STUDIES ON THE CYTOTOXIC POTENTIAL OF THE CRUDE VENOM AND PHOSPHOLIPASE A2 OBTAINED FROM *Crotalus durissus cascavella*

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Envenoming by *Crotalus durissus cascavella* leads to systemic alteration, being responsible for the primary cause of death after snakebite. This study aims on the evaluation of the cytotoxic potential of the crude venom (CV) and phospholipase A_2 (PLA_2) obtained from *C. d. cascavella* towards several tumor cell lines and mouse erythrocytes. The cytotoxic activity was accessed on 4 human tumor cell lines: HL-60 (leukemia), MDA-MB435 (breast), HCT-8 (colon) and SF-295 (nervous system) and quantified colorimetrically by the MTT assay after 72 hours incubation. Membrane damage was assayed on mouse erythrocytes after 1, 2 and 4 hours incubation. PLA_2 showed a hemolytic activity in a time-depend manner, although lacking a cytotoxic activity against the tumor cell lines. CV was strongly cytotoxic with mean inhibitory concentrations of: 1.56; 3.01; 3.30; and 0.66µg/mL on HL-60; MDA-MB435; HCT-8; and SF-295, respectively. On the other hand, CV did not show any hemolytic activity on the tested concentrations. Further studies are necessary to elucidate the mode of action of CV, as well as detailed PLA_2 kinetic understandings on the hemolytic activity.

KEY WORDS: snake venom, *Crotalus durissus cascavella*, cytotoxicity, hemolytic activity.

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STUDY OF SYSTEMIC AND LOCAL ALTERATIONS INDUCED BY NEUWIEDASE, A METALLOPROTEINASE ISOLATED FROM *Bothrops neuwiedi pauloensis* SNAKE VENOM

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The Viperidae snake venoms contain a large variety of proteins affecting the hemostatic system and cause severe local damage characterized by an acute inflammatory reaction and myonecrosis. Disturbances hemostatic, myonecrosis and acute inflammatory reactions induced by neuwiedase were studied. Proteolytic activity upon fibrinogen was evaluated “in vitro” and “in vivo”. Neuwiedase degraded the Aa and Bb chains of fibrinogen, dose and time-dependents patterns. The plasma fibrinogen level and platelets number from mice treated after 3 and 6 hours with 0,6 LD_{50} of neuwiedase decreased signifcantly. No hemorrhage was observed when neuwiedase was injected in the mice gastrocnemius muscle, but there was evident myoedema, myonecrosis and inflammatory reaction characterized by the presence of a leukocyte infiltrate. Administration of neuwiedase caused a significant increase of cytokines IL-1b, IL-6, IL-8, in the mice footpad, after 3 and 6 hours. Therefore neuwiedase contributes to the effect on hemostatic processes and local tissue damage caused by snake venom, so the understanding of these mechanisms caused by neuwiedase could help to find better treatments to victims and development to pharmacology approachs.

KEY WORDS: local tissue damage, neuwiedase, snake venom

FINANCIAL SUPPORT: CNPq, UFU

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COMPARATIVE STUDY OF TOXIC AND NON-TOXIC ENZYMATIC ACTIVITIES FROM CROTALIC AND BOTHROPIC BRAZILIAN SNAKE VENOMS

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Snake venoms are a complex mixture of toxic enzymes such as phospholipases A_2, proteolytic enzymes and other important enzymes such as hyaluronidase The present work had as goal to characterize the biological and enzymatic activities of these toxic enzymes such as coagulant, hemorrhagic, phospholipase A_2 and indirect hemolytic activities, and non-toxic enzymes such as hyaluronidases presents in some snake venoms from Triângulo Mineiro and Alto Paranaíba. For toxic enzymatic activity it was studied *Bothrops moojeni*, *Bothrops pauloensis* and *Crotalus durissus* sp. crude venoms. *Crotalus durissus* sp. was the most coagulant one, with a minimal coagulant dose of 3.67 µg of crude venom, followed by *Bothrops moojeni* and *Bothrops pauloensis* with 4.92 and 14.42 of minimal coagulant dose, respectively. Bothropic snake venom showed a strong hemorrhage however crotalic snake venom did not showed hemorrhage. For phospholipe A_2 tests, *Bothrops moojeni* and *Bothrops pauloensis* showed a strong activity with 122 and 111 U/mg/mL of specific activity respectively and *Crotalus durissus* sp. showed a lower activity with 36 U/mg/mL of specific activity. The hemolytic assay confirmed the last test but with some variability in time-dependent behavior. For hyaluronidase assays by tubidimetric and zymogram methods, *Crotalus durissus colillineatus* was the most active one, followed by *Bothrops jararaca*, *Bothrops moojeni, Crotalus durissus terrificus, Bothrops alternatus* and *Bothrops paulensis* snake venoms*. Bothrops jararacussu* showed this activity, but in less intense way. This work interesting results above bothropic and crotalic toxic and non-toxic activity.

KEY WORDS: comparative study, bothropic snake venom, crotalic snake venom

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HETEROLOGOUS EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF THE FIRST TWO DOMAINS OF DM43, AN ANTIHAEMORRHAGIC PROTEIN FROM *Didelphis marsupialis*

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Several animals are resistant to snake venoms due to the presence of neutralizing factors in their blood. An antihaemorrhagic glycoprotein named DM43 was isolated from serum of the South American opossum *D. marsupialis*. It inhibits snake venom metalloproteinases through non covalent complex formation with these enzymes. In a previous work, we cloned the cDNA coding for DM43 and showed that its deduced amino acid sequence was homologous either to oprin (an antihaemorragic protein isolated from *D.virginiana* serum) and to alpha1B-glycoprotein, a human plasma protein of unknown function, member of the immunoglobulin supergene family, which could be the orthologous gene of DM43 from humans. In the present study, we have used specific oligonucleotides to clone the cDNAs coding for the first (D1) or the second (D2) domains of DM43 in the prokaryotic expression plasmid pET102D/TOPO (Invitrogen). The identities of the cloned cDNAs were confirmed by DNA sequencing. Attempts to clone the third domain were unsuccessfull. To increase the solubility, the recombinant eukaryotic proteins were expressed as a fusion protein of D1 (or D2) and thioredoxin in BL21 Star (DE3) competent E. coli cells. The recombinant proteins were isolated by HisTrap FF crude Kit (GE Healthcare) and their partial amino acid sequences were confirmed by mass spectrometry. Preliminary results indicate that both D1 or D2 are able to inhibit the fibrinogenolytic activity of snake venom metalloproteinases, meaning that these domains fullfill the minimum structural requirements enabling the inhibitor to function.

KEY WORDS: DM43, recombinat, metalloproteinase.

FINANCIAL SUPPORT: Fiocruz, CNPq, Faperj.

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USE OF THE ANTITOXIN DM43 AS A TOOL FOR THE ANALYSIS OF SNAKE VENOM SUBPROTEOMES

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Snake venoms are complex mixtures of proteins and peptides with different biological activities, many of them very toxic. Several animals, including the opossum *Didelphis marsupialis*, are resistant to snake venoms due to the presence of neutralizing factors in their blood. An antihaemorrhagic protein named DM43 was isolated from opossum serum. It inhibits snake venom metalloproteinases through non covalent complex formation with these enzymes. In this study, we have used DM43 and proteomic techniques to explore snake venom subproteomes. Several venoms were chromatographed through an affinity column containing immobilized DM43. Bound fractions were analyzed either by SDS-PAGE and/or 2D-PAGE, followed by identification by MALDI-TOF/TOF MS. Following this methodology, we could classify venoms from *B. alternatus, B. asper, B. atrox, B. insularis, B. jararaca, B. jararacussu, B. moojeni, B. neuwiedi, C. adamanteus, C. atrox, C. d. terrificus, Lachesis muta muta* and *Naja naja atra* according to their relative content of metalloproteinases. Venom fractions not bound to DM43 column were composed basically of serine proteinases, C-type lectins, L-amino acid oxidases, nerve growth factor, metalloproteinases and/or some unidentified spots. Studied venoms presented important proteic variability, with frequent detection of multiple forms of the same protein and several members of the same protein family. DM43 affinity chromatography associated with proteomic techniques showed to be a useful tool to study proteins from snake venoms.

KEY WORDS: *D.marsupialis*, DM43, snake venoms, proteome.

FINANCIAL SUPPORT: FAPERJ, CNPq, FIOCRUZ -PDTIS.

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EFFECTS OF ^{60}Co GAMMA RADIATION ON *Bothrops jararacussu* BOTHROPSTOXIN 1

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ABSTRACTS: Snake venom are a complex mixture of toxic proteins and enzymes. *Bothrops* snake venoms have a great number of enzymes, such as phospholipases A_2 and L-aminoacid oxydase. The main symptoms of bothropic accident are hemorrhagy, edema and local tissue damage. Bothropstoxin 1 is a phopholipase A_2-like, basic myotoxin from *Bothrops jararacussu*, with a 13721 Da molecular weight, the active form being an homodimer. Protein irradiation has been successfully employed to attenuate snake venoms and toxins, promoting the loss of their biological activities while preserving immunological properties. When proteins are irradiated in solution, the major effect can be ascribed to the action of water radiolysis products, with the predominance of hydroxyl radical and aqueous electron. The extension of radiation damage can be studied and modified through the addition of "scavengers", molecules that act selectively removing each reactive species during the irradiation process. The most frequently used substances are nitrate ions and alcohol. In the present work, we investigated the effects of the above radicals on the structure and biological activity of bothropstoxin 1. This toxin was purified from crude snake venom by cation exchange chromatography. The toxin (2mg/mL) was then irradiated with 2 kGy gamma radiation in the presence or absence of scavengers substances. The scavengers used were sodium nitrate (e- aq.) and t-butilyc alcohol (OH). After irradiation the samples were analyzed by UV spectra, SDS PAGE and toxicity assays. Our results indicate that the toxicity of the native toxin was not modified by previous incubation with any of the scavengers, while the irradiated one with or without scavengers did not present detectable toxicity. SDS PAGE indicated that in the presence of scavengers, no modification of the migration pattern was observed, while, when irradiated *in natura*, major molecular weight alterations were detected. The UV spectra indicate that both the presence of scavenger and the effects of radiation promote structural modifications on the molecule.

