THE BLOOD-BRAIN BARRIER TO NEUROTOXINS AND NEUROTROPHINS

ABBA J. KASTIN AND WEIHONG PAN(1)

(1)Pennington Biomedical Research Center, Baton Rouge, Louisiana, USA

The blood-brain barrier (BBB) is the interface between the peripheral circulation and the central nervous system. This multi-dimensional structure is composed of specialized cerebral and spinal microvessels and surrounding cells and matrix. It serves important regulatory functions by differential permeability. It is also subject to modification by many pathophysiological factors. This talk will present ongoing BBB studies in our own laboratory and others, focusing on the following topics: (1) neurotoxins penetrating the BBB to induce neuropathology; (2) neurotoxins that increase the general permeability of the BBB or affect transport systems for other essential substances; (3) neurotrophins as pro-drugs to penetrate the BBB in either the native state or after modification by biotechnology; (4) circulating peptides that serve as either neurotoxins or trophic factors besides regulating behavior; and (5) how cytokine transport across the BBB affects neuroplasticity. Thus, the aim is to provide an overview of the regulatory nature of the BBB, and to show the importance of the endothelial interactions of some neurotoxins as well as trophic factors.

KEY WORDS: peptides; neurotoxins; blood-brain barrier; neurotrophic factors

FINANCIAL SUPPORT: NIH

CORRESPONDENCE TO: Abba J. Kastin, M.D., Pennington Biomedical Research Center, Baton Rouge, LA 70808, USA. Phone: 1 225 763 0266, Fax: 1 225 763 0265, Email: peptides@pbrc.edu

PROTEOMIC ANALYSIS OF PROTEINASES FROM *Bothrops* VENOMS

PAES LEME A.F., KITANO E.S., YAMASHIRO E.T., OLIVEIRA A.K., MENEZES M.C., PIMENTA D.C., KONNO K., ASSAKURA M.T., CAMARGO A.C.M., SERRANO S.M.T.

Laboratório Especial de Toxinologia Aplicada/CAT-CEPID, Instituto Butantan, 05503-900, São Paulo-SP.

The knowledge of venom proteome and its variability is important both in basic research and in pathologies associated with envenoming. Furthermore, the knowledge of the complexity and variety of specific targets of venom toxins may underscore new protein interactions. The *Bothrops* venom proteinase complexity was evaluated by comparison of venom from eight species (*jararaca, cotiara, moojeni, jararacussu, bilineatus, erythromelas, insularis, neuwiedi*). Subpopulations of venom proteins were analyzed by 2D gel electrophoresis (2D-PAGE) and specific antibodies in Western blot analyses, including serine proteinases and metalloproteinases, and by gelatin-zymography. In addition, the targets for proteolysis were evaluated by proteomic approaches using isolated proteinases from *B. jararaca* venom. *Bothrops* protease A (BPA) is a highly glycosylated thermo-stable serine proteinase from *B. jararaca* venom that shows high fibrin(ogen)olytic activity and prevention of venous thrombus formation in rats. The analysis of human plasma incubated with BPA followed by analysis by 2D-PAGE revealed various changes in the protein profile indicating that plasma proteins other than fibrinogen were degraded by BPA. HF3, a P-III class snake venom metalloproteinase (SVMP), is the most potent hemorrhagic toxin from *B. jararaca* venom. The hemorrhage caused by SVMPs can be the result of a synergistic effect of proteolytic degradation of capillary basement membranes and plasma proteins, and of inhibition of platelet aggregation. The target proteins for proteolysis by HF3 *in vivo* were evaluated by 2D-PAGE. Swiss mice were injected with HF3 and after 2h the dorsal skin was sectioned and the proteins were extracted and evaluated by 2D-PAGE. Moreover, gelatin-zymography of skin proteins showed *in vivo* activation of pro-metalloproteinases by HF3. These approaches have provided some insights into the complexity of venom proteinases and their *in vitro* and *in vivo* effects.

KEY WORDS: venom proteome, proteinase.

FINANCIAL SUPPORT: CNPq, CAT-CEPID/FAPESP.

SNAKE VENOM PHOSPHOLIPASES A_2 INHIBITORS: MEDICINAL CHEMISTRY

ANDREIMAR MARTINS SOARES(1)

(1)Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, FCFRP, Universidade de São Paulo, USP, Ribeirão Preto-SP, Brazil.

Phospholipases A_2 (PLA_2s) are commonly found in snake venoms and have extensively studied due to their pharmacological and physiopathological effects in living organisms. The high medical-scientific concern evoked by the involvement of these proteins in different biological processes promoted an increasing search for natural or artificial inhibitors, aiding at PLA_2s neutralization and a better understanding of their mode of action and their structure-function relationship. This article reports a minireview on natural and artificial inhibitors of enzymatic, toxic and pharmacological effects induced by snake venoms PLA_2s. These inhibitors act on PLA_2s through different mechanisms, most of them still not completely understood, including binding to specific domains, denaturation, modification of specific amino acid residues and others. Several substances have been evaluated to their effects against snake venoms and isolated toxins, including plant extracts and compounds from marine animals, mammals and snakes serum plasma, in addition to polyclonal antibodies and several synthetic molecules. Research involving these inhibitors may be useful to understand the mode of action of PLA_2s and their role in envenomations caused by snake bite. Furthermore, the biotechnological potential of PLA_2s inhibitors may provide therapeutic molecular models with antiophidian activity in supplementing to the conventional serum therapy against these multifunctional proteins.

KEY WORDS: phospholipases A_2, phospholipases A_2 inhibitors, natural and artificial inhibitors, snake venoms.

FINANCIAL SUPPORT: FAPESP and CNPq.

CORRESPONDENCE TO: Andreimar M. Soares, Depto. de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas – Ribeirão Preto, USP, Brazil. Phone: 55+ 16-36024714. E-mail: andreims@fcfrp.usp.br

THE CENTER FOR APPLIED TOXINOLOGY (CAT), A PLATFORM FOR DRUG DISCOVERY IN BRAZIL (PART I)

ANTONIO C. M. CAMARGO(1)

(1)CAT/CEPID–Fapesp at the Instituto Butantan

The Center for Applied Toxinology (CAT) is a multi-institutional research organization based at the Butantan Institute in São Paulo (Brazil), dedicated to the study of snake toxins and other poisonous organisms. CAT is one of 10 Centers for Research, Innovation and Dissemination (CEPID) (Portuguese: **Ce**ntro de **P**esquisa, **I**novação e **D**ifusão) created by a pioneering program of the Research Support Foundation of the State of São Paulo (FAPESP). The program was established to stimulate research, disseminate knowledge, and foster interaction between science and industry.

Nature's Blockbusters

During evolution, poisonous animals became specialized in affecting vital functions of their prey. For instance, venom from *Bothrops* or *Crotalus* snakes harm, respectively, the cardiovascular system and the central nervous tissue of their prey. However, snake venoms never limit their action to a single target molecule in order to affect an important physiological function of their prey. Both *Bothrops* and *Crotalus* snakes produce a number of toxins (mainly enzymes and peptides) that imbalance the physiological levels of hormones by disturbing the activity of critical enzymes, receptors or ion channels, thus disarranging the whole cardiovascular or nervous systems of their victims. Moreover, a toxin is frequently associated with a number of highly homologous molecules displaying distinct specificity toward the same target.

Venom glands can be seen as the R&D department of a natural pharmaceutical laboratory that helps the survival of species by mutating and selecting the most appropriate toxins to ensure that sufficient damage is caused to the prey's physiological system.

Due to its high target-specificity, venom toxins have been used increasingly as pharmacological tools and prototypes for drug development. While pharmaceutical companies spend millions of dollars searching for pharmacological compounds through extensive screening of chemical libraries, venomous animals, along millions

of years, have designed their own blockbuster drugs with the help of natural selection only.

THE CENTER FOR APPLIED TOXINOLOGY (CAT), A PLATFORM FOR DRUG DISCOVERY IN BRAZIL (PART II)

ANTONIO C. M. CAMARGO(1)

(1)CAT/CEPID–Fapesp at the Instituto Butantan

Nature's Blockbusters (Cont…)

In general, toxins form the main compounds found in venoms, and secretions from a variety of animals, plants, and microbial sources. Toxins can be used for defensive purposes by damage of the cardiovascular or the nervous systems, causing a number of effects such as, blood clotting and fibrinolysis, cell migration, inflammatory processes, paralysis, etc. At CAT, we take a multidisciplinary approach in the investigation of natural toxins that includes isolation and purification, studies of pharmacological actions, toxin structural determination and structure-function studies and its molecular and cell biology aspects. Several Brazilian institutions are involved with this endeavor that is centralized at the Butantan Institute. CAT also maintains active collaborations with foreign institutions in France, England, the USA, Germany, and Japan.

Technology Transfer

In Brazil, interaction between academia and industry often leaves much to be desired. There is little technological development outside of the Brazilian Universities, where an active, high quality basic scientific research environment trains qualified personnel, and stimulates brainpower in innovative thinking. A country may, if it so decides, build scientific and technical capabilities, but the maturation of such an effort requires time and continuity.

In order to reverse this picture, CAT has established a partnership with two Brazilian pharmaceutical enterprises. Within the scope of these partnerships, research findings obtained at CAT and evaluated for patent filing have been transferred to drug development in collaboration with some of the most important Brazilian pharmaceutical industries. A team of approximately 40 scientists and 25 graduated

students and pos docs responsible for different tasks such as conducting animal trials, isolation and characterization of new compounds, molecular and cell biology, pharmacokinetic studies, among others, helped the early stage of drug development. Toxin-based drugs affecting blood clotting, the cardiovascular system, pain perception, anti-proliferate compounds and immune suppression are among those subjected to pre-clinical trials as a result of the partnership between CAT and Brazilian pharmaceutical industries.

THE CENTER FOR APPLIED TOXINOLOGY (CAT), A PLATFORM FOR DRUG DISCOVERY IN BRAZIL (PART III)

ANTONIO C. M. CAMARGO

CAT/CEPID–Fapesp at the Instituto Butantan

Education

The educational activity at CAT aims at stimulating highly qualified professionals in the areas related to poisonous animals and their toxins so they can apply this knowledge to developing new pharmaceuticals and/or agrochemical products using biotechnological processes. Additionally, because we believe that scientific literacy is essential for a country economic development, together with Butantan, CAT has been actively involved with a toxin-related bioliteracy program that includes outreach programs and educational and cultural activities. “Museu Biológico”, for instance, exhibits both live and preserved specimens to about two hundred thousand visitors per year. The herpetological collection harbors some eighty thousand specimens of snakes and serves as a center for studies on distribution, taxonomy and evolution of snakes.

Together with Instituto Butantan, CAT has offered a variety of disciplines to some two thousand students a year. For that, the CAT team relies on 19 senior investigators, students and on international collaborators invited to teach some specialized disciplines. Some disciplines offered at CAT include molecular biology in toxinology, biology of amphibians, laboratory animals, and others. The disciplines are grouped according to a field of application such as (i) education in R&D, (ii) accident prevention, (iii) biology of venomous animals, and (iv) systematic and evolution. Many disciplines are taught at different levels, for school children, teenagers and the

general public, to undergraduate and graduate students, teachers and professionals. Additionally, CAT has published specialized books.

Through its partnerships and collaborations, the CAT initiative has opened new opportunities for innovation and for the young scientist interested in drug development. Indeed, when CAT was created 5 years ago, the Brazilian pharmaceutical industry was very skeptical about utilizing academic knowledge and technical-scientific know-how to generate prosperity and innovation. Today, the pharmaceutical companies have already hired some investigators and have shown interest in hiring an additional number of young PhDs.

At CAT, it is our earnest hope that our combined efforts will launch a new pharmaceutical R&D sector in Brazil.

DESIGNING A STRATEGY FOR DRUG DISCOVERY IN BRAZIL BASED ON ANIMAL TOXINS.

ANTONIO C. M. CAMARGO(1)

(1)CAT-CEPID-FAPESP, Instituto Butantan, São Paulo, SP.

The Center for Applied Toxinology (CAT-CEPID) performs cutting edge basic and applied research with an innovative character. Following earlier scientists like Rocha e Silva and Sergio Ferreira, animal toxins are being analyzed for their pharmacological properties. Venoms include a dmall number of structural frameworks. They are selective, potent and stable molecules, representing a unique source of leads and structural templates for new therapeutic agents. This Center of Research, Innovation and Dissemination has its headquarters at the Butantan Institute, and is associated with research laboratories of two São Paulo State Universities, UNESP and USP. (2001-2004), the infrastructure for Science & Technology was set up for prospecting toxins among poisonous animals. The aims and strategy based on (1) Providing conditions for modern science & technology development, (2) stimulating multi-disciplinary approaches, attracting scientists of distinct backgrounds, and (3) identifying and characterizing toxins of medical and pharmaceutical interests. Research facilities for Molecular and Cell Biology, Mass spectrometry, Protein chemistry and enzymology, and Proteomics helped increasing the impact-index of scientific publications, and filing 9 patents. Innovation activities aimed at establishing the intellectual property concept and rights among Brazilian biomedical scientists, understanding and applying the concepts and approaches of modern drug discovery, and stimulating lawyers to study Brazilian and international patent laws and economics applied to the pharmaceutical area. In addition actions were undertaken to stimulate the synergy among research institutions, government agencies and private enterprises for pharmaceutical and biotechnology innovations. In the second phase (2005-2009), the aims shifted to create an infrastructure for the early stage of drug development, involving toxin target identification, which will be performed in special laboratories for: Peptide synthesis and pre-formulation, bioassays, proteolytic enzyme inhibitors, and ion channel studies. Associated laboratories for structural biology and modeling, and bioinformatics are joining CAT, in order to investigate the molecular and cellular mechanisms of action of natural and synthetic molecules; to investigate the *in vivo*, *ex vivo* and *in vitro* properties of natural and synthetic molecules; and to help the pharmaceutical industries in the early stage of drug development.

FROM ENDOGENY TO INTERNATIONALIZATION

BENEDITO BARRAVIERA (1)

(1) Center for the Study of Venoms and Venomous Animals

Scientific journals are undoubtedly the greatest tools for Toxinology worldwide. Unfortunately, no more than 10 units are distributed for the five continents. In 1995, a group of researchers from The Center for the Study of Venoms and Venomous Animals launched a periodical on this subject. Since 2003, the editorial board of *The Journal of Venomous Animals and Toxins* decided to adopt a politics for the growth and internationalization of the journal. The first step was to change its title to *The Journal of Venomous Animals and Toxins including Tropical Diseases*, aiming at increasing its subject area. Then, a process to increase the frequency of publication from twice a year to three times a year and then four times a year was developed. This initiative was based on the minimum goals suggested by the international scientific community for journals of the biological area, which consists in publishing at least four numbers and 32 scientific articles per year. Moreover, endogeny, i.e. the publication of at least 70% papers from the journal's institution, has to be gradually reduced to promote internationalization. Thus, in 2003, we published two numbers containing 16 scientific articles: six (37.5%) from our Institution (UNESP), three (18.75%) from other Brazilian research institutions, and seven (43.75%) from different countries (Saudi Arabia, India, the United Kingdom and Egypt). In 2004, we published three numbers totalizing 22 papers. Out of these, 11 (50%) were from UNESP, six (27.27%) from other Brazilian research institutions, and five (22.72%) from different countries (Venezuela, Egypt, Colombia and India). In 2005, we achieved the desired four numbers per year containing 44 scientific articles. Out of these, 26 (59.09%) were from UNESP, 10 (22.72%) from other Brazilian research institutions, and eight (18.18%) from different countries (Saudi Arabia, India, Bulgaria, Turkey and Mexico). In 2006, we consolidated the four numbers per year and, again, published 44 papers: 15 (34.09%) from UNESP, five (11.36%) from other Brazilian research institutions, and 24 (54.54%) from different countries (India, Argentina, Iran, Egypt, Australia, Saudi Arabia, Turkey, Korea, Uruguay, Japan and Sudan). Now, we are encouraging international researchers to submit their papers in order to increase the participation of the international community in our periodical. Moreover, we intend to obtain important indexations, which will improve the consolidation and spreading of the publication. It must be emphasized that the contribution from UNESP, the virtual library SciELO and CNPq has been extremely important to the maintenance and growth of the journal.

CORRESPONDENCE TO: Benedito Barraviera, Caixa Postal, 576, 18.618-000 Botucatu SP Brazil. Email:bbviera@jvat.org.br

"INDUSTRIAL" SCREENING AND PREDICTION OF BIOACTIVE MOLECULES

CARLOS BLOCH JR.(1)

(1)Laboratório de Espectrometria de Massa; Embrapa Recursos Genéticos e Biotecnologia.

