Toxicon 55 (2010) 1125–1131

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Biochemical profile of dogs experimentally envenomed with *Tityus serrulatus* scorpion venom

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ARTICLE INFO

Article history:
Received 6 July 2009
Received in revised form
2 October 2009
Accepted 2 January 2010
Available online 7 January 2010

Keywords:
Tityus serrulatus
Envenomation
Blood analysis
Urine analysis
Dog

ABSTRACT

The aim of this study was to evaluate the canine blood and urinary profiles after envenomation by *Tityus serrulatus* venom. Twelve dogs were randomly distributed into two equal groups. Control group animals received 0.5 mL phosphate buffered saline (PBS) injected subcutaneously into the internal portion of the left thigh, whilst dogs in the envenomed group were injected with scorpion venom (250 μg/kg in 0.5 mL PBS). No significant alterations were detected in the urine of envenomed dogs. Levels of plasma glucose and serum urea, creatinine, total protein, potassium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), and amylase were determined. Semi-quantitative analysis of serum cardiac troponin I (cTnI) was performed using an immunochromatographic test. The concentrations of cortisol and insulin were determined using commercial radioimmunoassay kits. Increases in serum cortisol levels in experimental group animals coincided with hyperglycaemia and was probably a response to pain. Increased insulin levels were observed during the hyperglycaemic peaks. Envenomed dogs presented discreet increases in ALT, AST and CK, but no alterations in LDH, amylase, cTnI, urea, creatinine and potassium levels were observed. It was concluded that the venom of *T. serrulatus* induces blood and urinary biochemical changes in dogs.

1. Introduction

Scorpion stings represent an important and serious public health problem in certain regions of Brazil and in other parts of the world. In Brazil, approximately 10,000 human cases of scorpion sting are treated at hospital centres and notified annually, and 50% of these cases occur in Minas Gerais and São Paulo states. The scorpion species *Tityus serrulatus* is the most prevalent (95%) and accounts for fatal stings, especially among children (Soares et al., 2002; Cupo et al., 2003).

Scorpion venom comprises a complex mixture of short and long chain peptides associated with mucopolysaccharides and small amounts of hyaluronidases and neurotoxins (Gazarian et al., 2005). The mechanism of action of the toxins involves blockage of the ion channels of the neuronal membranes and release of neurotransmitters from the postganglionic nerve terminals. Some toxins stimulate the entry of sodium through the voltage- and non-voltage dependent sodium channels, whilst others obstruct the potassium channels and impede the return of this ion to the cell, thus causing its accumulation in the extra cellular medium. In either case, the result of such action is

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0041-0101/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2010.01.001

membrane depolarisation followed by an action potential and the massive release of neurotransmitters, in particular acetylcholine and noradrenaline from the postganglionic nerve terminals, and adrenaline from the adrenal medulla (Ismail, 1995). The effects can be adrenergic or cholinergic depending on the neurotransmitter and the type of neuron, and the symptoms may range from excruciating pain at the site of the sting to grave systemic effects (Cordeiro et al., 2006; Ribeiro et al., 2009).

Cardiovascular and haemodynamics alterations (congestive heart failure, pulmonary oedema and arterial hypertension) are the most severe consequences in this envenomation, usually followed by cardiac muscular lesions (Cupo et al., 2003, 2007). The occurrence of myocarditis after scorpion envenomation might be multifactorial, being caused by cardiotoxic effect of catecholamines (i.e. hypoxia), vasoconstriction and high oxygen demand, lactic acid accumulation (Raab, 1960; Murthy and Hase, 1994; Ismail, 1995; Sofer et al., 1996; Meki and El-Dean, 1998; Meki et al., 2003); by direct venom effect (Teixeira et al., 2001) and by cytokines (Magalhães et al., 1999). The cardiac troponin I (cTnI) concentration is highly correlated with the occurrence of alterations in electro and echocardiogram features and CK-MB and AST increase. The cTnI have been observed in moderate and serious scorpionism cases, in which is observed a 50-time increase compared to the normal value (Bucaretchi et al., 1995; Cupo and Hering, 2002; Cupo et al., 1994, 2003).

