
Pancreatic toxicity in mice elicited by *Tityus zulianus* and *Tityus discrepans* scorpion venoms

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Abstract. Envenoming by *Tityus discrepans* (TD) scorpions in northcentral Venezuela mainly causes pancreatic and gastrointestinal complications whereas the sting by *Tityus zulianus* (TZ) (western Venezuela) often produces respiratory arrest and death by pulmonary oedema. Since TZ pancreatic toxicity may have been overlooked, a study was carried out to evaluate TZ venom effect on mice pancreas. BALB/c mice were injected intraperitoneally with 0.5 mg protein/kg of either TD or TZ venoms. A significant increase in serum-amylase activity (IU/mL) was obtained after 3 h (20.6 ± 3.0 , $p < 0.05$) and 6 h (60.4 ± 3.0 , $p < 0.001$) of TZ venom injection compared to saline-inoculated mice. Increased amylase levels were also elicited by TD venom, although these were significantly lower than those induced by TZ. Light microscopy of pancreas from TZ-envenomed mice revealed interstitial oedema and vacuolization of acinar cells as soon as 1 h after injection. Differences in the time course of the pancreatic oedema (wet pancreas weight/body weight ratio) elicited by TD and TZ venoms suggest the existence of species-specific mechanisms for oedema formation. Taken together, these data suggest that the TZ venom contains components highly toxic to the mammalian pancreas, which may play a role in developing TZ-related pulmonary complications.

Acción del veneno de los escorpiones *Tityus zulianus* y *Tityus discrepans* sobre el páncreas murino.

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Palabras clave: Veneno de escorpión, *Tityus*, pancreatitis, Amilasa.

Resumen. El envenenamiento por escorpiones *Tityus discrepans* (TD) (centro-norte de Venezuela) causa principalmente complicaciones gastrointestinales y pancreáticas mientras que el envenenamiento por *Tityus zulianus* (TZ) (Sur del Lago de Maracaibo) puede producir paro respiratorio y la muerte por edema agudo de pulmón. En vista que la toxicidad cardiorrespiratoria puede tener su origen en el daño pancreático, este estudio tuvo como objetivo evaluar la pancreatotoxicidad inducida por TZ en ratones BALB/c. Los animales fueron inoculados intraperitonealmente con 0.5 mg de proteína/kg de los venenos de TD o TZ. Se registró un incremento significativo en la amilasa sérica (IU/mL) luego de efectuada la inoculación de TZ a las 3 horas ($20,6 \pm 3,0$, $p < 0,05$) y 6 horas ($60,4 \pm 3,0$, $p < 0,05$) en relación a controles inoculados con solución salina. Los niveles séricos de amilasa en ratones inoculados con TD igualmente resultaron incrementados, aunque éstos fueron significativamente inferiores a los obtenidos para TZ. Se observó mediante microscopía de luz, edema intersticial y vacuolización en páncreas de ratones 1 h luego de efectuado el inóculo con TZ. Las diferencias observadas en el curso temporal del edema pancreático (relación peso húmedo del páncreas/peso corporal) inducido por TD y TZ sugiere la existencia de mecanismos especie-específicos de generación de edema. Tomados en conjunto, estos datos sugieren la presencia en el veneno de TZ de componentes con elevada toxicidad a nivel del páncreas de mamíferos, los cuales pueden estar relacionados con las complicaciones respiratorias asociadas al cuadro clínico del envenenamiento por TZ.

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INTRODUCTION

Envenoming in humans by scorpions belonging to the family Buthidae leads to the development of a variety of symptoms such as abdominal distress (epigastric pain, nausea, and vomiting) and cardiorespiratory manifestations (for a review see 1). Amongst the abdominal effects, the development of acute oedematous pancreatitis is prominent, especially after stings by some species of the Neotropical genus *Tityus* (2-4). This genus is accounted for most deaths by scorpion sting in South

