ISOLATION AND CHARACTERIZATION OF A NEW COAGULANT FACTOR FROM *Bothrops pirajai* VENOM

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Snake venoms display a variety of proteins and peptides able to affect thrombosis and hemostasis, including serinoproteases which constitute 20% of the total proteins of the venom. A serinoprotease from *B. pirajai* snake venom was isolated and biochemically characterized. It was named piraserbin. Its high purity level was shown by SDS polyacrylamide gel electrophoreses under reducing conditions, showing a single chain with Mr 37,500, 12% neutral carbohydrate and pI 4.6. Its minimum coagulant dose was 1.75m g and its fibrinogenolytic activity was mainly upon the a chain of fibrinogen, and its fibrinolytic activity was observed only at high enzyme dose (100m g). It showed a high esterase activity upon TAME (15U/mg) and high amidolytic activity upon synthetic peptides S2302 (H-D-Pro-Phe-Arg-pNA-2 HCl) which is a substrate for plasma calicrein, and S 2238 (H-D-Phe-Pip-Arg-pNA-2HCl), substrate for thrombin. When assayed for cinin release, it did not show any activity. It was however able to induce platelet aggregation of 90% at 20m g/ml. N-terminal sequencing reveated 90% homology with crotalase (calicrein-like serinoprotease from Crotalus adamanteus venom).

KEY WORDS: serinoprotease, *Bothrops pirajai* venom, clotting activity, fibrinogenolytic activity.

FINALCIAL SUPPORT: FAPESP, CNPq

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EFFECT OF *Bothrops lanceolatus* FRACTIONS ON PLATELETS ADHESION TO FIBRINOGEN

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Snake venoms contain a wide range of components, many of wich affect haemostasis by activation or inhibition of platelets or coagulation factors. In this study, we investigated the inhibition of *Bothrops lanceolatus* fractions on platelets adhesion to fibrinogen. Human washed platelets adhesion was evaluated using fibrinogen-coated 96-well micro titer plates. Platelets and venom fractions were maintained in the plate for 15 and 30min. Adherent platelets were incubated with the acid-phosphatase substrate (p- nitrophenyl phosphate disodium) for 1h. The plate was read by micro plate reader set al 405nm. Results showed that *Bothrops lanceolatus* fractions inhibit human platelets adhesion to fibrinogen and this inhibition was lower when these fractions were heated 100^{o}C.

KEY WORDS: *Bothrops lanceolatus*, platelet adhesion

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HEMOLYTIC ACTIVITY OF *Bothrops lanceolatus* (FER DE LANCE) VENOM *in vitro*

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Bothrops lanceolatus venom contains a variety of enzymatic and biological activities. In this work, we investigated the hemolytic activity of this venom and its phospholipase A_2 (PLA_2). *Bothrops lanceolatus* venom (6.7 mg/ml) caused indirect hemolysis of cow, horse, rat and sheep erythrocytes, with horse erythrocytes being the most sensitive; no direct hemolysis was observed. The hemolysis in sheep erythrocytes was concentration-dependent (5-11.7 mg/ml) and was markedly attentuated by heating the venom for 30 min at temperatures >40^{o}C and by the PLA_2 inhibitor p-bromophenacyl bromide. An acidic PLA_2 purified from *B. lanceolatus* venom also caused hemolysis. The PLA_2 showed immunoprecipitin lines with antivenom against *B. lanceolatus*, which suggests that the enzymatic and hemolytic activities of this enzyme may be neutralized during antivenom therapy. These results indicate that *B. lanceolatus* venom and its PLA_2 can cause hemolysis *in vitro*.

KEY WORDS: *Bothrops lanceolatus*; Hemolytic activity; Phospholipase A_2; Snake venom

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NA+/K+ATPASE ACTIVITY AND EXPRESSION IN KIDNEY OF RATS TREATED WITH *Bothrops alternatus* SNAKE VENOM

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The ion pump Na+/K+-ATPase is widely expressed in renal tubules and has an important role in modulating sodium reabsorption, renal function and homeostasis of the extracellular compartment. In this study, we investigated the activity and expression of Na+/K+-ATPase in renal tissue of male Wistar rats (~250 g) 6, 24, 48 and 72 h and 7 days after the administration of 0.8 mg of venom/kg, i.v. At preset intervals, the rats were killed with an overdose of halothane and the kidneys were removed, frozen in liquid nitrogen and stored at -80oC until processed for Na+/K+-ATPase activity. Treatment with venom significantly increased Na+/K+-ATPase activity (nmol/mg/min) after 6 h (326.9+63.2; mean+S.D.) and 24 h (310.2+54.4) when compared to saline-treated (control) rats (208.4+34.2) (n=6 each). However, incubation of renal membrane preparations with venom (30-100 mg/ml) for 30-120 min at 37oC did not affect Na+/K+-ATPase activity. Quantitative real-time PCR revealed a significant increase ($p<0.05$) in Na+/K+-ATPase expression 6 h after the administration of 0.8 mg of venom/kg (expression level: 1.6+0.1 for the α1 subunit and 2.03+0.35 for the β1 subunit of the pump) when compared to saline-treated rats (1.16+0.2 and 1.01 + 0.16 for the α1 and β1 subunits, respectively). These results indicate that *B. alternatus* venom transiently and indirectly alters renal Na+/K+-ATPase activity in rats. This effect may contribute to renal damage following envenomation.

KEY WORDS: acute renal failure, *Bothrops alternatus*, kidney, Na/K-ATPase.

FINANCIAL SUPPORT: CNPq, FAPESP.

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HISTOLOGICAL AND FUNCTIONAL RENAL ALTERATIONS CAUSED BY *Bothrops alternatus* SNAKE VENOM IN RATS

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Acute renal failure (ARF) is one of the most serious complications of human envenomation by *Bothrops* snakes. The pathogenesis of ARF in snakebite envenomation may involve hemodynamic disturbances, immunological reactions and direct nephrotoxicity. In this study, we investigated the morphological and functional renal alterations caused by *Bothrops alternatus* venom in male Wistar rats (~250 g) 6, 24, 48 and 72 h and 7 and 15 days after the administration of 0.8 mg of venom/kg, i.v. (n=6/group). Creatinine and lithium clearances were used to estimate the glomerular filtration rate (GFR) and the proximal tubule output, respectively. Histological alterations were assessed by Sirius-red staining. Six hours after envenomation, there was an increase in urinary sodium excretion accompanied by a significant decrease in proximal sodium reabsorption. These initial changes were followed by a transient, rapid decrease in the GFR and a persistent increase in the post-proximal sodium excretion from the second day of envenomation onwards. The decrease in GFR was accompanied by morphological disturbances in the glomeruli such as lobulation of the capillary tufts with dilation of Bowman´s space. In addition, there was deposition of collagen around the glomeruli and proximal tubules, as well as peritubular capillary dilation with desquamation of cells into the tubule lumen. These results indicate that *B. alternatus* venom causes significant morphological and functional changes that may contribute to ARF following snakebite.

KEY WORDS: *Bothrops alternatus* venom, nephrotoxicity, renal function.

FINANCIAL SUPPORT: CNPq, FAPESP.

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ANTI-INFLAMMATORY ACTIVITY OF THE HYDROALCOHOLIC EXTRACT FROM *Eclipta prostrata* IN THE PAW OEDEMA INDUCED BY *B. moojeni* SNAKE VENOM

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Many Brazilian plants have been utilized in traditional medicine as active agents against various effects induced by snakebite. In this work we investigated the ability of the hydroalcoholic extract from *Eclipta prostata* to inhibit the edema induced by *Bothrops moojeni* venom (BmV). BmV was injected into hind paws and the change in volume over time was measured by plethysmometry. BmV (0.50 µg/paw) induced a significant oedema formation which peaked 3 h after venom injection. Mice were pretreated with the hydroalcoholic extract (500 mg/kg, i.p) and anti-oedema activity was compared with dexamethasone (0.5 mg/Kg i.p.), a phospholipase A2 inhibitor. The results showed that hydroalcoholic extract and dexamethasone produced similar anti-inflammatory effects, observed after the venom injection, with an oedema reduction of 69, 76, 92 % and 69, 95 e 89%, respectively at 60, 180 and 360 min. Histological sections of rat paw confirmed that the edematogenic activity was significantly reduced by dexamethasone and also by hydroalcoholic extract. In conclusion, the hydroalcoholic extract from *E. prostrata* markedly inhibited paw oedema induced by *B. moojeni* snake venom. *E. prostrata* contained abundant amount of the Wedelolactone, which has been reported as the constituent of anti-venom activity. A possible mechanism of action of Wedelolactone in inhibiting the phospholipase A2 activities of the venom might result from the polyphenolic nature of the compound.

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KEY WORDS: *Eclipta prostrata*; *Bothrops moojeni*; Edema-inducing activity.

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MIONECROSE AND INFLAMMATION INDUCED BY *Bothrops jararacussu* (Bjssu) VENOM IN THE GASTROCNEMIUS MUSCLE OF MICE

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Venoms from snakes of genus *Bothrops* cause pronounced local effects in the victims, which are represented mainly by edema, pain, haemorrhage and necrosis. In this work we examined the ability of the total venom of the snake Bjssu to cause morphologic damage, edema and cellular influx in the gastrocnemius muscle of mice. The myonecrosis was examined through histologic analysis 3 h after the venom injection, with the following doses 15, 30 and 60 mg, in the gastrocnemius muscle of Swiss mice. The leukocytes migration was studied counting the total and differential cells in the muscle. The edematogenic effect was evaluated by humid weight of the muscle 3 and 24 h after the injection of the venom or saline (control). The results demonstrate that the dose 30 and 60 mg caused exaggerate necrosis in muscle fiber reaching adjacent muscles. Therefore, the dose chosen for realization of this work was 15 mg. The venom of the snake Bjssu significantly increased the number of inflammatory cells 24 h after venom injection, polimorfonuclear leukocytes were the predominantly cells, mainly neutrophis (control 9,2 x 106 ± 2,3; Bjssu 66,4 x 106 ± 3,4). The venom induced a significant edema formation in the gastrocnemius muscle 3 h after its injection (control 0,15 ± 0,01 mg; Bjssu 0,28 ± 0,01 mg) and was unchanged over the following 24 h. The present data demonstrate that the venom of Bjssu caused myonecrosis and local inflammatory reaction, represented in this work by edema and cell migration, in the gastrocnemius muscle.

FINANCIAL SUPPORT : UNIVAP

KEY WORDS: myonecrosis; edema; leukocites migration; snake venom.

