Case 2: A 30-year-old female was bitten on the head by a green snake, while working in the field. She presented with pain, swelling and bleeding at the bite site, nine hours after the accident when she was admitted to the hospital. The accident was classified as mild and she received five vials of antivenom. CT at admission was normal. Case 3: A 12 years old female student was bitten in medial right thigh, where she presented an extensive edema and pain. She sought medical care four hours after the accident. The initial CT was incoagulable and the accident was classified as moderate. She received eight vials of antivenom. Case 4: A 43-year-old farmer was bitten on the head by a snake, when he was hunting. He denied any symptomatology and his physical examination was normal. The accident was classified as a dry bite. CT, blood parameters, and renal function were normal. **Methods:** Retrospective study of data obtained from medical records. **Results and Discussion:** Although the snakebites in the centripetal body are rare, this localization has variable severity. Some cases develop with local complication such as necrosis and infection, systemic complications such as airway obstruction due intense edema and uncommonly death, while others develop without any manifestations (dry bite). In general, these accidents occur in particular epidemiological situations: large and/or arboreal snakes, patient is lying down or sitting on the ground or the snake is in a ravine. Conclusion: Although this unusual bite site is rare, it is important to point out that this accident could be fatal and that preventive actions are very difficult.

Supported by: INCTTOX Program - CNPq, FAPESP

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

1.03 Effect of DM43 and venom peptides on the proteolytic activity of metalloproteinases of *Bothrops jararaca* venom

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Introduction: Snake venom metalloproteinases (SVMPs) play a significant role in envenomation, causing local hemorrhage and necrosis. SVMPs isolated from *B. jararaca* show different proteolytic and hemorrhagic activities: HF3 (P-III class) is highly glycosylated and is an extremely hemorrhagic SVMP; bothropasin (P-III class), which has a minor carbohydrate moiety, is a highly proteolytic protease; and BJ-PI (P-I class) is a potent proteolytic enzyme but is not hemorrhagic. Previous studies have shown that the protein DM43, isolated from *Didelphis marsupialis* serum, inhibited *B. jararaca* crude venom hydrolysis of casein, fibronectin and fibrinogen and the hemorrhagic effect of jararhagin, a P-III SVMP from *B. jararaca*. Bradykinin-potentiating peptides (BPPs) are involved in the symptoms of hypotension observed upon envenoming by *Bothrops* species. The venom tri-peptides pEQW and pEKW are known to inhibit SVMPs by occupying the S^{-1} pocket of the proteinase with the tryptophan residue. Recently, it was demonstrated that the venom from *Echis ocellatus* contains an unusual pHpG peptide which inhibits the venom hemorrhagic activity. **Objectives:** To analyze the effects of the venom peptides BPP10c, BPP5a, pEKW and pHpG1 and the protein DM43 on the proteolytic activity of HF3, bothropasin and BJ-PI. **Methods:** HF3, bothropasin and BJ-PI were preincubated with 5 mM of each peptide for 30 min at room temperature and incubated with casein, fibrinogen and collagen VI for 1 h at 37°C. Native and N-deglycosylated HF3 and native bothropasin were preincubated with DM43 (1:1 and 1:2, enzyme-to-inhibitor ratio) for 40 min at room temperature and incubated with fibrinogen for 1 h at 37°C. Proteins were incubated without enzyme under identical conditions. The reactions were stopped by adding the Laemmli sample buffer and submitted to SDS-PAGE (12% polyacrylamide gels). Gels were silver stained. **Results and Discussion:** BPP10c did not affect the degradation of fibrinogen and collagen VI by all SVMPs but enhanced the caseinolytic activity of BJ-PI. BPP5a altered the hydrolysis pattern of all SVMPs on fibrinogen. Moreover, it enhanced the caseinolytic activity of BJ-PI but did not affect the degradation of collagen VI by SVMPs. pHpG1 affected the degradation of collagen VI and fibrinogen but did not affect the caseinolytic activity of the SVMPs. DM43 inhibited the fibrinogenolytic activity of bothropasin but had no effect on native and N-deglycosylated HF3. These data indicate that the SVMPs are differently affected by the peptides and DM43 probably due to their diverse tertiary structures.

Supported by: FAPESP

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

1.04 Effects of pre-natal exposure to *Tityus bahiensis* scorpion venom on the reproductive development of pregnant dams and on the development of pups

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Introduction: Scorpion envenoming is a public health problem. In Brazil, the scorpion *Tityus serrulatus* is considered the most dangerous, but a large number of exposures also occur with *Tityus bahiensis*. There are quite a few studies in literature about the toxic effects of this venom. **Objectives:** The objective of this work was to determine possible toxicological effects of the *Tityus bahiensis* scorpion venom on maternal reproductive development and on the development of pups, when administered to pregnant dams. **Methods:** The dose of the venom used was 2.5 mg/Kg. The venom was administered to two groups on the 5th (GD5, n=5) or on the 10th (GD10, n=10) gestational day. The control group (C, n=10) received 1.46% NaCl on both days. On the 21st gestational day, the pups were taken out through a laparotomy and divided into three groups that received specific treatments for skeletal, visceral and histological analyses. On the following day after laparotomy, the pups selected for skeletal analysis had their organs removed and weighted. **Results and Discussion:** No changes in maternal weight were observed during the gestational period. There were no alterations in the number of implantations (C=7.1±0.4; GD5=6.0±0.3; GD10=6.5±0.3), reabsorptions (C=0.4±0.1; GD5=0.2±0.1; GD10=0.1±0.06), corpus luteum (C=7.4±0.4; GD5=7.0±0.4; GD10=7.1±0.3), on the number of pups (C=6.6±0.3; GD5=5.8±0.4; GD10=6.4±0.4), or in the number of live pups (C=6.6±0.3; GD5=5.7±0.5; GD10=6.3±0.4). There were no changes in uterus weight (C=99.5±4.3; GD5=95.2±6.6; GD10=94.8±3.6). Also there were no changes to the weight of the pups (C=5.1±0.1; GD5=5.7±0.04; GD10=5.8±0.4). There was an alteration in the weight of the placentas in GD5 and in GD10 (C=0.49±0.01; GD5=0.54±0*; GD10=0.53±0.01*). In relation to the development of the pups, some changes were observed, such as in heart weight (C=0.04±0.01; GD5=0.06±0.0*1; GD10=0.05±0.01*) and lung weight (C=0.14±0.01; GD5=0.15±0.01*; GD10=0.15±0.01*) on GD5 and GD10, and in liver weight (C=0.47±0.01*; GD10=0.58±0.01*) on GD10. The moderate envenomation by *Tityus bahiensis* scorpion venom causes subtle changes in maternal reproductive development and in fetal development.

Supported by: CAPES, Fundação Butantan

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

1.05 Detection of proteins in the microvesicles present in *Crotalus durissus terrificus* snake venom

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Introduction: Microvesicles are known structures that shed from the surface of many cells. These small specific structures have proteases or specific inhibitors that can modulate the activation of zymogen and can therefore play an important physiological and pathological role. Numerous electron-dense microvesicles (40 – 80 nm in diameter) are observed on the luminal side of secretory cells of the venom gland and in the venom of *Crotalus durissus terrificus*. Microvesicles found in the *Crotalus durissus terrificus* venom originate from microvilli by fragmentation or membrane budding and have intramembranous particles on the cytoplasmic leaftet, suggesting the presence of transmembrane proteins. **Objectives:** The aim of this study was to determine the presence of proteins in the microvesicles present in *Crotalus durissus terrificus* venom. **Methods:** The venom used was manually extracted from *Crotalus durissus terrificus* maintained in the Laboratory of Herpetology at Instituto Butantan. A volume of 5.0 ml of venom was diluted with cold PBS (1:4) and centrifuged at 150 g for 15 min at 4°C to eliminate cell debris. The supernatant was ultracentrifuged at 200,000 g for 60 min at 4°C, and the pellet was resuspended in 20 ml of cold PBS and ultracentrifuged again under the same conditions. The resulting pellet was processed for morphological analysis using transmission electron microscopy or for protein analysis by one-dimensional (1-DE) and two-dimensional (2-DE) electrophoresis. The density of the spots in 2-DE was quantified using ImageMaster 2D Platinum 7. **Results and Discussion:** Morphological analysis showed that after ultracentrifugations the microvesicles kept their morphology. Analysis of protein profile of microvesicle extracts in 1-DE showed s band of approximately 66 kDa and bands between 116 and 200 kDa which were not found in the protein profile of the venom. Analysis of the protein spots in the 2-DE images showed many stained spots that are present in microvesicles extract but not in the venom. Only some spots are present in both microvesicles extract and venom. The proteins detected only in microvesicles are: 1) approximately 180 kDa with PI around 4; 2) ranging from 60 to 70 kDa with PI ranging from 7 to 9; 3) 11 kDa with PI 4; 4) approximately 10 kDa with PI ranging from 5 to 7 or 9 to 10. Our data showed that the microvesicles have proteins that differ from the venom proteins, suggesting that these proteins could have important role such as regulating the activity of toxins from the venom, or even having a biological activity that contributes to the pathology of the envenoming.

