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Effect of clonidine in mice injected with *Tityus discrepans* scorpion venom

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ABSTRACT

A study was conducted to assess the effect of clonidine (α_2-adrenoceptor selective agonist) on glycemia, serum and urine α-amylase, blood urea nitrogen (BUN), serum creatinine, white blood cell count, kidney histology and zymogen granule content in pancreatic acini, in mice under the effect of *Tityus discrepans* (*Td*) scorpion venom. BALB/c male mice (20 ± 2 g, $n = 7$–11) were intraperitoneally (ip) injected with a sublethal dose (1 μg/g) of *Td* venom, and were treated (ip) with 0.1 μg/g of clonidine (Catapresan®) or 0.9% NaCl 30 min after the venom injection, and then every 2 h. Six hours later, mice were anesthetized with diethylether and urine and blood samples were withdrawn by cystocentesis and cardiocentesis, respectively. Tissue samples were obtained and fixed immediately in buffered formalin (2%, pH 7.4) and then processed for stain H&E. *Td* venom did not cause hyperglycemia by itself. However, clonidine induced hyperglycemia, which was synergized by *Td* venom. Although the venom did not produce hyperamylasemia, clonidine significantly diminished serum α-amylase activity in envenomed mice. *Td* venom did not significantly increase urinary α-amylase activity, which was unaffected by clonidine. Morphometric analysis using microphotographs of pancreata from mice injected with *Td* venom showed a reduced zymogen granule content as judged by the acidophilic bidimensional area of acini. This effect was significantly reduced by clonidine. Kidney samples showed histological changes which were partially affected by the drug. Clonidine reduced the increase in BUN and serum creatinine concentration in envenomed mice. *Td* venom produced neutrophilia and lymphopenia, which were clonidine-resistant at the assayed dose. These results suggest that α_2-adrenoceptor selective agonists would be able to reduce some scorpion venom-induced renal and pancreatic disturbances, possibly through the inhibition of neurotransmitter release from presynaptic cholinergic and noradrenergic terminals, as well as from adrenal medulla.

1. Introduction

Scorpionism is a public health problem in tropical and subtropical countries (Chippaux and Goyffon, 2008). The lethality of scorpion venoms in mammals has been mainly associated with the presence of long-chained (59–76 aa, 6-8 kDa) peptides containing disulfide bridges, which are able to modify the functional properties of voltage-dependent sodium channels (Na_v) in excitable tissues (Chávez-Olórtegui and Kalapothakis, 1997), leading to

Abbreviations: ANS, autonomic nervous system; α_2-AR, α_2-adrenoceptor; BUN, Blood urea nitrogen; GFR, glomerular filtration rate; *Td*, *Tityus discrepans*; WBC, white blood cells.

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repetitive neuronal depolarizations. The resultant massive release of neurotransmitters is responsible for an exacerbation of the autonomic nervous system (ANS) function (Freire-Maia, 1995), which contributes to a widespread inflammatory response and multi-organ failure (D'Suze et al., 2004).

Although the use of antivenoms has been widely recognized as the first choice of treatment to victims stung by scorpions (Boyer et al., 2009; De Rezende et al., 1995), variations in the polymorphic regions of the structure of Na_v-specific scorpion toxins, which lead to poor cross-reactivity, hamper the production of antivenoms with a broader range of specificity (Delori et al., 1981; Gazarian et al., 2005).

The pharmacological management of scorpion envenomation has included vasodilators, sedatives, prokinetics, antiemetics, anesthetics, antipyretics, α- and β-adrenoceptor (α- and β-AR) antagonists, and glucocorticoids (Ismail et al., 1992; Amitai, 1998). However, to our knowledge, the effect of α_2-AR agonists in mammals under scorpion envenomation has not been widely studied. Since α_2-AR agonists are able to inhibit the cholinergic and noradrenergic neurotransmission in several organs (Green et al., 1979; Grundström et al., 1981; Langer, 1980), as well as catecholamine release from adrenal medulla (Zhou et al., 1994), a study was conducted to assess the effect of clonidine on several biochemical, hematological and histological changes induced by *Td* venom in mice.