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MOLECULAR ASPECTS OF THE TOXINS IDENTIFIED IN THE TRANSCRIPTOME OF THE COLUBRIDAE SNAKE *Philodryas olfersii*

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The interest about venoms of Colubridae, a family of snakes relatively neglected in the venom studies, is increasing due to its importance in human envenoming accidents. *Philodryas olfersii*, an important species of this group and the one involved in most of the accidents in Brazil, has a Duvernoy's gland that produces a secretion exhibiting high hemorrhagic activity. The analysis of a transcriptomic database from *P. olfersii* Duvernoy's gland revealed that this species shares its toxins classes with the Viperidae family. Here we describe some particular features of these toxins, their sequence structures, similarities and phylogenetic relationships. The predominant toxins were the metalloproteases, the firsts described in a Colubridae and shown to belong to the P-III type. The serine proteases are trypsin-like as in Viperidae. Four different C-type lectin chains and slightly different Cystein Rich Secretory Proteins (CRISPs) isoforms were identified. We observed the high expression of a C-type natriuretic peptide precursor (CNP) and its phylogenetic analysis showed it as a linker between the multifunctional Bradykinin Potentiating Peptides (BPPs) precursor found in Viperidae and the CNP precursor found in Elapidae snakes. The *P. olfesii* dbEST provides an overview of a Colubridae toxin structures, some insights of their evolution and supports the important role of metalloproteases in the hemorrhagic effect elicited during *P. olfersii* envenoming.

KEY WORDS: *Philodryas olfersii*, Colubridae toxins, transcriptome, Duvernoy's gland, CNPs, metalloprotease

FINANCIAL SUPPORT: FAPESP, Fundação Butantan

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A PROFILE OF THE COLUBRIDAE SNAKE *Philodryas olfersii* VENOM PROTEOME REVEALED FROM A DUVERNOY´S GLAND TRANSCRIPTOME

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Philodryas olfersii, a representative species of the Colubridae family of snakes, has a Duvernoy's gland that produces a secretion exhibiting high hemorrhagic activity. The local effects of envenomations by this species are similar to those produced by *Bothrops* species. A cDNA library from *P. olfersii* venom gland was constructed for the generation of an Expressed Sequence Tags database (dbEST) aiming to characterize its transcriptome. We obtained 2194 ESTs with an average of 415.7 bp that were grouped in 1285 clusters. About 35% of all transcripts were classified into non-toxins and 35% were not identified (no match on public database). Among the clusters matching cellular proteins, the major part represents molecules involved in gene and protein expression and secretion, such as proteins disulfide isomerase (PDI), reflecting the specialization of this tissue for toxin synthesis. The remaining 30% of all transcripts revealed the presence of the major toxin classes from the Viperidae family. Metalloproteases, serine proteases, C-type lectins, Cystein Rich Secretory Proteins (CRISPs), and C-type natriuretic peptides (CNPs) were found. Some of the corresponding proteins were detected in a 2-D gel of the venom by mass spectrometry identification. Together, the transcriptomic and proteomic data showed a low complexity of the venom when compared to other snake families. The *P. olfesii* dbEST is the first effort to massively identify cDNA sequences from a Colubridae species, revealing some features of its venom composition.

KEY WORDS: *Philodryas olfersii*, transcriptome, Colubridae snake, Duvernoy's gland

FINANCIAL SUPPORT: FAPESP, Fundação Butantan

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L-AMINO ACID OXIDASE ISOLATED FROM *Bothrops moojeni* SNAKE VENON PRESENT SELECTIVE CYTOTOXIC ACTIVITY AGAINST TUMORS

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L-amino acid oxidase (LAAO) is flavoenzyme which catalyses the stereospecific oxidative deamination of an L-amino acid in an a-keto acid along with the production of ammonia and hydrogen peroxide. This enzyme presents important biological proprieties as platelet aggregation, mediation of inflammatory signs, bactericidal activity, anti-viral activity and cytoxicity against tumor cells. In this work we evaluated the cytotoxic activity of LAAO isolated from *Bothrops moojeni* (BmooLAAO-I) against 4 tumor cell lines (JURKAT, SK-BR-3, B16F10 and EAT) and also towards human peripheral blood mononuclear cells (PBMC). Herein to address the mechanism of LAAO antitumor properties, participation ROS generation was evaluated and the cell death by induction of necrosis or apoptosis. Our results shown that BmooLAAO-I at concentration of 1-0.01mg/mL presented potent cytotoxic activity against all tumor cell tested as dose dependent manner. Importantly, LAAO showed a significant cytotoxic activity to PBMC only at high concentrations (1mg/mL) thus suggesting that this enzyme have a selective cytotoxicity to tumor cells. Also we verify that ROS is generated by tumor cells cultured in the presence of LAAO and that LAAO cytotoxic activity was abolished by the presence of catalase, indicating that ROS generation must be relevant to LAAO anti-tumor activity. Finally the cell death assays showed that LAAO induced predominantly necrosis and little apoptosis on tumor cells as time dependent manner, thus suggesting that LAAO kill tumor cells by at least two different pathways.

KEY WORDS: L-amino acid oxidase, *Bothrops moojeni* snake venom, anti-tumor activity, reactive oxygen species, necrosis, apoptosis.

FINANCIAL SUPPORT: FAPESP, CNPq, FCFRP-USP.

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ANTITUMORAL AND MICROBICIDE POTENTIAL Of L-AMINO ACID OXIDASES ISOLATED FROM *Bothrops* SNAKE VENOMS

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L-amino acid oxidases (LAAO, E. C. 1. 4. 3, 2) are flavoenzymes, which catalyze the oxidative deamination of L-amino acids to ketoacids producing ammonia and hydrogen peroxide. Recently we isolated three LAAOs from snake venoms, namely BmooLAAO-I, BaltLAAO-I and BjussuLAAO-I, from *Bothrops moojeni, B. alternatus* and *B. jararacussu*, respectively. We are showing now their antitumoral activity and microbicide potential. The *in vitro* cytotoxic effect of the enzymes was evaluated on the tumoral lines B16F10, SK-BR 3 and JURKAT, bacteria lines (*E. coli*, *S. aureus*, and *P. aeruginosa*), fungi lines (*Candida albicans* and *Peniccillium* sp.), and parasites (*Leishmania* sp. and *Trypanosoma cruzi*). Our results clearly show that LAAOs display potent selective cytotoxic activity on tumoral lines JURKAT, SK-BR-3 and B16F10, but not on blood mononuclear cells. In addition, the cytotoxic activity of BmooLAAO-I and BaltLAAO-I are carried out through induction of apoptosis in tumoral cells by means of the production of oxygen reactive intermediates. Similarly, both enzymes showed to be cytotoxic upon bacteria, fungi, and parasites, catalase drastically inhibiting all activities. This set of date indicates that LAAOs display selective cytotoxic activity, mainly upon tumors, with a high potential for future utilization in clinical therapeutics.

KEY WORDS: *Bothrops* venoms, L-amino acid oxidases, antitumoral activitiy, microbicide potential and biotechnological applications.

FINANCIAL SUPPORT: FAPESP, CNPq.

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PURIFICATION AND SOME PHYSICO-CHEMICAL PROPERTIES OF A 5'NUCLEOTIDASE ISOLATED FROM *Lachesis muta* SNAKE VENOM

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Snake venoms are formed by a mixture of active principles responsible for diverse pharmacological properties. A 5'-nucleotidase was isolated from *Lachesis muta* snake venom through gel-filtration and ion exchange chromatographies. Electrophoresis of enzyme revealed a single band protein with an apparent molecular mass of 25 kDa in the presence or not of 2-mercaptoethanol, indicating that the protein contained no subunits. The isoelectric point was estimated to be 4.7. No alkaline or acidic phosphatase, pyrophosphatase or adenylate kinase activities were detected on isolated 5'-nucleotidase. The enzyme had a pH optimun in the range of 7.0-8.0 and optimum temperature from 35ºC to 40^{o}C. 5'-nucleotidase activity was inhibited by Cu2+, Mn2+, Zn2+ whereas Ca2+, Mg2+ or Co2+ preserved its activity. Indeed, EDTA inhibited its activity. Besides AMP, 5'-nucleotidase cleaved other nucleoside monophosphates, such as UMP and CMP with potencies of 93 and 89 % when compared to AMP hydrolysis. In contrast to IMP or GMP, with potencies of 11 and 5 %. The enzyme did not hydrolyze ATP or other nucleoside triphosphates and a low activity upon ADP was observed. 5'-nucleotidase inhibited ADP-induced platelet aggregation. This soluble 5'-nucleotidase may play a special role in the degradation of endogenous nucleotides from prey tissues that in turn released adenosine may regulate activities in many targets, such as nervous system or be important on immobilizing and digestion of prey.

KEY WORDS: *Lachesis muta*, 5'-nucleotidase, purification, platelet aggregation, snake venom.

FINNANCIAL SUPPORT: FAPERJ, FAPEMIG, FAPERGS, CNPq.

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LYSOPHOSPHATIDYLCHOLINE PRODUCED BY THE PHOSPHOLIPASE A2 ISOLATED FROM *Lachesis muta* SNAKE VENOM MODULATES NATURAL KILLER ACTIVITY AS A PROTEIN KINASE C EFFECTOR

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In a previous report we showed that the phospholipase A2 isolated from *Lachesis muta* snake venom, denoted LM-PLA2-I, displayed some pharmacological effects. Here, we examined an immunomodulatory effect on natural killer (NK) activity of lymphocyte. Pre-incubation of cells (5x106 cell/mL) for 1 h with 45 mg/mL LM-PLA2-I plus 60 mg/mL phosphatidylcholine (PC) stimulated the lymphocyte NK activity against [51Cr] K562 target cells, as 10 nM phorbol myristate, (PMA), the selective activator of protein kinase C (PKC) did. In both cases, this was accompanied by activation of transcription factor kB (NFkB), an increase in PKC activity and translocation of PKC from the cytoplasma into the plasma membrane. Similarly, the incubation with commercial lysophosphatidylcholine (lyso-PC) reproduced all these effects. Addition of stausrosporin and p-bromophenacyl bromide, inhibitors of PKC and LM-PLA2-I, respectively, abolished stimulation of all the activities. However, whereas PMA protected lymphocytes to enter in apoptosis, lyso-PC did not. Autophosphorylation of PKC isoforms indicated that lyso-PC stimulated the phosphorylation of PKC delta, differently of PMA. Taken together, these results strongly suggest that the enzymatic acitivity of phospholipase A2 present in *Lachesis muta* venom activates NK citotoxicity through a mechanism involving PKC pathway.

KEY WORDS: lysophosphatidylcholine; phospholipase A2; *Lachesis muta*; snake venom, lymphocytes, natural killer activity.

FINACIAL SUPPORT: CNPq, FAPERJ, FUJB, FAPERGS

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STRUCTURAL ANALYSIS OF A THEORETICAL MODEL OF *BjussuSP-I*, A SERINE PROTEASE FROM *Bothrops jararacussu* VENOM

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Snake venom serine proteases (SVSPs) are enzymes which affect the haemostatic system and belong to the trypsin family S1 (clan SA). Their actions on many components of the coagulation cascade and other systems and cells cause a disorder of the haemostatic system, contributing thus for the immobilization and death of the preys. A theoretical model of BjussuSP-I, a high-catalytic SVSP isolated from the *Bothrops jararacussu* venom, was built using threading techniques and submitted to a molecular dynamics (MD) simulation in order to improve the initial quality of the model and to obtain insights into the functional mechanisms of this molecule. The “open” disposition of 37, 60, 70, 99, 148, 174, and 218-loops in the theoretical model could explain part of the high proteolytic activity of BjussuSP-I since such conformation seems favoring the contact of the substrates with the catalytic site triad formed by the residues His57, Asp102, and Ser195. In addition, the good quality of the final theoretical BjussuSP-I model obtained after the MD simulation shows the potential importance of the acquired information to the understanding of the features relative to BjussuSP-I and other serine proteases.