A novel methodology for antimicrobial and other bioactive peptide screening have been described. A set of 3D plots based on Physical-Chemical properties is initially used to identify, in the 3D space, the specific volume in which a given biological activity may be represented. Each activity volume in the 3D space is primarily determined by its respective cluster of amino acid sequences obtained from natural occurring peptides with well established biological functions and graphically represented as Physical-Chemistry spheres. Entire proteins (or even genomes) are randomly digested *in silico* producing peptides that can be analyzed according to their clustering tendencies into each specific activity volume in the 3D space plot. The putative function attributed to a given amino acid sequence must be evaluated by *in vitro* and *in vivo* assays after chemical synthesis and/or gene expression of the peptides. Practical examples of this technique will be given as well as biological activity evaluations.

CORRESPONDENCE TO: E-mail: cbloch@cenargen.embrapa.br

TOXINOLOGY AND BIODIVERSITY OF THE CAATINGA

JARED C., ANTONIAZZI M.M.(1)

(1)Laboratory of Cell Biology, Instituto Butantan, São Paulo.

The Caatinga, the semi-arid region, constitutes a morphoclimatical domain embracing about 800,000 km², representing 70% of the north-eastern area and 11% of the Brazilian territory. It is characterized by high temperatures and limited, unpredictable rainfall (300-800 mm/year) from January to March. The soil is sandy and rocky and the landscape is marked by sparse emergences of naked igneous rocks. The vegetation is composed by an expressive proportion of endemic species and is characterized by thorny, xeromorphic bushes and small trees with small leaves, and by an abundance of cacti. For most of the year the landscape remains brownish. After the rare occasions when rain falls, the vegetation rapidly becomes green and flourishes for a brief time. Because the rivers are all temporary, during the dry seasons, water supply is only available in weirs or in waterholes, usually dug at the riversides or in the dry riverbeds. In the fauna there is also a considerable number of endemic species. The herpetofauna is particularly abundant with 97 reptile species and 45 amphibian species, representing an important source for toxinological studies. Birds are represented by around 200 species. Mammals are less abundant with a few endemic species. The Caatinga is being severely affected by human activity and, due to the substitution of natural vegetation for plantations, the soil is passing through a gradual process of desertification. This brings serious consequences for maintenance of biodiversity, in special of the endemic species which are more susceptible to environmental modifications. In the last two decades, our group has dedicated special attention to the amphibian fauna of the Caatinga, studying many species and integrating data about their natural history, skin morphology and cutaneous secretion toxinology. With these studies we intend to contribute to the general knowledge of the amphibians of the semi-arid and, in particular, to indicate promissing species to be studied by the toxinological viewpoint.

KEY WORDS: Caatinga, semi-arid, biodiversity, toxinology, cutaneous secretions.

FINANCIAL SUPPORT: CNPq.

CORRESPONDENCE TO: Carlos Jared, Instituto Butantan, 05503-900, São Paulo, Phone: 11 3726-7222 ext. 2234, E-mail: jared@usp.br

HOMO AND HETERODIMERIC SNAKE VENOM PHOSPHOLIPASE A_2S: STRUCTURE AND INHIBITION

CHRISTIAN BETZEL[1], TEJ P. SINGH[2], R.K. ARNI[3,4] AND NICOLAY GENOV[5]

[1]Department of Biochemistry and Molecularbiology, University of Hamburg, c/o DESY, Notkestrasse 85, 22603 Hamburg, Germany

[2]Department of Biophysics, All India Institute of Medical Sciences, New Delhi 110029, India

[3]Department of Physics, IBILCE/UNESP, SJRP, Brazil & [4]Center for Applied Toxinology, CAT-CEPID, São Paulo, SP, Brazil.

[4]Institute of Organic Chemistry, Bulgarian Academy of Sciences, "Akad. G. Bonchev"-str. bl. 9, Sofia 1113, Bulgaria

Phospholipases A_2 (PLA_2s) play an important role in a number of physiological processes such as phospholipid metabolism and remodelling, mediator production, homeostasis of cellular membranes, host defense and signal transduction. In the same time, they are involved in a number of inflammatory diseases. Snake venom PLA_2s exert additionally a wide variety of pharmacological activities and for all these reasons it is of high pharmacological and medical interest to develop specific inhibitors, which can be used for a rational design of anti-inflammatory drugs. We have determined the three-dimensional structures of a number of homo- and heterodimeric, snake venom PLA_2s, native as well as in complex with naturally occurring anti-inflammatory agents and synthetic inhibitors to high resolution, applying synchrotron radiation The structures revealed specific interactions that are important for the molecular recognition and this knowledge was applied for *de novo* design of inhibitors with improved potency.

Acknowledgements. Financially supported by CAPES/DAAD

IDENTIFICATION OF CONTINUOUS INTERACTION SITES IN PLA_2-COMPLEXES BY PEPTIDE ARRAYS

CONSUELO LATORRE FORTES-DIAS(1)

(1)Fundação Ezequiel Dias (FUNED), Belo Horizonte, MG, Brazil

Crotoxin, the main neurotoxin of *Crotalus durissus terrificus* snake venom, is a heterodimer formed by an acidic protein (CA) devoid of any biological activity per se and a basic, enzymatically active, PLA_2 counterpart (CB). The SPOT synthesis methodology was used to map possible interaction sites in two PLA2-based complexes: CA.CB (crotoxin) and CNF.CB, a nontoxic complex formed between CB and an endogenous PLA_2 inhibitor from *C. d. terrificus* plasma. Sets of overlapping dodecapeptides, spanning the whole primary structure of two main isoforms of CB (CB_1 and CB_2) were immobilized on a membrane and probed with CA or CNF previously conjugated to alkaline phosphatase. Main reacting peptide clusters were identified on the CBs as well as amino acid stretches susceptible to be involved in protein-protein interactions in the heterodimers. A highly reactive C-terminal cluster, exclusive to CB_1, might explain, at least partially, the differences in stability of crotoxin, depending on the CB isoform present, as well as the apparent preference of CNF for CB_2.

KEY WORDS: phospholipase A_2 inhibitor, crotoxin, phospholipase A_2, SPOT synthesis, *Crotalus durissus terrificus*

CORRESPONDENCE: R. Conde Pereira Carneiro 80, 30510-010, Belo Hte, MG. Phone: +55 31 33719485; e-mail: consuelo@funed. mg.gov.br

ISOLATION AND CHARACTERIZATION OF A NOVEL BRADYKININ POTENTIATING PEPTIDE (BPP) FROM THE SKIN SECRETION OF *Phyllomedusa hypochondrialis* (DAUDIN,1802).

Pimenta D.C.(1), Conceição, K. (1), Konno, K. (1), Melo, R.L. (1), Antoniazzi, M.M. (2), Jared, C. (2), Sciani, J.M. (3), Conceição, I.M. (3), Prezoto, B.C. (3), Camargo, A.C.M. (1)

(1) LETA (Laboratório Especial de Toxinologia Aplicada), Center for Applied Toxinology (CAT/CEPID). Instituto Butantan, Avenida Vital Brasil, 1500, São Paulo, SP, 05503-900, Brazil. (2) Departmento de Biologia Celular. Instituto Butantan, Avenida Vital Brasil, 1500, São Paulo, SP, 05503-900, Brazil. (3) Laboratório de Farmacologia. Instituto Butantan, Avenida Vital Brasil, 1500, São Paulo, SP, 05503-900, Brazil

Bradykinin potentiating peptides (BPPs) from *Bothrops jararaca* venom were first described in the middle of 1960s and were the first natural inhibitors of the angiotensin-converting enzyme (ACE). BPPs present a classical motif and can be recognized by their typical pyroglutamyl (Pyr) / proline rich sequences presenting, invariably, a proline residue at the C-terminus. In the present study, we describe the isolation and biological characterization of a novel BPP isolated from the skin secretion of the Brazilian tree-frog *Phyllomedusa hypochondrialis*. This new BPP, named Phypo Xa presents the sequence Pyr-Phe-Arg-Pro-Ser-Tyr-Gln-Ile-Pro-Pro and is able to potentiate Bradykinin activities *in vivo* and *in vitro*, as well as efficiently and competitively inhibit ACE. This is the first canonical BPP (i.e. Pyr-Aaan-Gln-Ile-Pro-Pro) to be found not only in the frog skin but also in any other natural source other than the snake venoms.

KEY WORDS: *Phyllomedusa hypochondrialis*, Bradykinin-Potentiating peptide, mass spectrometry, de novo sequencing, natural peptides, secretion, Bradykinin.

FINANCIAL SUPPORT: CAT/CEPID, FAPESP, CAPES, CNPq.

CORRESPONDENCE TO: Instituto Butantan - Laboratório Especial de Toxinologia Aplicada. Avenida Vital Brazil 1500, São Paulo – SP, 05503-900, Brazil, Phone/fax: + 55 11 3726-1024, e-mail: dcpimenta@butantan.gov.br

THE MAIN MECHANISMS INVOLVED IN THE ANTIBACTERIAL ACTIVITY OF RATTLESNAKEVENOM

DANIELA D.O. TOYAMA(1)

(1)Universidade Presbiteriana Mackenzie-UPM

Crotalus durisusus ssp and is the most important snake found in the South America and from its venom it has been isolated and characterized the presence of several fraction such as crotacetin, L-amino acid oxidase, thrombin like, PLA_2, crotapotins, convulxin and crotamine. We observed that whole venom of some Brazilian rattlesnakes such *Crotalus durissus terrificus* or *Crotalus durissus cascavella* display several kind of morphological modification on the bacterial cell or massive ultra structural alteration of cell cytoplasm, which are the resulting of actions of several fraction that found in this venom. The effect induced by some this fraction solely induces membrane destruction as in case of PLA_2, although our results support that catalytic activity is the main rout for membrane destruction. Whereas in case of lectins we observed that in some cases that this protein induced the membrane vesiculation and bacterial aggregation without specific membrane destruction in case of C-type lectin isolated from the Bothrops. In this context there is other fraction and their effect on the bacterial growth rate that will discuss in this presentation.

KEY WORDS: PLA_2, L-amino acid oxidase, rattlesnake, antibacterial.

FINANCIAL SUPPORT: FAPESP, CNPq.

DERMONECROSIS: SCIENTIFIC PATHS FOR NEW PROMISING TREATMENT ALTERNATIVES

DENISE V. TAMBOURGI(1)

(1)Laboratório de Imunoquímica, Instituto Butantan

Envenomation by spiders belonging to the *Loxosceles* genus (brown spider) often results in local dermonecrotic lesions. We have previously shown that *Loxosceles* sphingomyelinase D (SMase D), the venom component responsible for all the pathological effects, induced the expression of matrix metalloproteinases (MMPs) in rabbits and in human keratinocytic cells. We also showed that the SMase D induced apoptosis and MMP expression of keratinocytes was inhibited by tetracyclines. The aim of this study was to further investigated the ability of tetracyclines to inhibit or prevent the dermonecrotic lesion induced by *Loxosceles* venom *in vivo* and *in vitro* models. Primary cultures of rabbit fibroblasts incubated with increasing concentrations of venom or SMase D showed a decrease in cell viability, which was prevented by tetracyclines. *In vivo* experiments showed that topical treatments with tetracycline of rabbits, inoculated with crude *Loxosceles intermedia* venom or recombinant SMase D, significantly reduced the progression of the dermonecrotic lesion. Furthermore, tetracyclines also reduced the expression of MMP-2 and prevented the induction of MMP-9. Our results suggest that tetracycline may be an effective therapeutic agent for the treatment of cutaneous loxoscelism.

KEY WORDS: Dermonecrosis, Tetracycline, Sphingomyelinase, *Loxosceles* Spider Venom

FINANCIAL SUPPORT: FAPESP, CNPq and The Wellcome Trust

CORRESPONDENCE TO: Dr. Denise V. Tambourgi, Laboratório de Imunoquímica, Instituto Butantan, Av. Prof. Vital Brazil, 1500, CEP 05503-900, São Paulo, Brazil. Tel –55-11-3726-7222 ext. 2106. Fax: -55-11-3726-1505.
E-mail: dvtambourgi@butantan.gov.br

APPROACHES AND TASKS DIVISION FOR ACADEMY - INDUSTRY PARTNERSHIP ON NEW DRUGS DISCOVERY IN BRAZIL

EDUARDO PAGANI, MD, PhD(1)

(1)Clinical Trials Manager from Cristalia

The first word pharmaceutical companies proudly divulges that for every 10,000 new synthetic compounds screened, 250 start pre-clinical studies, 5 arrive to the clinical phases and only one is finally marketed. This "compound killing" approach costs over $800,000,000.00. Many argue that this number is artificially inflated. The real number could lowered to a half, but hardly to a tenth of it. This is still too much for Brazilian sponsors to afford. Brazilian scientists and pharmaceutical companies could and should work together in order to propose a cheaper but trustable approach for new drugs discovery. Toxins allow for a "compound optimization" approach that could avoid exhaustive screening. Accidental poisoning by natural toxins produces clinical manifestations attributable to toxin's components. Some can be isolated and purified giving birth to drug candidates. The first task is to perform *in vitro* and *in vivo* experiments aimed to widen the comprehension on its biological effects. The demonstration of *any* effect in concentrations below 10^{-8} M is a strong indicator of specificity. Up to this point, the job was predominantly done by the academy, more tolerant to negative results after long time spending especially if these could be published. But, if a specific biological effect was consistently demonstrated, a putative target should be chosen and validated. It is the time for publication refraining, geting advice on patentability and looking for partnership with a pharmaceutical company. The next tasks are: toxicity and pharmacokinetic assessing. These studies are better performed by specialists. These can be in the academy or in a private institution. The industry can contract and support the service. This information will drive the compound's optimization. Could the molecule weight be lowered? Could anything be done to improve its bioavailability? What is the best way to write the patent? The industry can provide financial support as well as technical advice on these subjects. It also can perform a preliminary evaluation of marketability. After the formulation establishing by the industry, the clinical trials start. They are usually projected together by the academy and the industry, conducted at the academy and monitored and sponsored by the industry. The submission of the dossier to the health authorities is a bureaucratic and heavily regulated task better performed by the industry. The pharmacovigilance starts after product launching. This is also an industry's task.

PROTEOMIC ANALYSIS OF *OPISTHACANTHUS CAYAPORUM* SCORPION VENOM

ELISABETH FERRONI SCHWARTZ(1)

(1)Laboratório de Toxinologia, Universidade de Brasília, efschwa@unb.br

Many animals produce and secrete venoms used to their defense and/or to capture prey. Scorpion venom contains a large variety of active components such as peptides with 22-47 amino acid residues that recognize K^+ channels and peptides with 58-76 residues that affect Na^+ channels function. Both toxins are structurally related, adopting the "Cysteine Stabilized α/β motif" (Csαβ-motif), in which two strictly conserved disulfide bridges cross-link an alpha helix segment of the peptide with the second strand of an alpha-beta-beta minimum topology. These neurotoxins have played an important role as tools for the identification, purification, and functional characterization of voltage-gated ion channels. Another class of larger peptides containing 59-75 amino acid residues have also been described. This class is tightly folded by three disulfide bridges and show two structural domains: a linear N-terminus resembling cationic antimicrobial peptides and a Cysteine-rich C-terminus, with the consensus signature of Csαβ-motif, which is supposed to confer a K^+ channel blocking activity to some of these peptides. Non-disulfide-bridged peptides are a different class of scorpion venom peptides exhibiting antimicrobial, hemolytic, bradykinin-potentiating and immune-modulating activities. Phospholipases may also be present in substantial amounts in scorpion venom. The Ischnuridae family includes the genera *Opisthacanthus*, *Hadogenes* and *Cheloctonus* which are not venomous to man, and a sting from one of them should, at worst, be no more than a bee sting. Due to its lower toxicity there have been few reports on the venom of scorpions from the Ischnuridae family. This family is distributed through Africa, south-east Asia, Australia and South America and associated islands. *Opisthacanthus* occurs in the Caribbean, Central and South Americas, Africa and Madagascar. The genus *Opisthacanthus* presents a gondwanian pattern of distribution. In Brazil, the family Ischnuridae is represented by two species of *Opisthacanthus*. *O. cayaporum* is endemic to open savannas in the eastern Amazonia (south of the State of Pará and State of Tocantins). In this conference it will be presented the fractioning of the *O. cayaporum* crude venom, showing the complex profile of molecular masses of its components. Besides, it will also be presented the purification of the main peptide components, their N-terminals sequences, and the biological activities of some of them.

