BIOCHEMISTRY

Molecule factory

Partnership between the Applied Toxinology Center and a pharmaceutical company results in an analgesic that is more potent than morphine

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New active ingredients, discovered by a group of researchers from São Paulo, have shown great pharmacological potential for alleviating pain and controlling blood pressure, as indicated by tests carried out with molecules synthesized from the venom of the rattlesnake (*Crotalus terrificus*) and of the jararaca (*Bothrops jararaca*). Other pure molecules extracted from a sponge are capable of reducing tumors, but cannot yet be revealed because they are at a stage of research that calls for secrecy.

Since its creation, the Applied Toxinology Center (CAT), housed in the Butantan Institute, has already deposited six patent requests. The most recent deals with a substance obtained from rattlesnake venom, which, in a single dose, revealed an analgesic power 600 times more potent than morphine, an effect that is prolonged for up to five days without any side effects. The first patent, deposited in March 2001, derived from the study not only of the jararaca's venom, but also in this snake's brain, where 17 peptides were found that result from a chain of amino acids with antihypertensive properties baptized as Evasins (endogenous vasopeptidase inhibitors). The researches were carried out in partnership with the Brazilian pharmaceutical industry, represented by the Pharmaceutical Consortium (Coinfar), made up of the Laboratories Biolab-Sanus, União Química and Biosintética.

The studies that resulted in isolating the molecules responsible for the analgesia have covered a long road. Vital Brazil, who founded the Butantan Institute and was a pioneer in the study of snakes in Brazil and the Brazilian precursor in the medical application of animal toxins, showed the analgesic effect of rattlesnake venom at the beginning of the 20th century. Professor Antonio Carlos Martins de Camargo, the coordinator of the CAT, which is one of the ten Research, Innovation and Diffusion Center (Cepids) created by FAPESP in 2000, recalls that the researcher used, effectively, diluted rattlesnake venom on patients with chronic pains. "The venom used was very much diluted, almost homeopathic, but the patients felt very well", he says.

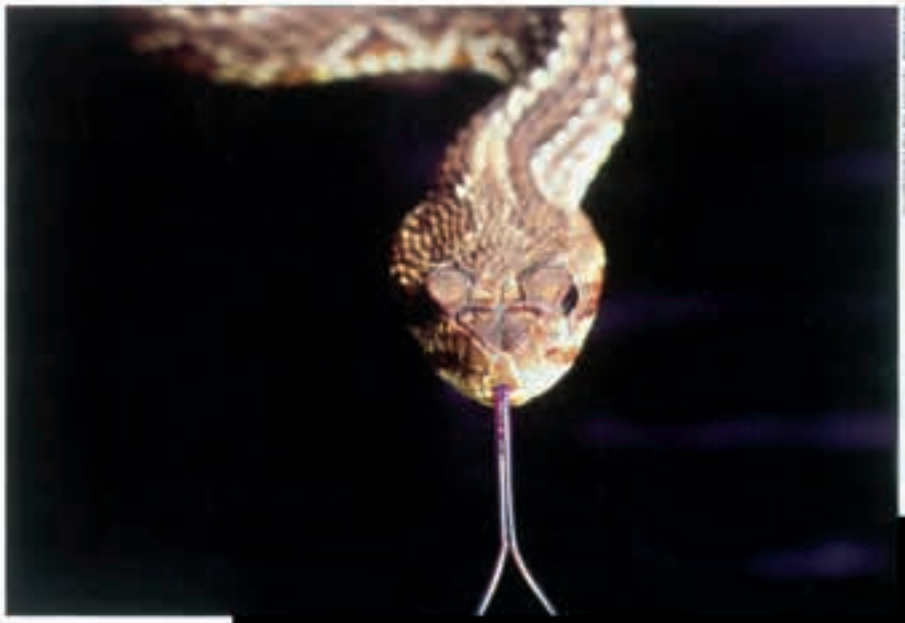
It was following these observations that researchers from Butantan managed to carry out a pharmacological characterization of the substance contained in the rattlesnake venom, but not the active ingredient, that is, the molecule or the molecules responsible for this effect. This only became possible, according to Camargo, with the creation of the CAT, which made it possible to set up the infrastructure needed for isolating, chemically identifying, and synthesizing the active substance.

Effects reproduced - As these molecules are minority components in the venom, isolating them and characterizing them is a very complex task, which calls for specialists in the subject and specific instruments, such as the mass spectroscopy apparatus, fundamental for determining the molecular structure. "After several failures, we managed to make it, thanks to the work of the researchers from the Butantan Institute, like Yara Cury and her postgraduate pupil Gizele Picolo, and of a Japanese researcher, Katsuhiro Konno, a specialist in purification who worked with us for three years as the holder of a scho-

Biolab's production line:
new pharmaceutical products
developed in Brazil

larship from FAPESP", Camargo says. "At the end of last year, we managed to arrive at one of the molecules responsible for the analgesic effect."

The molecule was isolated, its structure identified, and then synthesized. Tests proved that the synthetic molecule isolated reproduced the analgesic effects. "It has an effect similar to morphine, but is far more potent and lasting, and without any side effects identified up to this moment. Furthermore, the product is administered orally", he stresses. Morphine, on the other hand, which is standard for analgesia, acts for a short period of time. To get the same effect as the first dose administered the quantity taken has to be increased, which results in a cumulative effect on the organism and, in some cases, on dependency. The tests with the new analgesic were carried out on animals and still have to be confirmed in human beings, in the so-called phase 3 and 4 clinical tests.



