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THE CENTER FOR APPLIED TOXINOLOGY

RESEARCH, INNOVATION AND DISSEMINATION CENTERS (RIDC)

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Studies conducted by CAT on rattlesnake venom may result in important pharmaceutical innovations

The Center for Applied Toxinology is a multi-institutional research organization based at Butantan Institute in São Paulo, Brazil, dedicated to the study of animal and microbial toxins. It was established in 2000 to stimulate research, disseminate knowledge, and foster interaction between science and industry. Laboratories from the University of São Paulo (USP), State University of São Paulo (Unesp), and Federal University of São Paulo (Unifesp) take part in CAT's activities.

Due to their high target selectivity, venom toxins have been used successfully as pharmacological tools and prototypes for drug development. While pharmaceutical companies spend billions of dollars searching for pharmacological compounds through extensive screening of chemical libraries, venomous animals, during millions of years, have designed their own "pharmacological" tools with the help of molecular evolution.

Acting as a whole, the large variety of toxins present in animal venoms impair the homeostasis of the cardiovascular, the nervous, and the defense systems of the animal, causing dysfunction of blood clotting and pressure, neurological responses to stimuli, cell secretion, migration and adhesion, ultimately resulting in the animal paralysis or death. At CAT, we take a multidisciplinary approach by investigating each particular toxin, which includes isolation, purification, studies of pharmacological actions, structure determination, and structure-function studies of the molecular and cell biology aspects.

CAT has established partnerships with Brazilian pharmaceutical enterprises for drug development. Research findings, obtained at CAT and protected by patents, have been transferred for drug development to some of the most important Brazilian pharmaceutical industries. Toxin based drugs affecting blood clotting, the cardiovascular system, pain perception, anti-proliferative compounds, and immune suppression are being subjected to pre-clinical trials.

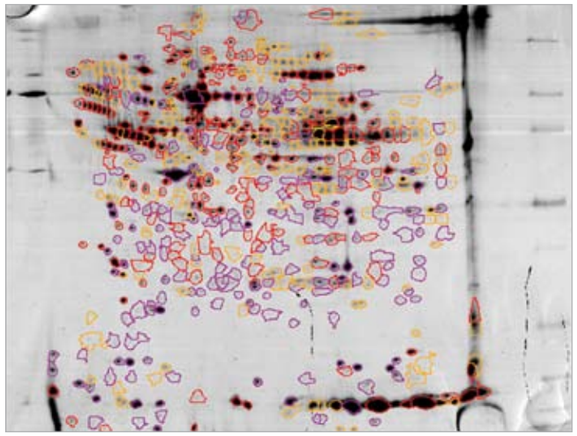
MAIN RESEARCH TOPICS

The toxins found in the venom of the snake *Bothrops jararaca* and their action on the cardiovascular system is one of the most advanced research areas of CAT, having already been studied since the late 1940's. It deals with the effects of the toxins on the control of blood pressure, coagulopathies, fibrinolysis, blood cell migration and adhesion. Involved in these studies are researchers working with proteomic techniques, synthesis of compounds derived from toxins, crystallography, molecular modeling, and a large number of biological assays. Molecular and cellular biology of these toxins, structure-activity relationship concerning their pathophysiological effects, their ability to identify pharmaceutical targets and/or biomarkers are also being investigated. Chemically modified snake venom toxins are being developed to treat dysfunctions of blood pressure regulation. Another research group is studying the toxins of a caterpillar, which have a strong action on coagulopathy processes.

Other research groups are devoted to the nociceptive effects of snake and fish toxins. These include: pain, analgesia, action on cell receptors and ion channels, cell migration and adhesion with special emphasis on neuronal and immune cells. Molecular and cellular mechanisms are under investigation, as well as their systemic action in animal models, both on isolated organs and cells. The main purpose is to use these toxins as lead molecules for the treatment of neuropathic pain and in inflammatory processes of the lung.