America and the Caribbean (5, 6). Acute pancreatitis has been diagnosed by elevated serum amylase (2) and by immunoreactive trypsin (7). At least in the case of *Tityus trinitatis* (range Trinidad and Tobago, West Indies), venom-induced pancreatitis appears to be the result of muscarinic hyperstimulation of pancreatic acini and constriction of the ampullary mechanism (8). However, the clinical consequences (including development of pancreatitis) following *Tityus* envenoming in humans seem to depend on the scorpion species involved. Thus, sting by *T. trinitatis* produces

myocarditis and pancreatitis as major complications (9) whereas pulmonary oedema is the most frequent finding following envenoming by the Brazilian scorpion, *Tityus serrulatus* (10). Similarly, pancreatitis is a common complication in children after sting by *Tityus discrepans*, from north-central Venezuela (3, 11, 12), whereas accidents involving *Tityus zulianus*, a highly toxic species inhabiting the rain forest areas south of Lake Maracaibo, western Venezuela (13-15), produce cardiorespiratory complications (16). Average mortality rate due to scorpion sting in Mérida State, where *T. zulianus* is prevalent, rank highest among Venezuelan endemic regions (2.50 cases per million inhabitants for 1996-2000; L. De Sousa and A.B., unpublished results). However, pancreatic function in *T. zulianus*-envenomed patients has not been evaluated due to the lack of technical resources (Dr J Arandia, Mérida Health Commission, personal communication). It is well established that severe acute pancreatitis is frequently associated with distant organ failure, including lung injury that may present clinically as adult respiratory distress syndrome (17, 18). Given the possibility that the lung injury derived from *T. zulianus* envenoming could have its origin in an overlooked acute pancreatitis, as mentioned previously, a study was carried out to assess the pancreatic toxicity of *T. zulianus* venom in a mouse model.

MATERIALS AND METHODS

Venoms

Adult *Tityus zulianus* scorpions were collected near Mesa Bolívar, Mérida State, western Venezuela. *Tityus discrepans* scorpions were captured near San Antonio de los Altos, Miranda State, northcentral Venezuela. Scorpions were classified according to the criteria of González-Sponga (19).

Venoms were obtained by manual stimulation of the telson (the scorpion's last caudal segment) (20), and immediately lyophilized at -50°C and 35 mBar of pressure. Lyophilized venom from 50-60 scorpions was pooled and reconstituted in 0.9% NaCl, centrifuged at 12,000 *g* to eliminate insoluble matter, and protein concentration determined in the supernatant according to Lowry *et al.* (21) using bovine serum albumin as standard.

Animals

All experiments were performed according to protocols approved by the Institutional Animal Care. Male BALB/c mice (20-22 g) were used throughout which were bred and housed in standard cages in a room with an ambient temperature of $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and 12-hour light-dark cycle. They were fed standard laboratory chow and had access to tap water *ad libitum*. Mice were randomly assigned to control or experimental groups.

Preparation of serum and tissue samples

Mice were injected intraperitoneally (i.p.) with *Tityus zulianus* or *Tityus discrepans* venoms at a dose (in 0.2 mL) of 0.5 mg protein/kg body weight. Such venom concentration is known to produce pancreas structural alterations in the case of *T. discrepans* (12) and is below the medium lethal dose for *T. discrepans* (2.51 mg/kg) (22) and *T. zulianus* (1.54 mg/kg) (15) venoms. Control animals were injected i.p. with 0.9% (w/v) NaCl. Venom-inoculated mice were divided into five groups ($n = 5$ per group) and blood (by cardiac puncture) and pancreatic tissue withdrawn at 0, 1, 3, 6, and 24 hours after injection. Whole blood was centrifuged at 4°C , and serum removed to determine α -amylase activity (in International Units (IU)/mL) at 37°C using 2-chloro-4-nitrophenyl-alpha-D-maltotrioxide as a substrate

(Eagle Diagnostics, DeSoto TX) according to Gella *et al.* (23). Tissue was removed on ice, weighed, and immediately fixed in 125 mM phosphate buffer (pH 7.4) containing 2% formaldehyde and processed for paraffin histology. Sections were stained with hematoxylin and eosine and examined by light microscopy by two independent observers who were not aware of the experimental conditions to avoid bias. The increase in pancreatic water content (measured as relative pancreatic weight, mg pancreas wet weight/g total mouse body weight) was determined to indicate the degree of pancreatic oedema (24). Data are shown as mean \pm standard error of the mean. Statistical analysis was performed using paired Student's *t* test to determine statistical significance.