ARANEISM AND SCORPIONISM IN THE STATE OF PARANÁ: AN EPIDEMIOLOGICAL APPROACH

MARQUES-DA-SILVA E.(1)

(1)Seção de Animais Peçonhentos, Centro de Produção e Pesquisa de Imunobiológicos – SESA - Paraná;

The epidemic and environmental surveillance of the accidents caused by venomous animals in the Paraná State is made by the Division of Zoonosis and Intoxications - Center of Environmental Health of the State Secretariat of Health. The Paraná State is composed of 399 municipal districts divided in 22 Health Regional Units (HRU) and there are also four Centers of Toxicological Information (CTI). Between 2001 and october 2006 60,502 accidents were notified by the System of Information of Offences of Notification (SINANW). From the total of notified accidents above, 74% (n=44,825) were caused by spiders and 3% (n=2,072) by scorpions. Among the accidents caused by spiders the genus *Loxosceles* (brown spiders) caused 70%, and spiders of the genus *Phoneutria* 8%. A matter of concern has been the increase in the number of accidents caused by scorpions during the period of time cited above. The occurrence of accidents caused by the scorpions *Tityus stigmurus*, *T. serrulatus*, and *Rhopalurus rochai* in different areas of the State is receiving the health professionals´ attention for this new reality. Due to the service of identification of poisonous arthropods made available to the HRUs now we can estimate the distribution of the species of higher toxicological importance in the municipal districts of the State, and evaluate its relationship with the registered epidemiological characteristics. The data on signs and registered symptoms reflect the quality of the service to the patient, driving the surveillance service to question factors such as the degree of toxicity of the animal and the therapeutics used. Trainings are provided seeking for the improvement in the quality of the service to the accident´s victim. Therefore, the attention to the progresses in the studies of biotoxins, either in the production of imunotherapics, or in the physiopathological actions related to each toxin or their fractions, is an important tool to the quality of the services of health.

KEY WORDS: epidemiology, venomous animals, *Loxosceles*, *Tityus*, *Phoneutria*, Paraná.

CORRESPONDENCE TO: EMANUEL MARQUES DA SILVA, Seção de Animais Peçonhentos, Centro de Produção e Pesquisa de Imunobiológicos (CPPI). R: Targino da Silva s/nº, Piraquara – Paraná – Brasil. CEP: 83302-160. Email: ems@pr.gov.br

NEUTRALIZATION OF AMAZONIAN CROTALINE SNAKE VENOMS BY BRAZILIAN ANTIVENOMS

MUNIZ E. G. (1), NORONHA M. D. N. (1), NASCIMENTO S. P. (2), SILVA-HAAD J. J (3), LÓPEZ-LOZANO J. L. (1)

(1) Fundação de Medicina Tropical do Amazonas, Amazonas, Brazil, (2) Instituto Nacional de Pesquisas da Amazônia, Boa Vista, Roraima, Brazil, (3) Hospital Regional de Letícia, Colômbia.

Efficacy of brazilian antivenoms to neutralized the several toxic effects induced by *Bothrops atrox* and *Crotalus durissus ruruima* venoms from Amazonas and Roraima State – Brazil, were studied. Antivenoms (AV) used were antibothropic (AB) or anticrotalic (AC) from Fundação Ezequiel Dias (FUNED), Instituto Butantan (IB), Instituto Vital Brazil (IVB) and polyvalent - IB/IBEX to neutralized *B. atrox* or *C.d.ruruima* venom activities. Potency of antivenoms against lethal activity of *B. atrox* venom was 2.4 to 3.5 mg venom/ml AV, but the minimum potency of AB recommended is 1ml neutralizing 5 mg of *B. jararaca* venom. *B. atrox* (Manaus region) defibrinating venom activity was neutralized by all antivenoms tested ED100 <125 µlAV/mg venom, but to *B. atrox* venom from Letícia region was <125 to 500 µlAV/mg venom. All antivenoms neutralized coagulant activity of *B. atrox* venoms ED < 25 µlAV/mg venom and 125 µlAV/mg venom from Manaus and Letícia regions, respectively. Hemorrhagic effect was neutralized by all antivenoms ED100 ranging 375 to 750 µlAV/mg venom. Antivenoms showed neutralizing ability against phospholipase activity ED100 375 to 750 µlAV/mg venom. *C. d. ruruima* venoms showed qualitative and quantitative differences in the biological activities with presence or absence of hemorrhagic and crotamine activities. Lethal activity was efficiently neutralized by AC and polyvalent antivenoms, 3.1 to 5.0 mg venom/mlAV, but AC antivenoms in 5µlAC/µg venom rations, showed low neutralization efficiency of hemorrhagic activity (3 to 50%). Our results showed that brazilian antivenoms have neutralizing activity on *B. atrox* or *C.d. ruruima* venoms but with quantitative variations on the biological venom activities studied. Clinical trials are required as the better criterion for the efficacy evaluation of antivenoms.

KEY WORDS: snake venoms, antivenoms.

FINANCIAL SUPPORT: FAPEAM, FINEP, FMT-AM.

CORRESPONDENCE TO: EMIRO MUNIZ, Centro de Ofidismo Prof. Paulo Friedrich Buhrnheim, FMTAM. E-mail: emiromuniz28@hotmail.com

ANTI-VENOMS: CURRENT USE AND PERSPECTIVES. THE ROLE OF LYOPHILIZATED SERUM

FRANÇA, F.O.(1)

(1)Hospital Vital Brazil do Instituto Butantan, Hospital das Clínicas da USP

The accidents caused by poisonous animals are one of the most neglected health problems mainly in the developing countries. Chippaux estimates more than 100,000 deaths per year caused by snake bites. Brazil has at least 28.000 snake bites with more than 100 deaths per year. The perspective to produce more effective and safe antivenoms, with acceptable cost, immediate and easy administration are essential to diminish the morbidity and lethality of these accidents. Nevertheless there are a lot of limitations to achieve these objectives. Besides the antivenom produced in Brazil has high quality, we need to overcome some obstacles to avoid complications and deaths. The first problem is the geographic distance between the place where the accidents frequently occurr and the health care centers. Almost 70% of deaths in Brazil has occurred in patients who received the antivenom 6 or more hours after the accident. The National Program to Control Poisonous Animal Accidents, developed since 1966, made possible the decentralization of the distribution of antivenom serum and has promoted training to hundreds of health workers to diminish the interval between the accident and the treatment. Many authors suggest the immediate administration of intramuscular antivenom where the accident occurred to neutralize the venom quickly, others have proposed the use of lyophilizated antivenom to avoid the necessity of constant temperature to maintain the neutralizant power of liquid antivenom. Nowadays snake antivenom is basically composed of horse Ig G polyclonal immunoglobulins and the production of homologous antivenom will permit the administration of antivenom without risk of anaphylaxis reactions. There are researchers that are working to improve the capacity of neutralization of snake venoms. The use of pepsin-digested antibodies antivenom is compared with the whole IgG antivenoms in order to verify which of them are more efficient. Another possibility is the production of monoclonal antibodies that are able to recognize antigenic determinants and theoretically could have a major role in the treatment of snake bites accidents. The discovery of the components that causes abnormalities in patients can lead the production of antivenoms that neutralize only the toxins that has a role in the human accidents. These objectives are not easy to achieve because antibodies derived from animal sera are one of the most misunderstood and undervalued pharmaceutical products. Another obstacle is the complexity of the envenoming. There are variations in venoms of snakes belonged to the same genus, and differences in venoms of snakes belonged to the same species or subspecies (It is common some variation in the venom constitution of snakes belonged to the same species but with different ages and from different geographic origins). The verification of this different approaches are extremely difficult in human accidents because it's almost impossible to control the variables that influence the evolution of snake bites. Aspects related with the snakes like species, age, length; with the patients like age, weight, the time between the accident and the treatment, the use of tourniquet and aspects related to the snake bite like the quantity of venom inoculated, the region of the body and the site of venom deposition (subcutaneous, muscle or direct in a blood vessel).

RENAL AND VASCULAR EFFECTS OF VENOMS FROM BRAZILIAN NORTHEAST ANIMALS

HELENA SERRA AZUL MONTEIRO(1)

(1)Depto. Fisiologia e Farmacologia, UFC, Brazil

Crotalus durissus cascavella (*C.d.c*) is a snake usually found in scrubland of Brazilian Northeast and its bite constitutes a public health problem. *C.d.c* venom in isolated perfused rat kidney method increased perfusion pressure (PP), urinary flow (UF), and decreased glomerular filtration rate (GFR), and percent of sodium tubular transport (%TNa^+).The proximal renal tubule was the major site for its toxic effect. Dexamethasone (Dexa) and Indomethacin (Indo) protected against effects on PP, UF, GFR, %TNa^+. Nifedipine reversed all functional changes. The infusion of the supernatant of macrophages stimulated with *C.d.c.* venom increased PP, GFR, UF and decreased %TNa^+. Dexa and quinacrine provided protection against the effect of the supernatant on GFR, UF, %TNa^+, and PP. Indo and nordiidroguaretic acid reversed all functional changes, except PP. These results suggest that macrophages stimulated with *C.d.c*. venom release mediators capable of promoting nephrotoxicity. Phospholipase A_2 and cyclooxygenase products are involved in these biologic effects. Convulxin had no effect and Gyroxin produced a minor effect compared to Crotoxin. Crotoxin is the main component responsible for acute nephrotoxicity, because it altered all evaluated renal parameters. *Bothrops erythromelas* venom on the renal perfusion decreased PP, RVR, GFR, and %TNa^+. The anti-bothropic factor incubated with this venom blocked the effects on PP, RVR, %TNa^+, and %TK^+. A higher concentration reversed all renal effects. *Tityus serrulatus* (Tsv) venom on the renal perfusion increased PP, RVR, and decreased GFR, and UF. TsV increased the basal perfusion pressure of isolated arteriolar mesenteric bed and affected renal haemodynamics, probably by a direct vasoconstriction effect, leading to a decrease on renal flow. Gamma toxin (gT) of *T. serrulatus* venom increased renal PP, RVR, GFR ,UF and decreased %TNa^+, %TK^+, %TCl^-.Gamma toxin also increased the basal PP on mesenteric bed. gT affected renal hemodynamics increasing vascular resistance and PP in isolated kidney and mesenteric bed, suggesting a direct action. *T. nattereri* (Niquim) venom was studied on renal perfusion and caused increases on PP, RVR, GFR and UF. A higher dose promoted alterations in all renal parameters.

KEY WORDS: renal and vascular effects, venoms

FINANCIAL SUPPORT: CNPQ, CAPES, FUNCAP

EPIDEMIOLOGY OF THE ACCIDENTS CAUSED BY ANIMALS OF TOXICOLOGICAL INTEREST IN RIO GRANDE DO SUL

HUDSON BARRETO ABELLA(1)

(1)Centro de Informação Toxicológica do Rio Grande do Sul, Fundação Estadual de Produção e Pesquisa Saúde, Secretaria Estadual de Saúde.

The Centro de Informação Toxicológica do Rio Grande do Sul is an unit of medical emergency that has as a goal the assistance to toxic accidents occurred in the State. It acts in 24-hour duty regimen through the free call number 08007213000 to all Brazil. In the period of 1980 to 2005 CIT/R took care of 286,478 occurrences. The total of human exhibitions which stood out in this period was of the accidents with medications (62,071 requests - 28.1%) and with venomous animals (61,172 requests - 27,7%). The occurrences seasonality with venomous animals was larger in hot months and smaller in the winter. Analyzing the records of 2005, 18.883 human intoxications are reported. The cases of human accidents with venomous animals represented 5.604 records where 29% had favorable evolution and 3% evolved to death. Highlights in this group: The accidents with spiders - 1.826 occurrences of which 520 reported as accidents by *Loxosceles* sp and 475 by *Phoneutria* sp; accidents with snakes - 1,010 occurrences reported of which 955 with the Bothrops sort; scorpions - 349 registers of which 286 with *Bothriurus bonariensis*, *Tityus serrulatus* 10, *Tityus costatus* 27; insects - 459; caterpillars - 878; aquatic - 14; other venomous animals - 264 and indeterminate venomous animals - 804. The age frame with larger number of records corresponds to people with age up to 19(3,865 registers - 69%). The anatomical region with larger number of occurrences reported with spiders and scorpions were the upper and lower limbs. In the accidents with snake it is the lower limbs (72%). In the accidents with caterpillars it is the upper limbs (62%).

KEY WORDS: Epidemiology, snake, scorpions, spider, animal poisoning

ELECTRONIC ADDRESS: hudson-barreto@fepps.rs.gov.br

SCARS OF THE MOLECULAR EVOLUTION OF SNAKE TOXINS REVEALED BY TRANSCRIPTOMIC STUDIES

JUNQUEIRA-DE-AZEVEDO I. L. M. (1,2,3); CHING, A. T. C. (1,2); LEÃO, L. I. (1,3); HO, P. L. (1,2,3)

(1) Centro de Biotecnologia, Instituto Butantan, São Paulo, SP, Brazil. (2) Interunidades em Biotecnologia and (3) Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, Brazil.

Snake toxins are largely known to undergo various evolutionary processes, such as gene duplication, intense paralogy, accelerated evolution, multiple recruitments of important scaffolds, simplification of multidomain structures and others. In the last years, a huge amount of transcriptomic data from snake venom glands, mainly provided by ESTs (Expressed Sequence Tags) initiatives, were made available. Besides profiling the toxin expression in such tissues and describing new toxins unlikely to be found by venom based analysis, these approaches also reveal unusual toxin variants and cellular transcripts, opening windows for the investigation of toxin and venom gland evolution and physiology. Analyzing the whole amount of data, publicly available and those from our databases generated from Viperidae, Elapidae and Colubridae species, we found some interesting features of toxin and non-toxin cDNAs that are discussed here. For instance, the metalloprotease sequences reveal scars of several kinds of evolutionary process, from gene duplication by a retrotransposon-like insert to the loss of domains by stop codon mutations and segment exchanges. In accordance, retrotransposon sequences are found in the untranslated regions of some toxin genes, and curiously, somatically transcribed at high levels in the venom glands. Old scaffolds of some very known toxins, such as the carbohydrate binding domains (CRD) of C-type lectins, appear to be recruited more than once in the venom evolution, and others, like the three-finger domain (3FTx) of alpha-neurotoxins, may be widespread in unlikely venoms, such as from a Viperidae. These and other observations reinforce the idea of a rapid evolution of the venom system and provide the evidences of the involved mechanisms.

KEY WORDS: transcriptome, venom, molecular evolution, retrotransposon.

FINANCIAL SUPPORT: FAPESP, Fundação Butantan

CORRESPONDENCE TO: Av. Vital Brasil, 1500, Instituto Butantan, 05503-900, São Paulo-SP, Brazil, Centro de Biotecnologia +55 11 37267222 ext 2244 email: ijuncaze@butantan.gov.br

TOXINOLOGY STUDIES IN AMAZONAS STATE- BRAZIL: STATE OF ART AND FUTURE

LOPEZ-LOZANO, J.L. (1,2), MUNIZ, E. G. (2), NORONHA, M.D.N. (1,2)

(1) Programa de Pós-graduação em Biotecnologia e Recursos Naturais - Universidade do Estado do Amazonas- UEA; (2) Centro de Ofidismo Prof. Paulo Friedrich Bürnheim / FMTAM.

Clinical and epidemiological data of human accident snake bite was the first toxinological study in Amazonas state, showing that *B. atrox* is the main snake specie in human accidents but other snake species with very low incidence. Histological effects produced by *Lachesis muta* venom in mice was studied using electronic microscopy. *Latrodectus curacaviensis* spider was detected in urban areas of Manaus city but no high human accidents were reported. Potency of Brazilian equine serum antivenoms against biological activities from *Bothrops*, *Crotalus* and *Lachesis* venoms were studied in mice and this experiment showed that are necessary more human clinical evidences to new equine serum against venoms from Amazonian snakes species. Proteomics studies about molecular composition from *Bothrops, Bothriopsis, Crotalus, Lachesis* and *Micrurus* venoms and structural analyses of venom toxins and ontogenetic changes of biological activities and molecular composition in *B. atrox* venom were studied. Clinical studies using lyophilezed polyvalent and plants extracts against *B. atrox* and *C. d. ruruima* venoms in mice are in course. Actually are starting studies about molecular composition and biological activities from amphibian, scorpions, spiders, wasp and insect larvae toxins, *B. atrox* venom gland transcriptome and toxin inhibitors in plasma from Amazonian snake species. Strictu sensu pos-graduation in Biotechnology and Natural Resources together genomic and proteomic technological platforms available in Genomic and Proteomic Amazonas Networks have a high impact to development of toxinology studies in Amazonas State. Biotechnological applications of toxins as anti-insect, antimicrobial, enzymes, immune response modulators and inhibitors of tumor metalloproteinases are in course.

KEY WORDS: Amazonia, toxins, biotechnology.