KEY WORDS: gamma radiation, structure alteration and Bothropstoxin 1

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EVALUATION OF PRIMARY AND TERTIARY STRUCTURE OF ^{60}Co IRRADIATED CROTAMINE, IN THE PRESENCE OR ABSENCE OF SCAVENGER SUBSTANCES

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Crotamine is a strongly basic 4,882 Da polypeptide, composed of 42 amino acids. This toxin produces skeletal muscle spasms leading to spastic paralysis of hind limbs in mice. Ionizing radiation, in aqueous solution, produces several highly reactive species. The most important are hydroxyl radical (OH·) and hydrated electron (e-aq.). Theses products interact with peptides and proteins causing several modifications such as fragmentation, aggregation, oxidation, amongst others. Some substances are used as scavengers for the selective removal of either (OH·) or (e-aq.), as for example t-butylic alcohol and nitrate ions. Crotamine was obtained by gel filtration followed by cation exchange chromatography. The scavengers used were sodium nitrate and t-butylic alcohol. Purified crotamine with or without scavengers was irradiated with 2,000 Gy using gamma rays emitted by a Gammacell 220 60 Co source and with a dose rate of 5,17 kGy/h. Following irradiation, the toxin was submitted to aminoacid analysis and solvent mediated fluorescence quenching. Our results indicate that irradiated crotamine suffered structural modifications with fluorophores being more exposed to the solvent. On the other hand, when the toxin was irradiated in the presence of t-butylic alcohol, the structural elements were preserved. In all cases, the primary structure did not present significative modifications. Concluding, irradiation of crotamine induces conformational changes with little or no effects on its primary structure. These results are in agreement with other works that showed that the loss of toxicity of irradiated toxins is due to conformational changes.

KEY WORDS: gamma radiation, crotamine, *Crotalus durissus terrificus*, structure alteration. .

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A C-TYPE LECTIN FROM THE VENOM OF *Bothrops jararacussu* (LACERDA,1884) (SERPENTES: VIPERIDAE) ADHERE TO EXTRACELLULAR MATRIX PROTEINS AND INDUCE THE ROLLING OF LEUKOCYTES

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The purification of the lectin of *B. jararacussu* venom was achieved using agarose-D-galactose affinity gel. Divalent cations were required for its activity, as their complete absence reduced hemagglutination. The lectin was more effective at neutral pH range with total loss of activity at pH below 4.0 and above 9.0. The agglutinating activity remained stable up to 60 min at 25º C, but was increased when the lectin was left for at least 15 min at 35°C. Adhesion assays to extracellular matrix glycoproteins showed that the biotinylated lectin (0.039 to 5.0 µg/100µl) was capable to bind in a dose-dependent way to fibronectin and vitronectin. The binding was partially inhibited in the presence of D-galactose. The potential for the lectin (1.25 to 10 mg/30 ml) in leukocyte rolling and adhesion to endothelial cells in living microvessels was investigated using the intravital microscopy and showed that the lectin induced the increase of rolling and adherent leukocytes in a dose-responsive way, acting directly on endothelial cells of post capillary venules creating an adhesive surface for rolling a great number of leukocytes.

KEY WORDS: Lectin; Fibronectin; Vitronectin ; *Bothrops*; extracellular matrix, rolling, migration of leukocytes.

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INVESTIGATION OF THE ACTIVITY OF LECTINS PURIFIED FROM THE *Bothrops jararacussu* VENOM

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The venom extracted from *Bothrops* genus snakes are complex proteic mixtures with toxic and enzimatic proprieties. The lectins represent a small plece of such comples and are presented by an heterogein group of glycoprotein with the capacity of hemagglutination and to bind with high specifity to D-galactose. The macrophages are cells with higth fagocityc capacity, and are intimaty related to immunological process. Evidences suggest the participation of lectin-carbohidrates interaction with differentiation to macrophages and these calls efective functions. The purification of the lectin of *B. jararacussu* venom was achieved using agarose-D-galactose affinity gel. The macrophages were collected from the peritoneal cavity of mice, after inoculating 10ml of steril PBS (pH 7,4), followed by a rigorous massage and aspiration. The aspired was centrifugated for 10 minutes, 2000rpm, and 4°C. The pellet was ressuspented in Hanks solution. To test the adhesion capacity, the lectin (0,12 to 90mg.ml-1) was imobilized in a 96 well plate, followed by the addition of cells ($1x10^6$cells.mL-1). The adhesion was measured by violet crystal coloration, detecting by absorbance of 550nm. To verify bind innibition by carbohydrates, differents concentration (0,156 to 100mM) of D-manose, D-glucose, D-galactose and D-lactose were inserted to the assay. In the adhesion assay was verified the capacity of the peritoneal machophages to bind to lectin isolated and in the carbohidrates assays it wass observed the innibition of bind lectin-macrophage by D-lactose and a discret increase of that bind by D-manose.

KEY WORDS: Lectin; *Bothrops*; macophages, bind, innibition.

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A DEOXYRIBONUCLEASE II FROM *Bothrops alternatus* SNAKE VENOM

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Snake venoms contain a variety of enzymes that degrade nucleic acids and their constituents. Acidic deoxyribonucleases (DNase II) have been implicated in DNA fragmentation during apoptosis in mammals. In this work, we describe the purification and characterization of DNase II from *Bothrops alternatus* snake venom. DNase II was purified from *B. alternatus* venom using a combination of ion exchange and gel filtration chromatographies and enzymatic activity towards salmon testes DNA was determined based on the increase in absorbance at 260 nm. The specific activity of the purified enzyme was 1.9x103 units/mg compared to 36.1 units/mg for venom (purification factor = 51.2), with a protein yield of 1.75%. SDS-PAGE showed a single band with a molecular mass of 26.4 kDa that was unaffected by dithiothreitol or β-mercaptoethanol. Immunoblotting with affinity-purified IgG from commercial bothropic antivenom also gave a single protein band with the same molecular mass. The isoelectric point determined by 2D-gel electrophoresis was ~5.0. DNase II cleaved double-stranded DNA, denatured DNA and circular DNA from the plasmids pGEM and pBR 322, but there was no degradation of RNA. The enzyme was active in the pH range of 4.5-5.5, with an optimum at 4.7; activity was lost at >50oC. Enzymatic activity was inhibited by aurintricarboxylic acid (25 μM), iodoacetamide (1 mM), DTT (1 mM) and Zn2+ (10 mM). *Bothrops alternatus venom* contains a DNase II that shares several characteristics with mammalian acidic DNases. This enzyme could contribute to DNA degradation and apoptosis following envenomation.

KEY WORDS: *Bothrops alternatus*, DNase II, purification, 2D-PAGE, snake venom

FINANCIAL SUPPORT: CNPq, FAPESP.

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COMPARISON BETWEEN THE EFFECT OF AN RGD-DISINTEGRIN AND AN ECD-DISINTEGRIN FROM *Bothrops alternatus* ON THE EXPRESSION OF VEGF RECEPTORS IN HUMAN ENDOTELIAL CELLS

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Disintegrins are small cysteine-rich proteins with an RGD motif isolated from viperidae snake venoms. DisBa-01, a recombinant RGD-disintegrin, inhibits human microvascular endothelial cells (HMEC-1) proliferation in vitro. On the other hand, Alternagin-C (ALT-C), an ECD-disintegrin, induces human umbilical vascular endothelial cell (HUVEC) and HMEC-1 proliferation by up-regulating vascular endothelial growth factor (VEGF) expression. VEGF is a critical regulator of angiogenesis and its effects are mediated by two receptors, VEGFR-1 and VEGFR-2. VEGFR-2 mediates the VEGF-dependent mitogenic effect, while VEGFR-1 is usually considered as a decoy receptor. In this work, we analyzed the effect of ALT-C and DisBa-01 on the expression of VEGFR-1 and -2 in HMEC-1 by real-time PCR. HMEC-1 culture was treated with soluble ALT-C or DisBa-01 (1, 10, 100nM) for 4, 24, and 48h. Real-time PCR was accomplished based on detection of SYBR® Green. All samples were analyzed in duplicate and gene expression was normalized to β-actin expression. The comparative expression level was calculated by DDCt method. DisBa-01 down-regulated VEGFR-1 and VEGFR-2 expression in almost all tested concentrations. Only after 4 h, DisBa-01 (1nM) up-regulated equally both receptors. These effects are opposite to those observed for ALT-C, which up-regulated VEGFR-2 expression in almost all tested conditions. However, the highest effect was observed with the lowest ALT-C concentration (1nM) after 48h, about 10 times. Regarding VEGFR-1 expression, mRNA level was decreased by 1nM ALT-C after 48h and up-regulated in all others tested conditions, but less than of VEGFR-2. These results can explain at least in part how DisBa-01 inhibits and ALT-C induces endothelial cells proliferation. Moreover, they show significant differences between an RGD-disintegrin and an ECD-disintegrin from the same snake.

KEY WORDS: disintegrins, endothelial cells, cell proliferation, VEGFR expression.

FINANCIAL SUPPORT: FAPESP, CNPq.CORRESPONDENCE TO: HELOÍSA S. SELISTRE-DE-ARAÚJO, + 55 1633518333; hsaraujo@power.ufscar.br

MOLECULAR CLONING OF DIPEPTIDYL-PEPTIDASE IV (CD26) FROM *Bothrops alternatus* (URUTU) SNAKE VENOM GLAND cDNA

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Dipeptidyl-peptidase IV (DPP IV or CD26) cleaves a variety of peptides and may have a role in venom-induced hypotension. In this work, we isolated and sequenced a cDNA encoding DPP IV from a cDNA library prepared from poly (A)+ RNA of the venom gland of the snake *Bothrops alternatus*. Total RNA was extracted from venom glands three days after venom milking. A cDNA library was prepared using standard procedures and the DPP IV gene was cloned from this library. Random positive clones were sequenced using a Perkin-Elmer ABI Prism Model 310 sequencer. An open reading frame (ORF) sequence of 2286 base pairs encoded a mature, 762-amino acid protein with a calculated molecular mass of 86.2 kDa and a theoretical pI of 6.11. Multiple sequence alignment using Clustal X and phylogenetic analysis showed that this protein grouped with other DPP IV from various vertebrate species and was essentially identical to DPP IV from Gloydius blomhoffi brevicaudus (NCBI no. AB158224/158225). Computer modeling showed that the protein contained numerous α-helix and β-sheet regions, with the N-terminal region being highly conserved, as in other DPP IV. The active site contained Serine630, which is characteristic of this group of enzymes. DPP IV from *B. alternatus* venom is a high molecular mass protein that shares similarities with other venom and non-venom DPP IV. This is the first characterization of a DPP IV from *Bothrops* snake venoms.

KEY WORDS: *Bothrops alternatus*, dipeptidyl-peptidase IV, peptidases, venom gland library.

