Effects of *Androctonus crassicauda* Scorpion Venom on the Heart Tissue

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Abstract: The aim of this research was to determine the presence of cardiac injury in rabbit animal model with envenomed *Androctonus crassicauda* venom by serial measurements of cardiac troponin I and assessed the histopathologic changes of the heart for evaluation of the extent of damages. Experimental envenomations were performed by intravenous of 0.5 mL of PSS containing a scorpion venom. Blood samples were collected from each animal (0 min [control]) before envenomation. Blood samples were collected from each animal on the 1st, 3rd, 6th and 24th h after venom injection. Serum cardiac troponin I level determined using abbot architect troponin I kit (Abbott Laboratories, USA). Cardiac tissues taken from envenomed animals were for histopathological examination after 24 h. The scorpion venom led to significantly increased in serum cardiac troponin I enzyme activities at the 3rd, 6th and 24th h after injection when compared with those of the control group. *In vivo* effects of the venom observed such as salivation, lacrimation, deep dyspnea and tachypnea in rabbits. Histopathological examination of heart tissue was showed development of myocardial injury. This study demonstrated that *Androctonus crassicauda* venom may be cause development of myocardial injury. The using of cardiac troponin I as a follow-up criterion in the scorpion envenoming may be allow early establishing of the cardiac involvement.

Key words: Scorpion, *Androctonus crassicauda*, venom, heart, cardiac troponin I

INTRODUCTION

Scorpions do not harbor agents of disease. However, they are medically important arthropods since, they cause envenomations by stinging humans, most of the time to protect themselves. Several studies on scorpion sting cases emphasized that various clinical pictures are seen ranging local symptoms to serious autonomic and central nervous system symptoms, death due to cardio and respiratory failure, especially in children (Altinkaynak et al., 2002). The clinical symptoms of central and peripheral neurotoxicity, cardiotoxicity and metabolic alterations present in envenomed patients are assumed to be directly related to the concentration of toxins existing in the venom injected by the scorpion (Ozkan et al., 2008).

Scorpion venoms are known to stimulate both branches of the autonomic nervous system simultaneously with predominance of sympathetic stimulation and release of tissue and medullary catecholamines (Cupo et al., 1994; Ismail et al., 1994). Changes in myocyte membrane related to reversibly injury are considered sufficient for the release of cardiac troponins from the free cytosolic pool whereas in case of irreversible myocardial injury the source of troponin release is the structural damage of myocytes. Myocarditis associated cardiac Troponin I (cTnl) release has been reported with various studies including toxic venom related myocarditis (Meki et al., 1998). Cardiac troponins are highly specific markers of myocardial injury. It has been postulated that unlike other markers of myocardial injury, troponins could be elevated in reversible myocardial injury and the myocardial necrosis does not have to occur for troponins to be release from myocytes. Reversibly, injury related changes in myocyte membrane are considered sufficient for the release of cardiac troponins from the free cytosolic pool whereas in case of irreversible myocardial injury the source of troponin release is the structural damage of the myocytes (Chu et al., 2002; Correa et al., 1997; Ibrahim et al., 1996).

Scorpions as well as human envenomation cases are common in Turkey due to its geographical location, climate and socioeconomic structure (Altinkaynak et al., 2002; Chen et al., 2000). The majority of the envenomation symptoms are due to a massive release of catecholamines which play an important role in the pathogenesis of

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scorpion sting. They are released from the adrenal glands and postganglionic nerve endings and increasing the cardiac contractility or causing release of renin from the kidneys. Pulmonary edema as a result of scorpion envenomation is due to myocardial dysfunction (Cupo et al., 1994; Karnad, 1998; Dittrich et al., 2002; Freira-Maria, 1990; Ismail, 1993; Karakurt and Kocak, 2007).

The aim of this research was to determine the presence of cardiac injury in rabbit animal model with envenomed Androctonus crassicauda venom by serial measurements of cardiac Troponin I (cTnI). In addition, the role of the scorpion venom assessed the histopathologic changes of the heart for evaluation of the extent of damages.

MATERIALS AND METHODS

Scorpions: A. crassicauda specimens were collected from Southeastern Anatolia region (Sanliurfa), Turkey.

Experimental animals: Healthy New Zealand male rabbits (n=6; 2.8-3.0 kg) were used for determination of serum levels of cTnI. They were bred at the Refik Saydam Public Health Agency animal facilities (RSPHA). Rabbits were housed in temperature controlled rooms and received water and food ad libitum before being used for study. Throughout the experiment, animals were kept in the experiment room under standard conditions.

Venom: Venom was obtained from mature A. crassicauda scorpions by electrical stimulation of their telsons. The venom was dissolved with double distilled water and centrifuged at 14000 rpm for 15 min at 4°C. Supernatant was stored at -20°C until use. Supernatant was dissolved in physiologic saline solution (PSS; 0.9% chloride solution) to 2 A. crassicauda venoms/mL. Researchers were used for determination of the lethal dose of the scorpion venom was not performed due to determine in vivo effect of single venom.