FINANCIAL SUPPORT: FAPEAM, FINEP, FMTAM.

CORREPONDENSE TO: J. LUIS LÓPEZ-LOZANO, Centro de Ofidismo Prof. Paulo Friedrich Bürnheim, Fundação de Medicina Tropical do Amazonas, Manaus, Brasil. Phone: + 55 92 3238 4294. E-mail: luisiam@hotmail.com

EXPERIENCE IN THE TREATMENT OF ACCIDENTS CAUSED BY VENOMOUS ANIMALS IN THE STATE OF CEARA, BRAZIL

GUIMARÃES J.A. & GUIMARÃES, B.R.P.A.

Centro de Assistência Toxicológica, Instituto José Frota, Ceará, Brasil (CEATOX/IJF)

Having participated of the National Course of Ophidism and Other Venomous Animals, in the Butantan Institute in São Paulo, December 1987, sponsored for the Brazilian Health Ministery, we engage as a multiplying agent in clinic of accidents with venomous animals in the state of the Ceará. The training happened at a critical moment of the history of the ophidism in Brazil, arising in 1984 with the lack of anti-venom serum to supply the national demand. The results of the action of the government were the magnifying of knowledge for the improvement of the diagnosis and the outcomes of the treatments, thus diminishing the human suffering with sequels and mortality. Coming back of the Course, we assumed the coordination of the Center of Toxicological Assistance of the José Frota Institute (CEATOX/IJF), where we start to act as physician and advisor of the clinical cases related the venomous animals. In the ten first years of intense participation in the hospital, we had been involved in practically all the 26,313 registered cases of poisoning, between them 4,573 accidents due to venomous animals and 1,587 non-venomous ones. We diminish the lethality for 0.13%, due to good quality antivenin serum, as well as support of the authorities in the qualification of the health professionals. Significant facts occurred in this period, such as the first register of accident caused by *Lachesis* snake, coming from the Maciço de Baturité. Until then, such evidence had not been recognized by Health department and, therefore, we did not have the specific serum to treat the patient. It just arrived three days after the accident, when patient had been submitted to several blood transfusions due to a severe local hemorrhage since the first minutes after the accident, and the improvement of the patient only occurred six hours after the application of the antilaquetic serum. Another important register says respect the notification of cases of accidents with black-widow spiders (*Latrodectus curacaviensis*) in the State of the Ceará, being presented on VII Congress of the Associación Latinoamericana de Toxicologia. With the constant participation of many colleagues in the clinical practice we elaborate, in 2005, a spread sheet of data to facilitate to the correct diagnosis and the treatment of the accidents. This spread sheet was registered in the Self-Regulating Body of the Medicine Profession of the State of the Ceará (CRM/CE) in order to improve the adhesion of the health profissionals in the participation and attendance of victims of accidents due to venomous animals, independent to have previous specific training. Currently, we are developing partnerships with Universities and courses of the health area, in order to develop clinico-epidemiological research in the direction to optimize the attendance, as well as the behaviors that front to accidents caused by venomous animals in the State of the Ceara.

KEY WORDS: Ophidism, venomous animals, human accidents

CORRESPONDENCE TO: josebetiene@hotmail.com

IMPORTANCE OF THE BRAZILIAN COASTAL BIOME FOR TOXINOLOGY

JOSÉ CARLOS DE FREITAS(1)

(1)Departamento de Fisiologia, IB, USP, São Paulo, Brazil; Center for Applied Toxinology, CAT/CEPID- FAPESP, Instituto Butantan, São Paulo, Brazil; Email: jfreitas@usp.br Phone: 55-11-3091-7522. FAX: 55-11-3031-5028 Biosciences Institute,USP

During the last 15 years, our group have intensively collected species of marine organisms occurring along the Brazilian coast. We have been searching for toxins in a variety of ecological sites, as plankton, benthos and nekton. About 35 compounds were isolated and already identified, and some were novels, mainly from sponges, tunicates and sea-anemones found in São Sebastião channel, São Paulo State. Various bioassays are currently used, including hemolytic, anti-mitotic, neurotoxic, anti-inflammatory and cytotoxic. The evaluation, in some cases, follow up to binding assays and human cell line assays. By using four different techniques, as mouse bioassay, ELISA using T20G10 monoclonal antibody, HPLC and mass spectrometry, the toxicity in the extracts of *Colomesus asellus*, a freshwater pufferfish from the rivers in the Amazonian rain forest, was analysed and identified for the first time. The components responsible for its toxicity were only Paralytic shellfish Poisons (PSPs) Saxitoxin (STX), Gonyautoxin 2 (GTX 2) and GTX 3. These data were also confirmed by electrospray ionization mass spectrometry. An indirect competitive enzyme immunoassay using tetrodotoxin (TTX) showed very low affinity for *C. asellus* extracts, indicating that TTX and its analogues are not the main toxic components of these extracts. The antibody was efficient in detecting presence of TTX in a total extract of *Sphoeroides spengleri,* which is one of the most toxic puffer fish found in the Atlantic coast. Studies dealing with the venomous mollusk *Conus regius* from Fernando de Noronha Archipelagous (PE) and sea anemones *Bunodosoma caissarum* and *B. cangicum*, from São Paulo coast have shown a variety of biologically active peptides. We have determined the sequences of novel conotoxins from the Brazilian cone snail, *Conus regius*, and peptide neurotoxins from the sea anemones, *B. caissarum* and *B. cangicum*, by MS and NMR analyses. LAS390 is a low molecular weight and non-peptidic compound purified from *B.cangicum* venom that induces analgesia in rats. The characterization of this analgesic effect and the mechanisms involved in this effect is evaluated in pain threshold using the rat paw pressure test, applied before and at different times after treatments. LAS390 induces analgesic effect that is mediated by histamine and serotonin receptors and involves activation of voltage-gated K^+ channels. A new hemolytic toxin named caissarolysin I was isolated from the Brazilian sea anemone *B. caissarum* and identified as a protein of MW 19757, which lacks PLA2 activity but is recognized by antiserum against Equinatoxin II, a toxin isolated from *Actinia equina* of Mediterraneum Caissarolysin I has high hemolytic activity in human erythrocytes (ED50 = 0.270 µg/ml) and was inhibited by pre-incubation with sphingomyelin. Another study dealing with the dinoflagellate *Prorocentrum mexicanum* have shown that its organic extract (polar) blocked action potentials of crustacean isolated nerves. Also there are evidences of apoptotic events induced by *P. mexicanum* polar extract in T47D Cells. These type of activity have been observed for okadaic acid and yessotoxin, known cytotoxins found in dinoflagellates from the Pacific coast. This coming years we are going to extend our collections of marine organisms in São Pedro and São Paulo Rocks (RN), Fernando de Noronha Archypelagous (PE) and Antartic ocean to follow up these studies.

ACKNOWLEDGEMENTS: FAPESP, CNPq.

PRIMORDIA OF ANTIVENIN THERAPY IN OCCIDENTAL MEDICINE

JOÃO LUIZ COSTA CARDOSO(1)

(1)HOSPITAL VITAL BRAZIL, I.BUTANTAN, S.PAULO.

Historical aspects of antivenom treatment have been neglected in the literature. Cornelius Celsus, a Roman author during Emperor Tiberius (A.D. 14-37) wrote in the book De Medicina the basic guidelines to treat patients bitten by snake: " *Therefore first the limb is to be constricted above this kind of wound (…) make incisions with a scalpel around the wound*…"(1). Those instructions are until our days used as first aid measures. Many empirical procedures were advocated by different authors in these last 20 centuries including the utilization of plants, bones, secretions, mechanical manoeuvres, etc.. Fayrer (1872) did the first series of experiments in order to find a systemic treatment for snakebites in Asia. He wrote: " *To conceive an antidote to snake poison, one must imagine a substance subtle as to follow, overtake and neutralize the venom in the blood.*" (2) In 1878, Brunton & Fayrer confirmed that potassium permanganate completely destroyed, *in vitro*, the lethal activity of the cobra-poison (2). This practice was introduced in Brazil by Lacerda (1881) to treat snake bites envenoming. Otero et al (2000) performed a extensive study. screening the antivenom activity of about 100 different plants employed by traditional healers, in Colombia (3).

KEY WORDS: History of Medicine, antivenom therapy, traditional healers.

1- CELSUS. *DE MEDICINA* volume II, p.115. Loeb Classical Books, Harvard University Press, London, 1972.

2- HAWGOOD, BJ. T Sir Joseph Fayer MD FRS (1824-1907) Indian Medical Service:snakebite and mortality in British India. *Toxicon* , 1996,#2.pp.171-182.

3- OTERO P, R et al. *Plantas utilizadas contra mordeduras de serpientes en Antioquia y Chocó*. Medellin, 2000, 402p.

CORRESPONDENCE TO: JOÃO LUIZ COSTA CARDOSO. Instituto Butantan, Hospital Vital Brazil Email: jlcardoso@butantan.gov.br

PEPTIDE TOXINS FROM BRAZILIAN BIODIVERSITY

KATSUHIRO KONNO(1)

(1)Center for Applied Toxinology, Butantan Institute, São Paulo, Brazil

Brazil consists of a great variety of ecosystems, from tropical rain forests to temperate climate, making this country extremely rich in biodiversity. For example, there are approximately 40000 species of wild plants, which accounts for about 10% of all species existing on the planet. It is the case for venomous animals as well. The venomous animals such as snakes, scorpions and spiders often cause envenomation accidents to man, and therefore, they are very important for public health. On the other hand, these venoms can be a rich source of lead compounds for drug discovery as represented by bradykinin potentiating peptides (BPP) from the *Bothrops jararaca* snake venom. In this context, we have been investigating a variety of animal venoms from Brazilian biodiversity and found several peptide toxins which should be good candidates for drug lead. Reported herein will be a brief summary of our recent results: isolation, sequence determination and biological properties of these peptide toxins with special focus on crotalphine, a novel and potent analgesic peptide from the venom of the South American rattlesnake *Crotalus durissus terrificus*.

KEY WORDS: peptide toxins, Brazilian biodiversity Financial support: FAPESP, Coinfar.

CORRESPONDENCE TO: KATSUHIRO KONNO, Center for Applied Toxinology, Butantan Institute, São Paulo, Brazil. Tel & Fax:+55-11-3726-1024. Email: kk-gon@butantan.gov.br

MARINE ORGANISMS FROM THE NORTHEASTERN BRAZILIAN COAST AS A SOURCE OF NEW MOLECULES WITH PHARMACEUTICAL POTENTIAL

LETÍCIA VERAS COSTA-LOTUFO (1,2)

(1) Departamento de Fisiologia e Farmacologia, UFC, Fortaleza, CE; (2) Instituto de Ciências do Mar, UFC, Fortaleza, CE.

Studies with marine natural products showed a variety of organic compounds derived from marine species with known and novel biological activities. In fact, many aspects contributed to increase the interest in this research field, and the great biodiversity found in the marine environment is one of the most important. Brazil is the largest country in South America, with more than 8.500 Km of shore line faced to the Atlantic Ocean, is a gifted country in terms of marine biodiversity. The northeastern region of Brazil has the largest tropical coast extension of the country and remains virtually unexplored by research groups that study natural products. In the recent years, our research group started a screening program in the northeastern Brazilian coast to verify the biomedical potential of marine organisms, optimizing the efforts on the isolation and characterization of bioactive metabolites. In this program, we focus on the bioprospection of cytotoxic and anticancer compounds in ascidians, sponges, cnidarians and macroalgae collected off the coast of Ceará state. In a first approach, it was evaluated the cytotoxicity of 54 crude extracts on the following bioassays: 1) antiproliferative activity on cultured tumor cell lines; 2) hemolytic effect on mouse erythrocytes and 3) anti-mitotic activity on sea-urchin eggs. Based on these results, we selected 13 species to follow with the bioguided extract fractionation. The ascidian *Eudistoma vannamei* is the most promising one, leading to the isolation of aminoacids derivatives with very strong cytotoxicity (IC_{50} < 0,05 μg/mL). On the other hand, alginates isolated from *Sargassum vulgare* are also considered very promising since they strongly inhibited *in vivo* tumor progression after oral administration in mice. These data highlights the pharmaceutical potential of Brazilian marine organisms.

KEY WORDS: ascidians, sponges, algae, anticancer potential.

FINANCIAL SUPPORT: FUNCAP, CNPq, FINEP.

CORRESPONDENCE TO: Letícia V. Costa-Lotufo, Rua Cel Nunes de Melo, 1127, Rodolfo Teófilo, Fortaleza, Ceará, Brasil, CEP: 60430-270. Phone: + 55 85 3366 8255. Fax: + 55 85 3366 8333. Email: lvcosta@secrel.com.br

FROM TOXINS TO MEDICINES: THE GUANYLIN CASE

MANASSÉS C. FONTELES(1)

(1)Universidade Presbiteriana Mackenzie and Universidade Estadual do Ceará - Brazil

Our laboratory described in 1983 the first evidence of guanylin like peptides, when studing renal effects promoted by semipurified extracts of E. coli toxins, originated from fecal materials of children with diarrhea in Ceará, Brazil. This represents the evidence that many toxins can generate medicines that can be used to treat many diseases. The guanylin family of bioactive peptides consists of four endogenous peptides, including guanylin, uroguanylin, lymphoguanylin and renoguanylin, and one exogenous peptide toxin produced by enteric bacteria *E. coli* (Sta). This thermo stable toxin is the main responsible for traveler's diarrhea. These small cysteine-rich peptides activate cell-surface receptors, which have intrinsic guanylate cyclase activity, thus modulating cellular function via the intracellular second messenger, cyclic GMP. Membrane guanylate cyclase-C is an intestinal receptor for guanylin and uroguanylin that is responsible for stimulation of Cl^- and HCO_3^- secretion into the intestinal lumen. Guanylin and uroguanylin are produced within the intestinal mucosa to serve in a paracrine mechanism for regulation of intestinal fluid and electrolyte secretion. Enteric bacteria secrete peptide toxin that mimics uroguanylin and guanylin and activates the intestinal receptors in an uncontrolled fashion to produce secretory diarrhea. Opossum kidney guanylate cyclase is a key receptor in the renal tissues that may be responsible for the diuretic and natriuretic actions of uroguanylin in vivo and in vitro. Uroguanylin serves as an endocrine axis linking the intestine to the kidney where its natriuretic and diuretic actions contribute to the maintenance of Na balance following oral ingestion of NaCl. Lymphoguanylin is highly expressed in the kidney, myocardium and lymphoid tissues, where this unique peptide may act locally to regulate cyclic GMP levels in target cells. It is also produced by cells of the immune system where other physiological functions may be influenced by intracellular cyclic GMP. Observations of several species provided insights into cellular mechanisms involving guanylin peptides in intestinal diseases, such as colon cancer, diarrhea and in chronic renal diseases or cardiac disorders such as congestive heart failure, where guanylin and/or uroguanylin levels in the circulation and/or urine are pathologically elevated. Guanylin peptides are clearly involved in the regulation of salt and water homeostasis, but new findings indicate that these novel peptides have diverse physiological roles, in addition to those previously documented for control of intestinal and renal function, that may contribute to the physiopathology of salt sensitive hypertension and even cancer treatment as a promoter of apoptosis.

NATURAL PRODUCTS FROM BRAZIL AS SOURCE FOR NEW ANTICANCER DRUGS

MANOEL ODORICO DE MORAES FILHO(1)

(1)Clinical Pharmacology Unit and Experimental Oncology Laboratory, Department of Physiology and Pharmacology, Federal University of Ceará, Brazil.

The development of more effective drugs for treating patients with cancer has been a major human endeavor over the past 50 years, and 21st century now promises some dramatic new directions. Nature has provided many effective anticancer agents in current use, such as the microbial-derived drugs, dactinomycin bleomycin and doxorubicin, plant-derived drugs, vinblastine, irinotecan, topotecan, etoposide, and paclitaxel, and marine-derived drugs, citarabine and bryostatin. Therefore, search for novel antitumor agents continues providing convincing evidence that natural products could be a source of novel cancer chemotherapeutic agents and leads for synthetic modification. Although, cancer drug discovery has been, and continues to be, a process of serendipity, “screening” natural products remains one of the most important methods in cancer drug discovery. More recently, progress in molecular pharmacology has demonstrated that each anticancer drug has a unique molecular target. Presently, drug development has focused on natural product compounds that specifically inhibit and/or modify tumor-specific molecular biological changes (target-based drug development). These compounds include angiogenesis inhibitors and matrix metalloproteinase inhibitors. In Brazil, there are many examples of plant and marine organisms-derived molecules screened in the Experimental Oncology Laboratory of the Federal University of Ceará, Brazil, with potential to be developed as new anticancer drugs.