FINANCIAL SUPPORT: CAPES, CNPq, FAPESP

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COMPARATIVE STUDY OF ANTINOCICEPTIVE ACTIVITIES PRESENT IN THE *Crotalus durissus collilineatus* WHOLE VENOMS WITH AND WITHOUT CROTAMINE

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Comparative study of peripheral and central antinociceptive activities present in the whole *Crotalus durissus collilineatus* venoms with (*Cdc*$^{(+)}$ venom) and without crotamine (*Cdc*$^{(-)}$ venom) was realized. Both venoms presented peripheral antinociceptive activity, since when they were administered at 40, 60 and 80 μg/kg doses by intraperitoneal (i.p.) route significantly diminished the contortion numbers in the acetic acid-induced writhing test. Furthermore, naloxone (5 mg/Kg, i.p.) significantly blocked the inhibition of the contortions, showing that opiod receptors are involved in the peripheral antinociceptive activity of these venoms. *Cdc*$^{(+)}$ and *Cdc*$^{(-)}$ venoms (40, 60 and 80 μg/kg, i.p.) didn't change the reaction times in the tail-flick test, showing that they have no spinal antinociceptive activity. However, while the *Cdc*$^{(+)}$ venom presented central antinociceptive activity, as demonstrated by the increasing of the reaction times in the hot-plate test (40, 60 and 80 μg/kg, i.p.), the *Cdc*$^{(-)}$ venom presented no central antinociceptive activity. These results indicate that, although crotamine may be involved in the analgesic effects of *Cdc*$^{(+)}$ venom, other unknown substances acting in opioid receptors may be responsible for the peripheral analgesic effect of *Cdc*$^{(-)}$ venom.

KEY WORDS: *Crotalus durissus collilineatus*, crotamine, antinociceptive activities

FINANCIAL SUPPORT: FUNCAP, CAPES, CNPq

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

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Muscular necrosis is a serious consequence of *Bothrops* snake bit that may lead to permanent loss of tissue or function and require amputation of the affected member. Myonecrosis may be due to an indirect action as consequence of vessel degeneration and ischemia caused by hemorrhagic metalloproteases or by direct effect of myotoxic enzyme on plasma membrane of muscle cells. In this work, a myotoxin, named BmTx, was purified from the venom of the snake *Bothrops moojeni* by DEAE Sephacel, Sephadex G-75 and Heparin-agarose column chromatography. 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 15.7 kDa. BmTx is devoid of hemorrhagic, phospholipase A2, defibrinogenating and blood-clotting activities. Myonecrosis was determined by injecting mice i.m. purified enzyme (1.0 μg/g body weight) by histopathological analysis of injected muscle sections which were appropriately fixed for light microscopic examination 24 hr after infection. Examination of the sections of skeletal muscle clearly demonstrates that enzyme causes myonecrosis. There was intracellular edema and leukocytes were present in the connective tissue after 24 hr. The inflammatory reaction was evident microscopically. The high presence of macrophages and polymorphonuclear leucocytes suggest that phagocytosis of cell debris already had initiated. Muscle cells from control mice injected with 0.85% NaCl were normal in appearance.

KEY WORDS: *Bothrops moojeni*, myotoxin

FINANCIAL SUPPORT: UFU

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ANIMAL VENOMS AS A RICH SOURCE OF POTENTIAL THERAPEUTIC AGENTS – THE CONTRIBUTION OF BRAZILIAN RESEARCH

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Half a century after the Brazilian researcher Rocha e Silva, during his studies on the effects of *Bothrops jararaca* venom, discovered bradykinin, a finding that later led his student, Sérgio Ferreira, to find bradykinin potentiating peptides (BPPs, from *B. jararaca* venom), the prototypes of captopril (the potent inhibitor of the angiotensin converting enzyme), a highly successful drug that originated other widely used hypotensive agents, as enalapril and lisinopril – BPPs are still being used as prototypes of new medicines for controlling high blood pressure, as evasin, a Brazilian registered patent. And Brazilian research has revealed a lot of new potential therapeutic agents in animals venoms and protected by Brazilian patents. For example: the studies on analgesic effect of *Crotalus durissus terrificus* venom that led to the identification of enpak (endogenous pain killer, CNF021.03), an analgesic agent more potent than morphine; the investigations on severe haemorrhagic syndrome induced by the contact with the bristles of *Lonomia obliqua* caterpillars revealed the presence of lopap, a prothrombin activating protease that has been suggested to be used as an anti-thrombotic agent, and several others potential anti-thrombotic agents, most of them from snake venoms. To review these and other discoveries in the field of animals venoms with medical applications or therapeutic prospects performed in Brazil, we conducted a search in PubMed and Scielo databases. Revelant findings were summarized and discussed. These review already allows us to establish that, in Toxinology, most of the important findings reported in scientific literature began with studies on crude venoms actions, a lot of them screening methods that revealed biological effects of interest. In fact, animal venoms are rich sources of toxins that can exhibit a range of biological activities as a high degree of target specificity. These facts point out the importance of research on crude animals venoms and their toxins for science and therapeutics progress.

KEY WORDS: drug design, literature review, Toxinology.

SUPPORTED BY: CNPQ

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ACTIVITY OF A METHANOLIC FRACTION FROM *Casearia sylvestris* Sw. AGAINST *Bothrops jararacussu* VENOM AND BOTHROPSTOXIN-I IN NERVE MUSCLE PREPARATIONS

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Bothrops jararacussu (Bjssu) snake venom and bothropstoxin-I (BthTX-I) are myotoxic and cause neuromuscular blockade *in vitro*. *Casearia sylvestris* Sw. is a plant used in folk medicine as antisnake. In this work, we examined the ability of a methanolic fraction (MF) of leaves from this plant to neutralize the myotoxicity and neurotoxicity of Bjssu and BthTX-I in mouse phrenic nerve-diaphragm preparations. A MF of C. sylvestris leaves was obtained by the Soxhlet procedure. Myographic and histological analyses were done in preparations incubated with Bjssu or BthTX-I (40 mg/ml), a mixture of Bjssu or BthTX-I + MF (0.2 mg/ml), MF alone (0.2 mg/ml) or Tyrode solution (control) for 120 min. Bjssu (n=7) and BthTX-I (n=8) caused total neuromuscular blockade, whereas in the Bjssu +MF and BthTX-I + MF groups (n=6) the responses were similar to those of the control (n=9) and MF alone (n=6) groups, i.e., no neuromuscular blockade. The percentage of damaged muscle fibers per group (n=3 each) was: control - 5.6±1.6%, MF alone - 8.6±1.4%, Bjssu - 40.9±2.8%, Bjssu + MF- 9.7±1.6%, BthTX-I - 44.1±3.6% and BthTX-I + MF - 8.9±2.0% ($p<0.05$ for treated vs. non-treated groups, Student´s t-test). These results show that the methanolic fraction of *C. sylvestris* prevented the neuromuscular blockade and myonecrosis caused by Bjssu and BthTX-I.

KEY WORDS: antisnake activity, *Bothrops jararacussu*, bothropstoxin-I, *Casearia sylvestris* Sw., neuromuscular preparations.

FINANCIAL SUPPORT: FAPESP, PROBIC/UNISO, FAEPEX/UNICAMP

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CARDIOVASCULAR ALTERATIONS INDUCED BY *Bothrops alternatus* SNAKE VENOM

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Bothrops snake venoms produce a variety of local and systemic effects. In this work, we investigated the cardiovascular alterations caused by *Bothrops alternatus* snake venom in dogs. Male mongrel dogs (15-20 kg) were sedated with sodium thiopentone (30 mg/kg, i.v.), anesthetized with isoflurane (2% in 98% air) and cannulated for the measurement of hemodynamic parameters on a Dixtal digital recording system. Cardiac output (CO) and derived parameters were determined by thermodilution using a Swan-Ganz catheter. Venom (300 µg/kg, i.v., in 1 mL) was injected via the left femoral vein and systemic blood pressure was recorded from the left femoral artery. Venom caused a significant ($p<0.05$) decrease in mean arterial blood pressure and CO after 5 min (from 132±18 to 39±8.4 mmHg and from 6.5±2.8 to 1.9±0.5 L/min, respectively; n=3 each, mean+S.D.). The blood pressure recovered over the following 30-40 min but did not reach prevenom levels; CO showed no recovery after venom. There were no significant changes in heart rate, systemic vascular resistance or pulmonary vascular resistance throughout the experiments. Plasma levels of lactate dehydrogenase and creatine kinase were increased after 2 min (from 132±34 to 283±103 IU/L and from 26±2.9 to 96±77.2 IU/L, respectively; n=3) but returned to basal thereafter. Lactate levels showed a slight increase after 2 min (from 0.6±0.1 to 2.7±1.6), with no change in glucose. There were no significant changes in the blood gas (pO_2 and pCO_2) levels and pH, or in the levels of Na+ and K+. These results indicate that the cardiovascular alterations produced by *B. alternatus* venom are essentially vascular, with few metabolic effects.

KEY WORDS: blood pressure, cardiac output, creatine kinase, dogs, hemodynamics, lactate, lactate dehydrogenase.

FINANCIAL SUPPORT: FAPESP.

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IDENTIFICATION OF PUTATIVE ANTIGENIC CANDIDATES TO AN ANTIELAPIDIC SERUM BASED ON THE ANALYSIS OF *Micrurus corallinus* TRANSCRIPTOME

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The transcriptomic characterization of venom glands has proved to be a fast and efficient way to describe the general composition of toxins from these animals, at least regarding the gene expression related to them. We have generated 1438 “Expressed Sequence Tags” (ESTs) from *Micrurus corallinus* venom gland, a snake from Elapidae Family, commonly found in tropical forest areas. The 1438 sequences were grouped in 611 clusters that were built in a pipeline of softwares in LINUX system, specially adjusted to the characteristics of a project of medium scale ESTs generation for venom gland projects. Among these clusters, we have obtained 7 putative types of toxins that had their sequences partial or totally described for the first time. Likewise the transcripts related to toxins, the transcripts related to celular proteins represent each around 46% in this databank. The general proportion of toxins include: three-finger proteins (24%), phospholipases A_2 (PLA_2s) (16%), lectin type C (5%), and others. The databank allowed not only the identification of putative toxins, but also celular transcripts, being the majority probably involved in physiologicals functions. The major part of these molecules shows an involvement in gene and protein expression reflecting the high especialization of the tissue to toxin synthesis. In conclusion, the transcriptomic databank helped an analysis of gene expression and it allowed the identification of probable vaccines candidates to a future recombinant antielapidic serum.

