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Short communication

Phoneutria nigriventer spider toxin Tx2-6 causes priapism and death: A histopathological investigation in mice☆

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ABSTRACT

Phoneutria nigriventer spider bite causes priapism, an effect attributed to the peptide toxins Tx2-5 and Tx2-6 and involving nitric oxide. Tx2-6 (MW = 5287) is known to delay the inactivation of Sodium channels in the same fashion as many other venom toxins. In the present study we evaluated the i.p. dose that induces priapism and the other symptoms in mice. Animals killed by the toxin or crude venom (0.85 mg/kg) were autopsied and a pathological study of brain, lung, kidney, liver and heart was undertaken using standard techniques. The same protocol was employed with animals injected with crude venom. Results showed that priapism is the first sign of intoxication, followed by piloerection, abundant salivation and tremors. An i.p. injection of about 0.3 μg/kg induced only priapism with minimal side-effects. The most remarkable histological finding was a general vascular congestion in all organs studied. Penis showed no necrosis or damage. Lungs showed vascular congestion and alveolar hemorrhage. Heart showed also sub-endothelial hemorrhage. Brain showed only a mild edema and vascular congestion. Results obtained with crude venom closely resemble those of purified toxin. We conclude that Tx2-6 have profound effects on the vascular bed especially in lungs and heart, which may be the cause of death. Interestingly brain tissue was less affected and the observed edema may be attributed to respiratory impairment. To the best of our knowledge this is the first histopathological investigation on this toxin and venom suggesting a possible cause of death.

1. Introduction

The aggressive wandering spider *Phoneutria nigriventer* is responsible for several hundreds of human accidents in Brazil every year. The most remarkable sign of this spider bite is an excruciating local pain that usually extends from the bite site to the whole limb and demands treatment with local anesthetics. Other signs are muscle cramps and nausea. In less than 2% of the cases a severe intoxication may arise characterized by lung edema. Among the younger male victims it can be observed also a persistent penile erection but this symptom is considered very rare (Bucaretchi et al., 2000). The peptide toxins Tx2-5 and Tx2-6 of 5116 and 5287 Da respectively, are known to delay the inactivation of sodium channels (Araujo et al., 1993; Matavel et al., 2002), and were identified as the toxins that consistently induce penile erection in mice when injected i.p. (Troncone et al., 1998; Yonamine et al., 2004). Such effect seems to involve a nNOS-dependent mechanism, as we described earlier (Yonamine et al., 2004). A recent study

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employing brain c-fos expression mapping argued against the involvement of CNS in toxin-induced priapism, further confirmed by the ineffectiveness of intra-cerebral toxin injections (Troncone et al., 2011).

Erectile dysfunction has been reported to affect about 25% of the male population below 69 years and about 61% of those above this age (Bacon et al., 2003). The treatment of many of these cases has improved significantly with the introduction of phosphodiesterase inhibitors like sildenafil, tadalafil and others. Since these drugs have also important side-effects, some potential users cannot benefit of these treatments and remain untreated. Therefore, new drugs should be available to help these patients and the discovery of venom components that interfere positively with the erectile function represent potential new drug leads waiting for further development. Also, a better understanding of the mechanism by which the toxin produces erection may open unexpected new therapeutic strategies in this field.

1.1. Objectives

This study aims to describe the histopathological consequences of intoxication by Tx2-6 and crude *P. nigriventer* venom in order to propose a possible cause of death. Also, the dose and time frame of the erectogenic effect of Tx2-6 toxin by the i.p. route was investigated.

2. Methods

2.1. Toxin preparation

Tx2-6 toxin was purified as described elsewhere (Troncone et al., 1995, 1998). Briefly, crude desiccated venom was dissolved in 2% acetic acid, submitted to a Sephadex G50-f liquid chromatography, followed by RP-HPLC. Pure fractions were screened by mass spectrometry (Q-TOF – Micromass) and the component with the characteristic 5287 Da molecular weight was tested for activity and positively identified as Tx2-6. The toxin was then aliquoted, lyophilized and kept at −20 °C until use. Quantification of the peptide toxin was carried out by automated Edman degradation and the molar amount of the first identified amino acid was considered to calculate the net content of toxin.

2.2. Animals

Male Swiss mice with ages between 18 and 24 weeks breed in the animal facility of Instituto Butantan were used. Mice were kept in our temporary animal facility for two weeks before experimentation, with water and pellet food *ad libitum*, controlled ventilation, temperature and light period of 12/12 h. The experimental protocol was submitted to the Ethical Committee for Animal Research of Instituto Butantan under the number 453/08 and found in agreement with the Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation.

2.3. Dose-related symptoms assessment

A total of 52 mice were used to investigate how the intoxication by Tx2-6 develops. Groups of 19 animals were i.p. injected with doses of 0.3 or 0.6 μg/kg and groups of 7 animals were injected with doses of 1 or 3 μg/kg. Animals were examined for priapism, piloerection, salivation and death every 5 min by two investigators. Observation lasted 2.5 h as after this time the occurrence of death became unlikely. Animals were checked for survival 24 h later.

2.4. Histopathological examination

The histopathological consequences of intoxication by crude venom or Tx2-6 were investigated in 9 mice. They were divided in three groups: for control purposes three mice were injected with 0.25 ml of saline solution; three mice were injected i.p. with 0.85 mg/kg of crude *P. nigriventer* venom suspended in saline solution (1 ml/100 g of body weight) and the last three mice were injected i.p. with 0.6 μg/kg of Tx2-6 toxin, a dose that produces full penile erection as well as the other signs of intoxication and was lethal to most of the mice. Preliminary experiments demonstrated that subcutaneous and i.p. injections of toxin or venom rendered identical effects. After a maximum time of 2 h of observation, control saline-injected animals were sacrificed by cervical dislocation, as well as the surviving venom- or toxin-injected mice. Venom- and toxin-injected mice that died earlier were processed immediately after death. Brain, lungs, kidney, liver and heart were excised and formalin-fixed for further histological examination using routine H–E staining.

3. Results

The toxin Tx2-6 induced priapism, piloerection and salivation and the dose/responses of these effects are depicted in Fig. 1. Priapism was observed with lower doses of toxin and was usually the first sign to appear. Salivation and piloerection appeared later and persisted until death as well as priapism. With higher doses priapism was observed sooner and animals injected subcutaneously also showed priapism (data not shown). It was clear that if a higher dose of toxin or venom was injected the animals could die without showing all the symptoms described here. Also, lower doses could elicit only priapism. All the animals

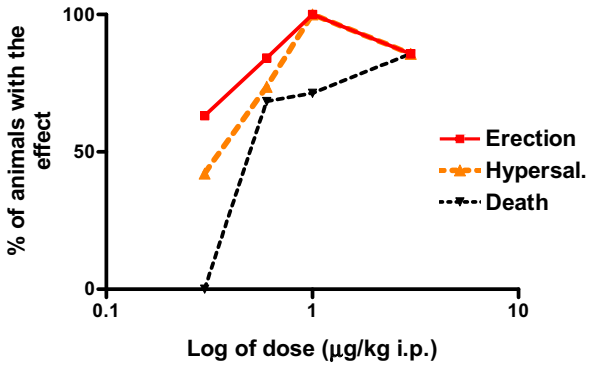

Fig. 1. Dose vs. response relationship of the three main signs of intoxication by Tx2-6. Doses of 0.3 and 0.6 μg/kg were injected i.p. in 19 mice; 7 mice were injected with the doses of 1 and 3 μg/kg.