

Full Article

Neutralizing effects of polyvalent antivenom on severe inflammatory response induced by *Mesobuthus eupeus* scorpion venom

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ABSTRACT

This study evaluated the effects of *Mesobuthus eupeus* (*Me*) scorpion venom on inflammatory response following injection. Additionally, the present study examined whether immunotherapy at specific time intervals would be effective on inflammatory response after *Me* venom inoculation. Animals were divided randomly into four groups: the first group received LD50 of venom and the second and third groups of animals; immunotherapy was performed in different time intervals and fourth group was considered as control group. *Me* venom inoculation is caused respiratory perturbations such as respiratory distress, respiration with open mouth, crepitation and finally respiratory arrest. *Me* inoculation is resulted in increased pro-inflammatory cytokines including TNF- α and IL-1. Venom injection also induced inflammatory response, characterized by significant increase in serum white blood cells and neutrophils at 30, 60 and 180 min following envenomation. Simultaneous administration of antivenom and venom prevented entirely clinical signs, cytokines and hematological changes. Delayed immunotherapy gradually ameliorated clinical features, cytokines changes and hematological abnormalities related to the envenomation. In conclusion, our observations indicate injection of *M. eupeus* scorpion venom induces severe inflammatory response which can be one of the causes of clinical complications. Additionally, immunotherapy beyond 1 h after envenomation with appropriate dose and route in victims with severe inflammatory response related to the *M. eupeus* scorpion envenomation is beneficial.

Keywords: *Mesobuthus eupeus* scorpion venom, inflammatory cells, cytokines, immunotherapy, rabbit

INTRODUCTION

Scorpionism represents a serious public health problem in tropical and subtropical countries.

Mesobuthus eupeus, *Androctonus crassicauda* and *Hemiscorpius lepturus* are involved with the highest number of victims in Iran (Dehghani *et al* 2009, Chitnis *et al* 1993, Mashak 2000, Radmanesh 1990). *Mesobuthus eupeus* (*Me*) is one of the major scorpions in Iran. Radmanesh demonstrated that 45% of all cases

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of scorpion stings in Iran were due to *M. eupeus* (Radmanesh 1990). Scorpion venom mainly consists of peptides that bind to specific sites on ion channels of the membrane cells inducing an increase in their permeability (Elgar et al 2006). These toxins induce a systemic inflammatory response with cytokines and chemokines elevation characterized by pulmonary edema that is the main cause of death among children (Magalhães et al 1999, Fukuhara et al 2003, Pessini et al 2003). In addition, inflammation related to the scorpion envenoming involves the acute-phase proteins, the complement and kinin systems and is also characterized by the leukocytosis (Pessini et al 2003, Fukuhara et al 2004, Bertazzi et al 2005, Coelho et al 2007). Envenoming by Buthidae family scorpions cause cardiac dysfunction and respiratory failure that can be fatal. The most common perturbation in human, especially in children, is pulmonary edema (Sofer & Gueron 1988, Cupo & Hering 2002, Meki & Mohey El-Dean 2003). Clinical reports of victims stung by *M. eupeus* depict severe pain, hyperemia, edema, thirst, dry mouth, hypotension, hypertension, increase of bronchial secretion, difficulty in breathing, tachycardia and cyanosis (Ozcan & Kat 2005). Immunotherapy is the specific treatment for scorpion envenoming because of the severity of scorpion envenoming and the rapid diffusion of its toxins. Unfortunately, the administration of antivenom remains empirical and their efficacy is controversial. Several factors such as the antibody form (Fab or F(ab')₂), the route and the time delay before antivenom administration can reduce the efficacy of immunotherapy (Ismail & Abd-Elsalem 1998, Hammoudi-Triki et al 2004). The aim of the present study was to characterize the systemic inflammatory response induced by subcutaneous administration of *M. eupeus* scorpion venom. We studied the production of cytokines participating in the inflammatory response, and neutrophil mobilization to blood. In this investigation, we evaluated efficacy of scorpion antivenom (SAV) to inhibit lethality and neutralizing potency on toxicity effects of *Me* venom. We also studied the improving ability of SAV on

systemic inflammatory response induced by *Me* scorpion venom injection in experimentally anesthetized rabbits after the intravenous administration of SAV at specific time intervals. The findings of this investigation can be new insight into treatment efficiency for envenomated patients.

MATERIALS AND METHODS

Venom and antivenom. *Mesobuthus eupeus* (*Me*) venom was supplied using electric shock at the telson of the scorpions in department of venomous animals and antivenom production, Razi Vaccine and Serum Research Institute (RVSRI). It was lyophilized and stored at 4°C. Also, scorpion polyvalent antivenom was provided by RVSRI. The polyvalent antivenom comprises a purified solution with F(ab')₂ fractions of equine immunoglobulins specifically for venoms of six dangerous scorpions in Iran. It was obtained from hyperimmune plasma of healthy horses that had been immunized with a mixture of venoms from six species of medically important scorpion species in Iran (*Odontobuthus doriae*, *Mesobuthus eupeus*, *Androctonus crassicauda*, *Buthotus (Hottentota) Saulcyi*, *Buthotus sach* and *H. lepturus*). Protein in the plasma mixture precipitated with ammonium sulfate, enzymatically digested with pepsin. This followed by dialysis, and finally formulated for use (Latifi & Tabatabai 1979).