FINANCIAL SUPPORT: FAPESP

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VASCULAR PERMEABILITY INDUCES BY *Bothrops leucurus* VENOM (SERPENTES; VIPERIDAE)

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The *Bothrops leucurus* snake has large distribution in the State of Bahia, being responsible for the majority of registered cases at the Metropolitan Region of Salvador. It causes severe systemic and local reactions, characterized by an acute inflammatory reaction. The aim of this study was to investigate the pro-inflammatory effects of *Bothrops leucurus* venom (BLV). Vascular permeability was investigated 5,15, 30, 60, 120 and 240 minutes after intravascular injection of BLV (30 or 50µg/mice) by measuring extravasation of Evans blue dye (EB, 20mg/kg, i.v.) in the Swiss mice peritoneal cavity. To examine mast cell degranulation, mice were injected i.p. with *BLV* or sterile saline (control). After 15 min, the mesentery of anesthetized animals was excised. The mast cells were fixed and stained with Toluidine blue solution followed by mounting on glass slides. The percentage of degranulated cells was counted on a light microscope (250X). The response was maximal within 15 min disappearing over 120 min. The vascular permeabity response was not modified by pretreatment with prometazine (5 mg/kg, i.p.), a histamine H1 receptor antagonist and indomethacin (4 mg/kg, i.v.), an inhibitor of the cyclo-oxygenase pathway. Also, dexamethasone (2 mg/kg, i.v.) failed to affect the vascular permeability extravasation induced by BLV. The venom did not degranulate mast cells confirming that histamine was not important to this event. These data suggest that local inflammation induced by *Bothrops leucurus* venom is controlled by different pharmacological systems than eicosanoids and histamine.

KEY WORDS: *Bothrops leucurus*, venom, inflammation

FINANCIAL SUPPORT: FAPESB

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DETERMINATION OF PRIMARY STRUCTURE OF TWO ISOFORMS 6-1 AND 6-2 PLA_2 D49 FROM *Bothrops jararacussu* SNAKE VENOM AND NEUROTOXIC CHARACTERIZATION USING *IN VITRO* NEUROMUSCULAR PREPARATION

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The objective was to purify and characterize the amino acid sequence of two new isoforms basic 6-1 (Bj-IV) and 6-2 (Bj-V) PLA_2 D49 purified from the *Bothrops jararacussu* venom. The isoforms 6-1 and 6-2 had a sequence of amino acids of 121 amino acid residues 6-1: DLFEWGQMIL KETGKNPFPY YGAYGCYCGW GGRGKPKDKD TDRCCYVHDC CYKKLTGCPK TDDRYSYSWL DLTIVCGEDD PCKELCECDK AIAVCFRENL GTYNKKYRYH LKPCKKADKP C and *pI* value 7.83 and 6-2: DLWQFGQMIL KETGKIPFPY YGAYGCYCGW GGRGGKPKDG TDRCCYVHDC CYKKLTGCPK TDDRYSYSWL DLTIVCGEDD PCKELCECDK AIAVCFRENL GTYNKKYRYH LKPCKKADKP C with a *pI* value of 7.99 Skeletal muscle preparations from the young chicken have been used previously in order to study the effects of toxins on neuromuscular transmission, providing an important opportunity to study the differentiated behaviour of a toxin before more than one model, because it shows differences in its sensibilities. Both isoforms have produced neuromuscular blockade in young chicken biventer cervicis nerve–muscle preparations in presence or absence of crotapotin crotalic (F3 e F4) indicating that catalytic activity was not essential for neuromuscular action in this preparation.

KEY WORDS: *Bothrops jararacussu*, PLA_2 D49, neuromuscular action, sequence of amino acids.

FINANCIAL SUPPORT: CAPES-CNPq, FAPESP.

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CITOTOXIC ACTIVITY OF CROTAMINE FROM *Crotalus durissus terrificus* SNAKE VENOM ON SARCOMA 180 AND MACROPHAGES

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Crotamine is non enzymatic myotoxin isolated from *Crotalus durissus terrificus* venom. The aims of this work were to evaluate the action of crotamine on Sarcoma 180 (S180) cell line and macrophage. The assays showed that crotamine incubated separately in different concentrations with S180 or macrophage, reduced the cellular viability in a dose dependent manner. Crotamine (1mg/mL) showed of 75% on S180 and 20% on macrophages. These results demonstrate that crotamine possess hight cytotoxic activity on the cellular lines tested in vitro

KEY WORD: crotamine, cytotoxicity and tumor cells.

FINANCIAL SUPPORT: FAPEMIG, CNPq, CAPES

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CITOTOXIC ACTIVITY OF MODIFIED AND NATIVE BTHTX-I FROM *Bothrops jararacussu* SNAKE VENOM ON SARCOMA 180, MACROPHAGES AND BACTERIAS

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BthTX-I are non enzymatic myotoxin isolated from *Bothrops jararacussu* venom. The aims of this work were to evaluate the action of BthTX-I (native or modified) on cells lines and on the bacterial proliferation; The *in vitro* assays showed that native and modified BthTX-I incubated separately in different concentrations with sarcoma 180 (S180) and macrophages, reduced the cellular viability in a dose dependent manner. BthTX-I (1mg/mL) produces 90% in macrophages, and S180; while BthTX-I BPB (1mg/mL) showed 20-40% of cytotoxicity on both cellular lines. The bactericidal activity of BthTX-I (0, 8 mg /mL) against *E. coli* and *S. aureus* was of approximately 100%, while the chemical modification reduced this activity These results demonstrate that BthTX-I and crotamine possess hight cytotoxic activity on the cellular lines tested in vitro and the cytotoxicity of BthTX-I after the modification by BPB was lower suggesting that the His residue has an important role in the cytotoxicity;

KEY WORDS: BthTX-I, cytotoxicity and tumor cells.

FINANCIAL SUPPORT: FAPEMIG, CNPq, CAPES

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STRUCTURAL STUDIES WITH BTHTX-II, A MYOTOXIC ASP49 PLA_2 WITH LOW CATALYTIC ACTIVITY FROM *Bothrops jararacussu* VENOM

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Phospholipases A_2 are components of *Bothrops* venoms and consists of a broad range of enzymes that catalyze the hydrolysis of the center *sn-2* ester bond of substrate phospholipids. A complete X-ray diffraction data set has been collected from BthTX-II, a basic myotoxic Asp49-PLA_2 with low catalytic activity, purified from *B. jararacussu* snake venom. Crystals of BthTX-II were obtained by hanging-drop vapour-diffusion method with the protein solution equilibrated against a reservoir solution containing 0.1M sodium citrate pH 5.6, 20% 2-propanol and 13% (w/v) polyethylene glycol 4000, at 291K after two months. X-ray diffraction data of a single BthTX-II crystal were collected at a wavelength of 1.427Å using a Synchrotron Radiation Source (LNLS, Campinas, Brazil). Data were processed using denzo/scalepack program at 2.13Å of resolution. The crystals belong to C2 space group with cell constants a=58.9, b=98.5, c=46.7Å and beta=125.9°. The data are 96.1% complete with R_{merge}=9.1% and I/sigma= 3.7. The volume of the unit cell is compatible with a dimer (V=2.0 $Å^3$/Da, 37.4% solvent content). The crystal structure was determined using molecular replacement techniques implemented by AMoRe program with the atomic coordinates of the PrTX-III (Asp49PLA_2) from *Bothrops pirajai*. The structure refinement and modeling is underway. The quaternary structure of BthTX-II resembles the myotoxin Asp49-PLA PrTX-III and all non-catalytic and myotoxic dimeric Lys49-PLA_2.

KEY WORDS: PLA_2, snake venom, BthTX-II, structure.

FINANCIAL SUPPORT: FAPESP, CNPq, FUNDUNESP and LNLS.

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BpirLAAO-I: A NEW L- AMINO ACID OXIDASE ISOLATED FROM *Bothrops pirajai* SNAKE VENOM

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In this work we describe the isolation of a new L-amino acid oxidase (LAAO) referred to as BpirLAAO-I from *Bothrops pirajai* snake venom, which was highly purified using a combination of molecular exclusion, affinity and hydrophobic chromatography steps. BpirLAAO-I homodimeric acid glycoprotein (approximate *Mr* and *pI* of 130,000 and 4,9, respectively) displays high specificity toward hydrophobic/aromatic amino acids, while deglycosylation does not alter its enzymatic activity. The N-terminalLAAo sequence of its first 49 amino acids presented a high similarity between a amino acid sequence with other LAAOs from *Bothrops* spp., *Crotalus* spp., *Calloselasma rhodostoma* and others. BpirLAAO-I induces time-dependent platelet aggregation, mouse paw edema, cytotoxic activity against Escherichia coli, Pseudomonas aeruginosa and also a typical fago (M13mp18) DNA fragmentation. Thus, BpirLAAO-I is a multifunctional protein with promising biotechnological and medical applications.

KEY WORDS: Bactericidal and cytotoxic effects, *Bothrops pirajai*, L-amino acid oxidase, Platelet aggregation, snake venom, structural analysis.

FINANCIAL SUPPORT: CAPES, FAPEMIG AND CNPq

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TL-Bnp: A NEW THROMBIN-LIKE ENZYME FROM *Bothrops neuwiedi pauloensis* SNAKE VENOM

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Snake venom serine proteases comprise several peptide hydrolases wich have a very important role in the formation and dissolution of blood clots. These toxins, mainly thrombin-like enzymes that affecting haemostasis have greatly used for assays of fibrinogen or fibrinogen-breakdown products and detection of fibrinogen dysfunction. The present work reports the biochemical properties and enzymatic activities of TL-Bnp a new thrombin-like enzyme isolated from *Bothrops neuwiedi pauloensis* snake venom. TL-Bnp has been purified in our laboratory and purity degree was monitored by high-performance liquid chromatography (HPLC). The TL-Bnp showed a major band with molecular weight of 34KDa under reduced conditions and 30KDa in the absence (by SDS-PAGE). Moreover, data suggested that TL-Bnp is a glicoprotein with basic properties. Samples of 75μg of bovine fibrinogen (1mg/mL PBS) were incubated with TL-Bnp at different doses, times and inhibitors at 37°C, pH 7.4. These activities were analysed by SDS-PAGE. TL-Bnp displayed dose and time dependent activity upon fibrinogen whose A-α chain was preferentially degraded. This activity was inhibited by PMSF, thus suggesting the presence of serine residue at active site. Fibrinolytic activity showed that TL-Bnp wasn't able to degrade fibrin clots *"in vitro"* however was able to induce an anticoagulant effect when it was injected by i.p. route in mice. This serine peptidase shares common features with other thrombin-like enzymes of the *Bothrops* genus and are attractive as a model for drug development for the treatment of thrombosis.