RESULTS

Serum amylase activity and interstitial oedema in mice injected with *Tityus* scorpion venoms

Both scorpion venoms were able to significantly increase serum α -amylase levels with respect to control animals, with a maximum at 6 hours after venom injection (Fig. 1A). However, elevated (14.8 ± 2.4 , $p < 0.05$) activity appeared as early as 1 hour in the case of *T. zulianus* and the activity measured at the peak of *T. zulianus* hyperamylasemia at 6 hours (60.4 ± 3.0), was 3-fold higher than the activity elicited by *T. discrepans* venom (21.6 ± 2.6). Activity in *T. zulianus*-envenomed mice remained elevated (4-fold higher than control) after 24 hours. Enzymatic activity in control animals remained unchanged at levels comparable to other studies (24, 25).

A further parameter of pancreatic damage in clinical as well as in experimental pancreatitis is the formation of intercellular fluid collections termed pancreatic oedema (24). As shown in Fig. 1B,

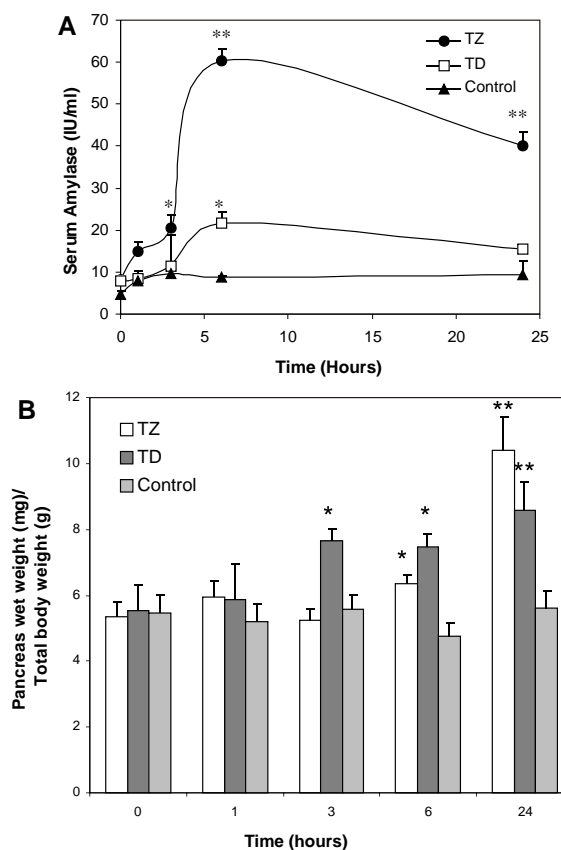


Fig. 1. Pancreatic damage elicited by *Tityus zulianus* and *Tityus discrepans* scorpion venoms. The time course over 24 hours is shown for (A) serum α -amylase activity and (B) pancreatic oedema. Enzyme activity was measured as indicated in *Materials and Methods*, and the increase in pancreatic water content was determined indirectly as the increase in pancreatic weight. Data points represent the means of five animals at each interval \pm standard error of the mean. *Significant differences ($p < 0.05$) between envenomed and control mice. **Significant differences ($p < 0.001$) between envenomed and control mice.

development of oedema reached significant levels at 3 hours of the *T. discrepans* pancreatitis evolution, and remained at approximately the same level up until 24 h of venom administration. In the case of *T. zulianus*, oedema started to develop at 6 h