Supported by: Fundação Butantan, FAPESP

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

1.06 Expression of adhesion molecules by different metalloproteases isolated from Bothrops: role of different domains

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Introduction: Snake venom metalloproteinases (SVMP) are major toxins involved in inflammatory reactions at the site of the bite in human envenoming. Depending on the domain composition, SVMP can be classified as P1 to P4. Three toxins were used: Jararhagin (JAR) and JAR-C, isolated from *B. jararaca* venom, and BnP1, isolated from B. neuwiedi venom. JAR, a P3 SVMP with a strong hemorrhagic activity, comprises catalytic, disintegrin-like, and cysteine-rich domains. JAR-C is a degraded form of JAR devoid only of the catalytic domain, with no hemorrhagic activity. BnP1, a weakly hemorrhagic P1 SVMP, has only the catalytic domain. **Objectives:** In this study, the participation of different domains of SVMP on alterations of leukocyte-endothelium interactions (LEI) in the microcirculation of the cremaster muscle of mice was evaluated as well as the expression of the adhesion molecules ICAM-1 (CD54) and PECAM-1 (CD31), responsible for adhesion and cell migration, respectively. **Methods:** JAR, JAR-C or BnP1 (0.5µg) or PBS (100µL) were injected into the scrotal sac of mice, and the microcirculation of cremaster was analyzed by intravital microscopy 2 or 24 h after the injections. A 100-µm segment of a post-capillary venule was observed for 5 min, and leukocytes rolling, adhered and emigrated were counted. The cremaster muscle was isolated and incubated with antibodies against CD54 and CD31 tagged with fluorescence, 2, 4 or 24 h after the toxin injections to evaluate the expression of integrins. **Results and Discussion:** A significant decrease in leukocyte rolling was observed 2 h after toxin injection. Adhered and emigrated leukocytes were increased at all times studied. There was a high increase in expression of CD54 in the first hours (2 and 4 h) analyzed and a decrease 24 h after the injection. Related to the CD31 immunostaining table, we observed a progressive increase related to time, when compared to the controls, with a similar distribution in the microcirculation in the period studied. It is suggested that the alterations observed in the microcirculation occur in the expression of the adhesion molecules CD54 and CD31.Despite differences in hemorrhagic activities and domain compositions of the three toxins used, the dose of toxins used induced alterations in leukocyte-endothelium interaction of similar magnitude. In conclusion, our results suggest that catalytic, disintegrin-like, and cysteine-rich domains of these *Bothrops* SVMP can induce alterations in leukocyte-endothelium interactions mediated by CD54 or CD31.

Supported by: FAPESP

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

1.07 Analysis of differential gene expression of venom gland of *Bothrops jararaca*

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Introduction: The venom gland of the Brazilian venomous snake *Bothrops jararaca* (Crotalinae, Viperidae) is an exocrine tissue related to the salivary gland. When the venom is released, the production of new venom is triggered by the activation of noradrenaline on both α1- and β-adrenoceptors. However, the genes involved and the regulation of venom production cycle are poorly known. Here we describe the construction and first analysis of nylon cDNA arrays aiming to identify the most differentially expressed genes of *Bothrops jararaca* venom glands. **Objectives:** The aim of the study was to identify the gene expression pattern of venom gland of *Bothrops jararaca*. **Methods:** The cDNAs arrays were constructed with 4608 clones from male and female B. jararaca cDNA libraries, using the methodology that consists of bacterial clones grown on a nylon membrane. The venom gland was removed from snakes in which venom was not manually extracted (0 days), and from snakes in which venom was extracted 1, 2, 4 and 15 days before sacrifice. We used 3 snakes, all adult males, for each experimental condition. RNA was extracted and converted into cDNA which was used as the probe for hybridization of cDNA arrays. For probe labeling and array hybridization, we used Direct Labeling and Detection System with CDP-Star (GE Healthcare).The image obtained was scanned and analyzed using Bzscan 2 software. All statistical analyses were performed using R language and the package LIMMA3 from the Bioconductor project. Clones with FDR values <0.05 and B-statistics >0 were deemed differentially expressed and selected for the next analysis. Gene expression patterns at the investigated times of venom gland cycle were identified using the Short Time-Series Expression Miner (STEM)4 tool. **Results and Discussion:** In the gene expression pattern, high level of transcripts were found from 1 to 2 days after manual extraction of venom, results which differ from previous data showing higher levels of transcripts from 4 to 8 days. More analyses are necessary to confirm or refute our results.

Supported by: FAPESP, CNPq

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

1.08 Action of sphingomyelinase D in loxosceles spider venom in human kidney cells

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Introduction: The *Loxosceles* spider (brown recluse) can be found worldwide, although its distribution is heavily concentrated particularly in the tropical urban regions of South America. Brown spider bites can cause dermonecrotic lesions and systemic reactions known as loxoscelism. Systemic manifestations include intravascular hemolysis, disseminated intravascular coagulation and acute renal failure. The venom factor responsible for both local and systemic manifestations is a phospholipase D, with sphingomyelin substrate specificity, called sphingomyelinase D (SMase D). **Objectives:** The aim of this study was to investigate the effects of *Loxosceles* spider venom and the SMase D toxin on the Complement (C) regulators expression and the C-resistance of kidney human cells (HK-2), as well as the possible involvement of endogenous metalloproteinases in the pathogenesis. **Methods:** Cells were incubated with *Loxosceles* venom or SMase D and the expression of complement regulators was assessed by flow cytometry; cell viability was analyzed by the MTT assay, and supernatants of these cells were also analyzed by zymography to verify the expression of endogenous metalloproteinases. **Results and Discussion:** A reduced expression of membrane co-factor protein (MCP) was observed, while expression of decay-accelerating factor (DAF) and CD59 was not affected. Analysis of other cell-surface molecules showed a reduced expression of the major histocompatibility complex I (MHCI), epithelial growth factor receptor (EGFR) and endothelial protein C receptor (EPCR). Removal of MCP, caused by HK-2 cell endogenous metalloproteinase activation, allows complement to be activated on the cell surface, as evidenced by increased deposition of C3 and C4, resulting in cell death, in the presence of normal human serum as source of C-components. Increased deposition of factor H (fH) and properdin, but not of C4-binding protein (C4BP), was observed on these cells after venom/toxin treatments, followed by incubation with normal serum. Zymography assays showed increased expression of MMP-9 after 24 and 48 h of the treatment; after 72 h, an increase in MMP-2 expression was also detected. Our data showed that *Loxosceles* venom and its SMase D are toxic to human renal cells, causing increased expression of endogenous metalloproteinases, which may contribute to cell death. Moreover, they suggest that a failure of complement regulation, allowing complement activation, may play a role in the pathogenesis of kidney failure, present in the systemic form of the human loxoscelism.