KEY WORDS: snake, venom, *Bothrops neuwiedi pauloensis*, serine protease, thrombin-like enzyme and fibrinogen activities.

FINANCIAL SUPPORT: CNPq, UFU.

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MACROPHAGES ACTIVATION BY *Bothrops jararacussu* VENOM

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In Brazil, cancer is considered a problem of public health, being the second cause of death. Immunotherapy is one of the several types of treatment where the immunological system is stimulated by means of Biological Response Modifiers (BRMs). Evaluating a system without treatment, where the cells of the tumor proliferate faster than the destructive capacity of the immune system, presumes that the therapeutical use of immunoestimulant agents could contribute to diminish the tumoral growth. Thus, natural substances, as snakes venoms, have been evaluated as potential BRM, being able to be used in the immunotherapy. For that reason, this work had the objective to evaluate the activation of macrophages of mice by *Bothrops jararacussu* venom. For such, cells from mice peritoneal cavity were collected and added to wells of 96-well plates. Culture medium containing different concentrations of the venom (10, 1, 0.1, 0.01 e 0.001 μg/ml) was added to the cells and the cellular viability, the NO production and fagocitosis were evaluated in different times, in independent experiments. Cells survived in concentrations below 10 μg/ml, for 24 and 48h, and showed no viability in 72h. For that, following experiments were carried through those periods of time. There was no significant nitric oxide production for the macrophages incubated with the venom. However, concerning fagocitosis it was possible to observe a considerable increase for all the tested concentrations of the venom, mainly in 24h, in a dose-responsive way, promoting an increase of fagocitosis in ~600% (10μg/ml). These results demonstrate that the venom do activate macrophages *in vitro*, so it becomes interesting to carry out experiments *in vivo* to verify if it can act as BRM, being able to be manipulated and to be used for pharmacological purpose, mainly in the immunotherapy, acting as a resource in the combat the tumors.

KEY WORDS: venom, *Bothrops jararacussu*, macrophages, immunotherapy.

FINANCIAL SUPPORT: PUCPR, CNPq.

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EFFECT OF THE VENOM OF *Bothrops jararacussu* ON THE ADHESION OF HeLa CELLS IN CULTURE

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Many cell types require the linking to the extracellular matrix (EM) to be able to proliferate and to differentiate. Recent studies demonstrate that the EM also functions as a factor of survival for many types of cells, taking part in the etiology and patogenesis of a great number of illnesses, including cancer. Studies have demonstrated that the lack of adhesion to a substratum activate a suicidal process in a number of cells taking them to death. Lectins from the venom of different species of *Bothrops* showed capacity to diminish the viability of cells of breast cancer and human ovarian carcinoma and to inhibit the growth of renal and hepatic tumoral cells. Therefore, this work had the aim to evaluate the influence of the crude venom of *Bothrops jararacussu* on the adhesion of tumoral HeLa cells in culture. For such, plates of culture were previously coated with laminin, vitronectin and fibronectin. HeLa cells were added together with culture medium containing venom samples in different concentrations (10, 1, 0,1, 0.01, 0.001 μg/ml). After 2 h, the adhered cells were stained with crystal violet dye previously to cellular lise for colorimetrically quatification by reading absorbance at 550 nm on a plate reader. Results demonstrated that the venom inhibited the adhesion of HeLa cells on all of the EM molecules tested, in the doses of 10, 1, and 0.1 μg/ml. Laminin was the matrix protein that suffered the greatest interference, once even in the lowest dose the reduction in adhesion was of ~85%. Concerning vitronectin and fibronectin, the results presented a dose-responsive correlation, diminishing gradually the cellular adhesion up to the maximum interference with 10 μg/ml (80%). In this context, the results are of note, as the ability of cancer cells in adhering to extracellular matrix molecules play a decisive step in tumoral development.

KEY WORDS: venom, *Bothrops jararacussu*, HeLa cells, cell adhesion.

FINANCIAL SUPPORT: PUCPR, CNPq.

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NEUTRALIZATION OF ENZYMATIC ACTIVITIES INDUCED BY VIPERIDAE SNAKE VENOMS BY *Schizolobium parahyba* AQUEOUS

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Animal venoms, including snake venoms, are complex mixtures of proteins that act by different mechanisms. Medicinal plants are source of many pharmacologically active compounds. *Schizolobium parahyba* (Sp) is used against ophidism by lay people of Minas Gerais, Brazil. The aim of this work was to investigate the ability of the aqueous extract of Sp (EV) to neutralize the main enzymatic and biological activities induced by crude venoms (CV) from Viperidae family or isolated toxins (Neuw, BnSP7 and PLA2). For neutralization assays, toxins and venoms were previously incubated with EV at different ratios (1:1. 1: 5, 1:10 and 1:50 w/w, venoms or toxins/extract) for 30 min at 37ºC. The clotting activity induced by *Bothrops neuviedi pauloensis* (Bnp), *Bothrops moojeni* (Bm) venoms were significantly inhibited by S.p at lower ratios 1:1 and 1:5 (w/w). However, the extract was able to increase the clotting time, prolonging it around four and eight, when the venoms of Bnp and Bm were preincubated with EV at 1:10 and 1:50 (w/w, venoms: extract), respectively. While for *Crotalus durissus terrificus* (CDT) venom significative inhibition was observed only in the ratio of 1:50 (w/w).The metalloproteinase neuwiedase (neuw) and Bnp were able to degrade the Aα and Bβ chains of bovine fibrinogen, this activity was partially inhibited by EV at ratios 1:10, 1:50 (w/w). However, the degradation of Aα chain was not inhibited by the extract. PLA2 activity of the venoms of Bnp, Bm and Cdt was completely inhibited by EV at ratio of 1:50 (w/w).The use of natural products, exclusively derived from plants, in alternative therapies for antiophidian drugs has been increased. The observations confirmed that the aqueous extract of Sp possesses potent snake venom neutralizing properties.

KEY WORDS: Inhibition, vegetal extract, snake venom

FINANCIAL SUPPORT: UFU and CAPES, FAPEMIG.

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NEUTRALIZATION OF PROTEASES FROM *Bothrops pauloensis* SNAKE VENOM BY THE AQUEOUS EXTRACT FROM *Stryphnodendron adstringens*

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Envenomation caused by snake venoms of the genus *Bothrops* induces many local effects such as myonecrose, edema and hemorrhage. Medicinal plants play a key role in world health, as they are source of many phamarcologically active compounds. This study shows the ability of the aqueous extract from *Stryphnodendron adstringens* (EVA) against the proteolitic activities induced by *Bothrops pauloensis* snake venom. EVA was prepared by decoction and lyophilized. The inhibitions of the activities coagulant, hemorrhagic and sanguine unclothing induced by the venom were assayed with incubation by 30 min to 37ºC in three ratios: 1:1, 1:5 and 1:10 (m/m; Bp/EVA). EVA inhibited the clotting activity, prolonging the time of coagulation of the plasma in 50%. The sanguine uncloting was inhibited significantly in the ratio of 1: 5 (m/m). The inhibition of the hemorrhagic activity for EVA was of 100% in the ratio of 1:1 (m/m).In conclusion, our results that the *Stryphnodendron adstringens* aqueous extract contains compounds that neutralize proteases present in snake venoms. Furthermore, snake venom inhibitors can be useful tools for the elucidation of the mechanisms of action of toxins.

KEY WORDS: inhibition, *Stryphnodendron adstringens*, *Bothrops pauloensis*, snake venom.

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TWO-STEP COLUMN CHROMATOGRAPHIC METHOD FOR SEPARATION AND PURIFICATION OF A MYOTOXIC ENZYME FROM VENOM OF *Bothrops alternatus*

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Muscular necrosis is a serious consequence of *Bothrops* snakebites that may lead to permanent loss of tissue or function and to require amputation of the affected member. Myonecrosis may be due to an indirect action as consequence of vessel degeneration and ischemia caused by hemorrhagic metalloproteases or by a direct effect of myotoxic enzyme on plasma membranes of muscle cells. In this work, a myotoxin (BaTx) was purified from the venom of the snake *Bothrops alternatus* by a combination of ion exchange chromatography using DEAE Sephacel resin and gel filtration chromatography using Sephadex-G75 resin. BaTx displays a molecular mass of 15 kDa as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) stained with coomassie blue, in the presence of dithiothreitol. The myotoxic activity was assayed on the basis of the morphological alterations induced by i.m. injections of 50 mg toxin in the right gastrocnemius skeletal muscle of Swiss mice. After 24 h the animals were sacrificed by deep anesthesia with ethyl ether and small section of the central region of the muscle was excised and soaked in fixing solution [4% formaldehyde (v/v)]. The material was then dehydrated by increasing concentrations of ethanol and processed for inclusion in paraffin. The resulting blocks were sliced in 5 mm thick section, stained with hematoxylin/eosin and examined under a light microscope. The toxin led to a series of drastic degenerative events. The hemorrhage, muscular fatty degeneration, myonecrosis and inflammatory reaction were evident microscopically. The high presence of macrophages and polymorphonuclear leucocytes suggests that phagocytosis of cell debris already had initiated.

KEY WORDS: *Bothrops alternatus*, myotoxin, phospholipase A_2.

FINANCIAL SUPPORT: UFU

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HISTOLOGICAL ALTERATIONS CAUSED BY *Bothrops leucurus* VENOMS FROM DIFFERENT GEOGRAPHIC REGIONS

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Snake venoms vary in their biochemical composition and pharmacological profile, not only between different species, but also within a single species and in snakes of different ages. In the present work, a comparative study was performed on histological alterations of *B. leucurus* venoms from specimens at different geographic regions of Bahia, Brazil. The venoms tested and describes in this study were obtained from snakes collected from Deciduous forest and/or degraded Atlantic forest (B3 and B4 venoms) and Caatinga (B2 venom). The histological alterations of viscus (heart, lung, liver and kidney) were assayed on the basis of the morphological alterations induced by i.p. injections of 300 mg each venom in Swiss mice. After 24 h the animals were sacrificed by deep anesthesia with ethyl ether and small section of the central region of the each viscus was excised and soaked in fixing solution [4% formaldehyde (v/v)]. The material was then dehydrated by increasing concentrations of ethanol and processed for inclusion in paraffin. The resulting blocks were sliced in 5 mm thick section, stained with hematoxylin/eosin and examined under a light microscope. In general the lesions were similar to three venoms. The hearts did not demonstrate apparent injuries. In the lung was observed sanguine vases contend hemolysed cells and high number of leukocytes, mainly with B4 venom. Leukocyte migration from the blood into the lung has been suggested as being responsible for the increase of lymphocytes in the bronchoalveolar lavage and bronchial mucosa in human asthma, but so far there has been no direct proof. In the liver was observed light fatty degeneration and hemolysis signal. In the kidneys was observed hemolysis signals and hemorrhage in the medullary tubes. All injuries apparently were more intense with B4 venom.