Tityus zulianus

Tityus discrepans

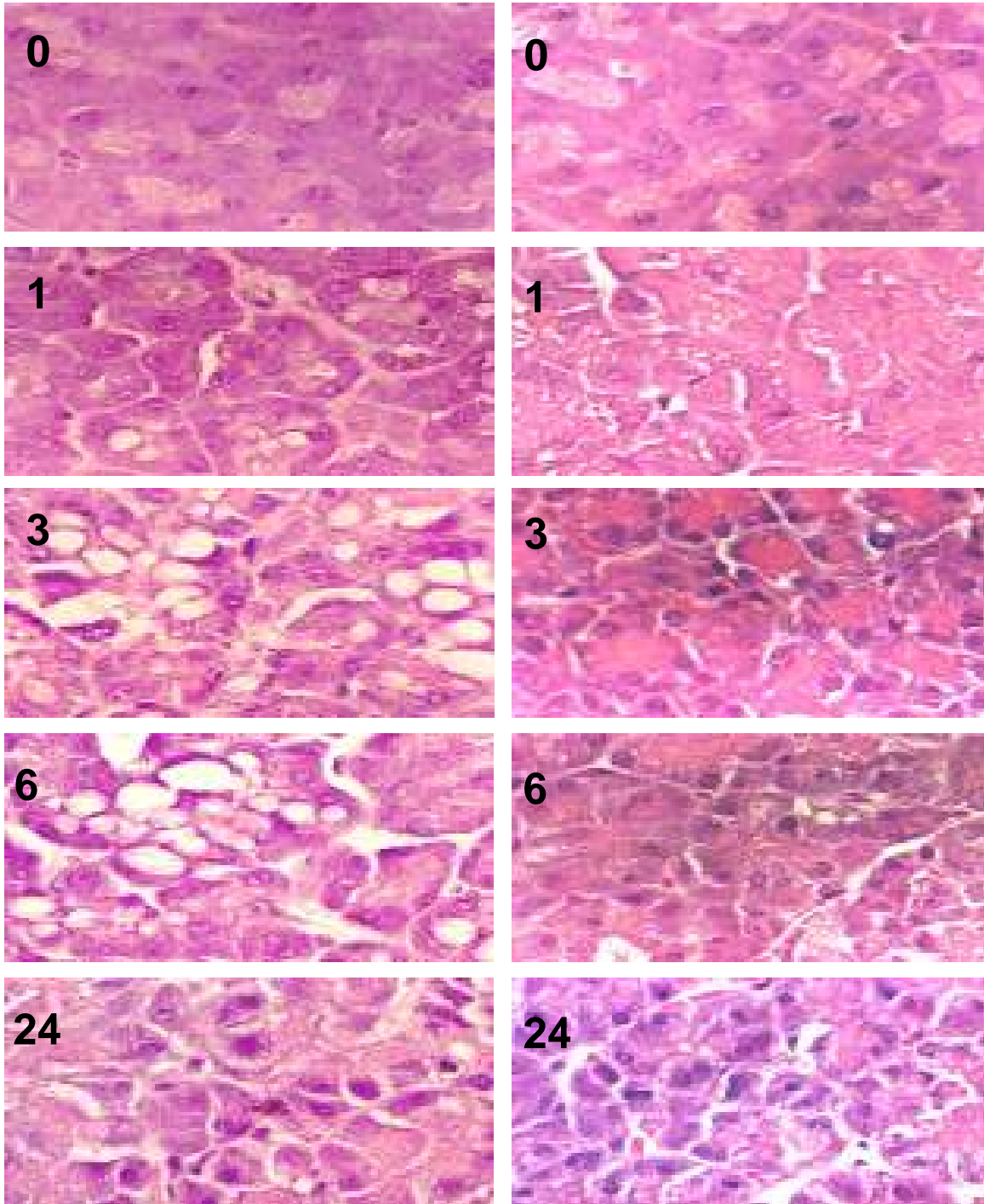


Fig. 2. Pancreas histology at various times after *T. zulianus* (left panels) or *T. discrepans* (right panels) venom injection. Tissue was stained with hematoxylin and eosin. Tissue from three mice in each group was examined and representative results are shown (original magnification, x312).

after inoculation and increased to the highest level elicited in this study (2-fold vs control, $p < 0.001$) at 24 h of evolution.

Signs developed within 10-20 minutes after inoculation were (in *T. zulianus*) sialorrhea, apnea, diaphoresis, and spasticity of rear limbs. Diarrhea, sialorrhea, and diaphoresis were observed in *T. discrepans*-envenomed mice. No deaths were recorded as a result of venom administration during the experiments.

Light Microscopy of Pancreas from Envenomed Mice

Fig. 2 shows pancreatic tissue obtained from *T. zulianus* and *T. discrepans* envenomed mice at various times. Formation of cytoplasmic vacuoles and interlobular oedema is evident in *T. zulianus*-envenomed animals as early as 1 hour after venom injection; the number and size of vacuoles roughly increased from this time up to 6 hours post-injection. Tissue collected from *T. discrepans*-envenomed mice only showed moderate interstitial oedema and smaller cytoplasmic vacuoles starting at 3 hours post-injection. Vacuolization was negligible after 24 hours for both *T. discrepans* and *T. zulianus*, with acini showing nuclei in a peripheral location throughout the exocrine pancreas. Neither venom had any effect on the islets of Langerhans at the magnification used. Lymphocyte infiltration was only observed in pancreata from *T. zulianus*-envenomed mice as early as 1 hour after injection (data not shown).