Supported by: FAPESP, CNPq

1.09 Isolation of progenitor/stem cells from canine amniotic and allantoic fluids

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Introduction: Pet applied stem cell therapy is a rapidly growing market, which requires constant innovation and developing new technologies in order to expand the list of currently treated diseases. The dog is an excellent preclinical model for the study of diseases, pharmacological tests and new therapies for future application in humans. Thus, the canine model is a excellent model for isolation of (PS) cells from amniotic and allantoic liquids **Objectives:** We aimed at isolation and comparative characterization of (PS) cells from amniotic and allantoic liquids **Methods:** Therefore, ovarian hysterectomy technique was performed during company castration in order to isolate progenitor/stem (PS) cells from canine amniotic and allantoic fluids (CAFs). **Results and Discussion:** We showed that efficient CAF-PS cell harvesting occurs at 50 days of gestation. Different culture media were used and optimal conditions for culturing of these cells were established. CAF-PS cells expressed vimentin, nestin and cytokeratin-18 proteins, which were negative for Oct-4, a marker of pluripotent stem cells. PS-cells from canine amniotic fluid were able to undergo osteogenic and chondrogenic differentiation, while potential of PS cells from allantoic fluid was limited to chondrogenic differentiation. Our data suggest that CAF-PS cells are a source of PS cells with restricted differentiation, but these cells can be useful in the emerging field of regenerative veterinary medicine due to their low rejection following heterologous transplantation.

Supported by: CNPq

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

1.10 Transcriptomic and proteomic analysis of Duvernoy's glands of the colubrid snake *Phalotris mertensi*

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Introduction: The venom from members of the Colubridae family has been neglected for a long time, and currently little is known about this heterogeneous and diverse family. Many colubrid snakes have Duvernoy's glands, which are homologous to the venom gland, and this species can have opisthoglyphous or aglyphous dentition. *Phalotris mertensi,* a colubrid (Xenodontinae subfamily), popularly known as false coral occurs in the central region of Brazil and possesses Duvernoy's gland and opisthoglyphous teeth. In vitro assays with the venom of P. mertensi indicated that it acts on casein, fibrinogen and gelatin, and contains some enzymes. **Objectives:** This work aimed to characterize the transcriptome of Duvernoy's gland of the snake *P mertensii* and to use the ESTs generated database as a tool for the identification of proteins present in the venom by mass spectrometry. **Methods:** The transcriptome was determined by extraction of Duvernoy's glands of a snake followed by total RNA extraction with Trizol reagent (Invitrogen) and mRNA purification through oligo dT cellulose. The cDNA library was constructed with 5 μg mRNA using the Superscript Plasmid System for cDNA Synthesis and Cloning kit (Invitrogen), directionally cloned in the pSPORT-1 plasmid and transformed in Escherichia coli DH5 alpha cells. Plasmid DNA was isolated using alkaline lysis from randomly chosen clones and DNA was sequenced on an ABI 3100 sequencer using BigDye2 kit (Applied Biosystems) with standard 5' primer. After bioinformatics treatment, the ESTs were grouped into clusters using the program Cap3 and were annotated with Blast2Go program. **Results and Discussion:** As a result, we obtained 1540 ESTs, grouped into 777 clusters, 131 contigs and 646 singlets. Toxins represented about 38% of gene expression in Duvernoy's glands of this colubrid. Metalloproteases (SVMPs) were the most abundant toxins, accounting for 54% of the total toxins, followed by protease inhibitors and C-type lectins. The profile of toxin transcripts suggests the presence of a new abundant toxin (3% of all clones) similar to vertebrate lysosomal lipase A. Another 21% of gene expression was not identified, indicating that many components present in venoms of these animals have not yet been studied. The database generated was also used to identify venom components analyzed by mass spectrometry for spots obtained in two-dimensional electrophoresis of venom P mertensi. Mass spectrometry allowed the identification of most toxins found in the transcriptome. Further analyses are being carried out aiming to a better match the transcriptome and proteome sets.

Supported by: CNPq

1.11 Crotoxin inhibits the secretion of pro-inflammatory cytokines: effect on cell migration

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Introduction: *Crotalus durissus terrificus* snake venom (CdtV) modulates the inflammatory response, and the long-lasting anti-inflammatory effect of CdtV on inflammatory response induced by carrageenan (Cg) has been demonstrated. Other results showed that CdtV inhibits cytokine secretion, such as IL-α and IL-1β, by macrophages stimulated by phagocytosis or lipopolysaccharide (LPS). Recent data demonstrated that crotoxin (CTX), the main toxin of CdtV is responsible for this long-lasting anti-inflammatory effect. **Objectives:** The aim of this study was to evaluate histologically the inhibitory effect of CTX on the migration of cells to the subcutaneous tissue of footpads injected with Cg, and to investigate if this inhibitory effect involves the action of CTX on the secretion of pro-inflammatory cytokines. **Methods:** A single dose of CTX (0.89 μg/50 μL s.c.) or saline (50 μL) was administered 1 h before Cg injection in male Swiss mice (18-22g) (n=6). For histopathological analysis, the footpad was removed after 6 h of intraplantar injection of Cg (300 μg/50 μL) or saline (50 μL). The tissue was processed for light microscopy. Sections were obtained from paraffin embedded pieces (5 mm thick), stained with hematoxylin and eosin (H/E) and observed under Zeiss microscope (Axiolab) with pickup AxioCam MRC (Zeiss). To evaluate the effect of CTX on cytokine secretion, after 4 h of intraperitoneal injection of Cg (300 μg/200 μL) or saline (200 μL), peritoneal exudate was collected and the cytokines IL-1β, TNF-α and IL-6 were determined by ELISA, using reagent kits (R&D System). **Results and Discussion:** Histological analysis confirmed the inhibitory effect of CTX on cell migration. Tissue sections from animals treated with CTX 1 h before intraplantar injection of Cg showed significantly fewer areas of edema and consequent reduction of inflammatory infiltration (polymorphonuclear cells) compared to untreated animals (Saline+Cg). The peritoneal exudate of animals pretreated with CTX showed a significant decrease in the secretion of all pro-inflammatory cytokines evaluated, IL-1β, TNF-α and IL-6, when compared to untreated animals. The decrease in cytokine secretion observed in exudates from animals pretreated with CTX was 71%, 51% and 35% for IL-1β, TNF-α and IL-6, respectively. Our results showed for the first time, by histological analysis, the inhibitory effect of CTX on cell migration. We suggest that this inhibitory effect involves the action of CTX on the secretion of important pro-inflammatory cytokines that participate in the inflammatory response induced by Cg.

Supported by: CAPES, FAPESP, INCTTOX Program - CNPq, FAPESP

1.12 South American rattlesnake bite with soft-tissue infection in Santarém, Pará, Brazil

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Introduction: According to SINAN - Information System for Notification of Diseases - the number of snake bites in Brazil was 29,374 in 2006. During 2001 to 2006, Brazil reported 155,973 cases, and in Pará State's 22,886 cases, rattlesnake bites were responsible for 10,462 cases. The majority of snake bites in Brazil were caused by *Bothrops* (90.5%), followed by *Crotalus* (7.7%), *Lachesis* (1.4%) and *Micrurus* (0.4%). Despite the frequency and severity of rattlesnake bites, which are considered potentially more serious than *Bothrops* (lethality of 0.3% to 1.3%, respectively, in 2006), local complications are very rare with South American rattlesnake. **Objectives:** We report a case of a rattlesnake bite with secondary soft-tissue infection **Methods:** A retrospective study of data obtained from medical record was carried out. A 17-year-old patient, male, previously healthy, was bitten in the medial portion of the left foot by a rattlesnake in June, while working in the field. He did not use a tourniquet or placed substances on the site of the bite; he just washed the bite with water. After 120 min, he developed intense myalgia associated with change in visual acuity (in fact, bilateral ptosis) and on the injury site there was only local erythema, without bruising or bleeding. He reached the Santarém Municipal Hospital seven hours later and at admission he had bilateral ptosis and complained about decreased urine output, besides darkened urine. The initial laboratory results were: leukocytosis and left shift, CPK = 123,000, coagulation time normal, urea = 32 and creatinine = 3.2 mg/dl. The accident was classified as moderate and the patient received 10 ampules of anticrotalic antivenom. The patient was transferred to the intensive care unit for monitoring and for support treatment. After 1 day of admission, he had fever, progression of left lower limb edema and blister at the site of the bite. He was prescribed 2 g of chloramphenicol/day for the treatment of secondary soft-tissue infection. He remained hospitalized for 15 days and needed two sessions of hemodialysis. **Results and Discussion:** Patients bitten by a rattlesnake in South America usually do not show local changes. In contrast to what occurs with North American rattlesnake bites, widely described in literature, it is not common for patients bitten by South American rattlesnakes to develop soft-tissue infection. Soft-tissue infection is a widely known complication of *Bothrops* bites, probably because of the local acute inflammatory action and the oral fauna of snakes. Even in South American rattlesnakes, it is important to note the local clinical picture.