Experimental protocol: Experimental protocols for animal experiments were approved by the ethical committee of the RSPHA. Experimental envenomations were performed by intravenous (i.v.) of 0.5 mL of PSS containing one scorpion venom (A. crassicauda scorpion venom). Intravenous administrations were performed into the right ear marginal vein. Blood samples were collected from each animal (0 min control) before envenomation. Blood samples were collected from each animal on the 1st, 3rd, 6th and 24th h after venom injection. Animals were monitored for 24 h. Blood samples were collected from the left ear central artery. Plasma was collected after centrifugation at 1.500 g for 15 min and aliquots were immediately frozen and used later for biochemical analysis.

Biochemical analysis: Serum cTnI level determined using abbot Architect troponin I kit (Abbott Laboratories, USA). The architect STAT Troponin-I assay (Abbott Diagnostics) was a 2-step chemiluminescent microparticle immunoassay designed to detect cardiac Troponin I (cTnI) in serum and plasma. Detection limit of cTnI assay was approximately 0.002 ng mL⁻¹. Biochemical analyses were carried out at the Biochemistry Department of Ankara Numune Hospital.

Histological analysis: The animals were euthanized with cervical dislocation at 24th h after injection venom. For histopathological examination, cardiac tissues taken from envenomed animals were fixed in 10% formalin after macroscopical examination. The tissues were processed routinely and embedded in paraffin. Tissue sections from paraffin blocks were cut at 5 µm and stained with Haematoxylin-Eosin (HE).

Statistical analysis: Data analysis performed by Microsoft Excel 2007 (Microsoft Corporation, Seattle, USA) programs. Mean values and SD reported.

RESULTS AND DISCUSSION

Level of cardiac troponin I: Biomedical marker for myocardial injury was assayed to cardiac dysfunction. The effects of a single i.v. injection A. crassicauda venom determined on serum cTnI enzyme activities of rabbits (n=5) at 1st, 3rd, 6th and 24th h after the injection venom (Table 1). As shown Fig. 1, the scorpion venom led to significantly increased in serum cTnI enzyme activities at the 3rd, 6th and 24th h after injection when compared with those of the control group.

In vivo effect of the experimental envenomation: The symptoms of envenomation in rabbits were shown in the 1st 20 min after injection. Researchers observed

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>1st rabbit</th>
<th>2nd rabbit</th>
<th>3rd rabbit</th>
<th>4th rabbit</th>
<th>5th rabbit</th>
<th>cTnI</th>
<th>SD of cTnI</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.01</td>
<td>0.01</td>
<td>0.00</td>
<td>0.01</td>
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<td>0.006</td>
<td>0.01</td>
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<td>0.00</td>
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<td>0.00</td>
<td>0.02</td>
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<td>0.008</td>
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<td>3</td>
<td>0.03</td>
<td>0.41</td>
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<td>6</td>
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<td>0.90</td>
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<td>0.392</td>
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<td>24</td>
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<td>0.77</td>
<td>0.46</td>
<td>47.38</td>
<td>0.79</td>
<td>9.892</td>
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</tr>
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hypersalivation (Fig. 2) lacrimation, deep dyspnea and tachypnea in animals. During experiment, a rabbit died after 120 min. However, surviving rabbits after 24 h did not show abnormalities.

Macroscopical and histopathological findings

Macroscopical findings: Epicardial and all coronary vessels were filled and enlarged with blood related to congestion. When cut according to that from apex to basis of ventricles, septum interventricular and musculus papillaris were seen partially as pale pink colour. In addition, clots were attended in both of ventriculi of the heart.

Microscopical findings: Parenchymatous and hydropic degeneration became in almost all cytoplasm of myocytes besides of hyalin degeneration area containing pink granules in cytoplasm of some cardiac myocytes (Fig. 3A). There were many little vacuoles which had irregular shape and observed clearly. The picnotic nuclei were also seen with accompanied to vacuole degeneration in cytoplasm (Fig. 3B). However, necrotic myocytes which had roughly and homogeneously eosinophilic were rarely encountered amongst degenerated myofibrills. Some myofibrills were not striatated completely (Fig. 3C). There were hyperemic capillaries filled by eritocytes. Moreover, numerous erythrocytes were found at outside of capillaries and among myofibrills in most areas of myocardium. In addition, mononuclear cells infiltrations, commonly macrophages and rarely lymphocytes were attended, in some areas between myofibrills as well (Fig. 3D). However, it was not observed any findings in control group.

Important scorpion species threatening public health in Turkey are identified as Leirius quinquestriatus, A. crassicauda, Mesobuthus gibbosus and Mesobuthus eupeus of Buthidae family (Altinkaynak et al., 2002; Cesareli and Ozkan, 2010; De Roodt et al., 2009; Ucar and Tas, 2003). Scorpion stings are primarily due to accidents and in most cases the scorpion is neither seen nor identified (Magalhaes et al., 1999). In Turkey, Latifi and
Tabatabi (1979) reported two cases of children who suffered from myocarditis and pulmonary edema after scorpion stings. Ucar and Tas (2003) reported that two cases (1.17%) resulted in death in 1999. Altinkaya et al. (2002) and Bahoul et al. (2004) notified also 8.3% lethality due to cardio and respiratory failure. De-Matos et al. (2001) recorded that the patient died due to myocardial ischemia.