KEY WORDS: *Bothrops leucurus*, variability.

FINANCIAL SUPPORT: UFU

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Crotalus durissus terrificus VENOM INDUCES DNA DAMAGE IN GLIOBLASTOMA CELL LINE

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Glioblastoma multiforme (GMB) is the most commonly diagnosed primary tumour of the brain in humans and always culminates in death within 1–2 years of diagnosis. Despite decades of intensive clinical and laboratory research, progress has been slow, partly because of tumour heterogeneity. Several snake venoms and toxins have been referred to inhibit cancer cell adhesion, migration, tumour growth and metastases induced in experimental mice models. Previous reports have been shown therapeuticals effects of a *Crotalus durissus terrificus* toxin on skin, breast and lung tumours. In this study we examined the effect of crotalic venom (CV) on the DNA integrity, metabolic viability and clonogenicity of murine glioblastoma cells (RT2). RT2 cells were treated with different concentrations of crotalic venom (CV) for different times. Following 24 hours of treatment with 10mg/mL, monolayer cells acquired round shapes and formed cells suspension. Moreover, at the micromolar range, the venom inhibited mitochondrial metabolism and clonogenic proliferation (IC_{50} 2.4 mg/mL). DNA damage, measured by DNA ladder assay, could be observed one hour after the treatment with CV (1mg/mL) and increased along the time. RT2 cells were able to partially repair DNA damage induced by CV treatment. This repair attempt appears to be unsuccessful once RT2 cells metabolic viability and clonogenicity were lost. These results suggest the potential use of *Crotalus* sp. venom in tumour therapy. Further studies are in development to verify the specifics mechanisms of these anti-tumoral effects.

KEY WORDS: Glioblastoma, *Crotalus durissus terrificus* venom, tumour therapy.

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VENOMIC ANALYSES FROM *Bothrops* SPECIES: A NEW APPROACH TO DETERMINE TAXONOMICAL/PHYLOGENETIC RELATIONSHIPS BASED IN VENOM COMPLEXITIES.

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According to the Brazilian Health Ministry 19,000 to 22,000 snake envenom accidents occur each year. Most of these accidents are attributed to *Bothrops* species. This fact makes the taxonomical, immunological and venomic studies of this group extremely important. The taxonomical positioning of the species traditionally included in the *Bothrops* genus has been considerably modified in the past years. Such modifications have been based mostly on morphological characters, and on some biochemical traits (electrophoretic pattern of proteins from liver, kidney, heart, esqueletic muscle and blood plasma). In the present study, high performance liquid chromatography coupled to mass spectrometry (LC/MS) has been used to analyze the crude venom from 11 *Bothrops* species (*Bothrops jararaca*, *B. jararacussu*, *B. neuwiedi*, *B. taeniatus*, *B. brazili*, *B. erythromelas*, *B. moojeni*, *B. cotiara*, *B. atrox*, *B. leucurus* and *B. castenaldi*) in order to establish both taxonomical and phylogenetic relations between them. The large number of molecules found in these venoms has been clustered according to their physico-chemical properties (molecular mass and hidrophobicity), using the machine learning-based Weka software. In the assigned clusters the presence of some toxin structural families, such as BPPs, disintegrins, serineproteases and metalloproteases, could be verified. Then, a phenetic correlation tree has been generated from the clusterization process data, and was compared to existing taxonomical trees. In this way, we believe that this methodology is appropriated to determine the *Bothrops* venoms` complexities and to assess their taxonomical relationships as well.

KEYWORDS: venomic analyses, *Bothrops*, phylogenetic relationships

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PROTEOMIC ANALYSIS OF RAT CEREBRAL CORTEX SYNAPTOSOMES TREATED WITH CROTOXIN

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Crotoxin (CrTx), the main component from the venom of *Crotalus durissus terrificus* is a complex of two subunits (A and B). Subunit B is the toxic component, with phospholipase A2 (PLA2) activity. CrTx exerts its toxic action by blocking neuromuscular transmission. Component A, non-toxic, directs component B toward its specific sites, preventing its nonspecific binding and enhancing its blocking action on neuromuscular transmission. The PLA_2 induces mithocondrial swelling and increase O_2 consumption. Despite of various studies with CrTx its precise mode of action is still unknown. The objective of the present study is to characterize protein profile of synaptosomes from rat cerebral cortex treated with crotoxin, using proteomic approaches. We used 2D electrophoresis and MALDI-TOF-TOF analyses to search for qualitative and/or quantitative differences in proteins between crotoxin treated and non-treated synaptosomes. By analyzing total proteins separated in a non-linear pH range (3-10), we detected 42 differentially expressed proteins. From those, a total of eight spots were successfully identified by MALDI-TOF-TOF: creatine-kinase, beta-actin, rCRMP-1, Gapd, H+ transporting, Vdac-1, ATP-sintase and pyruvate kinase. These proteins are involved with mithocondrial swelling (Vdac-1), energetic metabolism (creatine-kinase, Gapd, H+ transporting, ATP-sintase and pyruvate-kinase) and cellular injury repair (rCRMP-1, beta-actin). We hope these results may contribute to understand some effects already described of crotoxin, i.e.apoptosis.

KEY WORDS: synaptosomes, crotoxin, proteomic analysis

FINANCIAL SUPPORT: FAPEMIG, CNPq, FINEP

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INFLUENCE OF THE POISON OF *Bothrops lanceolatus* ON THE SYSTEM IMMUNE FRONT TO ANTIGENS NON RELATED

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The main features associated with pit viper envenomations include the intense local lesions such as oedema, hemorrhagic complications, necrosis, acute renal failure and other effects. The severity of these reactions to snakebite depends on the degree of envenomation. These animal venoms have always inspired the researchers' curiosity due to the great number of accidents and your activities. Heterologous immune serums are produced to avoid the lethalness of venoms such as the other *Bothrops* sp. (90,5 percent of the accidents in Brazil; National Foundation of Health 1998). However, they can cause hypersensitivity and bring complications to the victim. It was observed at our laboratory that the immune serums made for the venom of *Bothrops lanceolatus* (BL) possess low levels of neutralizing antibodies when compared with other species of the genus. With the purpose of analyzing and increasing the amount of immune serum for the venom of (BL), the effect was tested from this venom front to the humoral immune answer to non-related antigens (Sheep of Erythrocytes[SE]). Previous inoculation of the venom of (BL) in the dose of 4,8µg/g;i.p 72 h before the primary(PI) and second(SI) incentives with (SE) i.p 2% in Swiss male mice(3 and 7 months). The levels of anti-(SE) antibodies were certain for the hemaglutination test in microplatelets. In (PI) it was reduced to 4,5 times of 1/118,4(control) for 1/26,4(n=5;P<0,05), and in (SI) it was reduced to 2,6 times of 1/4096 (control) for 1/1568 (n=4;P<0,05) when compared with the control (just SE). These results demonstrate there is a component in the venom of (BL) that reduces the production of reagents antibodies, which could justify a low effectiveness of the immune serum.

KEY WORDS: snake, antivenin, *Bothrops lanceolatus*, poison, poisoning

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DIMERIC LYS49-PHOSPHOLIPASES A_2: WHICH IS THE CORRECT BIOLOGICAL ASSEMBLY?

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Phospholipases A_2 are components of *Bothrops* venoms responsible for disruption of cell membrane integrity via hydrolysis of its phospholipids. Several myotoxic phospholipases A_2-like, which lack the catalytic activity upon phospholipids due to the D49K mutation, have been extensively structurally and biochemically characterized. The oligomeric conformation has been addressed as an important role to the development of their pharmacological activities. Based on the crystallographic and spectroscopic data, a dimer conformation formed mainly by polar contacts between the beta-wing and N-terminal helice, which exposes the hydrophobic channel or interfacial face, has been considered as the biological assembly. However, two recent Lys49-PLA_2s structures suggested an alternative dimeric conformation as the correct assembly. In order to study this discrepancy, we revised the crystal lattice of seven Lys49-PLA_2s and the presence of the alternative dimer was a common feature of all lattices analyzed. In this alternative conformation, the dimeric interface consists of non-polar contacts between the interfacial faces connecting the nominal active site which gives stability and high solubility of this protein in water. Regarding that these protein are very stable and water soluble, we proposed an alternative dimer conformation whose interface consists of non-polar contacts between the interfacial faces connecting the nominal active sites. This hypothesis, which explains the hydrodynamic behavior of Lys49-phospholipases A2-like, is based on small angle X-ray scattering, crystal structures of complexes with ligands, interface analysis and energy minimization.

KEY WORDS: Lys49-phospholipase A_2; myotoxin; crystal structure; bothropic venom; quaternary assembly; alternative dimer.

FINANCIAL SUPPORT: FAPESP, CNPq, FUNDUNESP

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ISOLATION AND CHARACTERIZATION OF NEW PLA_2 ISOFORM FROM THE *Crotalus durissus collilineatus* VENOM.

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In this article we isolated a new PLA_2 isoforms from the *Crotalus durissus collilineatus* venom collected from the specimens found in the São Paulo and Minas Gerais States. The venom showed the presence of L-amino acid oxidase that are absent in the venoms from the Goias region. This PLA_2 shows few amino acid replacements in comparison with other PLA_2 isolated from this venom. This protein induce a strong platelet aggregation using dosis of 3-5ug and induced a dose dependent edema using dosis of 3, 6 and 9ug by paw. Both effects were strongly inhibited by p-bromophenacyl bromide (p-BPB) that induced a not expected strong secondary modification of this protein in solution by circular dicroism. According these results we observed that p-BPB decreasing of alpha helix and increase of random coils in solution.

KEY WORDS: PLA_2, *Crotalus durissus collilineatus* and circular dicroism.

FINANCIAL SUPPORT: FAPESP, CNPq.