DISCUSSION

This work provides for the first time biochemical and histological evidence indicating that the venom produced by *Tityus zulianus* is highly toxic to the mammalian pancreas. The parallel increase in serum amylase levels and the acinar microscopic changes are signs of damage comparable to

conventional models of secretagogue-induced pancreatitis (26, 27).

Also evident from our data is the more potent secretagogue effect of *T. zulianus* venom over *T. discrepans* (4-fold higher *T. zulianus*-elicited amylase activity at the peak of hyperamylasemia) which is indicative of the presence in the former of species-specific components with greater pancreatic toxicity and/or shared toxins found in *T. zulianus* venom at higher concentration. Contribution of individual variations to these differences (28) should be considered minimal given that the venoms used were pooled from at least 50 scorpions. Interspecific differences in the pancreatic effect of *Tityus* venoms had already been noticed by Fletcher *et al.* (4), whom compared the release of newly synthesized secretory proteins by the Brazilian *Tityus serrulatus*, *Tityus bahiensis* and *Tityus stigmurus*, with *T. serrulatus* being the most potent secretagogue. Muscarinic hyperstimulation of the exocrine pancreas associated with premature enzyme activation, as determined for *T. trinitatis* (8) and *T. serrulatus* (29), is probably involved in the case of TZ and TD.

The differences in the onset and evolution of pancreatic oedema between TD- and TZ-envenomed mice (Fig. 1B) also imply the existence of differences in composition and/or mechanism of action between these venoms. In the rat model of caerulein-induced pancreatitis, oedema formation is initiated by tissue kallikrein (t-KK), a kininogenase catalyzing production of the endogenous kinins, bradykinin and kallidin, which have potent vasodilator and permeability-enhancing properties (30). The ensuing plasma protein extravasation supplies further kininogen and active plasma kallikrein to the tissue. Concomitantly, endogenous kallikrein inhibitors supplied by the inflammatory oedema can inhibit up to 99% of the activity of t-KK. Therefore, pan-

creatic oedema formation is the result of negative and positive feedback mechanisms controlling pancreatic kinin action and kinin generation (31). Recent evidence suggests that the kallikrein-kinin system is involved in the pathogenesis of envenomation by *Tityus serrulatus* in humans (32). TD- and TZ-specific mechanisms of pancreatic oedema generation and development could be invoked to explain, at least in part, the differences in time course between TD and TZ-induced oedema. For instance, a dissimilarity in kallikrein activation/inhibition as a result of structural and/or functional differences between TD and TZ venoms might be involved. In this sense, the presence of novel venom kallikrein inhibitors, as reported for *T. serrulatus* (33), should be explored.

It is known that the adult respiratory distress syndrome, among other distant organ failures, can be produced by pro- and anti-inflammatory mediators released from the pancreas during the course of acute pancreatitis (34, 35). In the light of the acute pancreatic toxicity of *T. zulianus* venom revealed in this work and the lung oedema developed in envenomed children, an investigation is now justified to determine (i) the role of the pancreas inflammation in *T. zulianus*-derived cardiorespiratory complications and (ii) evaluate whether a significant pancreatic damage can be produced in mild envenoming cases without a relevant lung compromise. The magnitude of the cardiopulmonary arrest after envenomation by *Tityus zulianus* (16), has possibly contributed to overlooking peripheral effects (including pancreatitis) in children stung by this species.

We have reported elsewhere (36) that *T. zulianus* venom (but not *T. discrepans*) contains components capable of sensitizing the response of the bovine trachealis smooth muscle to muscarinic agonists in at least an order of magnitude, in a similar fashion to iberiotoxin, a known blocker of

calcium-gated potassium channels (37). Taken together, the differences in pancreatic and tracheal effects of *T. zulianus* and *T. discrepans* suggest that the scorpion envenoming syndrome, at least within the genus *Tityus*, is species-specific. Such potential diversity in venom action and composition should constitute a warning for clinicians confronted with *Tityus* envenoming in endemic areas.

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