Supported by: FAPESP, Fundação Butantan

1.13 Juruin: an antimicrobial peptide from *Avicularia juruensis* venom

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Introduction: Antimicrobial peptides (AMPs) are the key elements of the innate immunity against bacteria and fungi in both the animal and plant kingdoms. AMPs are an extremely diverse group of small proteins that are considered together because of their native antimicrobial activity, and their function is essential to the animal immune response. Natural animal venoms are good sources of potential antimicrobial substances, and their venoms contain a large number of diverse biologically active components of various chemical structures, such as proteins, polypeptides and amines. Cystine knot toxins (CKTs) in spider venoms represent a rich source of novel ligands for various ion channels, and are among the most extensively studied constituents of spider venoms. They are small, compact molecules cross-linked by three to five disulfide bonds, ensuring greater stability in the conformation of the molecule that contains it, and therefore offers a high potential for applications in engineering proteins. They occur in a variety of peptides and proteins and are relatively common in small cysteine-rich toxins and small peptides. Toxins containing the cystine knot feature a range of biological activities, such as antimicrobial activities, anti-HIV potential and blocking of ion channels. CKTs have molecular masses ranging from 3.5 to 7 kDa. **Objectives:** The aim of the study was to identify antimicrobial peptides from *Avicularia juruensis* venom and its potential to become a new antibiotic. **Methods:** The venom was purified by reversed phase HPLC and the fractions obtained were submitted to antimicrobial activity assay (determined by a liquid growth inhibition assay against Gram-negative and -positive bacteria *Escherichia coli SBS363* and *Micrococcus luteus A270* and yeast *Candida albicans*). The fractions that showed antimicrobial activity were submitted to MALDI-TOF mass spectrometry to determine purity. The pure ones were submitted to Q-TOF mass spectrometry for sequencing. **Results and Discussion:** Juruin has a mass of 4,005 Da and showed antimicrobial activity against all the microorganisms tested, and was sequenced by Q-TOF mass spectrometry and "De Novo" sequencing. The probable sequence obtained is the 38 amino acid sequence FTCALSCNLKVNKGPCKGTNEGKCSGGWSCKFNVCVKT**.** BLAST analysis showed 71% similarity with cystine knot toxin from the Chinese bird spider *Chilobrachys jingzhao*.

Supported by: FAPESP, CNPq

1.14 Effects of Micrurus snake venoms on the human complement system

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Introduction: The family Elapidae is represented in the Americas by three genera of coral snakes: *Micruroides*, *Leptomicrurus* and *Micrurus*, the latter being the most abundant and diversified group. The genus *Micrurus* (Serpentes, Elapidae) comprises more than 120 species and subspecies distributed from the southern United States to the southern part of South America. *Micrurus* bites can cause death by muscle paralysis and further respiratory arrest a few hours after envenoming. Clinical observations show mainly neurotoxic symptoms, although other biological activities have also been experimentally observed, including cardiotoxicity, myotoxicity, hemolysis and edema. **Objectives:** The aim of this study was to analyze the action of venoms from 8 *Micrurus* species (*M. ibiboboca, M. lemniscatus, M. altirostris, M. spixii, M. surinamensis, M. corallinus, M. frontalis* e *M. hemprichii*) on the complement system (C) in *in vitro* tests. **Methods:** Samples of normal human serum, as source of complement components, were incubated with the snakes' venoms and the remaining complement activity was measured in conditions to develop classical, alternative or lectin pathways. **Results and Discussion:** All venoms were able to activate the classical but not the alternative complement pathway. This activation was in part associated with the cleavage of C1-INH by proteases present in these venoms, which disrupts complement activation control. To determine if the observed alteration in C-activity was caused by C-inhibition or C-activation/consumption, the generation of C-activation products C3a, C4a and C5a was measured. Moreover, C3a was generated in human serum treated with the venoms, not only through C-activation, but also through the direct cleavage of the C3 component, as determined using purified C3. These results suggest that *Micrurus* spp venoms can activate the complement system, generating large amounts of anaphylatoxins, which can play a role in the pathogenesis of human coral envenomation and may also contribute to the spread of other venom components, due to their vasodilatory effects.

Supported by: FAPESP

1.15 *Echinometra lucunter* (rock-boring urchin) accident: much more than a mechanical incident

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Introduction: The rock-boring sea urchin *Echinometra lucunter* can be found along the Western Central Atlantic shores, from the USA to most of Brazil. Its body is covered by calcified spines that are mainly involved in the defense of the animal, making this urchin responsible for about 50% of the accidents caused by marine animals in Brazil. Victims, however, have been treated mostly for the (swollen) mechanical consequences of the accident. The symptoms, on the other hand, usually surpass trauma and may be pathologically varied and last from spontaneous healing in a few days to painful consequences for weeks (for the worst untreated cases). Moreover, injuries may be complicated due to secondary infections or the development of a chronic inflammatory response, with granuloma formation. Based on these data, we mimicked the sea urchin accident by administering an aqueous extract of the spine into mice and rats (CEUAIB 438/07) and evaluated the main symptoms observed in the victims. **Objectives:** The aim of this study was to characterize the sea urchin accident by means of evaluating the main physiopathological symptoms. **Methods:** An aqueous extract of *E. lucunter* spines (10 μg) was injected in mice and after 2, 4, 24 and 48 h, the alterations in the microcirculation (rolling, adhered and emigrated leukocytes) were observed. Mouse paw edema formation was evaluated with a plethysmometer ¼, ½, 1, 2 and 3 h after injection (10 and 20 μg doses). In order to determine whether the extract would cause hyperalgesia, the pain threshold in rats, was measured before and after the administration of 10 or 20 μg, at times 1, 2 and 4 h, using a Ugo Basile® pressure apparatus. Hemorrhagic activity was determined by injection of the extract (10 and 20 μg) in the abdominal skin of mice, followed by exposure of the skin three hours after treatment in order to verify the extent of the hemorrhagic spots. The extract was also analyzed by SDS-PAGE and RP-HPLC, as an initial biochemical characterization. **Results and Discussion:** Our data clearly indicate that the sea urchin accident is indeed a pro-inflammatory event, triggered by toxins present in the spine which can cause edema and alterations in the microcirculation. Moreover, the extract demonstrated a hyperalgesic effect. The extract is also rich in proteins, as observed by SDS-PAGE, but also contains other molecules that can be analyzed by RP-HPLC. Altogether, these effects corroborate that an *E. lucunter* encounter is an accident and not an incident, as frequently reported by the victims.