KEY WORDS: BjussuSP-I, serine protease, molecular modeling, molecular dynamics simulation, snake venom, *Bothrops jararacussu*.

FINANCIAL SUPPORT: FAPESP, CAPES, CNPq, and FUNDUNESP.

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INHIBITORY PROFILE OF ANTI-MYOTOXIC FACTOR (BaMIP) OF *Bothrops asper* (SERPENTES: VIPERIDAE)

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In this work, the inhibitory profile of anti-myotoxic factor (BaMIP) derived from the blood of the snake *Bothrops asper*, known to neutralize the basic PLA2s myotoxins from its own venom, was determined. Neutralization of the enzymatic activity of PLA2s from different structural groups (I, II, and III) was determined using two different methods. Furthermore, in order to establish the pharmacological potential of this inhibitor against the toxic properties of PLA2s from different venom sources, panels of neutralization studies for the following toxic properties were performed: myotoxic activity, anticoagulant effect, edema and intraventricular lethality.Results indicate that BaMIP is highly specific for PLA2s towards group II and inhibits most of its PLA2s activity. On the other hand, its inhibitory effect against group I and III PLA2s is minimal, which correlates with the results of enzymatic activity. It was also determined that BaMIP particularly inhibits the toxic properties of group II PLA2s, but not that of group I and III PLA2s, which also correlate with the enzymatic results. The myotoxic effect as well as the edematogenic, anticoagulant, and lethal properties were all neutralized in group II PLA2s.

KEY WORDS: snake venom, phospholipases A2, inhibition, *Bothrops asper*, natural antitoxic factor, anti-myotoxic factor, BaMIP.

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THE VENOM OF *Bothrops asper* FROM PANAMA: BIOCHEMICAL CHARACTERIZATION AND TOXIC ACTIVITIES

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Bothrops asper is responsible for approximately half of the snakebite envenomations in Central America. Despite its medical relevance, only the venom of Costa Rican and Guatemala populations of this species has been studied to some detail, and there is very little information on intraspecies variability in venom composition and toxicity. Venom of *Bothrops asper* from Panama was analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis and isoelectric focusing, and its basic pharmacological activities were investigated with standard laboratory assays.Electrophoretic profile of *Bothrops asper* venom from Panama in SDS-PAGE showed the presence of several protein bands ranging from 16 to 90 kDa. The isoelectric focusing evidenced pattern of protein spots, ranging from acidic to highly basic components. The venom of *Bothrops asper* from Panama has lethal (LD50 ~ 55 mg/mouse, by the intraperitoneal route), hemorrhagic (Minimum Hemorrhagic Dose: 6,3 ± 3,6 mm), myotoxic (Minimum Myotoxic Dose: 10±1 mg), edema-forming (Minimum Edema-forming Dose:1±0,1 mg), coagulant (Minimum Coagulant Dose: 3,5±0,05 mg), fibrinolytic (Minimum Fibrinolytic Dose: 0,5 mg/mL), and phospholipase A2 (27,2 ± 0,45 mEq/mg.min) activities showing a similar toxicological profile to the previously described for *Bothrops asper* from Costa Rica and Guatemala.

KEY WORDS: snake venom, snake, *Bothrops asper*, Panama, biochemical characterization, toxic activities.

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A RETROSPECTIVE EPIDEMIOLOGICAL STUDY OF HUMAN ENVENOMATIONS DUE TO SNAKEBITES IN STATE OF CEARA, BRAZIL

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A thousand thirth-six snakebites on human patientes in Ceara State were documented retrospectively from January 1999 to July 2006. The majority was caused by non-venomous snakes (80%) and just 20% were attributed to venomous snakes. These latter are discussed here. Two hundred seven patients bitten by venomous snakes were studied. The majority of these patients was rural workers (83%), males (77.7%), with ages between 26 to 60 (42.7%). A hundred fifty-eight (76.3%) of the patients were diagnosed as having been bitten by snakes of the genus *Bothrops*, 25 (12%) by *Crotalus* and 21 (10.1%) by *Micrurus*. The main site of bite was the inferior members (feet, 64.0%, legs, 7.4%). Hands (14.8%), fingers (9.8%) and face (1.0%) also were found. When the time to search of treatment was analyzed, we found just 8.8% into the first hour after accident and 53.2% between 5-24h after envenomation. Clinical manifestations analysis showed some similarity with literature to bothropic accidents (edema, 36.6%; local pain, 23.4%), but to crotalic accidents we could observe local edema on 14.2% of the patients; mialgy was not related by the patients. Elapidic accidents presented local pain (24%) and edema (12.0%), “visual alterations” (12.0%) and cephaleia (16%). No death were notified at this period. May to August were the period of higher incidence of bothropic envenomations, whereas crotalic envenomations were more prevalent on February to April. Elapidic accidents were more common in July and August, in spite of some isolated occurrences in other months. This study showed that bothropic envenomations is more prevalent in Ceara state, and crotalic and elapidic envenomations possess some “regional” variations, perhaps being a consequence of tipical venom composition of northeast snake species. Further epidemiological studies must be developed in order to better understanding these results.

KEY WORDS: human envenomations, snakebites

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CLONING AND SEQUENCE ANALYSIS OF GYROXIN-LYKE FROM *Crotalus durissus terrificus* VENOM GLAND

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Gyroxin is an important toxin from *C. d. terrificus* venom however it is only partially characterized until now; it is a multifunctional toxin with thrombin-like activity and induces the barril rotation syndrome. A venom gland cDNA library from a single *C. d. terrificus* specimen was amplified with specific primers. The PCR product was cloned and sequenced. Gyroxin was synthesized as a prozymogen which 18 amino acids as a signal peptide, a propeptide of 6 and a matured protein with 238 amino acids. A clone with 895 bp length composed by a mature toxin-coding region of 717 bp and a 3'UTR of 178 bp length with a polyadenilation signal and poliA(+) tail. The predicted primary sequence of matured peptide displayed a relatively high amino acid homology to others members of the snake venom serine protease family (SVSPs) such as 92% similarity with crotalase and 82% with gyroxin analog (*Lachesis muta*). It contains 12 conserved cysteins, which form 6 SS bounds by similarity. A cluster analysis of an alignment with 32 typical SVSPs (including the gyroxin-like clone) generated a functional dendrogram organized in three major clusters: one with thrombin-like activity, other with kininogenase and the third one with plasminogen activators. The presumed sequence of mature gyroxin is close related to toxins from thrombin-like group. A three-dimensional model of gyroxin was built by homology modeling using TSV-PA (1BKY) crystal structure and AAV-SP-I (1OP0) and AAV-SP-II (1OP2) as template. Gyroxin model is useful to compare with thrombin and other SVSPs. The gyroxin cDNA sequence coding to mature toxin has been cloning into a dicistronic vector pED-gyroxin and expressing by mammalian cells CHO.DHFR-. Almost three others serine proteases are cloned and it will be analyzed.

KEY WORDS: gyroxin, *Crotalus*, serine protease, cloning, snake venom

FINANCIAL SUPPORT: FAPESP, CNPq

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OPTIMIZATION OF PURIFICATION AND ADDITIONAL CHARACTERIZATION OF DISBA-01, A RECOMBINANT RGD-DISINTEGRIN FROM *Bothrops alternatus*

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Disintegrins are a family of low molecular weight, RGD-containing peptides that bind specifically to integrins αIIbβ3, α5β1, and αvβ3 expressed on platelets and other cells including some tumor cells. DisBa-01 is a recombinant RGD-disintegrin, which interacts with αIIbβ3 integrin, inhibiting platelet aggregation and proliferation of endothelial and tumor cells. In this work, we have optimized the purification and further characterized the recombinant disintegrin structure through N-terminal sequencing and mass spectrometry. DisBa-01 was found in inclusion bodies and the solubilized toxin was firstly submitted to Ni-affinity chromatography in denaturating conditions. Next, DisBa-01 was submitted to ion exchange chromatography. The purified protein had its N-Terminal portion sequenced and its molecular mass determined by mass spectrometry. In addition, the polyhistidine tag was removed with thrombin. Polyclonal antibodies against DisBa-01 have also been produced in mice as a tool to localize RGD-disintegrins in crude venom. The use of the ion exchange chromatography as second step for DisBa-01 purification increased the purity degree and the protein yield. DisBa-01 had its 20 N-terminal residues sequenced confirming the predicted sequence. The mass spectrometry assays confirmed the protein mass as 11.658 Da. Thrombin cleavage was efficient and the polyclonal antibodies have shown to be highly specific to the protein with low cross reactivity. These results provided significant additional information for a better characterization of the recombinant RGD-disintegrin, which could be useful for the study of RGD-disintegrins and integrin interactions.

KEY WORDS: RGD-disintegrin recombinant, *Bothrops alternatus*, purification, N-terminal sequence, mass spectrometry.

FINANCIAL SUPPORT: CNPq

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FUNCTIONAL CHARACTERIZATION OF AN L-AMINO ACID OXIDASE ISOLATED FROM *Bothrops jararaca* SNAKE VENOM

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The L-amino acid oxidases (LAAO) are flavoenzymes found in different organisms, including snake venoms. In the present work, a BjarLAAO-I from *B. jararaca* venom was isolated and functional characterized. The *in vitro* cytotoxic effect of the enzyme was evaluated on the tumoral lines B16F10 and JURKAT, whereas, the inhibition of the Ehrlich ascites tumor (EAT) growth *in vivo* was analyzed, through the total and distinguishing number of cells and the spreading percentage and spontaneous release of hydrogen peroxide from peritoneal macrophages. The antiviral activity was analyzed through the inhibition of DENV-3 infecting C6/36 cells (Aedes albopictus). The bactericidal activity was accomplished in plates by the colony counting method, using of *E. coli* and *S. aureus*. The purified BjarLAAO-I presented a molecular weight of 60,000 (monomer) and about 130,000 (dimer) in reduced and non-reduced conditions, respectively. The BjarLAAO-I is an acid glycoprotein, pI~5.0 and N-terminal sequence showing close structural homology with other snake venom LAAOs. The enzyme revealed itself as a powerful antitumoral agent in vitro. BjarLAAO-I significantly inhibited EAT growth and increased initial influx of polymorphonuclear cells and the H_2O_2 spontaneous release from peritoneal macrophages. Cells infected with DENV-3 treated with BjarLAAO-I showed a decrease of 100, 100, and 10 fold in viral title for viral dilutions of 1:10, 1:20, and 1:100, respectively, when compared to the cells infected without treatment. In the same way, BjarLAAO-I was efficient in inhibiting the growth of Gram-negative and positive bacteria.

KEY WORDS: *Bothrops jararaca*, snake venom, functional activity.