Some metabolic disturbs are reported such as, hypersecretion and hypermotility of the digestive tract, causing excessive salivation, nausea, vomiting, abdominal pain, electrolytic imbalance and haemoconcentration, which leads to body dehydration (Bertazzi et al., 2003; Ribeiro et al., 2009). Pancreatitis caused by exocrine hypersecretion might be the result of the direct action of neurotoxins on pancreatic cells, and also, by the neurogenic activity affected by cholinergic hyperstimulation (Sankaran et al., 1983; Possani et al., 1992; Bucaretchi et al., 1995; Fletcher et al., 1996; Cupo et al., 2003). Some researchers (Fukuhara et al., 2003, 2004) have reported a correlation of the levels of kinins (bradykinin and kallikrein) with the production of cytokines (IL-1, IL-6 and TNF-α) and stimulation of the hepatic synthesis of acute-phase proteins in human patients.

Changes in cortisol and insulin levels observed in scorpion accidents are associated with the discharge of catecholamines and the stress produced by pain. One example of increased cortisol, accompanied of hyperglycaemia, was reported in a study involving canine inoculation with *Mesobuthus tamulus concanesis* venom (Murthy and Haghnazari, 1999). Ismail and Abd-Elsalam (1988) have stated that hyperglycaemia is caused by pancreatitis followed by insulin production and secretion decrease. Inhibition of insulin release and stimulation of glucagon secretion, directly or not, are often observed in cases of scorpionism. Such effects, gather with vasoconstriction, hypoxia and increased energy consumption, constitute the Energy Deficit Syndrome, the most severe outcome of scorpion envenomation (El-Asmar, 1984; Ismail and Abd-Elsalam, 1988; Murthy and Hase, 1994; Yugandhar et al., 1999).

Although dogs have been used in studies about physiological and pharmacological actions of scorpion venoms, especially for measuring cardiovascular parameters (Bartholomew et al., 1977, Murthy and Haghnazari, 1999; Tarasiuk and Sofer, 1999; Cordeiro et al., 2006), researches concerning a complete biochemical profile, after experimentally envenomation by *T. serrulatus,* are rare.

In the present report we investigated the *T. serrulatus* venom effects in cardiac, pancreatic, hepatic, renal and metabolic functions in dogs.

2. Material and methods

This research was approved on 12th June 2006 by the Ethical Committee for Animal Experimentation of the Universidade Federal de Minas Gerais (UFMG), under the protocol number 15/2006.

2.1. Animals and venom

The study population comprised 12 healthy (as determined by clinical and laboratory tests) adult male mongrel dogs (average weight 14.2 ± 5.4 kg) obtained from the Centres for Control of Zoonosis of the municipalities of Belo Horizonte and Betim, MG, Brazil. The experiment followed a random design with subdivided plots. Animals were distributed randomly into two equal groups. Those in the control group ($n = 6$) received 0.5 mL of phosphate buffered saline (PBS) injected subcutaneously into the internal portion of the left thigh. Dogs of the envenomed group ($n = 6$) were injected in a like manner with *T. serrulatus* venom (250 μg/kg ressuspended in 0.5 mL PBS) from a lyophilised pool originating from various scorpion specimens supplied by the Laboratory of Immunology and Biochemistry from Instituto de Ciências Biológicas (ICB) UFMG. The protein venom concentration was determined by using the Folin-Phenol Ciocalteau reagent (Lowry et al., 1951). The venom dose inoculation in was performed in a pilot experiment.

2.2. Samples and biochemical analyses

Urine samples were collected prior to the experiment and at 8, 24 and 48 h after venom inoculation using a urethral catheter. The density of each sample was determined using a urinometer (Ningbo Utech International CO LTDA, China), whilst pH, albumin, glucose, urobilinogen, bile salts and haemoglobin were assessed with the aid of reaction dipsticks (Uri-Test 10® – Inlab, Alemanha). Following centrifugation, the urine sediment was analysed under the optical microscope.

Blood samples were collected prior to the experiment and at 2, 6, 12, 24, 48 and 72 h after treatments by cephalic vein puncture, in tubes with anticoagulant sodium fluoride to obtain plasma to glucose measurements, and without anticoagulant, to collect serum and achievement biochemical profiles, either analysed immediately.

Plasma glucose and serum urea, creatinine, total protein, potassium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), and amylase were determined with