Supported by: FAPESP, CNPq, INCTTOX Program - CNPq, FAPESP

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

1.16 Effectiveness of serotherapy in pregnant mice after experimental evenomation by *Bothrops jararaca*

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Introduction: Snakebite accidents are considered a rare event among pregnant women, but serotharapy is indicated even when envenomation is not severe. However, antivenom can cause maternal adverse reactions, and consequently fetal death. Experimental *Bothrops jararaca* (Bj) envenomation can provoke marked morphological alterations in the antimesometrial (AM) region of uterus in pregnant mice which can culminate in the end of gestation. **Objectives:** This investigation aimed to verify whether *Bothrops* antivenom (BAV) could restore the normal morphology of the murine uterus after *Bothrops jararaca* (Bj) envenomation. **Methods:** On the morning of day 8 of pregnancy, animals received Bj venom (0.24 mg Bj venom/kg body weight) i.m., and after 3 h they were treated with BAV (Bj+BAV). Control groups received saline and were treated with BAV (Sal+BAV) or Bj venom (Bj). On day 9, uterine morphology was analyzed, especially at the maternal-fetal interface in the antimesometrial region. Plasma fibrinogen (Fg) was assayed in plasma samples of pregnant animals. Aiming to study the external appearance and the skeletal morphology of fetuses, as well as the incidence of fetal resorptions, another group of animals on day 8 received the same treatments mentioned above and was sacrificed on day 19. **Results and Discussion:** Histological analysis of most dams of the Bj+BAV group revealed the maternal and fetal tissues organized, similar to the uteri of dams of the Sal+BAV group. However, sometimes the antimesometrial region was not preserved in some implantation sites, showing hemorrhagic areas and a prominent inflammatory infiltrate at the maternal-fetal interface. Additionally, decidual cells (maternal) and trophoblastic giant cells (fetal) exhibited evident signs of necrosis. These findings were similar to the Bj group, indicating the possibly that BAV does not reach all implantations sites equally. Analyses of the external appearance and skeletal morphology of fetuses on day 19 showed no difference between groups; however, the dams that received Bj venom and were not treated with ABS showed smaller fetuses and a higher incidence of fetal resorption. Plasma Fg levels of the Bj+BAV group were similar to those of Sal+BAV group. However, experimental Bj envenomation showed lower plasma Fg levels. These findings show that although Bj venom induces a characteristic Fg consumption, BAV could re-establish plasma Fg levels, which might have contributed to the maintenance of the pregnancy.

Supported by: INCTTOX Program - CNPq, FAPESP

1.17 K49-PLA2 homologue in *Bothrops jararaca* venom

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Introduction: Endocrine effects of reptile venoms are in vogue and that from the lizard *Heloderma suspectum* is the most representative. This venom contains exendin-4 (EX-4), a peptide that gave rise to a new class of antidiabetic drugs with agonist activity on the glucagon-like peptide-1 receptors. Considering the phylogenetic proximity between snakes and lizards, we are prospecting for EX-4-like peptides in *Bothrops jararaca* venom (*vBj*). **Objectives:** The aim of this study was to describe a phospholipase A2 (PLA2) detected in this search. **Methods:** The supernatant of 10 mg *vBj*/2 mL 2 M HAc (centrifuged at 3,000Xg, 5 min, 4ºC) was chromatographed in G-50 column (46x1.5 cm, Vo=40.5 mL, flow rate=1.5 mL/min). The pooled fractions 31-45 (interval=15 min) was dried, resuspended in 1 mL (concentrated 1.5 times) and submitted to HPLC (C18 column, 4.6x250 mm, 5 µm particles) with linear gradient elution (0-100% solution B) formed from solution A (0.05/99.50 [v/v] TFA/water) to solution B (0.05/19.95/80.00 [v/v/v] TFA/water/acetonitrile) (240 min, flow rate=1 mL/min, Abs 214nm). Six peaks eluted near the retention time (RT) of EX-4 were dried, resuspended in EIA buffer and then analyzed by monoclonal anti-exendin-4 ELISA. ELISA was positive for all these peaks. These peaks pooled in 500 µL (concentrated 3 times) were then administered via ip, at a dose equivalent to 12 µg EX-4 /kg body wt in a 120-min oral glucose tolerance test (OGTT) in a rat model of streptozotocin-*diabetes mellitus*. As a result, the animals that received the pool had lower glycemia (monitored by Accu-Chek , Roche at 15, 30 and 60 min) when compared to controls. The peaks collected from the first HPLC were rechromatographed separately in the same column with a gradient of 0-40% of B for 18 min followed by 40-60% of B for 61.5 min (flow rate=1 mL/min). Five peaks (P1-P5) were then obtained and the first (P1) was analyzed by mass spectrometry (MS) including peptide mass fingerprint (PMF) search against database nrNCBI using MASCOT software. **Results and Discussion:** The linear mode MS analysis of P1 detected polypeptides with mw (Da) 13672.01 (+1), 6835.51 (+2) and 4548.05. The ion 6835.51 is probably the double charge of 13672.01. P1 was further reduced, alkylated and digested by trypsin resulting in 12 peptide ions. Half of these peptide ions covered 43% (MASCOT score of 433) of the amino acid sequence of K49-PLA2 from *B. pirajai* (chain A, piratoxin-II), which had not yet been reported in *vBj*. It is known that the catalytic mechanism of PLA2 involves the binding of Ca(2+) to Asp49, that the substitution Asp49Lys reduces the K49-PLA2 catalytic activity (Biochemistry 40; 28-36, 2001), and that the stimulation of insulin secretion is one of PLA2 activities not affected by catalytic inhibitors (Toxicon 54; 413-420, 2009). Thus, K49-PLA2 of *vBj* could be responsible for the lowering of glycemia observed in the OGTT test, but the existence of EX-4 cannot be discarded in the peak P1 containing K49-PLA2 homologue, as well as in the other four purified peaks of *vBj* with positive ELISA and hypoglycemic activity.

Supported by: FAPESP, CNPq

1.18 Specific antibodies and anti-inflammatory drugs decrease edema and nociception induced by *Potamotrygon motoro* stingray venom in a murine model

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Introduction: When stingrays are stepped on, they whip the tail to the stimulated dorsum site, as a defensive behavior, and insert the stinger into the victim's limb. The stinger is a mineralized structure covered by glandular and integumental tissues, where toxins (venom) are produced. Besides mechanical trauma, stingray venom causes an intense pain followed by edema, erythema and usually necrosis. There is so far no specific therapy for stingray envenomation, and treatment consists of the administration of analgesic, antipyretic and anti-inflammatory drugs. **Objectives:** The aim of this work was to determine the ability of specific antibodies and pharmacological inhibitors to decrease nociception and edema induced by *P. motoro* venom. **Methods:** Samples containing 25 µL of rabbit anti-*P. motoro* serum or its IgG purified (Protein A Sepharose) were incubated with 4, 8 and 16 µg of *P. motoro* venom (in 5 µL) for 30 min at 37 °C. After incubation with antibodies, the mixture was centrifuged and the supernatant injected into mouse footpad to evaluate nociception and edema formation. Normal rabbit serum and its purified IgG were used as controls. In order to assess pharmacological inhibition of toxic activities, dipyrone (200 mg/kg), indomethacin (4 mg/kg), etoriboxib (10 mg/kg) or PBS (control) were injected (100 µL) i.p. 30 min before injection of venom into the mouse footpad. Edema was measured at 0.5, 1, 2, 4, 24 and 48 h after venom injection by plethysmography. Nocipeptive activity was determined by recording the time of licking the injected paw by experimental animals over 30 min. **Results and Discussion:** Antibodies reduced nociception (in at least 50 %) at all doses tested. Purified IgG also partially diminished edema formation at all doses and time periods tested. All drugs neutralized nociception, but not totally, and dipyrone could decrease it at all doses tested reaching values as high as 90 % of reduction of nociception when using 16 µg of venom. These drugs also partially neutralized edema formation at early time periods. These results show the involvement of eicosanoids in stingray envenomation, which mediate local inflammatory reaction. In addition, specific antibodies neutralized edema and nociception, providing new perspectives for therapy.