FINANCIAL SUPPORT: CAPES, CNPq and FAPESP.

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NEUROMUSCULAR ACTIVITY OF A PHOSPHOLIPASE A_2 FROM *Bothrops marajoensis* VENOM

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Most snakebites in Brazil are caused by the genus *Bothrops*. *Bothrops marajoensis* is found in the savannah of Marajó Island in the State of Pará, and in some regions of Amapá State, Brazil. Despite its abundance in this region, little is know about the venom of this species. In this work, we isolated a phospholipase A2 (PLA2) from this venom and examined its neuromuscular activity *in vitro*. Fractionation of the venom by molecular exclusion and reverse-phase HPLC yielded 18 peaks, one of which, Bmaj-9, was pure and had a molecular mass of 13.8 kDa by SDS-PAGE that agreed with the amino acid analysis (13.6 kDa). Bmaj-9 contained 124 amino acids, with Arg + Lys accounting for 17% of the residues. The PLA_2 activity of Bmaj-9 was 7.9+0.5 nmoles/min compared to 2.3+1.1 nmoles/min for the venom. The neuromuscular activity of Bmaj-9 was examined in indirectly stimulated chick biventer cervicis muscle preparations mounted in a 5 ml organ bath containing aerated (95% O2 and 5% CO2) Krebs solution at 37ºC. Bmaj-9 (5 and 10 µg/ml) produced concentration-dependent blockade with the time for 50% blockade being 52.4±7.4 min (n=7, mean+SEM) and 30.1±8 min (n=6), respectively. Bmaj-9 did not significantly affect the contractures to exogenously applied ACh (110 mM) or KCl (20 mM). The lack of effect on the contractures to exogenous ACh suggested that Bmaj-9 acted presynaptically. This toxin was apparently not myotoxic since contractures to KCl were unaffected.

KEY WORDS: *Bothrops marajoensis*, myotoxicity neurotoxicity, phospholipase A_2.

FINANCIAL SUPPORT: CNPq, FAEPEX/UNICAMP

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ISOLATION AND FUNCTIONAL CHARACTERIZATION OF A-BJUSSUMIP, A PHOSPHOLIPASE A_2 INHIBITOR PROTEIN FROM *Bothrops jararacussu* SNAKE PLASMA

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A-BjussuMIP which neutralizes the enzymatic, toxic and pharmacological activities of various basic and acidic phospholipases A2 from the venoms of *Bothrops* species, was isolated from *Bothrops jararacussu* snake plasma by affinity chromatography using immobilized bothropstoxin-I (Lys49-BthTX-I) on Sepharose gel. Analysis of this inhibitor by size exclusion chromatography on Sephacryl S-200 revealed an apparent Mr around 120,000. Circular dichroism of BjussuMIP were measured. Biochemical characterization of this myotoxin a-inhibitor protein showed it to be an oligomeric glycoprotein with a Mr ~ 24,500 for the monomeric subunit. a-BjussuMIP was stable in the pH range from 3.5 to 10.0, between 4°C and 80°C, even after deglycosylation. The role of the carbohydrate moiety was investigated and found not to affect the *in vitro* function of the inhibitor. a-BjussuMIP has a wide range of inhibitory properties against basic and acidic PLA2s from *Bothrops* venoms (anti-PLA2, antimyotoxic, anti-edema and anticytotoxic). However, the inhibitor showed a reduced ability to neutralize the biological activities of crotoxin B, the PLA2 homologue associated with crotapotin in crotoxin complex from *Crotalus durissus terrificus* snake venom. Although it is unclear why a-BjussuMIP displays such a diverse inhibitory profile for the group II PLA2, identification of natural antivenom factors could be useful for the study of the venom toxicity mechanism and provide an alternative for treatment of ophidian envenomation.

KEY WORDS: *Bothrops jararacussu*, Inhibition, Phospholipase A2, BjussuMIP and snake plasma.

FINANCIAL SUPPORT: FAPESP, CNPq.

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MAPPING OF cDNA ENCODING ALPHA PLA_2 INHIBITORS IN ORGANS AND TISSUES OF *Crotalus durissus terrificus* (THE SOUTH AMERICAN RATTLESNAKE)

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PLA_2 inhibitors (PLIs) are widely distributed in the blood plasma of several snake species. They have been divided into three structural classes (α, β and γ) whose members may, in some cases, occur simultaneously in a single snake species. The alpha-PLIs display motifs similar to the carbohydrate recognition domain (CRD) of C-type lectins. Several authors have reported the presence of cDNA encoding PLIs in the liver of the snakes. In the present study we investigated the presence of cDNA encoding α-PLIs in distinct organs and tissues of *Crotalus durissus terrificus*. Total RNA was extracted from distinct organs and tissues of adult rattlesnakes and submitted to RT-PCR in the presence of specific oligonucleotides for that class of inhibitors. Major fragments of about 415 bp, corresponding to the mature sequence of a reference α-PLI have been amplified from several tissues. These results indicate the expression of α-PLI homologs in other tissues besides liver, and suggest, perhaps, additional functions for these proteins in the snake organism. The amplicons obtained are currently being sequenced to allow further homology and molecular studies.

KEY WORDS: cDNA, PLA2 inhibitor, *Crotalus durissus terrificus*

FINANCIAL SUPPORT: Fapemig and CNPq

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EFFECTS OF *Bothrops asper* AND *Bothrops jararaca* VENOMS ON LEUKOCYTE MEMBRANE-BOUND AND SOLUBLE NEUTRAL AMINOPEPTIDASE APN/CD13 ACTIVITY

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Aminopeptidases (APs) constitute a family of enzymes relevant for cell adhesion and modulation of the activity of inflammatory mediators released from leukocytes (Lf). Previous results from our laboratory demonstrated that Lf are central components in the inflammation induced by *Bothrops* snake venom. The aim of this study was to evaluate the effects of *Bothrops asper* (BaV) and *Bothrops jararaca* (BjV) venoms on Lf membrane-bound (M) and soluble (S) fractions of APN/CD13 activity. Swiss male mice received intraperitoneal (i.p.) injection of BaV or BjV (5 mg/mL) or a sterile PBS (control). At selected time intervals, peritoneal Lf were collected and the activity of APN/CD13 were measured by fluorogenic assay using naphthylamide-derivative substrate. The results represented as UP/mg of protein showed that both venoms increased M-APN/CD13 activity 24 and 48 h after i.p. injection. BaV and BjV increased S-APN/CD13 activity, respectively 6 and 24 h after i.p. injection. In contrast BaV, decreased M-APN/CD13 activity 12 h after injection. These results demonstrated that BaV and BjV can modulate the activity of Lf APN/CD13 in M and S fractions and the components responsible for these effects may differ for both venoms.

KEY WORDS: *Bothrops asper*, *Bothrops jararaca*, leukocyte, APN/CD13 activity, inflammation.

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ARTICULAR INCAPACITATION INDUCED BY METALLOPROTEINASE BAP1, ISOLATED FROM *Bothrops asper* SNAKE VENOM (VBA)

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Limitation of movement secondary to joint hyperalgesia is a serious burden to patients presenting inflammatory arthropaties. Elevated levels of matrix metalloproteinases (MMPs) in the synovial fluids of patients with arthritis have been implicated with the joint tissue destruction, however the involvement of these enzymes in joint pain is still unknown. The ability of BaP1, a metalloproteinase isolated from VBa, with high structural homology with MMPs and ADAMS, to induce hyperalgesia after its injection into rat knee joints, and the participation of the inflammatory mediator PGE2 was evaluated. Wistar male rats were injected intraarticular (i.ar.) with BaP1 or Bovine serum albumin (BSA-control) (0.025–0.030mM). At selected time intervals after these injections, articular incapacitation was evaluated using the rat-knee joint incapacitation test, and PGE2 concentrations were measured in the synovial washes of animals by EIA. In addition, groups of animals were pretreated with indomethacin (4mg/Kg) or vehicle (control) before i.ar. injection of BaP1 or BSA, and articular incapacitation was evaluated. Results showed that injection of BaP1 induced a dose-independent incapacitation with marked increased of paw elevation time (PET) from 1 to 6h after its injection, with maximum from 2 up to 6h. High amounts of PGE2 were detected in synovial fluids, at all periods of time studied (from 30min to 6h). Treatment of animals with indomethacin inhibited BaP1-induced articular incapacitation, at 3h after its injection. The release of PGE2 was abrogated in animals treated with indomethacin. These results show that BaP1 induces joint hyperalgesia, and PGE2 production after injection into rat knee joints. PGE2 may contribute to this effect induced by BaP1 in these joints.

KEY WORDS: metalloproteinase BaP1, hyperalgesia, arthritis, prostaglandins.

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DISTRIBUTION OF THROMBIN-LIKE ACTIVITY OF VENOMS ON BRAZILIAN *Bothrops* AND *Crotalus* SNAKES

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Disturbances of blood coagulation are very common in human envenomation by viperidae, either through direct fibrinogen activation, fibrinogenolysis or activation of the endogenous coagulation cascade. Clinicians uses to interpret blood incoagulability as resultant from injection of a big amount of venom. Of course, this criterion assumes that quantitatively the venom activities that induces incoagulability are quite uniform from specimen to specimen in a genus. To investigate this hypothesis we started by quantifying in venoms of individual specimens, the ability of coagulating pure bovine fibrinogen in conditions of reaction rates limited by the enzymatic step. Measurements were made by continuously recording the spectrophotometric changes at 450 mμ after the addition of venom to 3 mg/ml fibrinogen in 40mM phosphate buffer, pH 7.4. A thromboproteasic unit was defined as the amount of venom that causes an increase of 0.1 UA450nm/min. Results (Utp/mg) are presented in the order: species, range, average ± variation coefficient and number of observations: *B. jararaca* –adults (175 – 2048, 1002 ± 44%, 26); *B. jararaca* – youngs (173 – 1904, 583 ± 43%, 21); *B. neuwiedii* (167 – 2243, 978 ± 60%, 26); *B. moojeni* (613 – 1070, 857 ± 24%, 11); *B. alternatus* (5 – 598, 256 ± 57%, 23); *B. jararacussu* (59 – 830, 256 ± 67%, 20); *B. atrox* (3014 – 4667, 2); B. leucurus (1571 – 2506, 2) Crotalus durissus – adults (118 – 371, 195 ± 40%, 19); *Crotalus durissus* – youngs (116 – 608, 321 ± 60%, 7). Considering the thromboproteasic activities and the average amount of venom obtained by extraction in each species (data from FUNED serpentarium) we conclude: The criterion may be usefull from envenomations caused by *B. alternatus, B. jararacussu* and *C. durissus*. However for *B. jararaca* and *B. neuwiedii*, the criterion seems not directly aplicable due to higher variability between specimens.