IN VITRO AND *IN VIVO* EFFECTS OF A RECOMBINANT RGD-DISINTEGRIN FROM *Bothrops alternatus*

COMINETTI M. R. (1), RAMOS O. H. P. (2), CREPIN M. (3), BONNEFOY A. (3), SELISTRE DE ARAUJO H. S. (1)

(1) Departamento de Ciências Fisiológicas, Universidade Federal de São Carlos, SP, Brasil; (2) Divisão de Desenvolvimento Científico, Instituto Butantan, São Paulo, SP, Brasil; (3) INSERM, Unité 553, Paris, France.

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. In this work, we have produced and characterized a new recombinant RGD-disintegrin called Dis*Ba*-01, which interacts with αIIbβ3 integrin, inhibiting the platelet aggregation induced by different agonists and the proliferation of endothelial and tumor cells. Dis*Ba*-01 was tested *in vivo* using a metastasis model with B16F10-2B8 melanoma cells and C57BL6/j mice, Briefly, $5x10^5$ cells previously incubated with different concentrations of Dis*Ba*-01 (0, 0.05, 0.5, 2 or 4 mg/Kg) for 5 minutes were injected (100 μL) in the mice's tail vein. The homing and the evolution of pulmonary established metastasis were monitored at day 0, 1, 4, 12, 14 after luciferin injection (150 mg/Kg). By imaging and measuring the lung metastasis homing and the lung metastatic development of B16-F10 melanoma in C57Bl/6 mice we showed a strong antimetastatic activity of a single inoculation of Dis*Ba*-01 disintegrin. However, no direct cell toxicity was observed after a short treatment of melanoma cells with Dis*Ba*-01. Taking our results together, it is possible to conclude that Dis*Ba*-01 prevents melanoma metastasis by blocking adherence mechanisms of tumor cells to vessel endothelium. Since disintegrin peptides may be developed as antimetastatic agents, it is important to understand its functions *in vivo* in order to develop new strategies and new drugs which could be used in cancer therapy.

KEY WORDS: RGD-disintegrin, snake venom, metastasis, melanoma, cancer.

FINANCIAL SUPPORT: FAPESP.

CORRESPONDENCE TO: Dra. Heloísa S. Selistre de Araújo, Universidade Federal de São Carlos Departamento de Ciências Fisiológicas, Rod. Washington Luis, Km 235, São Carlos, SP, Brasil. Phone: + 55 16 33518333. Fax: + 55 16 33518327. Email: hsaraujo@power.ufscar.br

ISOLATION AND CHARACTERIZATION OF TWO NEW LECTIN TYPE C ISOLATED FROM THE *Bothrops erythromelas* VENOM.

TOYAMA, D.O.(1); ANTUNES, E.(2), CAMARGO, E.A. (2), MARANGONI, S.(3), CARNEIRO, E.M.(4) AND TOYAMA, M.H.(5)

(1) Universidade Mackenzie, São Paulo, (2) Departamento de Farmacologia, FMC, UNICAMP, (3) Departamento de Bioquímica, IB, UNICAMP, (4) Departamento de Fisiologia e Biofísica, IB, UNCAMP; (5) UNESP, CELP, São Vicente.

In this article we isolated two new lectin type C isoform from the venom of *Bothrops erythromelas* venom. Both proteins in the PAGE showed the presence of one electrophoretic band with molecular mass of 28kDa and in the presence of DTT we observed both proteins the presence of only 14kDa spot. Both proteins did not showed significative activity on the platelet aggregation but induce significative edema. The hamaglutination activity for both protein were inhibited by D-galactose, D-Lactose, that inhibited the edematogenic activity. Both lectin also induce strong increasing of the insulin secretion in the isolated β-cells by activation of PKC and PKA kinases and IP3 mobilization. These insulin secretion potencializations were inhibited by pre incubation of both proteins with lactose. Thus we conclude that both C-type lectin isolated from the *Botrhops erythromelas* venom was bind with specific membrane receptor mediated by specific recognition of D-galactose sugar residues.

KEY WORDS: lectin type C, *Bothrops erythromelas*, isulin secretion.

FINANCIAL SUPPORT: FAPESP, CNPq.

ISOLATION AND CHARACTERIZATION OF NEW PLA2 ISOFORM FROM THE *Crotalus durissus collilineatus* VENOM.

TOYAMA D.O.(1), ANTUNES, E.(2), CAMARGO E.A. (2), TOYAMA M.H.(3)

(1) Universidade Mackenzie, São Paulo, (2) Departamento de Farmacologia, FMC, UNICAMP, (3) UNESP, CELP, São Vicente

In this article we isolated a new PLA2 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 PLA2 shows few amino acid replacements in comparison with other PLA2 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: PLA2, *Crotalus durissus collilineatus* and circular dicroism.

FINANCIAL SUPPORT: FAPESP, CNPq.

EFFECTS OF POLYPHENOLIC COMPOUNDS ON THE STRUCTURE AND FUNCTION OF TOXINS.

TOYAMA, M.H. (1)

(1)UNESP, CELP, São Vicente

Polyphenolic compounds are wide spread substance in the nature, mainly found in the vegetable. These compounds are extensively used for production of relevant antioxidant compound that in some case they are used commercially as therapeutic compound. The main character of these compound flavonoids and its derivates is its strong antioxidant properties. Recently we investigate the effect of morin on the structure and function of PLA2 and we observed that morin induced significative modification of secondary structure of the protein but did not abolish its pharmacological action. In this presentation we present the effect of other flavonoids on the function and structure of PLA2 and other toxins. The main conclusion is the possibility of these compounds helps us to understand more of some structural or pharmacological actions of PLA2 and other toxins

KEY WORDS: PLA2, toxins, snake venom, flavonoids.

FINANCIAL SUPPORT: FAPESP, CNPq.

ACCIDENTS WITH CATERPILLARS OF THE GENUS *LONOMIA* REGISTRED AT THE RIO GRANDE DO SUL'S CENTRO DE INFORMAÇÃO TOXICOLÓGICA, IN PERIOD OF 1997 IN THE 2005.

MARIA DA GRAÇA BOUCINHA MARQUES(1)

(1)Centro de Informação Toxicológica do Rio Grande Do Sul, Fundação Estadual de Produção e Pesquisa Saúde, Secretaria Estadual de Saúde. Universidade Luterana do Brasil/RS.

The insect of the Lepidoptera Order can sum up to 165,000 species of butterflies and moths. For the agriculture, the caterpillars (immature form) represent significant damage in all continents as well as in the area of the public health due to accidents with either the caterpillar or its adult form. The accidents with caterpillar of the *Lonomia* sort represent a serious problem for public health in the South Region of Brazil, being these occurrences object of accompaniment and attention. An accidents occurrences retrospective analysis involving gender caterpillars *Lonomia* notified to the Emergence Room of CIT/RS in the period from 1997 to 2005 was made, summing to 1009 records, of which 984 occurred in the State and 25 in other places. The 5ª Coordenadoria Regional de Saúde (CRS), located in the mountain ridge region, with headquarters in Caxias do Sul, presented the biggest absolute number of accidents with *Lonomia* sp. 191 (19,4%). The accidents incidence caused by *Lonomia* was of 9,9/100.000 inhabitants for Rio Grande do Sul. Regionally, it can be seen that the 15ª CRS, Palmeira das Missões in the region of Alto Uruguai, north of the state, presents the biggest incidence with 48,7/100.000 inhabitants. Passo Fundo and Bento Gonçalves were the cities that stood out the most in absolute numbers of accidents. The biggest number of cases occured during summer. It has predominance of the 0 to the 19 years, mainly in the masculine sex. The anatomical region of more frequent contact was the upper limbs, mainly the hands. The lethality was 0.5% for accidents with *Lonomia* sp. in Rio Grande do Sul. As for the gravity of the accidents, it had larger incidence of light and moderated cases with evolution for the cure, despite the occurrence of seven deaths, in the evaluated period. In this context, the mapping of the risk areas, the knowledge diffusion, the optimization of the net that makes available specific immunobiological medicine, as well as the improvement of the Systems of Monitoring Epidemiology are important factors for the control of these accidents.

KEY WORDS: *Lonomia,* Epidemiology, Lepidoptera

ELECTRONIC ADDRESS: graboucinha@brturbo.com.br

ARTHROPOD VENOMS: OLD MOLECULES, SOME TREASURES FOR BIOTECHNOLOGY.

MARIA ELENA DE LIMA, ADRIANO M.C. PIMENTA

Lab. de Venenos e Toxinas Animais, Depto. de Bioquímica e Imunologia, ICB, UFMG.

Animal venoms have evolved in both predatory and defensive senses, which generate a massive repertory of specific-targeted molecules that act in synergy on the organism of the inflicted victim. Highly toxic compounds, most of them being well represented venom constituents, have been purified and characterized so far as neurotoxins, myotoxins, cardiotoxins, hemorrhagic factors, among others. Nevertheless, with the advent of proteomic approaches and increasing facilities to peptide synthesis, small molecules poorly represented in venoms and with micro-effects that are not easily visualized are being emerged. This “new generation” of molecules has been studied and their activities such as vasoactive, hormone-like, anti-microbial and others have been enlarging the known venom repertoire. We present some of the current works in our laboratory, in which we envisage the prospection, structural and functional characterization of peptides and/or proteins found in venoms from arthropods, as scorpions (*Tityus serrulatus*), spiders (*Lycosa erythrognatha* and *Phoneutria* spp), among others. We have focused our study on molecules that represent potential tools to be used in biotechnological processes. Anti-hypertensive peptides were identified in the venom of yellow scorpion and named *T. serrulatus* Hypotensins (*Ts*Hpt). The pharmacological activity of *Ts*Hpts was scrutinized, together with its synthetic analogs, in order to draw a map of its structure-function connections. Another example came from linear peptides from *L. erythrognatha* venom that elicits antimicrobial effects. Some peptides obtained from spiders (*i.e*. *Phoneutria sp*.) are very active on insects, being potential insecticides. In addition, a toxin of *P. nigriventer* causes sustained penile erection in rats including those with decreased function erectile (DOCA-Sal model). Structural and functional studies with these molecules, as well as their synthesis providing adequate material, are crucial to envisage biotechnological use.

KEY WORDS: Arthropod venom, *Tityus Serrulatus*, *Lycosa Erythrognatha*, *Phoneutria sp*

FINANCIAL SUPPORT: FAPEMIG/PRONEX, CNPq/FINEP.

CORRESPONDENCE TO: Maria Elena de Lima. Email. delima@icb.ufmg.br.

TOXINS FROM BRAZILIAN SAVANNAH SNAKES: INHIBITION, STRUCTURAL AND FUNCTIONAL CHARACTERIZATION.

HOMSI-BRANDEBURGO, M.I. (1); HAMAGUCHI, A.(1); RODRIGUES, V.M.(1).

(1) Laboratório de Toxinas Animais e Inibidores. Instituto de Genética e Bioquímica Universidade Federal de Uberlândia. Uberlândia – M.G. – Brasil.

The majority of ophidic accidents registered in the Clinical Hospital from Uberlândia, M.G, Brazil are caused mainly by *B. moojeni, B. neuwieidi* and *B*. *alternatus* snake venoms. *Bothrops* snake venoms induces release of pharmacologically active substances with pronounced local effects such as hemorrhage, edema and tissue necrosis. In addition, relevant haemostatic and hematological alterations are frequent. These venoms are complex mixtures of proteins, including phospholipase A2 (EC 3.1.1.4), myotoxins, hemorrhagic metalloproteinases, cytotoxins and others proteases that act by different mechanisms. Many of these toxins have been isolated, functional and structurally characterized by our group. The native people from our community utilize parts of plants (leaves, roots, bark, etc) to prepare infusion or tea to treat snake bite envenomation. Medicinal plant extracts, a rich source of nature inhibitors and pharmacologically active compounds, have been shown to antagonize the activity of some venoms and toxins. Our studies in this field have demonstrated that aqueous or methanolic extracts of *Casearia sylvestris, C. mariquitensis, C. gossypiosperma* and *C*. *grandiflora; Schizolobium parahyba* and *Stryphnodendron adstringes* include bioactive products such as flavonoids, catequins and poliphenols able to inhibit some these venoms and isolated toxins. The isolation and structural elucidation of these compounds will allow the understanding of the interaction with these toxins and, consequently, their inhibition mechanism. Furthermore, these inhibitors can be used as molecular models for development of new therapeutical agents in treatment of ophidian accidents.

FINANCIAL SUPPORT: FAPEMIG, CAPES, CNPq, UFU.

CORRESPONDENCE TO: MARIA INÊS HOMSI BRANDEBURGO, Instituto de Genética e Bioquímica, Universidade Federal de Uberlândia, Uberlândia, M.G. fone: (34) 3218.2203 R: 22; fax: (34) 3218.2203 R: 24. e-mail: homsi@ufu.br

CYTOSKELETON ALTERATIONS INDUCED BY *Geodia corticostylifera* CYCLIC PEPTIDES IN BREAST CANCER CELLS.

RANGEL M. (1,2) PFISTER S.C. (1) PRADO M.P. (1) FERREIRA R.A.S. (1) FREITAS J.C. (3) KONNO K. (2) SANTELLI G.M.M. (1).

(1) Depto. de Biologia Celular e do Desenvolvimento, Instituto de Ciências Biomédicas da USP, São Paulo, Brazil; (2) Lab. Especial de Toxinologia Aplicada, Instituto Butantan, São Paulo, Brazil; (3) Depto. de Fisiologia, Instituto de Biociências da USP.

The cyclic peptides geodiamolides A, B, H and I (1–4) from the sponge G. corticostylifera have anti-proliferative activity against human breast cancer cell lines (T47D and MCF7). Using fluorescence techniques and confocal microscopy, we found evidence that the geodiamolides A, B, H and I act by disorganizing actin filaments of T47D and MCF7 cancer cells, in a way similar to other depsipeptides (such as jaspamide and dolastatins), keeping the normal microtubule organization. The cytoskeleton proteins of normal cells lines (primary culture human fibroblasts and BRL3A rat liver epithelial cells) were not affected by the treatment as tumor cells were, thus indicating the biomedical potential of these compounds. At the present work we also investigated if the cytoskeleton alterations induced by sponge depsipeptides are associated with changes of connexin 43 (a membrane protein that form gap junction channels) assembly or degradation**.**
Normal liver (BRL3A) and hepatocarcinoma (HTC) cell lines were submitted to treatment with peptide solutions at different conditions. Immunofluorescence reactions and Lucifer Yellow assays by Scrape Loading and Dye Transfer (SL/DT) of the cells were analyzed under a confocal laser scanning microscope. The geodiamolides showed a probable role in enhancing or preserving GJC both in BRL3A and in HTC-transfected cells, however, the actin filaments disruption was more apparent in tumor cells than in normal ones. The treatment with proteasomal inhibitors enhanced gap junction plaques (GJP). Therefore, the geodiamolides could interfere with the delivery of connexins to the degradation structures, stabilizing connexins assembled and the accumulation of GJP.

KEY WORDS: cancer cell, cytoskeleton, marine sponge, geodiamolide, connexin, gap junction channels.

FINANCIAL SUPPORT: FAPESP and CNPq.

CORRESPONDENCE TO: Marisa Rangel Laboratorio Especial de Toxinologia Aplicada, Instituto Butantan, Av. Vital Brasil, 1500, CEP 05503-900 Sao Paulo,SP, Brazil. Phone: +55 11 37261024; fax: +55 11 37261024. E-mail: mrangel@butantan.gov.br

PITVIPERS OF CAATINGA: STRUCTURAL AND FUNCTIONAL ASPECTS OF THEIR TOXINS

GUARNIERI M.C.(1), DE ALBUQUERQUE-MODESTO J.C.(2), RÁDIS-BAPTISTA G. (3)

(1) Department of Zoology, Federal University of Pernambuco (UFPE), Brazil; (2) Nucleus of Biology, Academic Center of Vitória, UFPE, Brazil; (3) Department of Biochemistry and Laboratory of Immunopathology Keizo Asami, UFPE. Brazil.