Supported by: FAPESP

1.19 Effect of Piperaceae amide on *Biomphalaria glabrata* and *Schistosoma mansoni* stages

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Introduction: Schistosomiasis is an endemic parasitic disease. It occurs in 54 countries, mainly in South America, the Caribbean, Africa and east of the Mediterranean. In Brazil, it affects over 8 million people and about 30 million live in risk areas due to the presence of infected snails. One of the most efficient methods to control this disease is the application of molluscicides that eliminates or reduces the intermediate host population. The high cost of production, environmental pollution and resistance of snails to synthetic molluscicides have stimulated the study of molluscicides of plant origin. The species from the Piperaceae family have a diversity of chemical compounds, some with bioactive properties such as essential oils, unsaturated amides, pyrones, flavonoids, monoterpenes, sesquiterpenes, arylpropanoids and lignoids. **Objectives:** In the present study, the molluscicide and schistosomicidal actions of an amide from the genus *Piper* (Piperaceae) were evaluated on adult and embryos stages of *Biomphalaria glabrata*, miracidium and cercaria, the free-living larval stages of *Schistosoma mansoni.* **Methods:** The Piperaceae amide was evaluated at concentrations lower than 10 ppm to obtain LC_{90} (concentration producing 90% mortality) **Results and Discussion:** The amide was active at concentrations (LC_{90} of 7.18 ppm and 0.99 ppm for adult and embryo, respectively) lower than that recommended by OMS. However, at the same concentrations, the amide was not active on miracidia and cercaria. The molecular structure of the amide is being studied and modified in order to increase the molluscicide and schistosomicidal activities.

Supported by: FAPESP, CAPES

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

1.20 Inflammatory effects of patagonfibrase, a metalloproteinase isolated from the venom of Philodryas patagoniensis (Serpentes: Dipsadidae)

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Introduction: Patagonfibrase is a 57.5-kDa hemorrhagic metalloproteinase isolated from the venom of the South American rear-fanged snake *Philodryas patagoniensis*. Local inflammatory reactions are conspicuous signs of snakebites inflicted by this species. **Objectives:** Taking into consideration that most snake venom metalloproteinases, besides inducing hemorrhage and myonecrosis, play a relevant role in the complex and multifactorial inflammatory response characteristic of envenomation, this study dealt with the pro-inflammatory effects evoked by patagonfibrase. **Methods:** Male *Swiss* mice were intradermally injected into the right hind paw with patagonfibrase (0.1 μg/50 μL). The contralateral paw received the same volume of vehicle (50 mM Tris-HCl buffer, pH 7.4, containing 1 mM $CaCl_2$). Paw edema was determined by measuring paw thickness using a caliper at 0 (time before intraplantar injection), 45 min, and 1, 2, 4, 6 and 24 h after injection. Results were calculated as the difference in thickness of both paws, and edema was expressed as the percentage increase in paw thickness. In order to investigate, by intravital microscopy, the effects of patagonfibrase on the leukocyte-endothelium interactions in the microcirculation of the cremaster muscle, the enzyme (0.1 μg/100 μL) was injected s.c. into the scrotal sac of mice. At 2 (T2), 4 (T4) or 24 h (T24) after the injection, the cremaster was exposed. Ten minutes after the microcirculation exposure, a portion of 100 μm of a post-capillary vessel (20-40 μm diameter) was evaluated for 1 min, and rolling, adherent and emigrated leukocytes were counted. **Results and Discussion:** Patagonfibrase caused a time-dependent edema, which was accompanied by hemorrhage. The peak of edema was noticed as early as 45 min after injection. The enzyme induced cell recruitment with a significant decrease in leukocyte rolling (at all tested times after injection), a significant increase in cell adhesion to the endothelium surface (at T2 and T4), and cell migration to extravascular tissue (mainly at T4 and T24). The presence of 1 mM o-phenanthroline, which chelates metal ions, significantly inhibited the pro-inflammatory effects induced by patagonfibrase. Taken together, these results imply that patagonfibrase is an important contributor to local inflammation elicited by *Philodryas patagoniensis* envenomation.

Supported by: FAPESP, Fundação Butantan, CONICET

1.21 Disintegrin-like/cysteine-rich domains of the reprolysin HF3: site-directed mutagenesis reveals essential role of specific residues

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Introduction: HF3, a highly glycosylated protein, is the most potent hemorrhagic toxin isolated from *Bothrops jararaca* venom. The recombinant protein composed of its disintegrin-like/cysteine-rich domains (DC) inhibited collagen-induced platelet-aggregation. Moreover, native HF3 and DC activated alphaMbeta2-mediated phagocytosis of opsonized-zymosan particles by macrophages. The ECD sequence of the disintegrin-like (D) domain has been assigned as the disintegrin motif, and the hyper-variable region (HVR) of the cysteine-rich (C) domain was suggested to constitute a potential protein–protein adhesive interface. **Objectives:** The aims of this study were i) to evaluate the effect of recombinant D and C domains of HF3 as well as three peptides resembling its HVR on platelet aggregation, and ii) to investigate the role of specific residues of the putative ECD disintegrin motif and of the HVR of HF3 by site-directed mutagenesis. **Methods:** Recombinant (wild-type and mutant) DC, D16 or D18, and C proteins were obtained in fusion with GST in *E. coli* BL21 (DE3). Peptides based on the HVR of HF3 were synthesized by the solid phase peptide synthesis FMOC strategy. Charged residues of the disintegrin loop and of the HVR of HF3 were individually mutated to Ala to identify residues essential for the functionality of the DC domains. The recombinant proteins of non-catalytic domain of HF3 and synthetic peptides were tested for their ability to affect platelets. A suspension of washed platelets was preincubated with these proteins and peptides for 3 min and incubated with collagen I at 37°C for 8 min using an aggregometer. Alterations in the microcirculation were analyzed using intravital microscopy by transillumination of mouse cremaster muscle after topical application of GST-DC-D469A at 5 mM in PBS. **Results and Discussion:** The recombinant proteins GST-DC, GST-DC-D469A, GST-D16, GST-D18 and GST-C were purified by affinity chromatography and recognized by an anti-HF3 antibody. Recombinant D and C domains of HF3, expressed together or individually, and the HVR synthetic peptides inhibited collagen-induced platelet-aggregation. The mutation of the Asp residue of the ECD motif caused loss of the ability of the DC domains to affect platelet aggregation and to promote leukocyte rolling in the microcirculation. Moreover, the C domain and its HVR were demonstrated to be critical for HF3 to affect platelets and leukocytes; however, the disintegrin loop may be important for the functionality of the D domain in the context of the C domain.

Supported by: FAPESP

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

1.22 Africanized honey bee (*Apis mellifera*) venom profiling: seasonal variation of melittin and phospholipase A2 levels

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Introduction: The venom of the European honey bee, Apis mellifera, is composed basically of melittin, phospholipase A2, histamine, hyaluronidase, catecholamine and serotonin. While some of these components have been associated with allergic reactions, among several other symptoms, and sometimes leading to anaphylactic shock, mass stinging by the Africanized honey bee (AHB) causes serious toxic effects often leading to death, through a massive injection of venom. As AHB spreads through Brazil and the Americas, the number of mass stinging cases is rising, becoming a serious public health issue. The development of efficient serum-therapies has, therefore, become an urgent necessity. **Objectives:** The aim of this study was to carry out a biochemical characterization of the seasonal variation of the major components of the AHB venom, by analyzing the pooled venom composition of individuals pertaining to one specific hive over one year. **Methods:** The venom was collected by manual and electrical stimulation of bees from one specific hive at the Apiary of Botucatu School of Veterinary Medicine and Animal Husbandry (UNESP). The venom profiling and component purification was performed by RP-HPLC with an acetonitrile gradient in a C8 column. Mass spectrometry of the samples was performed in an ESI mass spectrometer (LCQDuo™, ThermoFinnigan, USA), equipped with a nanospray source and connected to nanoHPLC system. The venom profiling was correlated with climatic parameters obtained in Botucatu. Complete or N-terminal sequencing of purified peptides was performed by Edman degradation using a Shimadzu PPSQ-21 automated protein sequencer. **Results and Discussion:** It was possible to detect a seasonal variation on the venom contents of melittin and phospholipase A(2). Moreover, both compounds showed a synchronized variation of their levels, with an increased production in the same months. This variation does not correlate or synchronize with any climatic parameter. Data on the variation of the AHB venom composition is necessary to guide future intra- and interspecies studies. The production of specific AHB antivenom should take into account the possible regional variability of the venom composition due to climatic, seasonal and feeding factors. These variations could be either quantitative or qualitative. The compounds analyzed in this work showed a quantitative variation, but a closer inspection can reveal peaks that undergo qualitative variation through the year. The possible qualitative variations still need to be investigated compared with other bee colonies, and other regions.