KEY WORDS: thrombin-like activity, *Bothrops* and *Crotalus* venoms

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EFFECT OF 2 COUMARINS ON THE BIOLOGICAL ACTIVITY OF LYS 49 PLA_2 FROM THE *Bothrops jararacussu* VENOM

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Myonecrosis, edema and other biological manifestations, are conspicuous effects of *Bothrops* snake venoms, some of them caused by phospholipases A2 (PLA2s). Bothropstoxin-I (BthTx-I) is a Lys49-PLA2 from the venom of *Bothrops jararacussu*. This toxin accounts for approximately 35% of whole dried venom and responsible for several pharmacological and biological activities and have marginal enzymatic activity upon artificial substrates, due to the replacement of aspartic acid 49 for lysine at position 49. Coumarin is a chemical compound found in many plants and synthesized in chemical laboratory. It has clinical value as the precursor for several experimental anticoagulants. PLA2 was treated with coumarins and the PLA2-coumarin complex was purified by reverse phase HPLC column (µ-Bondapack C18). Thus essentially we conduct all experiments with BthTx-I, BthTx-I treated with coumarins. In this study, we demonstrate that both two types of synthetic coumarins cause significantly reduction in the BthTX-I pharmacological activity. When BthTX-I was treated with coumarins, we observe a reduction in edema activity on the mouse paw and decreasing of CK levels in presence of treated BthTx-I. These results suggesting that the coumarins cause some irreversible change in the PLA2.

KEY WORDS: Coumarin, *Bothrops jararacussu*, Lys 49 PLA_2, BthTx-I, Edema and CK

FINANCIAL SUPPORT: CAPES, FAPESP

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PLATELET AGGREGATION BY BASIC PHOSPHOLIPASE A_2 (BthTx-I) FROM *Bothrops jararacussu* VENOM

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Bothropstoxin-I (BthTx-I) is a Lys49-PLA2 from the venom of *Bothrops jararacussu*, composed of a single polypeptide chain of 121 amino acid residues (M r=13,720), containing one methionine and 14 half-cystines. Although deprived of any detectable PLA2 activity, BthTX-I reveals a high degree of sequence homology with Asp49-PLA2s and with other Lys49-myotoxins. In this work we test the activity of Bthtx-I in the platelet aggregation using human washed platelets. BthTx-I showed a strong platelet aggregation at doses of 5 and 15 µg/ ml with 28% and 71% of aggregation after 3 minutes of experiment. Upon chemical modification with p-bromophenacyl bromide, BthTX-I lost platelet aggregation activity, showing that the structural integrity of the protein is important for activity.

KEY WORDS: Platelet Aggregation, Basic Phospholipase A2, *Bothrops jararacussu*, Bothropstoxin-I.

FINANCIAL SUPPORT: CAPES, FAPESP, CNPq

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STRUCTURAL STUDIES OF PRTX-I, A LYS49 PLA_2 COMPLEXED WITH p-BROMOPHENACYL BROMIDE

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Phospholipases A_2 are among the main components of *Bothrops* venoms responsible for disruption of cell membrane integrity via hydrolysis of its phospholipids, culminating with cell death. Lys49PLA2 are catalytically inactive on artificial substrates yet maintain cytolytic and myotoxic activities and retain the ability to disrupt the integrity of both plasma membranes and model lipid bilayers by a poorly understood Ca2+-independent mechanism. PrTX-I is a basic myotoxic Lys49-PLA2, purified from *Bothrops pirajai* venom which is promotes blockade of neuromuscular transmission. Alkylation of His48 residue (active site) with p-bromophenacyl bromide (BPB) reduced 47% and 15% of its myotoxic and edema inducing activity, respectively. The neuromuscular blockage was completely absent in BPB-treated PrTX-I and the lethality (LD50) was strongly reduced. These toxic activities reduction is not totally understand and the structure of this complex may contribute to this study. Here, we report the crystallization, X-ray diffraction data collection, structure elucidation and preliminary analysis studies of PrTX-I complexed with BPB (PrTX-I-BPB). Crystals were obtained by hanging-drop vapour-diffusion method at 291K after four weeks. X-ray diffraction data of a single PrTX-I-BPB crystal were collected using a Synchrotron Radiation Source (LNLS, Campinas, Brazil). Data were processed at 2.3Å resolution. The crystals belong to P21 space group with cell constants a=38.62, b=70.02, c=43.82Å and β=102.62°. The volume of the unit cell is compatible with a dimer (Vm=2.141Å3/Da, 42.6% solvent content) and there is a BPB ligand at His48 for each monomer. The crystal structure was solved by molecular replacement techniques. The structure refinement and modeling is underway.

KEY WORDS: Lys49-PLA2; myotoxin; crystal structure; *Bothrops pirajai*; PrTX-I; p-bromophenacyl bromide; PrTX-I-BPB.

FINANCIAL SUPPORT: FAPESP, CNPq, FUNDUNESP, LNLS.

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Bothrops pirajai VENOM INDUCES AUTOLOGOUS COMPLEMENT ACTIVATION BY REMOVING GLYCOPHORIN C FROM HUMAN ERYTHROCYTE CELL SURFACE

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Bites by *Bothrops* snakes can induce severe clinical symptoms, including dermonecrosis, thrombosis, haemolysis and persistent inflammation. In the present study we have investigated the action of venoms from 19 species of *Bothrops* genus on the complement system (C) and its regulators. Human erythrocytes (E) were incubated with samples of venoms from 19 species of *Bothrops* snakes for 30 min at 37°C. Control samples were incubated with veronal buffer saline (VBS2+). The cells were washed, resuspended to the original volume in VBS2+, analysed in a haemolysis assay and prepared for flow cytometry. From the 19 bothropic species tested only the venom from *B. pirajai* was able to render E susceptible to lysis by autologous C. To assess whether the increased susceptibility to human C was caused by interference of the venom toxins with membrane regulators of C, E were analysed for the expression of DAF, CR1, and CD59 by flow cytometry. No change in expression of any of the regulators was observed after incubation of E with the whole venom. Although DAF, CR1, and CD59 are powerful inhibitors of C-mediated lysis, the abundantly expressed, heavily glycosylated E-membrane proteins known as glycophorins also contribute substantially to C resistance. E, incubated with *B. pirajai* venom were analysed for the expression of glycophorins by flow cytometry. A large reduction in the binding of anti-GPC antibodies recognizing extracellular epitope close to the membrane was observed after treatment of E with venom. These data suggest that *B. pirajai* venom promotes autologous C activation on human erythrocytes by inducing removal of GPC from the cell surface.

KEY WORDS: *Bothrops pirajai*, venom, Complement activation, glycophorins, erythrocytes.

FINANCIAL SUPPORT: CAT-CEPID/FAPESP, CNPq.

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LLLT INCREASES NEOVASCULARIZATION OF MOUSE GASTROCNEMIUS INJECTED WITH *Bothrops moojeni* VENOM

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Snake venoms contain toxins that induce coagulation, platelet aggregation, and severe myonecrosis. Here we investigate if low power laser therapy (LLLT) is able to minimize the local myotoxic acute effects of *B. moojeni* venom in the skeletal muscle and enhance regeneration in late periods of envenoming. Mice gastrocnemius was injected with 40 µg/ml of venom (n = 90) or 0.9% saline solution (n = 30). From venom group, 30 were unirradiated (V group), 30 were irradiated with HeNe (632.8 nm) laser (VHN) and 30 irradiated with GaAs (904 nm) (VGA). Anesthetized mice were killed at 3, 12, 24 h, 3, 7 and 21 d post injection, with one, one, two, four, eight and 22 irradiation sessions (4 J/cm-2 incident energy/session) given to each period of time, respectively (n = 5/period). Saline group (S) was unirradiated. After, the muscles were dissected, processed and immunohistochemistry for Flt-1 receptor (VEGF-R1) was done on paraffin sections. Counting of labeled capillaries was done for evaluating angiogenesis. The results show that the venom decreased the mean number of capillaries in the affected area, and that LLLT increased their number. The laser irradiation had no significant therapeutic effect in the first 12 h p.i. (degenerative stage). HeNe irradiation was more effective to increase vascularization than GaAs, but the number of myotubes was higher at 7 and 21 d p.i. in the VGA group, as was better the cytoarchitecture of the tissue. VEGF was positive in neutrophils of VHN and V groups, but GaAs depressed its expression. VEGF was expressed in macrophages, tenocytes, satellite cells and in normal, myonecrotic, and regenerating fibers besides being positive for capillaries. We conclude that the LLLT's photostimulation can enhance recovery of snake venom affected muscle.

KEY WORDS: Angiogenesis, HeNe and GaAs laser, immunohistochemistry, VEGF

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A ROLE FOR ADENOSINE RECEPTORS IN RAT SKIN EDEMA INDUCED BY *Bothrops alternatus* SNAKE VENOM

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Adenosine(Ad) and related compounds may mediate some actions of snake venoms. In this work, we examined the involvement of adenosine receptors (A) in edema caused by *Bothrops alternatus* venom. Male Wistar rats (~250 g) were anesthetized with thiopental(2mg/kg,i.p.) and venom(1-100µg/site), Ad(1-100 nmol/site) and its analogs and antagonists were injected intradermally (100µl/site) in shaved dorsal skin. Edema was expressed as the volume of plasma extravasation using human 125I-albumin as a marker. Venom and Ad produced dose-dependent edema. Theophylline (non-selective A receptor antagonist, 25nmol/site) significantly inhibited edema caused by Ad (before: 145±20 vs. after: 15±9µl, $p<0.001$; mean+SEM) and venom(163±15 vs. 56±6µl, $p<0.01$) (n=5 each). DPCPX(selective A1 antagonist, 3µmol/kg, i.v.) significantly inhibited edema induced by Ad(78±16 vs. 26±4 µl), CPA(selective A1 agonist, 50nmol/site) (94±16vs. 42±7µl) and venom (137±12 vs. 66±13µl) (n=5, $p<0.001$ each). DMPX(selective A2B antagonist) significantly inhibited the edema induced by Ad(145±19 vs. 62±8µl), NECA (selective A2B agonist, 3 nmol/site) (154±22 vs. 65±14µl) and venom(164±25 vs. 62±8 µl) (n=5, $p<0.01$ each). CSC(selective A2A antagonist) significantly inhibited the edema induced by Ad (78±13 vs. 34±6 µl), NECA(92±9 vs. 36±7 µl) and venom (109±10 vs. 40±5µl) (n=5, $p<0.01$ each). MRS1523(selective A3 antagonist) significantly inhibited the edema caused by Ad(141±15vs.77±7µl), IB-MECA (selective A3 agonist, 30 nmol/site) (126±4 vs. 74±5µl) and venom(140±8 vs. 96±8 µl) (n=5, $p<0.001$ each). These data indicate that the activation of A1, A2A, A2B and A3 receptors is involved in *B. alternatus* venom-induced edema in rat dorsal skin.