In Brazil, there are 27 species of snakes belonging to Viperidae family, of which six are found in Caatinga.This biome, with an area of approximatelly 800.000 km^2 and 10% of all Brazilian territory, encompasses seven states (CE, RN, PB, PE, SE, AL, BA, part of PI and MG).The Caatinga presents a semi-arid climate, with a period of irregular rain, xerophytic and decidous vegetation, as well as a particular biodiversity. In the state of Pernambuco, the Viperidae species found in Caatinga are *Bothriopsis bilineata*, *Bothrops neuwiedi*, *B. leucurus*, *B. erythromelas* and *Crotalus durissus cascavella*. These two latter are the main responsible for envenomation in Pernambuco. Our group has particularly dedicated to the investigation of *B. erythromelas* and *C. d. cascavella* venom, by purification, characterization and molecular cloning of their toxins. Recently, we have isolated and cloned a novel acidic Asp49 phospholipase A_2 from *B. erythromelas*, with a MW of 13,649.57 Da, as estimated by mass spectrometry (MALDI-ToF). The complete BE-I-PLA2 cDNA possesses 457 bp and encodes a protein with significant sequence similarity to many other snake venom phospholipases A_2. When tested in platelet rich plasma, the enzyme showed a potent inhibitory effect on aggregation induced by arachidonic acid and collagen, but not ADP. Moreover, BE-I-PLA2 stimulated endothelial cells to release prostaglandin I_2, suggesting an increase of its potential anti-platelet activity *in vivo*. For now, we are determining the exact mechanism of action of BE-I-PLA2 in the inhibition of platelet aggregation, and characterizing a new isoform of this PLA2. In other study, from *C. d. cascavella* venom gland, we have cloned several cDNA precursors of convulxin (CVX) subunit homologs. One of them, it was named crotacetin (CTC) beta-subunit and it predicts a polypeptide with a tridimensional topology very similar to other subunits of CVX-like snake toxins. Crotacetin was further purified from the venom of several *C. durissus*, but it is quantitative predominant in the venom of *C. durissus cascavella*. Functional analysis indicates that CTC induces platelet aggregation, and, importantly, exhibits an antimicrobial activity against Gram-positive and -negative bacteria, comparable with CVX. Our ongoing projects include the purification and cloning of hemorragic and factor X activator metalloproteases, neurotoxic PLA2s, and C-type lectins from *B. leucurus* and *C. d. Cascavella* venoms.

KEY WORDS: PLA2, C-type lectin, *Bothrops*, *Crotalus*, platelets, snake toxin.

CORRESPONDENCE TO: Rua Nestor Chaves, s/n, Cidade Universitária, Recife-PE, CEP: 52.011-330. E-mail: mcg@ufpe.br

FINANCIAL SUPPORT: CAPES, CNPq, FAPESP.

GENOTOXIC EFFECT OF CROTOXIN IN PANCREATIC TUMOR CELL AR42J BY USING MICRONUCLEUS ASSAY

SUZUKI M. F., CASARE M., SPENCER P. J.

Instituto de Pesquisas Energéticas e Nucleares, IPEN – CNEN/SP

Snake venoms are rich source of several bioactive compounds that possess therapeutic potentials. Fractions of snake venoms can modulate cell proliferation, cell growth and cell cycle. The genotoxic activity of venom fractions can be analysed by using micronucleus assay. The cytokinesis-block micronucleus (CBMN) assay was developed as a system for measuring micronuclei (MNi) originated from DNA fragmentation. The pancreatic adenocarcinoma cells, AR42J, were exposed to crotoxin, the main toxin of the South American rattlesnake Crotalus durissus terrificus, isolated and purified by molecular exclusion chromatography and pI precipitation. The cells were washed after 24 h exposure and cultivated for 48 h. The micronucleated cells incresed at the concentration of 3 and 14 μg/ml. This genotoxic effect of crotoxin was similar to that observed in AR42J cells irradiated with 1 Gy of 90Sr (β particle emitter) at a dose rate of 0.13 Gy/min and 1 Gy of 60Co (gamma rays) at the dose rate of 0.24Gy/min. The detection of genotoxic effect of crotoxin in AR42J cells by using the micronucleus assay in vitro was demonstrated and can be applied for the screening of other venom fractions.

KEY WORDS: genotoxicity, micronucleus assay, snake venom.

CORRESPONDENCE TO: Miriam Fussae Suzuki, Centro de Biotecnologia, Instituto de Pesquisas Energéticas e Nucleares, IPEN – CNEN/SP, Av. Prof. Lineu Prestes 2242, Cidade Universitária, São Paulo – SP, 05508-000, Brazil. Phone: + 55 3816 9236. Fax: + 55 3816 92

THERAPEUTIC AND/OR BIOTECHNOLOGICAL APPLICATIONS FOR NATURAL TOXINS

MIRIAN A. F. HAYASHI(1)

(1)Departamento de Farmacologia, Universidade Federal de São Paulo (UNIFESP)

Venom is a rich source of biochemically active enzymes, proteins, peptides and low molecular weight substances. This vast array of toxins consists of a very sophisticated mechanism for prey capture for the venomous creatures. These substances target an immense number of receptors and membrane proteins with high affinity, selectivity and potency, and can serve as potential drugs or scaffolds for drug design. The pharmaceutical industry has recognized the enormous potential inherent in these venom peptides and has begun to exploit the selectivity and sensitivity fine tuned by evolution. In the last few years, our group has dedicated to the study and characterization of snake toxins with potential pharmaceutical and/or biotechnological applications. The employment of modern technology involving expertise of different scientific areas, such as molecular and cellular biology, biochemistry, electrophysiology, mass spectrometry analysis, between others, allowed us to identify new targets and describe surprising molecular features for well-described toxins, namely, bradykinin-potentiating peptides (BPPs), and crotamine, from *Bothrops jararaca* and *Crotalus durissus terrificus*, respectively. In conclusion, besides the mechanism of action of each toxin family being different, it seems that the nature has evolved the venoms into a huge pharmacological library of active compounds with high selectivity and affinity. So forth, the study of venom toxin and its mechanism of action can be a powerful scientific tool to the discovery of new physiological pathways and also of new therapeutic compounds.

FINANCIAL SUPPORT: FAPESP (CAT-CEPID program).

ACYLPOLYAMINE TOXINS FROM NEPHILINAE SPIDER VENOMS AS MODELS FOR THE DEVELOPMENT OF NEUROPHARMACEUTICALS

PALMA, M.S. (1), CESAR-TOGNOLLI, L.M.M.(1), SALAMONI, S.(2), COSTA, J.C.(2)

(1) Lab. Structural Biology & Zoochemistry, CEIS/Dept. Biology, IBRC.UNESP, Rio Claro, SP, Brazil; (2) Lab. Neurosciences, IPB-PUCRS, Porto Alegre, RS, Brazil

The acylpolyamine toxins from Nephilinae spiders have been found to block glutamate excitatory activity. Epilepsy has been studied *in vitro*, mostly on rat hippocampus, through brain slices techniques. A series of these toxins have been synthesized by using manual solid phase protocols, following a combinatorial strategy. Structure / Activity Relationship studies have been performed with these toxins in order to verify the effect of the each different structural moiety on the epileptiform activity induced by magnesium-free medium in rat CA1 hippocampal neurons. Experiments were performed on hippocampus slices of control and pilocarpine-treated Wistar rats, prepared and maintained *in vitro*. Epileptiform activity was induced through omission of magnesium ion from the artificial cerebrospinal fluid (0-Mg2+ ACSF) superfusate and iontophoretic application of N-methyl-D-aspartate (NMDA). Intracellular recordings were obtained from CA1 pyramidal neurons both of control and epileptic rats. Passive membrane properties were analyzed before and after perfusion with the 0-Mg2+ ACSF and the application of the toxins. During the ictal-like activity, the acylpolyamines were applied by pressure ejection, abolishing this activity. This effect was completely reversed during the washout period when the slices were formerly perfused with artificial cerebrospinal fluid (ACSF) and again with 0-Mg2+ ACSF. Our results suggest that the acylpolyamine toxins from Nephilinae spider venoms are potent blockers of the induced epileptiform activity at different levels, according to each different structural moiety under consideration and be used as models of the development novel neuropharmaceuticals.

KEY-WORDS: orb-web-spider venom; acylpolyamine toxins; glutamate-receptor blocker; anti-epileptic action.

FINANCIAL SUPPORT: BIOprospecTA-FAPESP / CNPq

COMPARATIVE STUDY OF BIOLOGICAL AND BIOCHEMICAL ACTIVITIES OF VENOMS OF BRAZILIAN FISHES WITH MEDICAL IMPORTANCE

BOLETINI-SANTOS D[1], ERLICHMAN C[1], KADURA A[1], RAMOS AD[1], CONCEIÇÃO K[2], PIMENTA DC[2], CHARVET-ALMEIDA P[3], FIGUEIREDO SG[4], HADDAD JRV[5]; LIMA C[1], LOPES-FERREIRA M[1].

[1]Lab. Imunopatologia, Instituto Butantan, [2]Lab. Especial de Toxinologia Aplicada (CAT/CEPID), Instituto Butantan, São Paulo; [3]Universidade Federal da Paraíba; [4]Dep. Ciências Fisiológicas, Universidade Federal do Espírito Santo, Vitória; [5]Dep. Dermatologia, Universidade Estadual Paulista. Botucatu.

In Brazilian waters many species of venomous fishes are found, but 5 species are frequently involved with human accidents: *Potamotrygon orbignyi* (*Po*), *Cathrops spixii* (*Cs*), *Pseudoplatystoma fasciatum* (*Pf*), *Scorpaena plumieri* (*Sp*), and *Thalassophryne nattereri* (*Tn*). Usually, the clinical features induced by these fish venoms are pain, edema, and necrosis. In this work we compared the biological and biochemical activities induced by these different fish venoms. Toxic activities (nociception-10 µg, edema -10 µg, necrosis -30 µg) and alterations in the microcirculatory net were induced in Swiss mice by application of 10 µg of venoms, which were analysed by 12% SDS-PAGE and chromatography. Gelatinase, phospholipase A_2 (PLA_2) and proteolytic activity were also determined. *Tn* induced the highest level of nociception and edema, and the venoms of *Sp* and *Po* induced the lowest levels of nociception or edema, respectively. Only *Tn* venom induced necrosis and hemolitic activity. Alterations in microcirculation were not observed after application of *Sp* venom. All venoms presented proteolytic activity, and *Tn* venom was devoid of PLA_2. The SDS-PAGE and zymography showed a high similarity between *Pf* and *Cs* venoms. The chromatography shows that *Po*, *Cs*, *Pf*, and *Tn* presented a similar band around 18 kDa and *Sp* presented proteins with high molecular weight (> 100 kDa). This is the first study that compares the toxic activities and biochemical characteristics of the venoms from the major Brazilian venomous fishes: the venom of *Tn* showed the highest toxic activities and *Sp* venom presented the lowest, but with systemic effects.

FINANCIAL SUPPORT: Fapesp and FUNDAP.

ATTENUATION OF SNAKE VENOMS BY IONIZING RADIATION: A RETROSPECTIVE

NANCI DO NASCIMENTO

IPEN/CNEN-SP

Snake envenomation is a serious public health problem in Brazil, with around 20.000 cases reported every year. Serum therapy is the only known treatment, the antiserum being produced by immunizing horses with crude venom. Obviously, this treatment induces deleterious effects, impairing the animals lifespan. Ionizing radiation has been known as an effective tool to attenuate venoms and toxins since the early 70´s. Our group, as well as others around the world, has been working on the effects of ionizing radiation as a tool to improve antisera production by attenuating venom toxicity without affecting its immunogenic properties. The first experiments were performed using crude bothropic and crotalic venom. The results were rather promising, with a toxicity decrease of around five folds and with these toxoids inducing protective antibodies with no detectable deleterious effects on serum-producing animals. The next step was to further characterize the effects of radiation on the venom components, aiming to identify the mechanism of toxicity attenuation. Purified toxins were used as probes to analyze the functional and structural modifications induced by radiation. All the experiments indicate that the toxins, following irradiation, undergo structural modifications, such as oxidation and unfolding, associated with a dose-dependent loss of enzymatic activity. These alterations lead to a toxicity decrease, while enhancing phagocytosis by antigen presenting cells trough receptors specialized in the removal of oxidized proteins. Also, unfolding promotes exposure of neoepitopes, increasing the repertoire of cell surface receptors able to recognize these modified biomolecules. Pilot experiments performed on goats indicate that irradiated venoms induced high titers of neutralizing antibodies, without any detectable toxic manifestations.

POST-GRADUATION IN TOXINOLOGY – A FUTURE PERSPECTIVE

NANCI DO NASCIMENTO

IPEN/CNEN-SP

Toxinology is a multidisciplinary research area, looking for detection, isolation and characterization of toxins, biological products produced by a living being that prejudice or affects negatively others organisms, some of them affecting directly men. Those soluble products are produced from bacteria to Metazoa, and are powerful tools for the study of the cell biology and physiology. This poor defined research area involves a large set of biomedical researchers; most of them specialized in biochemistry, immunology and pharmacology. There are few specialized scientific journals and looking for publications in the last 40 years, we found ~200000 articles dealing with toxins in PubMed, with at least 50000 articles only in Metazoan toxins, as insects and snakes. The scientific production is dispersed in those above cited area, and at my knowledge, there were few efforts to introduce Toxinology as a scientific field area in Brazilian agencies, as CAPES and CNPq. The teaching of Toxinology at graduation level is also sporadic, mainly associated to the main field area, as biochemistry or immunology or pharmacology, without a specific course, even in Pharmacy or Biological Science baccalaureate programs. At post-graduation, the first efforts had resulted in some experiences in one or two year specialization programs in dedicated institutions. There are large numbers of dissertations and thesis related to toxins, but associated to post-graduations programs from those above cited areas. Recently, several interdisciplinary programs have been introduced as biotechnology and vaccinology, dealing with integration of several established scientific areas, a perfect place for Toxinology post-graduation, but a long way must be pursued. The first step must be the definition of Toxinology as isolated scientific area or sub area in scientific funding agencies, by our society with support of Toxinology research centers. The second step must be the inclusion of disciplines in regular courses in Universities with established Toxinology research centers. Finally, all efforts must be made in order to introduce specific post-graduation M.Sc. and Ph.D. programs in large universities, by integration of several disciplines under a new perspective, resulting in manpower growth in this strategic area, improving our efficiency in the management of those powerful toxins, in their use or in environmental or human accidents.

IMPORTANCE OF THE BRAZILIAN CERRADO BIOME FOR TOXINOLOGY

NELSON J. JR. SILVA(1)

(1)Department of Biology and Master Program in Environmental Sciences and Health, Catholic University of Goiás, Goiânia, GO, Brazil.

Among the most distinctive dry habitats of the Neotropical region the Cerrado biome is one of the most seriously threatened, representing 1/8 of the total area of South America and 23% of Brazil. It comprises a complex biogeographic domain that includes an incredible number of target species for toxinological studies including invertebrates (insects, myriapods, and arachnids) and vertebrates (fishes, amphibians, and reptiles) which are still unknown of its approximate total diversity. As an example, new species of amphibians and reptiles are described at a rate of 5 to 10 species per year for Cerrado alone. Similarly, the Cerrado's flora is one of the richest in terms of species with several different vegetation formations according to number and density of vascular species that may reach 300 to 450 species per hectare. The Cerrado is the only biome that contacts all other biomes of Brazil. Nonetheless, as the new agriculture frontier this biome is being rapidly transformed into giant monocultures (mainly soy bean and sugar cane) with a diversity rate loss (plants and animals) not yet evaluated. Also, the Cerrado's drainage basins have the highest potential for hydroelectric energy with more than a hundred power plants planned or being installed. With the flooding of power plant reservoirs tens of thousands animals are directly affected and subject of complex and controversial rescue operations that can potentially offer number and diversity of invertebrates and vertebrates for toxinological studies. A modest contribution is being given to few institutions (e.g. Instituto Butantan, IVB, and FUNED) since the 70's. In the other hand, there is a lack of interest from institutions and researchers to carry on prospective studies in a wider range of species other than the ones used for antivenin production. During the last twenty years approximately 70% of the natural formations of the Cerrado was modified by human activities and one way to describe and value its biodiversity is also through systematic toxinological studies and not only taxonomy and ecology. (2.088)

KEY WORDS: Cerrado, biodiversity, toxinology, environmental impact.

CORRESPONDENCE TO:

Nelson Jorge da Silva Jr., Universidade Católica de Goiás, Mestrado em Ciências Ambientais e Saúde, Rua 232, 128 – 3º andar – Área V, Setor Universitário, CEP 74605-140, Goiânia, GO, Brazil.

NEW CARDIOTONIC FROM *Bufo paracnemis* PAROTOID SECRETION

ARNAUD-BATISTA, F.J.; FONTELES, M.C.; SANTOS, C.F.; REFOSCO. R.M.; OLIVEIRA, R.G.; COSTA, P.P.C.; CARDI, B.A.; CARVALHO, K.M.; NASCIMENTO, N.R.F.

Superior Institute of Biomedicine, State University of Ceara.