Supported by: FAPESP, CNPq

1.23 N-acetyl-l-cysteine (NAC) affects renal function, aminopeptidases and oxidative stress in *Bothrops jararaca* envenomation in mice

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Introduction: NAC is a thiol antioxidant for which there are few reports of side effects. NAC has been reported to be effective in the prevention of acute renal failure (ARF). **Objectives:** This study aimed to contribute to the understanding of the mechanisms and consequences of the nephrotoxic effect of *Bothrops jararaca* venom (*vBj*) and to evaluate the possibility of introducing NAC as a coadjuvant in *Bothrops* antivenom therapy. **Methods:** The effects of NAC on hematocrit, protein, classical parameters of renal function, aminopeptidase activities and redox status were measured in mice with ARF induced by LD50 of *vBj* (protocol approved by the Ethics Committee of the Instituto Butantan, 492/08). **Results and Discussion:** NAC affected oxidative stress (GSSG/GSH index), uricemia, proteinemia and creatinuria (full restoration) and uremia (ameliorated) in envenomed animals. Alone or combined with *vBj*, NAC caused the reduction of protein content of membrane fractions of the cortex and renal medulla, increased proteinuria and reduced urinary excretion of urea. However, urinary hyperosmolality, also typical of *Bothrops* envenomation, was aggravated by NAC. Remarkable is the ability of NAC to affect the levels of aminopeptidase activity in renal tissue. In the soluble fraction of the renal cortex and medulla of envenomed animals NAC restored normal levels of APB, APN and PIP, but increased the DPPIV. In the membrane fraction of renal cortex, NAC restored normal levels of APN and PIP, but increased CAP, PAP and DPPIV of envenomed animals. In the membrane fraction of renal medulla, NAC restored normal levels of APA, APN and PIP, ameliorated the decrease of CAP, but increased PAP and DPPIV.

These data allow us to outline the pattern of action of NAC on renal function, aminopeptidase activity and renal oxidative stress in normal and *vBj* envenomed mice, showing significant beneficial effects and suggesting the convenience of the clinical evaluation of the association of this agent with the serotherapy of this envenomation.

Supported by: FAPESP, CNPq

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

1.24 Effects of allopurinol and probenecid on lethality and acute renal failure induced by *Crotalus durissus terrificus* envenomation in mice

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Introduction: Acute renal failure (ARF) is one of the most serious complications of rattlesnake envenomation. The venom of *Crotalus durissus terrificus* (*vCdt*) at a dose of 80% LD50 produces oxidative stress and 100% incidence of hyperuricemia and urinary hypo-osmolality, suggesting that the latter two can be early signs of direct nephrotoxic action (without myotoxicity) of *vCdt* (Toxicon 52:445-54, 2008). **Objectives:** This study aimed to evaluate the relevance of these alterations as mechanisms of induction of ARF by *vCdt*. **Methods:** The effects of uricostatic (allopurinol) and uricosuric (probenecid) drugs on hematocrit, protein, renal function parameters (osmolality, creatinine, uric acid and urea) and oxidative stress (GSSG/GSH) were measured in mice envenomed with 80% LD50 of *vCdt* (protocol 717/10 approved by the Ethics Committee of the Instituto Butantan). **Results and Discussion:** Allopurinol restored uricemia, osmolality, and the redox status of renal tissue and, above all, surprisingly completely abolished the lethality of 80%LD50 of v*Cdt* in mice without treatment with antivenom. In turn, probenecid also restored the level of uric acid in plasma and its excretion in urine and the renal redox status, but simultaneously caused hypercreatinemia, hypocreatinuria and urinary hypo-osmolality in envenomed mice.
Hyperuricemia and urinary hypo-osmolality seem to be the two main causes of mortality of ARF induced by the direct nephrotoxic action of v*Cdt*. Hyperuricemia in envenomed mice should be predominantly generated by increased production of uric acid, since allopurinol is uricostatic and showed higher therapeutic efficacy than that due to the uricosuric action of probenecid. However, it is also possible that the effectiveness of probenecid in *vCdt* envenomation is compromised only due to the hypercreatinemia, hypocreatinuria and urinary hypo-osmolality caused by this drug. Consequently, these data allow us to recommend a clinical evaluation of the use of allopurinol in rattlesnake envenomation, which seems to be an important complementary approach to antivenom therapy.

Supported by: FAPESP, CNPq

1.25 *Bothrops jararaca* envenomation elicits low uterine fibrinogen

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Introduction: Besides the classic function in hemostasis, fibrinogen (Fg) is also involved in the maintenance of gestation. *Bothrops* snakebites usually cause plasma fibrinogen consumption, but it is not clear whether hypofibrinogenemia causes gestational interruption. **Objectives:** This investigation aimed to evaluate the presence of Fg in the uterus of pregnant mice submitted to *Bothrops jararaca* (Bj) envenomation. **Methods:** On the morning of day 8 of pregnancy, animals were submitted to a single i.m. injection of 0.48 mg Bj venom/kg body weight. The control group received saline (i.m.). On day 9, implantation sites were: (a) isolated and immediately immersed in Bouin fixative solution for morphological evaluation; (b) immersed in 4% PFA in phosphate buffer for evaluation of Fg expression in uterine tissue; and (c) homogenized in solution to evaluate Fg expression by immunoblotting. Plasma fibrinogen was also assayed in plasma samples of pregnant animals. **Results and Discussion:** At the antimesometrial region, uterine analysis of control dams showed trophoblastic and mature decidual cells with morphological characteristics similar to those of animals submitted to no treatment. However, among some animals in the Bj venom group, the maternal and embryonic tissues were disorganized, showing trophoblastic and mature decidual cells with clear signs of cell death, hemorrhagic areas and inflammatory infiltrate. Immunohistochemistry revealed that the antimesometrial area showed positive reaction to Fg, which was scattered over the extracellular matrix; however, the reaction was more intense in the saline group than in the Bj group. Imunoblotting analysis showed that Fg bands in the Bj group were fainter than in the saline group, and a correlation between these results and those obtained with the immunohistochemistry was observed. These findings show that Bj venom evokes a decrease in fibrinogen in plasma and in maternal uterine tissue.

Supported by: INCTTOX Program - CNPq, FAPESP

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

1.26 Ontogeny in the venom proteins of neonates of *Crotalus durissus terrificus* and *Crotalus durissus collilineatus*

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Introduction: Venomous snakes are widespread in tropical and subtropical regions of the world. Snake venoms are mixtures of biologically active substances, most of which are enzymes or non-enzymatic polypeptides. There is reason to believe that envenomation behavior may vary with ontogeny, the composition and properties of which vary with age and the supply of available venom increases exponentially with growth. Individuals from different families, genera and species even from the same species differ in the constituents of their venoms. The intraspecific variations are present in relation to season, habitat, age of the specimen, sexual dimorphism and diet. Some researchers have conducted comparative studies on the venom constituents of males and females of the same species, some female snakes have shown similarity in the production of a component (crotamine) in the venom, which is absent in males of the same species. **Objectives:** The objective of this research was to determine whether there is any variability in the venom composition of *Crotalus durissus terrificus* and *Crotalus durissus collilineatus* neonates and if it is related to factors associated with sex, ontogenic development, geographical origin and/or individual variation. **Methods:** Twenty *Crotalus durissus terrificus* from three different litters (1st litter= 3 males and 4 females; 2nd litter = 5 males and 4 females; 3rd litter= 4 females); and seventeen *Crotalus durissus collilineatus* all from the same litter (6 males and 11 females) were used in this experiment. The first extraction was performed when the rattlesnakes were 24 months old and the 7th at 42 months. Protein levels were quantified by a biochemical method (Biuret) and then submitted to electrophoresis. The proteins were separated by SDS-PAGE (T=10.-20.0%) and stained with 0.25% Coomassie solution to analyze the proteins present in the gels. **Results and Discussion:** In the venom of the genus *Crotalus durissus,* there is variation between crotamine-positive and crotamine-negative protein, depending on the snake's geographic distribution. Investigating the litter's venoms in the two subspecies at different ages, the results showed that both subspecies of *Crotalus durissus* possess a variety of crotamine-positive and -negative proteins, present in males and females. The patterns of crotamine-positive and -negative protein are constant in the litters, independent of the animal age.