KEY WORDS: adenosine, purinergic receptors, rat skin edema

FINANCIAL SUPPORT: CAPES, FAEPEX-UNICAMP

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PHARMACOLOGICAL CHARACTERIZATION BY RAT SKIN EDEMA CAUSED BY A SUGAR-BINDING LECTIN FROM *Bothrops jararacussu* SNAKE VENOM

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A sugar-binding lectin from *Bothrops jararacussu* snake venom induces mouse hind paw edema. In this work, we investigated the mediators involved in rat skin edema induced by this protein. Male Wistar rats (~250 g) were anesthetized (sodium pentobarbital, 50 mg/kg, i.p.) and the dorsal skin was shaved for the injection of test agents. Edema formation was expressed as the volume (µl) of protein extravasation using human 125I-albumin. Venom or lectin (1-100 µg/site, i.d.) was injected alone or after pretreating rats with drugs (total volume/site = 100 µl). Venom and lectin produced maximal edema at >30 µg/site and 100 µg/site, respectively (n=4 each). L-NAME(100 nmol/site) significantly inhibited ($p<0.05$, ANOVA and Bonferroni test) edema caused by venom (30 µg/site) (191±26 vs. 68±15 µl, mean±S.E.M.) and lectin (50 µg/site) (74±15 vs. 18±6 µl). Indomethacin(10 mg/kg, i.p.) also inhibited venom- (247±15 vs. 138±12 µl) and lectin- (102±5 vs. 49±3 µl, $p<0.05$) induced edema. Mepyramine(5 mg/kg, i.v.) inhibited edema caused by venom (181±14 vs. 74±5 µl, $p<0.001$) and lectin (64±8 vs. 31±6 µl, $p<0.05$), as did Cyproheptadine(2 mg/kg, i.p.) (211±29 vs. 97±11 µl, $p<0.05$, and 128±11 vs. 45±10 µl respectively). Hoe-140(0.6 mg/kg, i.v.) inhibited venom- (173±15 vs. 108±12 µl, $p<0.01$) and lectin- (64±8 vs. 38±6 µl, $p<0.05$) induced edema, whereas SR140333(1 nmol/site) inhibited edema caused by lectin (65±23 vs. 23±5 µl, $p<0.05$) but not venom (184±23 vs. 150±20 µl) (n=4 in all cases). The edema caused by *B. jararacussu* venom lectin is multifactorial, involving arachidonic acid metabolites, bradykinin, histamine, nitric oxide, and substance P.

KEY WORDS: arachidonic acid metabolites, histamine, lectin, nitric oxide, tachykinin.

FINANCIAL SUPPORT:CAPES, CNPq, FAPESP.

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INFLAMMATORY MEDIATORS INVOLVED IN PHOSPHOLIPASE A_2-INDUCED ACUTE PANCREATITIS

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Phospholipases A2 (PLA2) induce acute pancreatitis (AP) when injected into the common bile duct of rats*. We have investigated the inflammatory mediators involved in PLA2-induced AP. AP was induced by the injection of PLA2 from *Naja mocambica mocambica* venom (PLA2-Nmm; 300 mg/kg) into the common bile duct of Wistar rats (220-260g, n=5-8). Four h later, the pancreatic plasma protein extravasation (PPE), pancreatic and lung myeloperoxidase (MPO), serum amylase (sAM). The PLA2-Nmm induced a marked increase in PPE (65%), pancreatic MPO (309%), lung MPO (34%) and sAM (155%). Pretreatment with the B2 receptor antagonist Icatibant (100 nmol/kg) reduced significantly the PPE, pancreatic MPO, lung MPO and sAM. The neurokinin-1 (NK1) receptor antagonist SR140333 (120 nmol/kg) reduced the PPE and pancreatic MPO, but did not affect the lung MPO and sAM. The PAF receptor antagonist PCA4248 (5 mg/kg) decreased significantly the lung MPO, without causing any change in the other parameters. The non-selective NO inhibition with L-NAME (20 mg/kg) reduced significantly the PPE, but rather caused an increase in the pancreatic MPO. The lung MPO and sAM were not significantly affected by L-NAME. Aminoguanidine (inducible NO synthase inhibitor, 50 mg/kg) treatment had no effect in any parameter evaluetad. Indomethacin (cyclooxygenase (COX) inhibitor, 5mg/kg) only decreased significantly the lung MPO. In conclusion, AP induced by exogenous PLA2 involves the activation of B2 and NK1 receptors, endothelial NO synthase-derived NO and COX-2 metabolites in the pancreatic tissue. The remote lung injury seems to involve COX-1 metabolites and the activation of B2 and PAF receptors.

KEY WORDS: phospholipases A2, acute pancreatitis, inflammatory mediators

REFERENCE: *Camargo EA et al., Toxicon, 2005, 46;921-6

FINANCIAL SUPPORT: FAPESP

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PHOSPHOLIPASES A_2-INDUCED AMYLASE SECRETION OF ISOLATED PANCREATIC ACINI

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Phospholipases A2- (PLA2s) from snake venoms induce acute pancreatitis when injected into the common bile duct of rats, a process that is characterized by pancreatic and pulmonary inflammation and hiperamylasemia*. As the initial stimulus for acute pancreatitis is thought to be the premature activation of pancreatic enzymes, such as trypsin or amylase, in the pancreatic tissue, we have investigated the ability of PLA2 to cause amylase secretion in isolated pancreatic acini (PA), in order to clarify its role in acute pancreatitis. The pancreatic tissue was digested with collagenase and the resulting isolated PA were incubated (1 h, 37°C) with different PLA2s. The amylase secretion was assayed by commercial kit. PLA2 from Naja mocambique mocambique venom (high catalytic activity) induced a significant amylase secretion (7.1±0.3; 8.2±0.3; 15.2±1.7; 16.8±2.5%, for 0.01; 0.03; 0.1 and 1 mg/mL respectively) when compared with vehicle-treated group (4.9±0.4%). Piratoxin-I (Lys-49 PLA2-homologue from *B. pirajai* venom, devoid of catalytic activity) also increased amylase secretion, (8.2±1.6 %; for 1 mg/mL). Similarly, Bothropstoxin-II (Asp-49 PLA2-homologue from *B. jararacussu* venom, with moderate catalytic activity) enhanced amylase secretion (10.5±0.9%; for 1 mg/mL). At the concentration used above, none of PLA2s assayed induced cell death, as evaluated by the MTT assay. In conclusion, PLA2s induce amylase secretion from PA, a process that may depend on the catalytic activity of these enzymes.

REFERENCE: *Camargo EA et al., Toxicon, 2005, 46;921-6

KEY WORDS: phospholipases A2, catalytic activity, acute pancreatitis, amylase secretion.

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ANTI-VENOM POTENCIAL OF *Vernonia scorpioides* EXTRACT AGAINST *Bothrops jararaca* VENOM (BjV)

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Many works have been realized testing plants extracts as alternative methods for the neutralization of snakes envenenomation. The venom of the South American *Bothrops jararaca* cause edema, hemorrhage and alterations in blood coagulation. Antivenom is the usual treatment by envenenomations, however it is not efficient to neutralize the edema forming activity and cellular influx. In this work, we compared the capacity of the vegetal species *Vernonia scorpioides* (Asteraceae) extract against the ability of antivenom neutralization. Venom was obtained from adult specimens of *B. jararaca* maintained in the Serpentarium at UNIVAP and lyophilized. The paw edema was induced through a sub-plantar injection of BjV (0,3 µg/paw) in the right hindpaw and measured with a plethysmometer before and 30, 60 and 120 min after BjV injection. The induction of inflammatory reaction in the peritoneal cavity was induced by BjV (5 µg/mouse) and the inflammatory exudate was withdrawn after washing the peritoneal cavity for determine total cells counts in neubauer chamber and differential leukocyte counts were performed on stained Instant Prove cell smears. All animals was treated with V. scorpioides extract (0,45 g/kg) in the peritoneal cavity 30 min before the venom injection, immediately after induction and 30 min later for both methods utilized in this work. Results showed that the extract reduced the edema forming activity (50,9%) in the animals treated before the BjV ($p<0,05$) injection. The leukocyte influx induced by BjV was not significant when compared to the groups of animals treated with plant extract or the antivenom. In conclusion the extract of V. scorpioides was able to reduced the edema forming activity but do not neutralize the leukocyte influx induced by *B. jararaca* envenenomation.

KEY WORDS: *Bothrops jararaca*, *Vernonia scorpioides*, plant extract, inflammation, neutralization

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THE ROLE OF INDOMETHACIN ON RENAL EFFECTS INDUCED BY CROTOXIN FRACTION ISOLATED OF *Crotalus durissus collilineatus* VENOM

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Ophidian accidents caused by the subspecie *Crotalus durrissus collilineatus* are responsible for high morbity. Acute renal failure is a common complication observed in these accidents. Crotoxin, the main fraction of crotalic venom, has been reported as responsible for some of the nephrotoxic effects induced by the venom. The aim of this work was to study the alterations produced by blockage with indomethacin in perfused kidney from Wistar rats, perfused with crotoxin fraction of *C. d. collilineatus* venom. Isolated kidneys from Wistar rats weighing 240-300g were perfused with Krebs-Henseileit solution containing 6% of bovine serum albumin for 120 min. Crotoxin (CTX) was added after 30 min of the beginning of the experimental time. Indomethacin (INDO) was added to system 30 min before the crotoxin (1mg; n=6). The data were analyzed by ANOVA and Student's t-test ($p<0,05$). The results shown in the last 30min of experiment an increased in glomerular filtration rate of kidneys perfused with INDO followed by CTX administration (INDO+CTX(120)=0.76±0.2; CTX(120)=0.32±0.08; INDO(120)=0.38±0.04 mL.g -1.min -1) and an increase in urinary flow (INDO+CTX(120)= 0.3±0.07; CTX(120)=0.14±0.05; INDO(120)= 0.12±0.01 mL.g -1.min -1). Perfusion pressure and renal vascular resistance remained constant throughout the experiments. It was observed no effects in sodium and potassium tubular transport. The administration of indomethacin before the crotoxin in perfusion rat kidney altered the renal parameters of crotoxin fraction of *C. d. collilineatus venom*.

KEY WORDS: indomethacin, *C.d. collilineatus*, venom, renal effects.

FINANCIAL SUPPORT: CAPES, CNPq.

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CYTOTOXIC EFFECTS OF CROTOXIN ON MURINE MELANOMA CELLS

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Crotoxin is the most abundant and active toxin from the venom of the Brazilian rattlesnake *Crotalus durissus terrificus*. It has been described as a neurotoxin involved in pre-synaptic blockade of neuromuscular junctions. Some authors have also attributed to this toxin a direct cytotoxic effect, and have suggested its use as a therapeutical agent against tumoural cells. In the present work, we investigated the cytotoxicity of crotoxin on a murine melanoma cell line and on normal fibroblasts. Cells (5 x 105 /ml) were grown in 96 wells microplates. After 24 hours, the cells were incubated with increasing concentrations of crotoxin, ranging from 12.5 to 400 mg/ml, previously diluted in RPMI with 10% calf serum. After 48 hours, the toxin containing medium was replaced by MTT in saline phosphate buffer and incubated for 3 hours. The formazan crystals were then solubilized with DMSO and the absorbance was read at 540 nm. Our results indicate that crotoxin is toxic to both cell strains, however the tumour cells appear to be more sensitive than the fibroblasts. Indded, while 100 mg/ml of crotoxin were sufficient to abolish the cell viability of the melanoma cells, about 200 mg/ml were necessary to elicit the same effect on fibroblasts.