MORPHOLOGICAL, BIOCHEMICAL AND PHARMACOLOGICAL STUDY OF *THE* INFRALABIAL GLANDS OF *Sibynomorphus mikanii* (COLUBRIDAE, DIPSADINAE)

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Despite infralabial glands being common among snakes, little is known about their morphology and function. They are generally homogeneous and mucous, but among dipsadines they are mainly serous and very developed. This is possibly related to the fact that some species of the group feed on soft and viscous invertebrates. This work aims the morphological study of *S. mikanii* infralabial glands and the biochemical and pharmacological characterization of its secretion, obtained by tissue maceration and glandular tissue culture (explant). The glands are divided in two portions: a more developed one, constituted by simple epithelium, with seromucous cells organized in acini, and a smaller one, constituted exclusively by mucous acini, which are directly connected to the ducts. SDS-PAGE profile from the homogenized tissue showed that the secretion is composed by proteins between 45 and 60 KDa, with a 25 KDa major protein, which is the most evident band appearing in glandular tissue culture. This protein probably corresponds to the major product secreted by the gland and presents a significant gelatinolytic activity. HPLC resulted in five main peaks, the most significant showing molecular weight of 24.761 KDa, which corroborates the results obtained by SDS-PAGE. The topic application on mice cremaster muscle of this purified major protein, when observed by intravital microscopy, caused alteration in microcirculation, which was evidenced by an increase in leukocyte “rolling”. The results indicate that the glands may present other functions besides food lubrication, as for example, chemical prey immobilization.

KEY WORDS: infralabial glands, Dipsadinae, *Sibynomorphus mikanii*

FINANCIAL SUPPORT: CAPES, FAPESP, CNPq, Fundação Butantan

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CHARACTERIZATION OF ENZYMATIC AND INFLAMMATORY ACTIVITIES OF VENOMS OF THE AMAZONIAN SNAKES *Bothriopsis bilineata* AND *Bothriopsis taeniata* (SERPENTES: VIPERIDAE)

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Snake venom is a complex mixture containing diverse protein components with different structures and functions that are used for prey immobilization and possibly death. Snake venoms from the family Viperidae cause pronounced local and systemic effects, such as pain, edema, hemorrhage and necrosis. We investigated some enzymatic and biological activities in venoms of two Amazonian snakes, *Bothriopsis bilineata* and *Bothriopsis taeniata*. Here we show that both venoms presented high enzymatic activities for the proteases kallikrein, thrombin and plasmin. These venoms lacked acetylcholinesterase activity, and exhibited low levels of trypsin, cathepsin C and leucine aminopeptidase activities. Protease activity against substrate Hide Power Azure was weakly detectable in both venoms. *B. taeniata* and *B. bilineata* crude venoms were able to induce neutrophil recruitment into peritoneal cavity of mice 4 hours after injection. The neutrophil recruitment induced by *B. taeniata* venom was accompanied by hemorrhage. Both venoms were also able to induce edema formation in mice hind paws. *B. taeniata* venom-induced edema presented a marked hemorrhage, suggesting that this venom has hemorrhagic activity. Our study shows that *B. taeniata* and *B. bilineata* venoms induce a pronounced inflammatory reaction, with leukocyte recruitment, edema formation and hemorrhage, which parallels to a high proteolytic activity found in these venoms.

KEY WORDS: snake venom, enzymes, neutrophil recruitment, edema, *Bothriopsis bilineata*, *Bothriopsis taeniata*.

FINANCIAL SUPPORT: CAPES, PUCRS, UFRJ.

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NEUTRALIZATION OF SNAKE VENOM PHOSPHOLIPASE A_2 TOXINS BY AQUEOUS EXTRACT OF *Casearia sylvestris* (FLACOURTIACEAE) IN MOUSE NEUROMUSCULAR PREPARATION

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Although specific antivenom is the mainstay of medical treatment for snakebite envenomations, it is important to search for different venom inhibitors that could complement or substitute for the action of antivenoms. Aqueous extract of *Casearia sylvestris* (Cs) has been shown to inhibit enzymatic and biological properties of several snake venoms and purified phospholipase A_2 (PLA_2) toxins. The skeletal neuromuscular junction is one of the major targets of PLA_2 toxins. In this work we evaluated the influence of Cs aqueous extract upon the neuromuscular blocking and the muscle damaging activities of some PLA2s [crotoxin (CTX) from *C. durissus terrificus*, bothropstoxin-I (BthTX-I) from *B. jararacussu*, piratoxin-I (PrTX-I) from *B. pirajai* and myotoxin-II (MjTX-II) from *B. moojeni*] in mice phrenic-diaphragm preparations. Data (mean ± SEM, n = 3 to 8) were analyzed by ANOVA ($p<0.05$). CTX (0.5 mM) and all others PLA_2 (1.0 mM) induced irreversible and time-dependent blockade of twitches. Except CTX, all PLA_2s induced significant indices of muscle damage, assessed by microscopic analysis. Preincubation of BthTX-I, PrTX-I or MjTX-II with Cs (1:5 w/w, for 30 min at 37°C) significantly prevented the neuromuscular blockade of preparations exposed to the mixtures for 90 min; the extent of protection ranged from 93% to 97%. The Cs extract also neutralized the muscle damage (protection of 80% to 94%). Higher concentration of the extract (1:10 w/w) was necessary to neutralize the neuromuscular blockade induced by CTX in 90%. These findings expanded the spectrum of Cs antivenom activities, evidencing that it could be a good source of potentially useful PLA_2 inhibitors.

KEY WORDS: plant extract; snake venom; neuromuscular junction.

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INTRAVITAL MICROSCOPY IN THE STUDY OF MICE MICROCIRCULATION UNDER NATIVE AND IRRADIATED CROTAMINE EFFECTS

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Ionizing radiation, in aqueous solution, produces several highly reactive species. The most important are hydroxyl radical (OH·) and hydrated electron (e-aq.). Theses products interact with peptides and proteins causing several modifications. Intravital Microscopy is a technique that allows evaluating cellular events occurring in the microcirculation, as well as verifies the contraction or dilation of post capillary venules. Thus, the evaluation of CTM and CTMi in the microcirculation alterations have been studied. The crotamine (CTM) is a 4,882 Da strongly basic polypeptide that produces skeletal muscle spasms leading to spastic paralysis of hind limbs in mice. Purified CTM from *C.d.terrificus* venom was irradiated with 2 kGy of ^{60}Co gamma rays (CTMi). The microcirculation was evaluated by intravital microscopy by exposition of cremaster muscle, and ten minutes later, the toxin was applied directly in the muscle at 1 and 10mg/10mL concentrations. The cellular evaluation (rolling and adhered cells) was observed at the first, fifth and tenth minute after contact with the toxin. The diameter post capillary venules had been also evaluated after contact with the toxin. The results showed decreasing of rolling cells in the presence of both concentration of CTM 1mg (5 min: 25%: 10 min: 58 %) and 10 mg (5 min: 4%; 10 min: 35%), without significant changes for adhered cells when compared with control group (NaCl, 015M). On the other hand, CTMi 1mg showed increasing of rolling cells (5 min: 11,5%: 10 min: 12%) without no changing in the number of adhered cells. With 10 mg of CTMi it was observed a decreasing of rolling cells at 5 minutes (23%) and at 10 minutes (14%) while the adhered cells have increased significantly (5 minutes: 56% and 10 minutes: 130%). The contraction profile is time and concentration dependent for CTM, but to CTMi it was observed a dilatation process for both concentration of toxin. Therefore, this work showed that irradiation process causes inversion in the effects of native crotamine on post capillary venules contraction.

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PERIPHERAL ANTINOCICEPTIVE FACTOR EXTRACTED FROM *Crotalus durissus collilineatus* VENOM

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Previous works have shown the presence of analgesic activity in rattlesnake venoms or in substances isolated from them, indicating that they may be important scientific tools to understanding the pain physiopatology or molecular models to discovery news therapeutic substances. In this work was isolated a factor from *Crotalus durissus collilineatus* venom with peripheral analgesic effect and its involvement with opioid system was observed. The factor was purified by HPLC with a C18 column (25 x 250mm), eluted with a flow of 4,5ml/min and using a 0-40% linear gradient of acetonitrile for 40 min. The factor presented peripheral antinociceptive activity, since when it was administered at 1-10 μg/kg doses by intraperitoneal (i.p.) route significantly diminished the contortion numbers in the acetic acid-induced writhing test. Furthermore, naloxone (5 mg/Kg, i.p.) significantly prevented the inhibition of the contortions, showing that opiod receptors are involved in the peripheral antinociceptive activity of this factor. However, the factor didn't change the reaction times in the tail-flick and hot-plate tests, showing that the factor has no spinal or central antinociceptive activity, respectively. This factor may be used as tool to study the peripheral pain mechanism and/or to develop new therapeutics to be used in peripheral control of the pain, without the side effects caused by the classical opioids on the central nervous system, such as tolerance and withdrawal syndrome.

KEY WORDS: snake, venom, antinociceptive factor, peripheral antinociceptive action, *Crotalus durissus collilineatus*

FINANCIAL SUPPORT: FUNCAP

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ISOLATION OF A NOVEL FIBRINOGENOLYTIC TOXIN FROM THE VENOM OF *Pseudechis australis*

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Australian elapids have always been known for their highly neurotoxic venoms. Indeed, most of the clinical observation point towards respiratory failure and other neurotoxic syndromes. In recent experiments, we were able to identify an immunoreactive band, recognized by polyclonal antibodies raised against jararhagin, a metalloproteinase from *Bothrops jararaca*. Such data are suggestive of the presence of toxins involved in the modulation of haemostasy, which might not be detected by clinical diagnosis, since neurotoxic symptoms predominate. In the present work, our goal was to identify eventual fibrinogenolytic enzymes in *P. australis* venom. 50 mg of venom were dissolved in 50 mM tris/150 mM NaCl pH 8 and injected into a benzamidin sepharose (5x100 mm) column. The unbound fraction was eluted with a 5 column volume wash with the same buffer. The bound fraction was eluted with 100 mM pH 3 glycine and immediately dyalized against PBS containing 1 mM CaCl2.Purity was assayed by reverse phase chromatography, using a C4 (Vydac) column and a 0-100% CH3CN gradient in 30 minutes, after an initial wash step with 50 mM pH 7 sodium phosphate. Fibrinogenolytic activity was assayed by incubating the toxin with bovine fibrinogen for different times, up to 24 hours. The resulting supernatants were analyzed by RP-HPLC for the presence of fibrinopeptides. Our data indicate that the isolated fraction was 95% pure and with high fibrinogenolytic activity.