Supported by: INCTTOX Program - CNPq, FAPESP

1.27 Antibody modeling and expression of human anti-crotoxin single-chain fragment variable (ScFv)

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Introduction: Antibody molecules bind antigens with high affinity and specificity by synergistically using multiple noncovalent forces. The combining site (paratope), whose shape is complementary to the epitope on the antigen, is made up of the hypervariable regions, also called complementarity determining regions (CDRs). The CDRs in the light and in the heavy chains fold into structures that are stabilized by the b-sheet framework of the variable domains. 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. Due to the difficulty in obtaining crystals suitable for the structural elucidation of antibody fragments in complex with proteins, other information about the key residues involved in the interaction are very useful. The human neutralizing recombinant anti-crotoxin scFv-6 was isolated by phage display technology from a naive library of more than 10^{10} scFv clones. **Objectives:** The aim of this study was to analyze the structure of anti-crotoxin scFv-6 and to express it in the periplasm of bacteria in order to obtain this molecule in its soluble and functional form. **Methods:** With computer-aided homology modeling using Modeller 9v5 program, the structural/functional relevant regions of heavy and light chains, CDRs, were defined. In each step of modeling, about a hundred models were generated, and the one with the best energy was selected. scFv-6 coding sequence was cloned into pET20b+ vector and the construction was used to transform C43 bacteria. The production of scFv was accomplished using 0.5 mM IPTG and growth condition at 37°C for 4 h. After expression, soluble scFv was recovered from the periplasm by osmotic shock and further purified with nickel resin. ELISA, SDS-PAGE and Western blotting were used to evaluate the purity of the sample. **Results and Discussion:** The modeled structure of ScFv showed the common features of a classical antibody. Its antigen binding surface exhibits electropositive and electronegative potentials that can be related to crotoxin recognition. The yield of expressed scFv-6 was 2.3 mg/L. The purified protein showed an expected band around 30 kDa, soluble and specific for crotoxin. Advantageous mutations of scFv will be generated by site-directed mutagenesis. Original and mutants scFv will then be biochemically characterized regarding their affinity and ability to neutralize crotoxin and venom toxic activities.

Supported by: FAPESP, CNPq, PAP/SES

1.28 Crotoxin modifies intracellular signaling involved in phagocytosis by neutrophils

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Introduction: Previous studies showed that *Crotalus durissus terrificus* venom (CdtV) inhibits the phagocytic activity of macrophages and neutrophils and that crotoxin (CTX), the main component of CdtV, is responsible for this effect. In macrophages, CTX causes reorganization of the actin cytoskeleton and inhibition of phosphotyrosine. **Objectives:** Considering that the signaling pathways for phagocytosis both in macrophages and neutrophils have some differences and that the mechanisms involved in the inhibitory effect of CTX on phagocytosis by neutrophils is still unknown, the aim of this study was to investigate the in vitro effect of CTX on tyrosine phosphorylation and actin polymerization on nascent and mature phagosomes. **Methods:** Neutrophils were obtained from the peritoneal cavity of male Wistar rats (CEUAIB 705/10) 4 h after the intraperitoneal administration of carrageenan (4.5 mg/kg). Neutrophils ($1x10^6$ cells/mL) were incubated (1 h) with CdtV (0.5 μg/mL) or CTX (0.08 μg/mL) and then submitted to phagocytosis of opsonized zymosan for 5 or 15 min. Next, neutrophils were fixed and permeabilized. Incubation with primary antibody against phosphotyrosine was performed overnight. The cells were then incubated with the secondary antibody FITC-labeled and stained with rhodamine-phalloidin. Nuclei were stained with DAPI. Slides were mounted and observed by confocal microscopy. **Results and Discussion:** The incubation of neutrophils with CTX induced a marked reduction in staining of phosphotyrosine (97%) and F-actin (73%) in neutrophils during phagocytosis at 5 min, when compared to the controls. Similarly, when phagocytosis was performed for 15 min, CTX reduced the content of F-actin (86%) in relation to controls. The same effect was observed when neutrophils were incubated with crude CdtV. During phagocytosis, the engulfment of the particle begins with nascent phagosome formation, which occurs at 5 min and is complete in approximately 15 min with maturation of the phagosome. In nascent phagosomes, an increase in tyrosine phosphorylation and actin polymerization is observed; this polymerization leads to phagosome maturation. Unlike what occurs in macrophages, our results demonstrate that CTX inhibits tyrosine phosphorylation and consequently actin polymerization. The results presented herein may contribute to explaining the inhibitory effect of CdtV, particularly CTX, on phagocytosis by neutrophils. Furthermore, taking into account the importance of these phagocytes in the inflammatory response, these results contribute to the elucidation of the mechanisms involved in the anti-inflammatory effect of CTX, which has been reported in the literature.

Supported by: FAPESP, CAPES, INCTTOX Program - CNPq, FAPESP

XII Reunião Científica Anual do Instituto Butantan
01 a 03 de Dezembro de 2010, São Paulo, SP

1.29 Hybrid offspring from *Bothrops erythromelas* and *Bothrops neuwiedi* snakes: characterization and biological activities of their venoms during development

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Introduction: Hybridization between species is a natural process, which can lead to the merging of taxa, to the reinforcement of behavioral barriers to mating, or even to the emergence of new hybrid species. In fact, interspecific hybridization has been shown to play a major role in the formation of new species. Natural hybridization between *Bothrops* spp has been already reported, but such investigations were restricted to morphological analyses of hybrids. Although grouped in the same taxonomical category, the snakes *B. erythromelas*, distributed in northeastern Brazil, and *B. neuwiedi*, occurring in southern states in Brazil, have striking differences in their venom composition, particularly regarding the absence of thrombin-like enzymes in *B. erythromelas* venom. **Objectives:** The aim of this study was to characterize the biological activities of venoms from hybrid snakes between *B. erythromelas* and *B. neuwiedi* with regard to their development. **Methods:** A male *B. neuwiedi* was mated with a female *B. erythromelas* on June 8, 2006, and 15 hybrids were born on February 22, 2007. At three-month intervals, venom was milked from parents and hybrids, lyophilized and used for analyses. The following tests were carried out in venom samples from hybrids and parents: protein assay (bicinchoninic acid method); determination of minimum coagulant dose (MCD) in bovine plasma and fibrinogen; and assays of collagenolytic (using azocoll) and amidolytic activity (using BAPNA). **Results and Discussion:** Protein concentration increased in hybrid venoms over time, reaching values similar to those of the father when hybrids were 2 years old (yo). Amidolytic and proteolytic activities were reduced in newborn venom samples, and progressively increased as snakes grew, reaching values similar to those of the father when hybrids were 1 yo and 6 months old, respectively. Using bovine plasma, lower MCD values were noticed in newborn venoms, close to *B. erythromelas* venom, and reached values similar to those of the father venom when they were 1 yo. The assay of thrombin-like activity showed that hybrid venoms do not exhibit such activity during the first 6 months after birth, beginning to exhibit it when they are 1 yo; similar thrombin-like activity as in the father was noticed when hybrids were 3 yo. Our findings show that there are important changes in the composition and activity of hybrid venoms over their development. Hybrid venoms imitate *B. erythromelas* venom when the offspring is young, and become similar to *B. neuwiedi* venom as hybrids grow older. In addition, our results show that *B. erythromelas* and *B. neuwiedi* are not well-differentiated species.

Supported by: FAPESP, Fundação Butantan