KEYWORDS: Crotoxin, melanoma, cytotoxicity.

FINANCIAL SUPPORT: PIBIC/CNPq

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CELL ADHESION ASSAYS BY A METALLOPROTEASE FROM SNAKE VENOM

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Leucurolysin-B (Leuc-B) is a P-III class snake venom metalloproteinase isolated from *Bothrops leucurus* (white tailed jararaca) venom. Leuc-B (55KDa) is a multidomain glycoprotein including N-terminal Zn2+- containing enzymatic domain, a disintegrin-like domain (ECD sequence instead of the typical RGD motif) and a cysteine-rich C-terminal domain (Sanchez et al., in preparation). The aim of this work was to evaluate the effect of Leuc-B on the adhesion of GP220 (human gastric carcinoma) cells to laminin, fibronectin, type I collagen and fibrinogen in a concentration dependent manner. At a concentration of 4.5µg/well, Leuc-B displayed 66% inhibition of cell adhesion to laminin and fibronectin while approximately 62% inhibition was observed against fibrinogen and type I collagen, respectively. In addition, cytotoxicity effects were observed with both samples, Leuc-B and crude venom. Our results suggest that the disintegrin-like domain of Leuc-B may be responsible for the ability to block GP220 cell adhesion.

KEY WORDS: metalloproteinase, *Bothrops leucurus*, cell adhesion, desintegrin

FINANCIAL SUPPORT: CNPq AND FAPEMIG

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Bothrops jararaca VENOM PROTEOME

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Mortality and morbidity data indicate that snake bite envenomation remains a public health problem in Brazil. Approximately 85% of reported envenomations are caused by bothropic species and most of these specifically by *Bothrops jararaca*. The determination of *B. jararaca* venom proteome could shed some light on its mechanism of action by unraveling previously unknown proteic components (toxins, enzymes, biologically active peptides) that could not be detected by traditional protein chemistry . As an aid to protein identification we have generated expressed sequence tags (ESTs) from a venomous gland cDNA library and made the data available as an EST sequence database. The elucidation of the proteome followed the use of 2D-PAGE to separate proteins and mass spectrometry analyses of tryptic hydrolysates of gel spots to identify these proteins. Venom separation in the first dimension of 2D-PAGE was performed in 3-10 NL and 4-7 pH ranges yielding 337 and 357 spots, respectively. Several spots have been identified and represent toxins already known to be present in this snake venom. *B. jararaca* venom was also submitted to size-exclusion chromatography and the three pools obtained were submitted to 2D-PAGE (pH 4-7) in order to determine this venom subproteomes. Results indicate enrichment in different regions of the gel (as compared to whole venom gel) as well as the appearance of unexpected low molecular weight components in the first eluting fractions (pool I), indicating the possible interaction of these molecules with high molecular weight venom components in their native state and/or artifactual data due to abnormal behavior (eg. interaction with column resin) during size exclusion chromatography. The use of proteomic techniques can be a very productive approach to snake venom studies, allowing better understanding of venom complexity and toxic properties and leading to more effective antivenom therapy in the near future.

KEY WORDS: *Bothrops jararaca*, venom, proteome.

SUPPORT: FAPERJ, CNPq and Fiocruz.

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COMPARATIVE STUDY OF CYTOTOXICITY BETWEEN PLA_2 D49 FROM THE *Crotalus durissus ruruima* AND *Crotalus durissus cumanensis* SNAKE VENOMS, IN CELLULAR CULTURE OF MYOBLASTS AND MYOTUBES (C2C12)

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A comparative study cytotoxicity, in cellular culture of myoblasts and myotubes (C2C12) was realized with the objective of establishing likeness and/or differences in relation to the cytotoxicity between PLA_2 D49 from the *Crotalus durissus ruruima* and *Crotalus durissus cumanensis* snake venoms.Variable amount of toxin (10, 20 and 40mg) were diluted in assay medium (Dulbecco's Modified Eagle's Medium supplemented with 1% fetal calf serum) and then added to the cells cultures growing in 96-well plates, in a total volume of 150 ml/well. Controls for 0 and 100% toxicity consisted of assay medium, and 0.1% Triton X-100 in assay medium, respectively. After 3 h of incubation at 37 °C, a supernatant aliquot was collected for the determination of lactic dehydrogenase (LDH; EC 1.1.1.27) activity released from damaged cells, using a colorimetric end-point assay (Sigma 500C). The experiments were carried out in triplicate.The PLA_2 of the *Crotalus durissus ruruima* not lised "in vitro" skeletal muscle myoblasts and myotubes with a dose of 40 mg/well (40 mg/150 ml), in contrast to the PLA_2 from *Crotalus durissus cumanensis* that showed a high cell lysis in myoblasts and myotubes, with the same dose, this suggests that the myotoxic action is support by the concept of catalytic-independent mechanisms exerted by this type of proteins (1).The PLA_2 display a more restricted cytotoxic profile on cells in culture, mainly affecting differentiated skeletal muscle myotubes, which are cytotoxic on most of differentiated myotubes that on myoblasts.

KEY WORDS: *Crotalus durissus ruruima*, *Crotalus durissus cumanensis*, PLA_2, Myoblasts, Myotubes.

1- Gutiérrez, J.M., and Ownby, C.L. Skeletal muscle degeneration induced by venom phospholipases A2: insights into the mechanisms of local and systemic myotoxicity. Toxicon ,2003, 42, 915–931.

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BIOCHEMICAL, PHARMACOLOGICAL AND STRUCTURAL CHARACTERIZATION OF A NEW PLA_2 FROM *Crotalus durissus ruruim*a SNAKE VENOM

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The objective was to purify and partially characterize a novel PLA_2 neurotoxin “in vitro” from *Crotalus durissus ruruima* snake venom. A new PLA_2 was purified from *Crotalus durissus ruruima* snake venom by molecular exclusion chromatography followed by analytical reverse phase HPLC. The PLA_2 (14.75 kDa by MALDI-TOF mass spectrometry). In this experiment the PLA_2 produced neuromuscular blockade in young chicken biventer cervicis nerve-muscle preparations in presence and absence of crotapotin, indicating that crotapotin was not essential for neuromuscular action in this preparation and its neurotoxic activity was reduced by the treatment of this protein, after acetylation of the Lysine basic amino acid residues and the treated PLA_2 with 4-bromophenacyl bromide (BPB) that also induced a significant reduction of the neuromuscular effects. In contrast, in mouse phrenic nerve-diaphragm preparations, the neuromuscular blockade produced by the same concentration of toxin was dependent of crotapotin. These results show that the biochemical and structural properties of the new PLA_2 from *Crotalus durissus ruruima* are similar to those of the crotalics PLA_2, but that the neurotoxicity and the requirement of crotapotin to form the crotoxin complex that varies according to the neuromuscular preparation.

KEY WORDS: *Crotalus durissus r*uruima, HPLC, PLA2, Crotapotin.

FINANCIAL SUPPORT: CAPES-CNPq, FAPESP.

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PURIFICATION AND PARTIAL CHARACTERIZATION OF A METALLOPROTEINASE FROM *Bothrops moojeni* (CAIÇACA) VENOM

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Snake venom glands are a rich source of bioactive molecules such as peptides, proteins and enzymes that show important pharmacological activity leading to in local and systemic effects as pain, edema, bleeding and muscle necrosis. Many of the components of bothropic venom are proteolytic enzymes. In the present work, a protease enzyme, here denoted Btha, was purified by DEAE Sephacel, Sephadex G-75 and Heparin-agarose column chromatography from the venom of *Bothrops moojeni*. The enzyme was purified to homogeneity as judged by its migration profile in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) stained with coomassie blue, and had a molecular mass of about 24,5 kDa. The hydrolytic activity on bovine fibrinogen and fibrin was evaluated by SDS-PAGE after incubating in (pH 4.0 – 10.0) buffer with 5 mg of Btha and 25 mL fibrinogen solution (3.0 mg/mL) or fibrin clot (10 units bovine thrombin in 50 mL fibrinogen solution 1.5 mg/mL) in different times (1 – 15 min) at 37 ºC. The enzyme cleaves the Aa-chain of fibrinogen first, followed by the Bb-chain, and shows no effects on g-chains. On fibrin, the enzyme hydrolyzed only the b-chain, leaving the a-chain and g-dimer apparently untouched. It was devoid of phospholipase A_2, hemorrhagic and thrombin-like activities. Unlike many venom enzymes, it is stable at pHs between 3 and 9 and resists heating at 70 °C for 15 min. The inhibitory effects of EDTA on the fibrinogenolytic activity suggest that Btha is a metalloproteinase and inhibition by β-mercaptoethanol revealed the important role of the disulfide bonds in the stabilization of the native structure. Aprotinin and benzamidine, specific serine proteinase inhibitor had no effect on Btha activity.

KEY WORDS: *Bothrops moojeni*, metalloproteinase, fibrinogenolytic activity

FINANCIAL SUPPORT: UFU

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PURIFICATION AND PARTIAL CHARACTERIZATION OF AN ACIDIC PHOSPHOLIPASE A_2 FROM *Bothrops moojeni* (CAIÇACA) VENOM

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Phospholipase A_2 (PLA2 EC 3.1.1.4), a major component of snake venom specifically catalyzes the hydrolysis of fatty acid ester bonds at position 2 of 1,2-diacyl-sn-3-phosphoglycerides in presence of calcium. In addition to the digestion of prey, PLA2 exhibit wide varieties of pharmacological effects such as neurotoxicity, cardiotoxicity, myotoxicity, necrotic, anticoagulant, hypotensive, hemolytic, haemorrhage and edema etc. In this work, an acid phospholipase A2 was purified from venom of the snake *Bothrops moojeni* by a combination of gel ion exchange on DEAE Sephacel, gel filtration on Sephadex-G75 and Phenyl Sepharose CL-4B chromatography. sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) stained with coomassie blue in the presence of β-mercaptoethanol showed that the enzyme is a single chain polypeptide with a Mw = 15.5 kDa. The PLA2 activity was determined by potentiometric titration using an egg yolk emulsion, which contained phosphatidylcholine, as a substrate. Specific phospholipase activity of the purified enzyme was 1,316 mEq/min/mg. The indirect hemolytic activity was assayed in agarose gel, using egg yolk emulsion and erythrocytes human as substrate. After incubated for 20 hours at 37°C, increasing concentrations of enzyme (2.5, 5, 10 and 25 mg) diffused into the gel and cleared the erythrocyte by haemolysis, forming a halo of 1.76, 2.06, 2.08 and 2.16 cm of diameter, respectively. In conclusion, we purified a potent acid phospholipase A2 from venom of the snake *Bothrops moojeni*.