KEY WORDS: *Pseudechis australis*; serine proteinase; fibrinogenolysis

FINANCIAL SUPPORT: IPEN.

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ISOLATION AND CHARACTERIZATION OF DELTA TOXIN FROM THE VENOM OF *Crotalus durissus terrificus*

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The *Crotalus durissus terrificus* venom has been so far described as being of low complexity, with four major components described: convulxin, gyroxin, crotoxin and crotamine. In 1980, Vital Brazil predicted the existence of a toxin which could be involved in platelet aggregation, and named it delta toxin. However, this toxin has never been isolated or characterized. The aim of the present work was to purify and characterize this new venom component. After FPLC size exclusion chromatography followed by reverse phase HPLC, an homogeneous fraction was obtained, with a molecular weight of 14,074.92 Da. When analyzed by SDS-PAGE, this toxin presented molecular weight of 14 kDa, while in 2D gels, spots around 40 kDa and with an isoelectrical point between 4 and 5 were obtained. After trypsin digestion and mass fingerprinting, the fragments were submitted to the swissprot databank showing high homology with trocarin, a prothrombin activator from *Tropidechis carinatus*. These data were further confirmed by aminoacid analysis. The toxin was tested for its ability to activate factor II and X using synthetic substrates. Our data indicate a direct activation of factor X. The same toxin also behaved as a potent direct platelet aggregation activator on washed platelets. Assays with specific inhibitors indicate that neither metalloproteinase, nor serinoproteinase or lectin domains are involved in the aggregating activity, since EDTA, benzamidin and D-galactose did not inhibit the toxin. Our data also indicate that it is probably a homotrimer with the subunities linked by hydrophobic and/or electrostatic interactions.

KEY WORDS: factor X activating, platelet aggregation, delta toxin.

FINANCIAL SUPPORT: CAPES

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L-AMINO ACID OXIDASES ISOLATED FROM *Bothrops* SNAKE VENON PRESENT SELECTIVE CYTOTOXIC ACTIVITY AGAINST TUMORS: THE DEPENDANCE OF OXIGEN REACTIVE INTERMEDIATES (ROS) GENERATION AND INDUCTION OF CELL DEATH BY NECROSIS AND APOPTOSIS

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L-amino acid oxidase (L-AAO) 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. The L-AAO is widely distributed in many different organisms and snake venoms. 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 L-AAO isolated from *Bothrops alternatus* (BaltLAAO) or *Bothrops moojeni* (BmooLAAO) 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 both BaltLAAO and BmooLAAO 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* snake venom, anti-tumor activity, reactive oxygen species, necrosis, apoptosis.

FINANCIAL SUPPORT: CNPq, FCFRP-USP

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VENOME COMPARATIVE ANALYSIS AND IDENTIFICATION OF NOVEL PROTEINS FROM SOUTH-AMERICAN CORAL SNAKE

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The *Micrurus* genus, so-called coral snakes (Serpentes, Elapidae), comprises 61 species distributed from the USA to South America. From the toxinological viewpoint, these venoms diplay neurotoxic, hemolytic, myotoxic and cardiotoxic activities. Our goal was to analyse the protein composition of *Micrurus frontalis* and *M. lemniscatus* and to identify components from *M. lemniscatus* venom. The comparative proteomic analysis of *M. frontalis* and *M. lemniscatus* was accomplished by reversed phase chromatography and electrospray ionization mass determination (ESI-Q-TOF/MS). To assess the venom proteomic profiles from *M. lemniscatus* we used a combination strategy which inclued two-dimensional liquid chromatography, mass spectrometry and N-terminal sequence analyses. The crude venom of the *M. frontalis* and *M. lemniscatus* showed large structural diversity when observed by ESI-Q-TOF/MS. However, *M. leminiscatus* venom diplays some molecular masses that are not observed in *M. frontalis* venom, such as molecular species at 4 and 8 kDa ranges. After N-terminal sequence determination, several proteins from *M. lemniscatus* showed strong similarity with others proteins from Elapidae venoms available at public protein databanks. The 6,678.02 Da protein showed 80% of the similarity with citotoxins and cardiotoxins from Naja naja venom. Neurotoxins from severals snakes display high similarity degree with 7,526.73 Da protein whereas 13,238.00 Da was shown to be similar to phospholipases. The higher protein observed in *M. lemniscatus* venom has a molecular mass of 22,532.74 Da and is probably an isoform of the kunitz inhibitors. The comparative proteomic analysis of these two venoms is a comprehensible catalogue of the secreted proteins. Among the totality of proteins from *M. lemniscatus* venom were identified as neurotoxins, citotoxins, kunitiz inhibitors and phospholipases isoforms.

KEY WORDS: Proteomics, *Micrurus*, ESI-Q-TOF/MS.

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ANTAGONISM OF THE CARDIOTOXIC ACTIVITY OF *Bothrops jararacussu* VENOM BY A SYNTHETIC COUMESTAN NAMED LQB93

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Bothrops snakebite induces local damage with edema, hemorrhage and myonecrosis and these effect are not well understood and poorly neutralized by antivenoms. We investigated the *in vitro* cardiotoxic activity of *B. jararacussu* crude venom and the antivenom effect of a synthetic coumestan named LQB93 in rats. The cardiotoxicity of the venom was carried out in isolated rat hearts in a Langendorff preparation continuously perfused (2-5 mL/min) with physiological saline solution (PSS) at 37°C. The heart tension and the electrocardiogram (EKG) were continuously recorded. When we added the crude venom (2.5-10µg/mL) to the perfusion solution, it's induced a progressive negative inotropic effect, decreasing the tension down (1.2 ±0.04 to 0.43 ± 0.03 g/cm, n=4), finaly abolished EKG waves, both after 15 min. When we added LQB93 (10 µM) to the venom, the coumestan decreased circa of 80% of the venom cardiotoxic effect (1.21±0.06 to 0.93±0.03 g/cm, n=4). The heart was removed from the Langendorff apparatus; both the auricles and root of aorta were excised out and the ventricles were sliced into uniform sections of 2-3 mm thickness. The slices were incubated in a triphenyl tetrazolium chloride solution (TTC, 1%) at 37 oC (pH 7.4) during 4 min. At the end of the incubation time, the heart slices were placed in a fixative solution, which not only fixes, but also enhances the damage area. The normal myocardium was stained brick red, while the infarcted portion remained unstained. Infarct size was measured by WCIF Image J program. The damaged area expressed in as % of total area, were: 1) *B. jararacussu* (10 µg/mL), 55 % of infarct area; 2) LQB93 10 µM (alone) and plus venom (10µg/mL) + LQB93 10 µM, 10 % of infarct area. The association of LQB93 to the crude venom prevents the cardiac arrest and contracture as well as the myocites damage.

KEY WORDS: *Bothrops* venom Cardiotoxicity and coumestans

SUPPORT: CNPq, FAPERJ, PRONEX, CAPES, FUJB-UFRJ.

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PURIFICATION AND PARTIAL BIOCHEMICAL CHARACTERIZATION OF A NOVEL NEUROTOXIN FROM *Bothrops pauloensis* SNAKE VENOM

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The venoms of several *Bothrops* species cause neuromuscular blockade *in vivo* and signs of neurotoxicity *in vivo*. The aim of this work was to purify and partially characterize a novel neurotoxin from *Bothrops pauloensis* venom. *Bothrops pauloensis* venom was fractionated by reverse phase HPLC on a m-Bondapack C-18 column, using only one chromatographic step. This fractionation resulted in 17 peaks (Bp-1 to Bp-17), one of which (Bp-12) gave a single band with a molecular mass of 13 kDa in PAGE-Tricine electrophoresis and was devoid of phospholipase A2 activity. Amino acid analysis yielded 10 Asp, 7 Thr, 5 Ser, 7 Glu, 7 Pro, 8 Gly, 5 Ala, 14 Cys, 4 Val, 1 Met, 3 Ile, 11 Leu, 12 Tyr, 2 Phe, 19 Lys, 2 His and 5 Arg. The N-terminal sequence of the first 21 residues was SLFELGKMIL QETGKNPAKSL and showed extensive homology (95%) with highly conserved amino acid sequences in Lys49 PLA_2 homologs of other *Bothrops* venoms; the degree of homology was lower when compared with Asp49 PLA_2. These results indicate that Bp-12 is a protein that is structurally homologous to PLA_2.

KEY WORDS: amino acid, *Bothrops pauloensis*, Lys49 phospholipase A_2.

FINANCIAL SUPPORT: CNPq.

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PHARMACOLOGICAL CHARACTERIZATION OF A NOVEL FRACTION (Bp-12) FROM *Bothrops pauloensis* SNAKE VENOM

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The venoms of several *Bothrops* species cause neuromuscular blockade *in vivo* and signs of neurotoxicity *in vivo*. The aim of this study was examine the pharmacological actions of a novel fraction (Bp-12) isolated from *Bothrops* pauloensis snake venom. Myotoxicity was assessed in mice based on the increase in serum creatine kinase (CK) levels and edema formation was assayed in mouse hind paws. Neuromuscular blockade was studied using conventional myographic techniques in mouse phrenic nerve-diaphragm (PND) preparations incubated with Tyrode solution (control) or Bp-12 (10-50 µg/mL) for 120 min at 37oC; the effect on the membrane resting potential was studied by standard electrophysiological methods. Bp-12 (5-20 µg/mL, via im) was weakly myotoxic since it produced only a small increase in plasma CK levels (225.35 ± 36.9U/L, n=5, $p<0.05$ from control). However, this fraction produced dose-dependent mouse paw edema, with increases in volume of 9.7 ± 4.7% (5 µg/paw), 15.8 ± 4.6% (10 µg/paw) and 20.7 ± 4.6% (20 µg/paw) after 24 h (n=5 each, $p<0.05$). The time for 50% paralysis by Bp-12 in PND preparations was 45.1 ± 6.6 min and 16.3 ± 6.6 min for 20 µg/ml and 50 µg/ml, respectively (n=5 each). At 50 µg/ml, Bp-12 produced neuromuscular blockade total without altering the membrane resting potential (-85 ± 2.5mV, n=6, $p>0.05$ from control). These results show that Bp-12 has neurotoxic and myotoxic activities that probably contribute to the effects of the crude venom.

KEY WORDS: *Bothrops pauloensis*, myotoxicity, neuromuscular blockade, phrenic nerve-diaphragm.

FINANCIAL SUPPORT: CNPq.

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