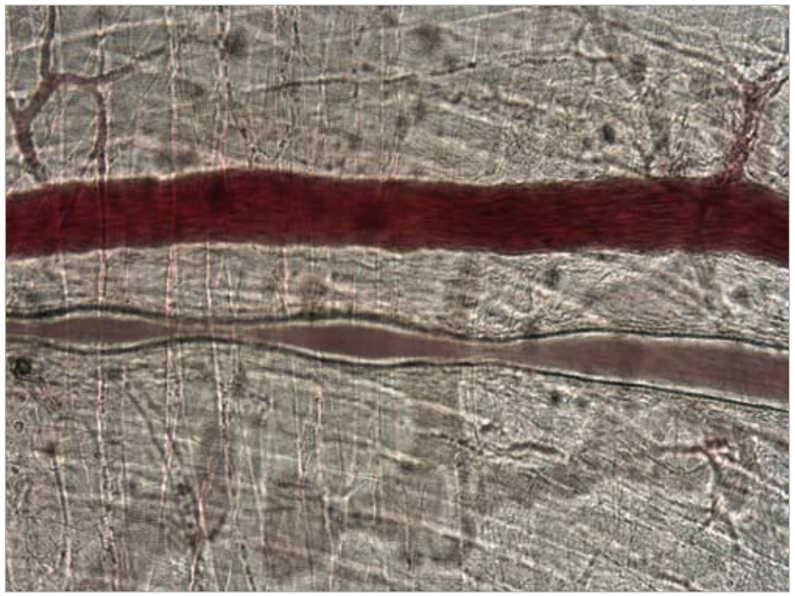
A third research area is concerned with arthropod toxins, which act on cellular proliferation and tumor growth, and are also being studied. Molecules have been isolated which have a strong effect on diverse tumor cells, and at present the mechanisms of action and the targets are being determined.

Finally, another group is investigating the embryogenesis of the central nervous system and the pathophysiological mechanisms related to neuronal migration, synaptogenesis, and neuritogenesis. A protein, fully characterized in our laboratory, was demonstrated to be essential for the cerebral cortex development. Several neuropathologies have been correlated to dysfunctions of this protein. It is thus being used as a target for drugs and toxin action.



2D Electrophoresis analysis of mouse skin proteins after injection of hemorrhagic toxin

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES



Fluorescence assisted cell sorter (FACS) at the Immunopharmacology Facility

Toxinology research projects have an obvious potential for pharmaceutical innovations and since its start in 2001, when CAT was created from scratch, the group efforts focused on basic research allied to protection of intellectual property, mobilization of additional funds for research (private sector investment), and the enhancement of partnerships with the industry.

The most representative publications reported findings on the following subjects: i) the cardiovascular effects of bradykinin potentiating peptides; ii) the antinociceptive effect of *Crotalus durissus terrificus* venom mediated by ions channels; iii) the structural basis for the activity of spider sphingomyelinases D; iv) sea anemone toxins activity on sodium channels; v) the gene delivery into cells by crotamine from *C. d. terrificus*; vi) the prothrombin activator from *Lonomia obliqua*; vi) the inhibition of NUDEL activity by disrupted-in-schizophrenia 1; vii) the function of non-catalytic domains of venom metalloproteinases.

Concerning the patents applied by CAT so far, we highlight the following pharmaceutical applications under development: 1) Evasin Project. We are finishing up the basic pre-clinical evaluation steps by using laboratory animals treated with various different synthetic peptides. Progress in the research with these molecules showed that the cardiovascular action of the peptides takes effect on a target different from the angiotensin-converting enzyme. 2) Enpak Project. A patent was filed concerning an analgesic compound, which is more potent than morphine and does not cause addiction. It is active, when taken orally, and shows long duration. This molecule, named Enpak (endogenous pain killer) was isolated and completely characterized and synthesized in our laboratories. 3) Lopap Project. Several aspects of the prothrombin activating activity of the bristles of the caterpillar *Lonomia obliqua*, assigned to a novel protease called Lopap, for *Lonomia obliqua* prothrombine activator protease. Recombinant Lopap, its use in diagnostic kits and as an agent causing fibrinogen depletion, are in basic pre-clinical evaluation steps 4) Amblyomin Project. A novel Kunitz-type protease inhibitor of factor FXa was identified in the salivary gland of the tick *Amblyoma cajennense*. Its therapeutic effect was shown on mice with dorsal melanomas and pulmonary metastasis.