Rattlesnake venom: analgesic effect will be tested on humans

Right away in the preclinical tests carried out with the antihypertensives obtained from jararaca venom, new properties that are not to be found in the first patent were discovered and patented. Tests were carried out that showed important differences between these and the antihypertensives of this class that exist in the market. These differences may improve the quality of the treatment of hypertensive individuals.

Yet another activity of these molecules not related to the one that was first identified is often discovered. This is the case of one of the Evasins that influences the permeability of ion channels and modifies the response of the stimuli, such as happens, for example, with the stimulus that leads to muscle contraction. This property may have another therapeutic application besides the hypertensive activity, such as in the treatment of diseases that affect the central nervous system. The new discoveries, as well as the paths followed by the synthesized molecule inside the organism, resulted in two other patents, filed in Brazil, in the United States, in the European Community, and in Japan.

At the current stage of the research, the best Evasins, which have greater effectiveness and fewer side effects, are being picked to develop the antihypertensives. The Federal University of Minas Gerais (UFMG) has been working along two lines. One of them deals with the search for formulations capable of making effective their administration orally, since the peptides are destroyed by the digestive tract. The other is assessing the antihypertensive action of four of the 17 new peptides found in the jararaca's venom. Genetically modified rats with hypertension, bred in the laboratory, are given dosages of each one of them and are subject to full time monitoring.

Promising results - The results achieved up until now, both with the analgesic and with the hypertensive, have been very promising, as the partners from the industry attest. "We went far beyond where we imagined at the outset", says Cleiton de Castro Marques, a vice-president of the Castro Marques Group, which includes Biolab-Sanus and União Química, two of the companies taking part in the consortium. The beginning of this partnership took place after a talk between Professor Camargo and physician Márcio Falci, the medical director of Biosintética, the other member of Coinfar. "When he talked to me about the kind of project he was developing, I glimpsed the possibility of having, at a single place in Brazil, new products being discovered in an intense and rapid manner", says Falci. This was the beginning of the partnership between the pharmaceutical industry and the CAT. "We perceived the possibility of having a basis for developing a line of pharmaceutical products that could end up reaching the market", says José Fernando Leme Magalhães, the Castro Marques Group's corporate director for strategic affairs.

The consortium was formed because it would be more difficult for each one of the companies individually to have the prowess to accompany the ramifications of the project. Within this scenario, the businessmen began to look with more attention at the details of the products in transformation. Up until now, each one of the three companies that make up the consortium has now put US$ 1 million of its own funds into the research, to a total of US$ 3 million. But from here onwards, the spending

José Carlos Martins Archive

Toxins from sponges show anticarcinogenic properties in tests

will be higher, with the certification of the tests and putting together the dossier that will be forwarded to the Brazilian and international regulatory agencies, for the product to be approved.

This form of work and new investments should also serve for one of the most recent novelties to have left the CAT's benches, with the support of Coinfar. The product, whose preliminary results are heartening, has anticarcinogenic properties and is obtained from sponges. "The tests with the pure molecule caused an extremely significant reduction in some kinds of tumors", Camargo says.

Sponges are very simple invertebrate animals that live stuck to rocks and other marine organisms. To feed themselves and to grow, they produce toxins that put their predators to flight from the places they inhabit. Knowing about this, the researchers went after these toxins to look for possible applications for them. This molecule was isolated and its structure determined by mass spectrometry. The next step is to get it in synthetic form. The studies are part of a program of the CAT for developing toxins from marine animals, with an application in many areas. "Just to mention one example, AZT, an antiviral used in the treatment of Aids, was produced from the toxin of a sponge", Camargo says.

With this broad range of research, Butantan's Toxinology Center is described by Castro Marques as a molecule factory. With the good news from the CAT, Biolab and União Química are committed to investing in research and development, to improve their competitiveness. This year, the group set aside 5.3% of their sales in the pharmaceutical area, which in 2003 were R$ 419 million, for research. The money injected will therefore amount to R$ 22.2 million. The investment covers everything from innovative products to the development of new pharmaceutical forms, new concepts, and clinical studies.

THE PROJECT

Applied Toxinology Center (CAT)

MODALITY
Research, Innovation and Diffusion Centers (Cepids)

COORDINATOR
Antonio Carlos Martins de Camargo – Butantan Institute

INVESTMENT
US$ 1,300,000.00

The partnership between the companies and research centers started nine years ago, with the Institute of Nuclear Energy and Research (Ipen), and resulted in Bandgel, a hydrogel for the treatment of burns. The product acts as a protective barrier, making possible a rapid recovery of the tissues at the place of the burn.

Business philosophy - "We have reached the point today at which we have received more proposals than our capacity for investing", Castro Marques reports. For him, research is of prime important for the industry in Brazil. Since the Law on Patents came into force in Brazil in 1996, the domestic companies began to be concerned with the need for developing new products, as the major international companies do. "We have to invest and to create technology, because the market will be made up, on the one side, by innovative companies, and, on the other, by companies making generic and similar medicines and popular products", he says. The same philosophy provides the bearings for Biosintética, which has been working in partnership with universities since 1993, to develop products. Every year, it invests 2% of its net sales of about R$ 260 million in research, which works out at R$ 5.2 million.

According to Falci, who is from Biosintética, one of the results of the partnership with the CAT, besides the promising drugs, was the laying of the bases for defining a policy for the pharmaceutical industry. The first pillar was the creation of the Pharmaceutical Innovation Management Agency (Agif), which brings together specialists capable of making a well protected patent, and has the task of identifying bottlenecks in the route by which discoveries are transformed into products.

For Magalhães, the objective of innovation is the market. And success is the greatest stimulus. "I am sure that when one of these innovative products reaches the market, many other companies are also going to want to invest." The capacity for putting onto the market a new product, with a higher added value and commercial appeal motivates the competition. •