NEUROTOXINS

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The Phoneutria nigriventer is a wandering solitary spider from South America and is responsible for many accidents with arthropod in São Paulo state. Its venom is very potent and contains innumerous neurotoxic peptides. Among them, one stands out for the toxicity and lethality - the TX2-5.

Several studies described its action on voltage dependent sodium channels. The main action observed in frog muscle sodium channels are the delay in inactivation. Sodium conductance and steady-state inactivation are shifted to more negative potentials and also a net reduction in current amplitude. Male mice injected with this toxin present a dramatic intoxication with two early signs: penile erection and hypersalivation, followed by death, with signs of severe respiratory distress. Severe human accidents involving this spider and scorpions as well, are characterized by lung edema, therefore, it seems plausible to assume that death is caused by lung edema. Since penile erection is triggered by central as well as peripheral mechanisms, a pharmacokinetic study employing iodinated toxin was performed in order to determine whether the toxin could penetrate the blood-brain barrier. Also, physiological penile erection involves non-adrenergic non-cholinergic transmission (NANC) and the mediation by nitric oxide have been demonstrated.

The TX2-5 was isolated, from the total venom, by gel filtration chromatography and RP-HPLC. The analysis of toxin purity was carried out by mass spectrometry (MALDI-TOF) and the identity confirmed by Edman amino acid analyses method. The TX2-5 radioiodination was carried out by a classic method of chloramine T. The biodistribution analysis suggested an hepatic metabolization, renal clearance and redistribution after 1 hour. Following a sequence there are the organs with with large vascularization such as lung, spleen, heart and liver. A low kinetic of radiotracer occurred in muscle, testicles and brain and high kinetic of radiotracer occurred in lung, liver, heart and spleen.

In this study, the pretreatment with de non-selective nitric oxide synthase (NOS) inhibitor L-NAME reduces the penile erection and partially protects from the lethal effects of TX2-5; but the pre-treatment with the nNOS selective inhibitor 7-NI completely abolishes all the toxic effects of TX2-5, including penile erection and death suggesting that nNOS is the major player in this intoxication.

Toxins from other animals that affect sodium channels similarly as TX2-5 and induce similar toxic syndromes may have as a major common outcome the activation of nitric oxide synthase.

USE OF NUCLEAR TECHNIQUES FOR BIODISTRIBUTION STUDIES AND ANTIGENS MODIFICATIONS

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Leishmaniasis are a complex of diseases caused by protozoan parasite Leishmania, (FIG.1) with a prevalence of 12 million cases, causing disease ranging from skin lesions in cutaneous leishmaniasis to a progressive and frequently fatal hepatosplenomegaly in visceral leishmaniasis (VL). Co-infection with HIV makes VL a priority for the World Health Organization. Pentavalent antimonials, as Glucantime and Pentostam, are the main drugs recommended in the treatment of all forms of leishmaniasis. Despite several gaps on the knowledge of action, toxicity and pharmacokinetics, pentavalent antimonials have been used over 60 years ago. The definition of a pharmacokinetic profile of antimony may suggest a better therapeutic protocol for doses, administration interval and duration of the antimonial therapy, reducing resistance, relapse and severe side effects.

Therefore, we devised to obtain a radiotracer, by neutron activation of Glucantime, resulting in radioactive antimony salts, useful as radiotracer, allowing easy determination in animals, determining its biocompatibility and its biodistribution in experimentally infected mice. This compound maintains the same antileishmanial activity as the native one. In its biodistribution studies, it was found higher uptake in the liver of healthy or infected mice and was mostly eliminated by biliary excretion, with a small and fast proportion of the drug excreted in the kidney. We hope that our data support new studies in the construction of radiotracers and that the antimony biodistribution data provide insights in the systemic effect of antimony salts in these neglected diseases.

On the other hand we also have studied the effects of gamma radiation on proteins and the capacity of the immune system to recognize modified macromolecules evaluating some immunological aspects of mice exposed to native or irradiated protein. Our results indicate that radiation promoted modifications on both the molecules. According to our results, the modified toxin was 5 folds less toxic than its native counterpart. Sera of animals immunized with the native and irradiated proteins were analyzed in order to evaluate levels of IgG, as well as to quantify specific isotypes. While the native proteins induced a predominant Th2 response, the irradiated molecules apparently promoted a switch towards a Th1 pattern.

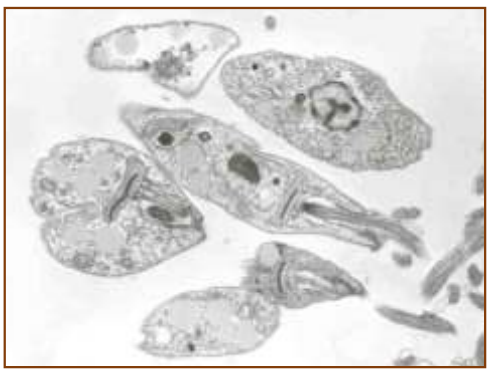

FIGURE 1 - Ultrastructure of Leishmania amazonensis.