Digitalis glycosides are widely used to treat cardiac failure but a narrow therapeutic index limits its use. A new cardiotonic was isolated from *Bufo paracnemis* by HPLC using the following protocol. 1g of the venom was dissolved in 80% ethanol (V/V), centrifuged at 10.000g during 30 min and the supernatant was eluted with HPLC-grade water using a linear gradient of 0-40% acetonitrile on a C18 column. The peak 3 (P3) was then lyophilized and diluted in 5% DMSO in saline (V/V) for pharmacological experiments. The cardiotonic activity was evaluated in the isolated guinea-pig heart perfused and on the electrically driven guinea-pig left atrium. P3 (10, 100 and 1000 mg.ml-1) induced an increase in force by 23.5 ± 8.3 %; 50 ± 14.7% and 279.2 ± 35.7 % in the perfused heart. The addition of P3 up to 100 mg.ml-1 to electrically stimulated left atria with submaximal pulses (5 ms, 2.5 Hz) induced a maximal increase in force of 769.5 ± 126 % (CE50 = 128.9 [59.5-279.6] mg.ml-1) compared with 408.3 ± 72.2 % (CE50 = 0.14 [0.09-0.22] mg.ml-1) achieved with digoxin. We also evaluated the effects of P3 on selected electrocardiographic parameters on the rat EKG. For this purpose, male Wistar-Kyoto rats (300-350g) were anesthetized and infused with (300 mg.Kg-1.min-1) P3 or digoxine and EKG acquired by subcutaneous platinum electrodes. The infusion of digoxine induced classical signs of intoxication: bradycardia (311±10 bpm vs. 258±7.7 bpm before arrhythmias), increase in PR segment duration (13.4 ±3.4 ms control vs. 33.4 ± 6.7 ms, before severe arrhythmia initiated), J-point depression (since 1st minute of infusion). Other findings include, T depression, second-grade atrio-ventricular block (AVB) under 4-5 min infusion and cardiac arrest (at 6-7 min) leading animals to death. On the other hand, the infusion of P3 during 20 minutes was devoided of EKG alterations. This results supports that P3 is a promising cardiotonic agent with larger safety index than digitals.

KEY WORDS: Cardiac failure, pharmacological therapy

SUPPORT: FUNCAP, CNPqCorrespondence to: Nilberto R. F. do Nascimento, Instituto Superior de Biomedicina, UECE, Fortaleza, Ceará, (85) 3101-9836, E-mail: nilberto@uece.br.

UNIVERSITY-COMPANY INTEGRATION - AN ENTREPRENEURIAL VISION

OGARI DE CASTRO PACHECO

Laboratório Cristália

The concept of “University-Company Integration” is ancient and amply recommended as an obvious receipt for rationalization of the available resources in the country, insufficient as in the most developing countries. However, there is an abyss between the speech and the daily experience.
The scientific production, with any discussion is definitely concentrated, basically, in the Universities and due to a question of an already implemented model are transformed in “papers”, published in Brazil as well as abroad, without being converted in tangible asset (as useful products) in the majority of time.
Referring to the Pharmaceutical Industry we must emphasize that its scientific production is performed in an almost exclusively way in the headquarters, at the country of origin, whenever we refer to the transnational companies; concerning the domestic companies’ participation, the influence is not significant, really derisory (compared to the global production).
This is the picture / scenery as a flash with no refining of the current situation.

THE WITHDRAWAL

In our understanding there is any recipe and universal magic that could be applied to everyone. We begin by searching the support for projects in the University, projects ones that we had begun by our own initiative inside our company and achieving to determined point of developing that we could not perform some tests by ourselves. The day by day relationship ended up to show us two very evident things. a) An expressive number of interesting projects in course in the Universities. b) A huge necessity of the Universities by support.
The growing necessity to analyze the merit of the applied projects candidates to receive for support ended inspiring us on the creation of a Scientific Council which is current formed by 10 professionals with different backgrounds seeking the following objectives:

1) To give credibility by the worth of the Council composition as well as the transparency which guides their judgment.
2) Multiply the consultations aiming the natural diffusion of the performed work.
3) Orientation and follow up a lot more efficient

PRATICAL EXAMPLES

I) Dantroleno Case
II) Ketamine Case
III) Novabupi Case
IV) Niquim Case
V) Paracaine Case
VI) Snake anesthetic Case
VII) Sea Cucumber Case

PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST BOTHROPSTOXIN-1, A K49 MYOTOXIN

PATRICK J. SPENCER(1)

(1)IPEN/CNEN-SP

Ophidic accidents are a relevant health problem in Brazil, with around 20000 cases reported every year. About 90% of the accidents are caused by snakes belonging to the *Bothrops* genus. Within this genus, some species present a specific myotoxic component which is involved in the onset of severe local injuries. These myotoxins are not neutralized by the autologous anti-serum. On the other hand the polyvalent antiserum has been reported to be efficient against the myotoxic component. A tool able to discriminate between miotoxic and non-miotoxic venoms would be of great value for the proper treatment of the patients. In the present work, we investigated monoclonal antibodies raised against bothropstoxin-1, a myotoxin from *Bothrops jararacussu*. The antibodies were investigated by surface plasmon resonance (Biacore) and immunoenzimatic assays. Also, an *in vitro* myotoxic assay was standardized and used for neutralization tests. Our results indicate that the antibodies are highly specific for myotoxins, enabling the development of a diferential diagnostic kit. Also, the incubation of indeferentiated and diferentiated myoblasts with bothropstoxin indicates that upon maturation, the cells become more sensitive to the toxin, suggesting the involvment of a receptor.

EFFECT OF DIFFERENT AGENTS ON TONGUE, SKIN AND PAW EDEMA INDUCED BY *Dieffenbachia picta* Schott *in vivo*

PAULO A. MELO(1)

(1)Departamento de Farmacologia Básica e Clínica ICB, CCS Universidade Federal do Rio de Janeiro.

We have investigated the tongue, paw and skin edema induced by *D. picta* juice in mice and the effect of some local anesthetic agents (benzocaine lidocaine and bupivacaine). Tongue edema was induced by topical application of *D. picta* juice and it was evaluated with a digital tachymeter during 120 min. We also investigated the skin edema by using i.v. injection of Evan's blue dye (2.5% solution; 50 mg/kg) 15 min. before the intradermal injection of of *D. picta* juice. Skin edema was evaluated by measuring the dyed plasma extravasation and accumulation in the skin in a spectrophometer. The tongue edema reached the maximum at 60 min. after topical application of 0,1 ml of *D. picta* crude juice in the control mice, and it was completely inhibited by topical application of benzocaine and partially inhibited by lidocaine (circa of 30%). Preincubation of *D. picta* juice with lidocaine, decrease the skin edema and alkaline bupivacaine completely abolished it. These local anesthetic effects are indicative that the functional integrity of the afferent sensitive fibers and terminal axons could be involved in the genesis of the acute edema and the inflammatory response induced by *D. picta* juice in mice.

- *Dieffenbachia picta*, tongue edema and inflammation,
- local anesthetics and eugenol

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

CORRESPONDENCE TO: PAULO A. MELO, Departamento de Farmacologia Básica e Clínica ICB, CCS Universidade Federal do Rio de Janeiro, 21941-590, Rio de Janeiro, RJ, Brazil. E-mail: pamelo@farmaco.ufrj.br.

FUNCTIONAL DIVERSITY OF TOXINS FROM SPIDERS OF THE GENUS *Phoneutria*

CAMPOS F.V. (1), LÚCIO A.D. (1), MATAVEL A. (1), LEÃO R.M. (1), KUSHMERICK C. (1), CRUZ J.S. (1), CORDEIRO M.N. (2), RICHARDSON M. (2), DINIZ C.R. (1,2) AND BEIRÃO P.S.L. (1)

(1) Department of Biochemistry and Immunology, ICB-UFMG, Belo Horizonte, MG; (2) Fundação Ezequiel Dias, Belo Horizonte, MG, Brazil

Spiders of the genus Phoneutria are prevalent over wide areas of Brazil, and their venoms contain a rich variety of peptides, most with activities on ion channels. After the pioneering work of Dr. C. R. Diniz, a number of investigators have been engaged in the determination of the structural and functional diversity of toxins contained in the venom of the spider Phoneutria nigriventer. Five fractions of polypetide toxins (mw. 3.5-8.5 kDa) have been described in this venom, including an insect-specific toxin. More recently, a small (4 kDa) family of neurotoxins has been identified in three species of the same genus: Phoneutria nigriventer, Phoneutria reidyi and Phoneutria keyserlingi. Preliminary work suggested that these toxins act on ion channels. We have focused our interest in the mechanism of action of the toxins, by determining the channels they target and their mechanisms of action. We have used the patch clamp technique, in the whole cell configuration, and appropriate cell types to directly measure currents carried through Na, Ca and K channels, and have used biophysical and pharmacological markers to identify the subtypes of channels that were affected. Most of the toxins found in the fraction PhTx2 have strong inhibitory effect on Na channel inactivation. This effect can account for the prevailing excitatory effect of the crude venom. In contrast, fraction PhTx3 is more diverse, containing toxins that inhibit Ca channels (w-PnTx3-3) and A-type K channels (PnTx3-1). Toxins that belong to the 4 kDa family inhibit L-type Ca channels, with different efficacies (PRTx27C3>PNTx27C4≈PNTx26An0C3, toxin PKTx32C4 having no effect). Since the overall inhibition of Ca channels is frequency-dependent, we propose that PhTx2 toxins potentiate the effect of PhTx3 fraction.

KEY WORDS: spider venom, neurotoxin, peptide, ion channel

FINANCIAL SUPPORT: CNPq and FAPEMIG

CORRESPONDENCE TO: PAULO BEIRÃO, Depto. Bioquímica ICB-UFMG. Phone: 55 31 3499-2663 / Fax: 55 31 3499-2614; email: pslb@ufmg.br

INHIBITION AND ACTIVATION OF THE BLOOD COAGULATION CASCADE BY VENOM AND SALIVA PROTEINS: MECHANISMS AND POTENTIAL CANDIDATES FOR DRUG DESIGN

RAGHUVIR K. ARNI(1)

(1)Structural Biology Group - Department of Physics - IBILCE/UNESP

Haemostasis, an intricate and balanced cascade of reactions that involves a number of serine proteinases and macromolecular cofactors which are responsible for maintaining the fluidity of blood under physiological conditions. Worm, snake, leech, tick and lizard gland secretions contain a wide variety of haemostatically-active proteins, which interfere at diferent levels in the coagulation cascade and fibrinolytic system and serve as promising drugs for the treatment of haemostatic disorders. Nematode anticoagulant protein NAPc2 from the hematophagous nematode *Ancylostoma caninum* has been targeted for the control and regulation of thrombosis and is significantly more potent than low molecular weight heparin. NAPc2 only partially inhibits the amidolytic activity of FXa and prevents the formation of thrombin by FXa at a site distinct and remote from the active site with the resultant binary complex inactivating the TF-FVIIa complex with a K_i = 8.4 pM. The crystal structure of the NAPc2 complexed with human FXa reveals the location of this novel site. The homologous protein NAP5 inhibits FXa by binding at both the active site and at the exosite. Molecular modeling of Ixolaris, an double Kunitz domain anticoagulant from the hard tick Ixodes scapularis, which is a vector for Lyme´s disease indicates that it binds to the FXa exosite and inhibits the interaction of FXa with the tissue factor/ F VIIa complex. Structural results indicate that both NAP5, NAPc2 and Ixolaris could serve as the basis for the design of powerful anticoagulants. Another promising anticoagulant protein for the treatment of haemostatic disorders is the protein C activator from *Agkistrodon contortrix contortrix* (Protac), a glycosylated single-chain serine proteinase, which activates the protein C pathway without relying on thrombomodulin and due to the direct and fast-action in protein C activation, Protac has found a broad application in diagnostic practice for the determination of disorders in the PC pathway. The crystal structures of native and inhibited Protac indicate the pivotal roles played by the positively charged belt and the strategic positioning of the three carbohydrate moieties surrounding the catalytic site in protein C recognition, binding, and activation.

Acknowledgements: We are grateful to FAPESP, CAPES, CNPq, DAAD, CEPID, Pentapharm Ltd., Wyeth Research and CORVAS International Inc for financial support and samples.

PHARMACEUTICAL INDUSTRY AND UNIVERSITY PARTNERSHIP

REGINA SCIVOLETTO(1)

(1)Pharmaceutical Innovation Management Agency/UNIEMP

Every innovation involves technical, scientific and commercial knowledge and abilities. Thus requires a partnership between researchers and companies. In Brazil, development of knowledge is mainly done in universities and research institutes. In developed countries, science, technology and production maintain information flow between them making possible efficient development. Legislation in these countries not only allow but foster this interaction. On the other hand, in developing countries there are big barriers in these exchanges such that each activity is isolated from the other.To revert this situation it is necessary to implement: 1- entrepreneurship; 2- establishing communication between agents of each step; 3- proper infrastructure for each stage of development; 4- adopting proper legislation. The Permanent Forum for University-Company Relations - UNIEMP Institute - has, as one of its functions, a nucleus - Pharmaceutical Innovation Management Agency (AGIF) - whose mission is to facilitate relations between the innovation and development agents in Brazil so as to lead the country to become a generator of new pharmaceuticals. Founded in 1992 by regents and an entrepreneur, it was created as a non profit organization, starting with technologies. Ten years later, AGIF was founded due to a specific demand from the pharmaceutical industry. This new group was designed to implement the steps 1 through 4 above. To put in action cooperation between the segments, AGIF identifies potential candidates for each case, prospects scientific and technological opportunities, evaluates the market, helps the researchers in matters of intellectual property and contracts, participates in meetings and government commissions with the intent to assist and counsel the ministries involved in the implementation of policies in the area of pharmaceuticals. The AGIF/UNIEMP also administrates research and development projects with incentive agencies.

Bothrops leucurus Wagler, 1824 (SERPENTES; VIPERIDAE): NATURAL HISTORY, VENOM AND ENVENOMATION

REJANE M. LIRA-DA-SILVA(1)

(1)Department of Zoology, Federal University of Bahia, Bahia, Brazil.

This work is to survey the natural history, venom and envenomation of the *Bothrops leucurus*. This snake is popular know as “white-tailed lancehead”, has a large distribution in the coast Brazilian, from Maranhão to Espírito Santo, where it occurs in the variety of forested and disturbed habitats including scrubs, and even cultivated fields. *B. leucurus* may be found in the ambient of the humid, sub-humid, dry and semi-arid clime, and at vertical range from sea level to 1200 m. Is terrestrial, medium-sized (250–1840mm) (females tend to be larger and heavier than males), mainly nocturnal and active most of the year. Preys are mostly rodents for adult snakes and frogs/lizards for juveniles, indicating an ontogenetic shift in diet. Caudal luring behavior occurs in juveniles that usually have conspicuous, pale tail tips. Date about the reproductive biology demonstrated that *B. leucurus* is viviparous specie, pregnancy (four months) and birth occurs of the winter and summer season, respectively and litter medium size is 19 young. It’s venom is characterized a high number of metals, a high activity of fibrinolytic, proteolytic, hemorragic and edematogenic and a low coagulant activity and it has an important capacity of grabbing myonecrosis. The myotoxicity of venom caused muscle damage and release of CK into blood plasma. The peak of liberation of CK was detected at 6, 3-6 and 3 hours for 25, 50 and 100 μg/ml, respectively and morphological analysis revealed that the venom affected a large number of muscle fibers as show by widespread and varying degrees of necrosis. The *B. leucurus* poison features an electrophoretic profile joined with 4 parts and a chromatographic with 7 peaks; it has a capacity of inhibiting the neuromuscular transmission in an irreversible way and dependent-dosis, because of its post-junccionals actions, and in low concentrations it has a pre-synaptic action. This snake is responsible for the majority of the ophidic accidents at Bahia, Brazil. The envenomation is characterized by local manifestations (pain, edema, heat, numbness, erythema, ecchymosis, phlyctena), coagulations disturbances (delayed coagulation, ingoagulability blood), general manifestations (headache, dizziness), digestive symptoms (vomit, nausea), urinary symptoms (oliguria, anuria, haematuria), disturbances haemorrhagic (haemorrhage, hematemesis, hemoptysis), cardiovascular disturbances (hypotension, bradicardia) and neurologic symptoms (cloude vision, tremors).

KEY WORDS: venom, envenomation, *Bothrops leucurus***.**

FINANCIAL SUPPORT: CAPES.

]CORRESPONDENCE TO: REJÂNE MARIA LIRA-DA-SILVA, Department of Zoology, Federal University of Bahia, Campus Universitário de Ondina, Salvador, Bahia, Brazil, 40170-210. Phone: + 55 71 3263-6564. Fax: + 55 71 3263-6511. Email: rejane@ufba.br

CONTINUED EDUCATION AND UNIVERSITY EXTENSION IN TOXINOLOGY (PARTE I)

RUI SEABRA FERREIRA JUNIOR(1)

(1)Researcher from the Center for the Study of Venoms and Venomous Animals (CEVAP). São Paulo State University – UNESP

Who needs continued education?