In the Middle East, it is known that common scorpion stings were caused by A. crassicauda, L. quinquestriatus and the remainder were caused by unidentified scorpions (Ismaiel, 1995). However, scorpions can also control the venom flow so some sting incidents are venomless or only mild envenomations. Therefore, in current research, the animals were experimentally envenomated with venom of extracted from a single A. crassicauda scorpion.

Evidence of cardiac injury thus far obtained includes in addition to the clinical manifestations, increased serum level of enzymes CK and its CK-MB fraction, LDH, AST, electrocardiographic changes. A direct effect of scorpion venom on myocardium has been postulated in few studies (Barbouche et al., 1996; Benvenuti et al., 2002; Bertazzi et al., 2003). The presence of cardiac enzymes in the blood serum is usually used diagnosis of tissue injuries. In many studies both in Turkey and other countries, the researchers reported an increase in the levels of LDH, CK, CK-MB and cTnI in patients stung by scorpion and suffering from cardiac lesions (Cupo and Hering, 2002; Cupo et al., 1994; De-Matos et al., 2001 Hermann et al., 2001; Latifi and Tabatabi, 1979; Meki et al., 1998; Pipelzadeh et al., 2006; Sahin et al., 2004). Among biomedical marker for myocardial injury, cardiac troponins are highly specific marker therefore currently considered the gold standard for his diagnosis. Some researchers have also reported that ALT and AST activities in the serum change during scorpion envenomation (Isbister et al., 2003; Omran and Abdel-Rahman, 1992; Osmaya-Romero et al., 2001; Pirgon et al., 2005). Correa et al. (1997) and Cupo and Hering (2002) notified that hemorrhage and edema in myocardium were described after injection of sublethal doses of scorpion venom. In the present study, high levels of enzyme cTnI in sera of envenommed animals are most likely a consequence of myocardial damage. The scorpion venom led to significantly increased in serum cTnI enzyme activities at the 3rd, 6th and 24th h after single intravenous (i.v.) injection A. crassicauda venom.

In the pathogenesis of scorpion envenomation, number of inflammatory mediators, consisting of prostaglandins, cytokines and nitric oxide besides of complement activation, bradykinin-kallikrein responsible generally for acute inflammatory reaction (Bawaskar, 2005; Cesarelli and Ozkan, 2010; Dimirov et al., 2001; Gueron and Ovssyshcher, 1987; Meki et al., 2003; Mirakhradi et al., 2006). As a result of releasing of many mediators, number of pathological changes which include degenerative or necrotic alterations with inflammatory reaction happened at primarily cardiovascular system (Benvenuti et al., 2002; Ismael, 2003) and consecutively in skin and kidney (Pipelzadeh et al., 2007). Freira-Maria (1990), Fukuimura et al. (2003), Cupo et al. (1994) and Daisley et al. (1999) for the pathogenetic mechanism of scorpion envenomation account for releasing of neurotransmitters such as catecholamines and acetylcholine in especially myocardial lesions. There is a fact that toxic and ischemic changes become to result of both directly to cytokine and indirectly to catecholamines (Deshpande and Alex, 2000). Benvenuti et al. (2002) and Bertazzi et al. (2003) describe to hemorrhage and coagulative myocytolysis and irregular cross band formation in the cytoplasm of cardiomoyocytes in Titus serrulatus envenomation. They do not point out direct toxic effect of scorpion toxin into myocardium because not detected any inflammatory reaction or thrombosis or detected only coagulation necrosis in cardiomoyocytes. They ascribed this pathologic finding to large amount of catecholamine in circulation. However, Adi-Bessalem et al. (2008) considered that prominent interstitial edema, hemorrhage, inflammatory cell infiltration containing leukocytes and fewer macrophages and lymphocytes as a consequences of activated inflammatory reaction. In the our study, it is evaluated as the result of severe acute inflammatory reaction because seen hemorrhage and a few cell infiltration besides of clearly degenerative and necrotic changes. As a result, it is attended in this study, that clinic, pathologic and enzymatic changes at blood due to myocardial injury after A. crassicauda envenomation are accounted with releasing of number of inflammatory mediators, cytokines and complement system activation. However, it is thought to be supported with further enzymatic analysis with immunohistopathological methods as for detecting to differ inflammatory mediators and cytokines in cardiac tissues.

CONCLUSION

This study demonstrated development of myocardial injury in rabbit animal model. Altered cTnI dosage should always be considered a warning sign and the patient should be carefully monitored in terms of cardiorespiratory system. The patient may be prevent from events that will occur later.
REFERENCES


Fukuhara, Y.D., M.L. Reis, R. Dellalibera-Joviliano, F.Q. Cunha and E.A. Donadi, 2003. Increased levels of IL-1β, IL-6, IL-8, IL-10 and TNF-α in patients moderately or severely envenomed by Tityus serrulatus scorpion sting. Toxicon, 41: 49-55.