KEY WORDS: *Bothrops moojeni*, phospholipase A_2

FINANCIAL SUPPORT: UFU

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PARTIAL BIOCHEMICAL CHARACTERIZATION OF Bp-13 FROM *Bothrops pauloensis* SNAKE VENOM AND ITS NEUROTOXICITY IN MOUSE PHRENIC NERVE-DIAPHRAGM PREPARATIONS

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Bothrops pauloensis occurs in the center and southwest of São Paulo state, Brazil. In this work, we describe the partial biochemical and pharmacological characterization of Bp-13 from this venom. The fractionation of *Bothrops pauloensis* venom by reverse phase HPLC yielded 18 peaks (Bp-1 to Bp-18), one of which, Bp-13, was pure. Bp-13 was a basic, 14 kDa (by SDS-PAGE) polypeptide with phospholipase activity. The neuromuscular activity of Bp-13 was tested in indirectly stimulated mouse phrenic nerve-diaphragm preparations incubated in aerated (95% O2 and 5% CO2) Tyrode solution for 120 min at 37ºC. Bp-13 (10-100 mg/ml) produced irreversible blockade, with the time for 50% blockade at concentrations of 50 and 100 mg/ml were 28±3 min (n=8) and 18±1 min (n=3), respectively. Lower concentrations (20 and 10 mg/ml) produced 50% and 35% blockade, respectively, after 120 min. These results suggest that Bp-13 is a PLA2 protein that can produce neuromuscular blockade in mouse phrenic nerve-diaphragm preparations. The contribution of this toxin to the effects of envenomation by *B. pauloensis* remains to be determined.

KEY WORDS: *Bothrops pauloensis*, neuromuscular blockade, neurotransmission

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PRE-SYNAPTIC NEUROTOXICITY IN THE AUTONOMIC NERVOUS SYSTEM OF *Bothrops marajoensis* VENOM

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Bothrops is a genus of highly venomous pitvipers found in Central America, South America and the Caribbean. *Bothrops marajoensis* is a snake found in “Marajó” island located in North Brazil. The present study was undertaken in order to study the effects of the crude venom of *Bothrops marajoensis* in the noradrenergic-peptidergic neurotransmission in the mouse vas deferens. *Vas deferens* was mounted in 5ml organ baths for isometric recordings added cumulatively in the bath (0.01 to 30µg/ml) and the effects of each recorded during 3min.In other set of experiments nalaxone (10µM) on yohinbine (10µM) were added to the bath at the peak of the effects elicited by *Bothrops marajoensis*. In order to test whether the effects were post-synaptic *Bothrops marajoensis* as probed against Noradrenaline (10µM), ATP (30 µM) or carbacol (cch) evoked contraction. *Bothrops marajoensis* induced a dose-related inhibition of neurogenic contarctions in with in the mouse *vas deferens* a maximal response attained at 80.6+- 92%. this effect was neither reserved by naloxone, by yohimbine.in the other set of experimentes the additions of Bmwv inhibited neurogenic contractions by 68+- 8% compared with no significant decrease in carbacol, Noradrenaline or ATP evoked contraction (in normal Krebs or with guanetidine 10µM and phentolamine 100µM. This work is the first demonstration of presynaptic inhibition of autonomic neurotransmission by a bothrops snake venom. This effect is neither dependent of opioid or a2 pre-sinaptic agonism and is probably dependent or a PLA_2 activity.

KEY WORDS: *Bothrops marajoensis*, mouse *vas deferens*, snake venom.

FINANCIAL SUPPORT: Capes, CNPq.

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FUNCTIONAL ALTERATIONS CAUSED BY THE VENOM OF *Crotalus durissus ruruima* AND ITS FRACTION, CROTOXIN AND PHOSPHOLIPASE A_2

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This snake is found in different parts of world, and is responsible for many clinical cases of envenoming. This study was designed do determine the effect of *Crotallus durissus ruruima* (Cdru) and its fraction, crotoxin (CTX) and phospholipase A_2 (FLA2) venom on spreading (SP), phagocytic (Phag) and fungicidal activity (FA) with *C. albicans* of peritoneal macrophage. Male wister mice (20-30g) were exsanguinated under ether anesthesia and injected crude venom Cdru (20mg/Kg), CTX (10mg/Kg), and PLA_2 (10mg/Kg) venom into the peritoneal cavity by two hours. The peritoneal cavity was washed with 3 mL PBS. After a massage of the abdominal wall, the peritoneal fluid was collected. Statistical analyses were performed by Student t-tests (*p £ 0,05).The following results were observed when we compared the different functional activities: SP: C- 39,8% the agreements with venom on Cdru -15,0%*, CTX – 16,0%* and FLA2 – 16,8%*; Phag: C (30'-39.2%, 60'-36.8, 90'-38.8%, 120'-36.8%) the agreements with venom Cdru (30'-29.8%*, 60'-29.8%*, 90'-15.0%*, 120'-15.0%), CTX (30'-27.2%, 60'-28.3%*, 90'-16.0%, 120'-16.0%*), FLA2 (30'-34.8%, 60'-35.5%, 90'-16.7%, 120'-16.7%) and FA - C (30'-34.7%, 60'-36.2%, 90'-34.7%, 120'-34.5%) the agreements with venom Cdru (30'-36.1%, 60'-37.6%, 90'-35.7%, 120'-34.2%), CTX (30'-16.0%*, 60'-15.2%*, 90'-16.7%*, 120'-16.7%*) and FLA2 (30'-23.3%, 60'-22.2%, 90'-20.8%, 120'-20.8%). The present results suggest that CTX causes a direct inhibition of macrophage spreading and phagocytic activities and may contribute to the inhibitory effect of crotalid venom on macrophage function.

KEY WORDS: spreading, phagocytic, fungicidal activity.

FINANCIAL SUPPORT: CNPq, FUNCAP.

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FUNCTIONAL ALTERATIONS CAUSED BY THE OF *C.d.cascavella* VENOMS OF CEARÁ AND MARANHÃO

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This study was designed do determine the effect of *Crotallus durissus cascavella* - Ceará (Cdc-Ce) and *Crotallus durissus cascavella* -Maranhão (Cdc-Ma) venom on spreading (SP), phagocytic (Phag) and fungicidal activity (FA) with *Candida albicans* of peritoneal macrophage. Male wister mice (20-32g) were exsanguinated under ether anesthesia and injected Cdc-Ce(120mg/Kg) and Cdc-Ma(50mg/Kg) venoms into the peritoneal cavity by two hours. The peritoneal cavity was washed with 3 mL PBS. After a massage of the abdominal wall, the peritoneal fluid was collected. Statistical analyses were performed by Student t-tests (*p £ 0,05). The following results were observed: SP: C- 39.8% the agreements with venom on Cdc-Ce-8.8%* and Cdc-Ma-18.8%*; Phag: C (30'-34.7%, 60'-39.2, 90'-34.8%,120'-36.8%) the agreements with venom Cdc-Ce (30'-29.3%*, 60'-28.2%*, 90'-13.7%*,120'-13.7%*) and Cdc-Ma (30' – 32.1%*, 60' – 32.8%*, 90' – 18.8%*,120' – 18.8%*) and FA - C (30'-34.7%, 60'-36.2%, 90'-34.7% and 120'-34.5%) the agreements with venom Cdc-Ce (30'-31.7%, 60'-32.%*, 90'-29.7%*,120'-31.8%*) and Cdc-Ma (30'-33.7%, 60' – 33.8%, 90'-32.5%,120'-33.5%). The venom reduced the Cdc-Ce (64.8%) e Cdc-Ma (52.7%). Activities of peritoneal macrophages the Cdc-Ce in the all study time, with relationship to the fungicidal activity there was just alteration in the time of 60, 90 and 120 minute. The present results suggest that Cdc-Ce causes a direct inhibition of macrophage spreading and phagocytic activities and may contribute to the inhibitory effect of crotalid venom on fungicidal activity, phagocytic.

KEY WORDS: spreading, phagocytic, fungicidal activity.

FINANCIAL SUPPORT: CNPq, FUNCAP.

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ASPECTS OF THE IMMUNE RESPONSE AGAINST TOXIC PROTEIN IRRADIATED WITH ^{60}Co GAMMA RAYS

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ABSTRACT: Ionizing radiation has been successfully employed to modify the immunological properties of biomolecules. Very promising results were obtained when crude animal venoms, as well as isolated toxins, were treated with ^{60}Co gamma rays, yielding toxoids with good immunogenicity. The obtention of modified antigens with lower toxicity and preserved or improved immunogenicity can be useful. Ionizing radiation has proven to be a powerful tool to attenuate snake venoms toxicity without affecting and even increasing their immunogenic properties. However, little is known about the modifications that irradiated molecules undergo and even less about the immunological response that such antigens elicit. In the present work, we investigated the immunological behavior of bothropstoxin-I, a K49 phospholipase, before and after irradiation. Structural modifications of the toxin were investigated by SDS-PAGE. Isogenic mice were immunized with either the native or the irradiated toxin. The circulating antibodies were isotyped and titrated by ELISA. According to our data, irradiation promoted structural modifications in the toxin, characterized by higher molecular weight forms of the protein (aggregates and oligomers). Our data indicate that irradiated toxins were immunogenic and the antibodies elicited by them were able to recognize the native toxin in ELISA. These results indicate that irradiation of toxic proteins can promote significant modifications in their structures, but still retain many of the original antigenic and immunological properties of native proteins. Also, our data indicate that the irradiated protein induced higher titers of IgG2a and IgG2b, suggesting that Th1 cells were predominantly involved in the immune response.

KEY WORDS: immunological, ionizing radiation, bothropstoxin-1.

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STRUCTURE ALTERATION AND IMMUNOLOGICAL PROPERTIES OF BOTHROPSTOXIN-I IRRADIATED WITH ^{60}Co GAMMA RAYS

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Promising results were obtained when crude animal venoms, as well as isolated toxins, were treated with gamma rays, yielding toxoids with good immunogenicity. These toxins, when submitted of gamma radiation, in aqueous solution, present structural modifications. This occurs due to reactions with radiolysis products of water. Some scavengers substances, remove selectively these products. However, little is known about the modifications that irradiated molecules undergo and even less about the immunological response that such antigens elicit. In the present work, we investigated the immunological behavior of bothropstoxin-I, before and after irradiation, with or without scavenger substances. Structural modifications of the toxin were investigated by SDS-PAGE. Isogenic mice were immunized with either the native or the irradiated toxin. The circulating antibodies were titrated by ELISA. The characterization of bothropstoxin-I, native or irradiated, and specific antibodies was performed by Western blot. According to our data, irradiation promoted structural modifications in the toxin, characterized by higher molecular weight forms of the protein (aggregates and oligomers). Irradiated toxins, alone or in presence of $NaNO_3$, were immunogenic and the antibodies elicited by them were able to recognize the native toxin. On the other hand, when the toxin was irradiated in presence of t-butanol, a discrete reduction in antibodies levels was observed, suggesting a role of hydroxil radicals in the modulation of immune response. Irradiated bothropstoxin-1 elicited antibodies responsive to both toxins forms, as demonstrated by Western blot. These results indicate that irradiation of toxic proteins can promote significant modifications in their structures, still retaining many of the original antigenic and immunological properties of their native counterparts.

KEY WORDS: Ionizing radiation, bothropstoxin-1, scavengers substances.

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