For ends of this exposition, the concept of continued education is restricted the formal activities of teach-learning, offered for carrying people of heading of conclusion of a superior course, with exception of programs of Master and PhD degree. One observes that these activities can in such a way be offered by institutions of superior education as for other types of entities, such as supplying of systems and companies of consultoria. The participation of entities not-colleges student, occupies an increasing space in the environment of the continued education. A broad concept of continued education exists, where the requirement of the previous conclusion of superior course is not established. Even though universities come offering increasingly courses where this requirement is not necessary.

How to make possible programs of continued education?

It is the practical international that the accomplishment of programs of continued education is remunerated for the entity that offers them. This if applies not only to the cases where the ofertante is a private company, but also the entities without lucrative purpose, such as foundations and public institutions of superior education. In this last case, the resources proceeding from the governmental budget are primordially destined to cover the costs of the after-graduation and graduation courses.

CONTINUED EDUCATION AND UNIVERSITY EXTENSION IN TOXINOLOGY (PARTE II)

RUI SEABRA FERREIRA JUNIOR(1)

(1)Researcher from the Center for the Study of Venoms and Venomous Animals (CEVAP). São Paulo State University – UNESP

How to make possible programs of continued education? (Cont…)

Exactly the costs of the accomplishment of basic research require arrive in port external of agencies such as the state foundations of support to the research, the CNPq or the FINEP. In this way, the accomplishment of activities of continued education must necessarily auto-be financed. It has some quarrel on the form to defray them, if for the cost delinquent or the total cost (that it implies, for example, in paying a tax for the use of the academic installations). The trend is to defray all the necessary insumos. On the other hand, it has that if to remember that price is different of cost, that is, a price that is adjusted to the power of purchase of the plaintiffs and to the value must be established who the course will add to the participant ones and its sponsors. An explosion of the long-distance teaching (LDT)

is happening in Brazil and in the world. With the challenges of acting in a society where the information disseminates in a fast and globalized way, the new profile of the professional is to search for knowledge.

CONTINUED EDUCATION AND UNIVERSITY EXTENSION IN TOXINOLOGY (PARTE III)

RUI SEABRA FERREIRA JUNIOR(1)

Researcher from the Center for the Study of Venoms and Venomous Animals (CEVAP). São Paulo State University – UNESP

How to make possible programs of continued education? (Cont…)

This new educational paradigm, which privilege the learning and not the teaching anymore, and, finally, the necessity of fulfilling an increasing demand for the continuing teaching of Toxinology, leaded the Center for the Study of Venoms and Venomous Animals (CEVAP), from São Paulo State University (UNESP), to search for models that would satisfy these needs. Hence, in 2003, the long-distance extension course on “Accidents with venomous animals” was created, that it allows access to CEVAP’s media library, comprised of videos, CD-Roms, DVD-Roms, books, textbooks, and a website (www.cevap.unesp.br).
The student enrols on the course online, and receives by post the obligatory didactic material with 2 books and 2 CD-Roms, and a password for the virtual classroom, where he/she will find tools for synchronal and non-synchronal interaction with the other students and the professors, complementary didactic material and tests online. With this modality of University Extension, CEVAP provided an organized educational program, in which, the professor and the student are physically separated by time or geographically. In addition, professionals that live in areas distant from the big centers can keep updated.

CORRESPONDENCE TO: Rui Seabra Ferreira Junior, Fazenda Experimental Lageado- UNESP, Rua José Barbosa de Barros, 1780, Botucatu, SP, CEP: 18610-307. Tel/Fax: (14) 3814-5555 / 3811-7241 e 3814-5446. Email: rseabra@cevap.org.br

THE LESSON FROM SNAKE VENOM: ION TRANSPORT AND GLYCOLYSIS ARE COUPLED THROUGH THE PUMPING RATE OF THE NA/KATPASE

FERNANDES DE LIMA,V.M.[1]; HANKE,W.[2]; CAMILLO,M.A.[3]; SPENCER,P.[3] AND NASCIMENTO,N. [3]

1- ANVISA/Centro de Biologia Molecular IPEN-CENEN/São Paulo; 2- Membrane Physiology Division, Institute of Zoophysiology, 230 Hohenheim University, Stuttgart, Germany; 3- Centro de Biologia Molecular, Instituto de Pesquisa Nuclear (IPEN) CENEN/ São Paulo, Brasil

Electrochemical waves are very commom in biology and in excitable tissue (muscle and brain) they are the key to understand their physiology. The *in vitro* retina model provides a very usefull experimental tool to investigate the spread of exciation waves in central nervous system(CNS). One of the reasons is the marked intrinsic optical signal (IOS) associated with retinal waves. The optical profile of excitation waves in retinae, contain information about the state of membrane channels, transporters (pumps) and the rate of ATP synthesis through glycolsysis in the following way: the rise of the first peak (light scatter) is associated with eletrochemical gradients expenditure, its recovery with increased pumping rate in order to restore these gradients with the consequent ATP breakdown. The rise of the second peak and its amplitude is associated with the increase in the metabolic glycolysis and lactic acid output toward the extracellular space by the glial cells. We have just found that two of the three main toxins of the rattlesnake venom (***Crotalus durissus terrificus***), gyroxin and crotamine have the Na/KATPase has a target and that by slowing down the ion transport, they modulate glycolysis. The effect in the metabolism is similar to lowering the temperature of the preparation from 30 to 20 degrees Celsius. This finding confirm the reports about the receptor role of the sodium pump and rises some interesting questions: these two protein have no homology either in primary or terciary structure, and yet, they share a comom target. Could each one have a different isoform of the pump as the main target? Could these toxins mimic a endogenous glycoside modulation? The exquisite sensisitivity of the tissue to ouabain, appears to indicate that this is true. Furthermore, the third main toxin, crotoxin, also modulates the retinal glial pump, but in different way: it brings “spontaneous” waves with a lacking second optical component, a situation similar to accelerating the pump to its maximun rate **before** the wave such that the frontwave cannot accelerate it further. This effect on the optical profile is similar to rising the potassium in the bath solution to 20 mEq/l, what brings the glial pump to its maxium rate. Gyroxin in high dosis kills the tissue; ouabain, a cardiac glycosideo, also kills. This toxic effect is a consequence of excitotoxicity- prolonged depolarization and high calcium activity within cells leds to lysis. However, ouabain in the retina also kills in low dosis (10 nM). This toxic effect looks different at macroscopic level, probably envolving a different mechanism. In contrast, gyroxin at low dosis is not toxic and appears even protective to the tissue. The same is true for crotamine even at high concentration. Excitotoxicity also spreads in the tissue, but the spread is not wavelike, it “jumps” ahead and invades quiescent tissue in patches at different velocities. By using a small drop (50 μl) of concentrated solution we could follow the spread of excitotoxic response in the presence of high dosis of ouabain and

gyroxin and could compare the tissue response to both substances. The spread is similar but the final outcome differs: oubain toxicity is stronger than the gyroxin toxicity. For the first time non-wavelike spread of excitation is documented in detail. This finding will be of interest to the theorists in the field.

THE OBSERVATION OF CLINICAL MANIFESTATIONS AND THE STUDY OF TOXINS OF AQUATIC VENOMOUS ANIMALS: WHAT IS BEING MADE AND WHAT IS INTENDED TO MAKE

HADDAD JR, V (1,2)

(1) Faculdade de Medicina de Botucatu – Universidade Estadual Paulista, Brazil; (2) Hospital Vital Brazil – Instituto Butantan, São Paulo, Brasil.

Envenomation mechanisms vary from species to species of aquatic venomous animals, involving various apparatuses and venomous glandular tissue which present different accident risks, causing a lack of uniform prevention measures. Although not recent, the development of venomous apparatus in fish seems rudimentary, using structures such as fins and rays to inoculate toxins, rarely having specialized structures for this. It is possible to perceive signs and symptoms of an envenoming caused for fish in an human injury, what take us to the other phase: the laboratorial toxin research. This intermeshing is basic in this type of study: it involves clinical field research of supposedly venomous fish, information of the fishermen on the intensity of the accidents and therapeutical measures used, which will be transmitted to the experimental researcher. In turn, this identifies toxins, proves the action of these in laboratory animals and, especially, reproduces the effects observed. Venomous aquatic animals and their venoms have only recently been afforded attention and new studies, not just because of clinical problems in humans but also for the possibility of discovering new active substances of immense pharmacological potential. This presentation will show some examples of this interaction between experimental and clinical research.

KEY WORDS: venomous animals, aquatic animals**,** venomous fish, cnidarians, injuries in humans, treatment.

FINANCIAL SUPPORT: none.

CORRESPONDENCE TO: Vidal Haddad Junior

Caixa Postal 557 - 18618-000 – Botucatu – SP – Brasil (haddadjr@fmb.unesp.br)

THERAPEUTIC MEASURES FOR THE ACCIDENTS FOR AQUATIC ANIMALS: LITTLE TO MAKE, GUILT OF WHO?

HADDAD JR, V (1,2)

(1) Faculdade de Medicina de Botucatu – Universidade Estadual Paulista, Brazil; (2) Hospital Vital Brazil – Instituto Butantan, São Paulo, Brasil.

The injuries caused by venomous marine and freshwater fish are associated with local manifestations as edema, erythema and intense pain. Systemic manifestations as cardiac arrhythmias or respiratory failure are rare in those accidents (they are observed in accidents caused by Scorpaenidae and stingrays). The envenomation for fish is not considered a priority by the Health Systems, as are those caused for snakes, spiders and scorpion bites. Anti-venom serum is produced only for a genus of Indo-Pacific fish (genus *Synanceja*, family Scorpaenidae), although some envenomations caused by Brazilian fish are known to cause serious consequences for victims. In Brazil, the only measures of treatment are pointed to the first aids of the victim. The use of immersion of the injured place in hot water is a good measure to control the pain, but do not intervenes with the necrotic effect of the venom.

KEY WORDS: venomous animals, aquatic animals, venomous fish, cnidarians, injuries in humans.

FINANCIAL SUPPORT: none.

CORRESPONDENCE TO: Vidal Haddad Junior, Caixa Postal 557 - 18618-000 – Botucatu – SP – Brasil (haddadjr@fmb.unesp.br)

CLINICAL EVIDENCES INDICATING ENVENOMING IN INJURIES CAUSED FOR FRESHWATER AND MARINE FISH: WHEN IS VALID TO SEARCH

HADDAD JR, V (1,2)

(1) Faculdade de Medicina de Botucatu – Universidade Estadual Paulista, Brazil; (2) Hospital Vital Brazil – Instituto Butantan, São Paulo, Brasil.

Venomous marine and freshwater fish are capable of to provoke serious injuries in humans. The main signs and symptoms associated to envenoming are local edema, erythema and intense pain, with possibility of development of vesiles, blisters and cutaneous necrosis. Systemic manifestations as fever, malaise, cold sweating, cardiac arrhythmias or respiratory failure can be observed in those accidents (they are observed mainly in accidents caused by Scorpaenidae and stingrays). When there is a wound in a ray of fin or other acute structure of one fish, there will be a moderate inflammation, caused by crinotoxins and occasionally for bacterial action. The persistence of the inflammation and the onset of very important pain and necrosis should alert the clinical for envenomation. The pain that is not proportional to the wound and this fact must be the great acknowledgment of that the injury can be associated to an envenoming. The research of the toxins in these fish generally is positive and it can bring new sources of studies for the treatment and identification of substances of pharmacological potential.

KEY WORDS: venomous animals, aquatic animals, venomous fish, cnidarians, injuries in humans.

FINANCIAL SUPPORT: none.

CORRESPONDENCE TO: Vidal Haddad Junior
Caixa Postal 557 - 18618-000 – Botucatu – SP – Brasil (haddadjr@fmb.unesp.br)

POTENTIAL ANALGESIC EFFECTS OF ANIMAL TOXINS: FROM BASIC RESEARCH TO CLINICAL APPLICATION

YARA CURY(1)

(1)Laboratory of Pathophysiology, Butantan Institute, São Paulo, Brazil

Pain is a major symptom of many different diseases. Identification of mechanisms and molecular components responsible for pain generation has contributed to the advance in pain control as well as to the selection of new targets for designing novel analgesic drugs. The potential use of natural products as analgesics is well documented. In this context, the selectivity / specificity of animal toxins have enabled their use as potential therapeutics in the treatment of pain, making them candidates for the development of new analgesic drugs. Experimental data have indicated that spider and scorpions peptide toxins induce analgesia by selectively inhibiting voltage-gated ion channels, ASIC channels, or glutamate receptors. Amphibian skin alkaloids that are potent agonists of nicotinic acetylcholine receptor and amphibian peptide opioids have been shown to produce analgesia in humans and animals. The marine environment has proven to be a very rich source of compounds endowed with potent antiinflammatory and analgesic activities. Prialt® (ziconotide intrathecal infusion, Elan Pharmaceuticals Inc.) is the first peptide derived from studies with *Conus* toxins, which was approved by the United States Food and Drug Administration for use in humans as analgesic. Snake venoms comprise a complex mixture of active substances, which can display toxic activities. Despite of their toxicity and based on their biological actions, snake venom components have been used as therapeutic agents as well as scientific tools for the comprehension of physiological and pathophysiological processes. Several lines of evidence indicate that various elapid and viperid venoms display central and peripheral analgesia, mediated by opioidergic or cholinergic mechanisms. The analgesic effect of these venoms has been credit to neurotoxins, small myotoxins, as well as to non-toxic venom constituents. Recently, crotalphine, a novel κ- and δ-opioid receptor agonist, was obtained from the venom of the snake *Crotalus durissus terrificus*. This peptide displays potent and long-lasting analgesic effect, being able to inhibit inflammatory, neuropathic and cancer pain. The pre-clinical trials with crotalphine are now in progress.

KEY WORDS: Animal toxins, analgesia, ion channels, opioid receptors, cholinergic receptors, NA transporter

CORRESPONDENCE TO : YARA CURY, Laboratory of Pathophysiology, Butantan Institute, Sao Paulo, Brazil, Phone: +55 11 37267222. Fax: +55 11 37261505. Email: yarac@attglobal.net

ANT (HYMENOPTERA:FORMICIDAE) VENOMS FROM CAATINGA BIOME: AN UNEXPLORED BIODIVERSITY

AUTHORS: QUINET Y. (1), DE BISEAU J.C. (2)

(1) Laboratório de Entomologia, Instituto Superior de Ciências Biomédicas, Universidade Estadual do Ceará, Fortaleza, Brazil, (2) Laboratoire d'Eco-Ethologie Evolutive, Université Libre de Bruxelles, Bruxelles, Belgium

Chemical defence is the most important defence mechanism in ants, and includes injection, spraying or topical application of venons. However, among nearly 11.000 known ant species, the defensive compounds of hardly tens of them have been analysed, mostly in a purely chemical way. Chemical composition and/or biological properties of defensive compounds still remain unknown in many ant groups. With more than 2000 ant species, Brazil has one of the most diverse ant fauna. However, important Brazilian biomes, like "caatinga", remain poorly investigated. Our study aimed to investigate the chemical diversity and biological functions of the venoms in the *Crematogaster* genus, one of the most successfull groups of ants, with more than 400 species. Instead of injecting venons, as most primitive ants do, *Crematogaster* ants use their modified sting to accumulate venom, and applicate it, topically, on the integument of enemies The analysis of the venom produced by species of the "caatinga" biome showed a high chemical diversity including long unsaturated hydrocarbon chains with primary acetate function, furanocembranoid diterpenes, and triacylglycerols, quite differing from the venoms generally found in other ants. Topical application of those venoms on the body of ant and termite target species showed toxic properties. More direct repelent properties were also shown, while in *C. montezumia*, venom has sticky properties. In addition, the *Crematogaster* venoms presented antibacterial properties, at least in one of the studied species (*C.* sp. prox. *abstinens*). The chemical and functional diversity of *Crematogaster* venoms emphasize the importance of ants as a group with high potentialities for the discovery of new natural products, as well as the need to invest research effort on important and still neglected biomes, like caatinga.

KEY WORDS: ants, venoms, caatinga, biodiversity, *Crematogaster*

FINANCIAL SUPPORT: CNPq.

CORRESPONDENCE TO: QUINET YVES, Laboratório de Entomologia, Universidade Estadual do Ceará, Fortaleza, Brazil. Phone: + 55 85 31019749. Fax: + 55 85 31012810. Email: yvesq@terra.com.br