2. Materials and methods

2.1. Mice, scorpion venom, and drug

BALB/c male mice (20 ± 2 g, $n = 7$–11) were obtained from the Instituto Venezolano de Investigaciones Científicas (IVIC) and received water and food *ad libitum* up to the day of the experiment, when only access to tap water was allowed. Adult *Td* scorpions were collected near San Antonio de Los Altos, Miranda State (10°20′N, 67°45′W), and identified according to the criteria reported by González-Sponga (2005). The scorpions were fed *Acheta domesticus* (Orthoptera, Gryllidae) once a week. *Td* venom was extracted by manual stimulation of the telson from 20 scorpions as described by Zlotkin and Shulov (1969), freeze-dried at 50 mbar and −40 °C and stored at −80 °C. Then, the venom was resuspended in 0.9% NaCl, centrifuged at 12000 × *g* for 10 min to eliminate insoluble matter, and maintained at 4 °C in the dark until use. The protein content was determined in the supernatant according to Lowry et al. (1951). Clonidine (Catapresan®) was purchased from Boheringer-Ingelheim (Germany).

2.2. Experimental procedures

All experiments were carried out in accordance with the current ethical guidelines for the care of laboratory animals and the ethical guidelines for investigations in conscious animals set by the Bioethics Committee of the Facultad de Ciencias Veterinarias, Universidad Central de Venezuela. Mice were randomly assigned to each of the following four experimental groups: A, control (0.9% NaCl, saline); B, clonidine (0.1 μg/g); C, *Td* venom (1 μg/g) and D, *Td* venom plus clonidine. The intraperitoneal injections were carried out in a total volume of 0.1 mL. In groups A and C, saline injections were repeated every 2 h, from 30 min after the first injection with saline and venom, respectively. In groups B and D, clonidine doses were repeated every 2 h, from 30 min after the first injection with saline and venom, respectively. This experimental protocol was designed to ensure a prolonged effect of clonidine (Conway and Jarrott, 1980). After 6 h, mice were anesthetized with diethylether, and urine, blood and tissue samples were obtained by cystocentesis, cardiocentesis and necropsia, respectively. Serum was obtained by centrifugation of the clotted blood at 3000 × *g* and maintained at 4 °C until use, 24 h later. Tissue samples were immediately fixed in buffered formalin (2%, pH 7.4).

2.3. Clinical chemistry assays and white blood cell (WBC) count

Glycemia, serum and urine α-amylase, BUN and serum creatinine were assessed by enzymatic colorimetric methods (Cromatest®, Linear Chemicals, Spain). Absorbance was determined in a Statfax® 1904 chemistry analyzer, and the results were compared with commercially available standards.

Blood samples were diluted in Turk's solution (1:20) and WBC count was performed in Neubauer's chamber. Differential count was obtained through observation of blood smears with an OLYMPUS® CX41 microscope.

2.4. Histopathology and processing of images

Fixed pancreata and kidneys were processed by a routine paraffin-embedded procedure and H&E staining. Microphotographs were randomly taken in 13–18 frames of pancreata from 7 mice with a Panasonic® LUMIX® DMC LX-3 digital camera, properly fixed to an OLYMPUS® CX41 microscope. Representative kidneys microphotographs were taken in a similar way as previously described. Images of pancreata were processed with the software ImageJ (public domain, National Institutes of Health, Bethesda, MA, USA), as described by D'Suze et al. (2004), with several modifications. Briefly, color images were changed to 16 bits images (*Image → Type → 16-bit*). Then the background was inverted and adjusted (*Image → Adjust → Threshold → Dark background*) to consider only the acidophilic central area of acini, which is related to zymogen granule content (Fletcher et al., 1994). A unique frame of 72000 μm^2 was used in all images. Finally, the *Analyse* tool was used to retrieve the corresponding bidimensional area (*Analyse → Measure*). The scale was previously set using a Neubauer's chamber microphotograph, taken in identical conditions as abovementioned.

2.5. Statistics

All results are shown as the arithmetic mean ± standard error of the mean (mean ± SEM) and were analyzed through Student's *t* test or variance analysis (ANOVA) followed by Bonferroni *post hoc* test (Graphpad Prism®). The results were considered statistically significant when $p \leq 0.05$.