Experimental protocol. Twenty for male New Zealand white rabbits with an average weight of 2 ± 0.2 kg were distributed in 3 groups (n=6) in this investigation. All animals were housed under conditions of controlled light (12 h light, 12 h dark), temperature (24 ± 1 °C) and humidity ($55 \pm 5\%$), with standard chow diet and water available ad libitum. Anesthesia was performed with ketamin (50 mg/kg) and xylazine (5 mg/kg). All animals were kept in according to the recommendation of the animal care committee of the Tehran University based on the 'Guide for Care and Use of Laboratory Animals' (NIH US publication 86-23, revised 1985). Then, LD₅₀ (4.5 mg/ kg) of *M. eupeus* scorpion venom was injected

subcutaneously into the first group animals. Simultaneously, venom (subcutaneously 1 ml of an ultra-pure water solution containing 4.5 mg/kg of *Me* venom) and 5 ml antivenom (intravenously) were administered in six rabbits considered as the second group. In the third group of animals, 5 ml of antivenom, was injected intravenously 60 min after *Me* venom inoculation. In time matched control experiments (forth group), animals were administered with normal saline. Heparinized cannula was inserted into the left central ear artery and rabbits were bled at different time intervals (0, 30, 60 and 180 min) after venom inoculation to evaluate blood cells and serum cytokines. At the end of the experiment, the animals were sacrificed by an overdose of diethyl ether.

Differential count of blood cells. Hematological parameters were performed using a hemocytometer (ADVIA, Hematology system). Leukocyte populations were identified and counted using Giemsa staining. The results represent the mean \pm standard deviation (SD) per percentage of cell suspension.

Quantification of serum cytokines. Serum cytokines levels of TNF- α and IL-1 were determined by sandwich ELISA kits according to the kit protocol (Biosource international, Europe). Serum cytokine concentrations were determined using standard curve established with the appropriate recombinant cytokines (expressed in pg/mL).

Statistical analysis. The statistical significance of differences between the groups were performed using One-way analysis of variance (ANOVA) followed by Tukey's test and for intragroup comparisons, paired Student's t test was made for parametric data and these data are shown as mean \pm S.D.

RESULTS

Effect of *Me* venom and immunotherapy on serum TNF- α and IL1. Table 1 summarizes cytokines (TNF- α and IL1) concentration following injection of *Me* venom and antivenom administration. In the first group of animals, *Me* venom induced a significant increase on serum TNF- α at 30, 60 and 180 min when

compared to the baseline. In the second group of rabbits, simultaneous administration of venom and antivenom prevented any change in TNF- α level. In the third group of animals, TNF- α concentration showed significant increase at 0.5, 1 h following venom injection but delay immunotherapy inhibited further rise at 3h. On the other hand, venom injection caused 18-fold increase on serum IL1 up to 3h. In the 2nd group, there was no alteration on IL1 serum concentration following simultaneous administration of venom and antivenom. Delayed immunotherapy in third group neutralized and reversed increased IL1 serum level related to the *Me* envenomation within 3 h.

Table 1. Effects of *Mesobuthus eupeus* scorpion venom and immunotherapy (SAV0 and SAV60) on TNF- α and IL1 serum levels.

Variable	Means \pm SD			
	0	0.5 h	1 h	3 h
TNF-α(pg/ml)				
venom	67.4 \pm 5.2	102.1 \pm 7.5*	274.9 \pm 11.3*	430.5 \pm 16.3*
SAV0	53.8 \pm 3.3	68.4 \pm 4.3**	48.1 \pm 6.5**	74.6 \pm 4.2**
SAV60	63.3 \pm 5.7	118.8 \pm 8.1	286.1 \pm 14.1	161.9 \pm 8.1**
Control	65.1 \pm 4.9	61.2 \pm 3.6	68.3 \pm 3.2	59.1 \pm 4.4
IL1 (pg/ml)				
venom	5.3 \pm 0.7	45.6 \pm 4.6*	68.4 \pm 7.1*	90.3 \pm 8.9*
SAV0	3.7 \pm 0.3	6.4 \pm 1.1**	4.2 \pm 0.9**	3.5 \pm 0.8**
SAV60	4.6 \pm 0.3	37.9 \pm 4.3	72.1 \pm 7.4	69.2 \pm 8.1**
Control	3.8 \pm 0.5	4.5 \pm 0.7	5.7 \pm 0.2	4.9 \pm 0.8

Mean values (\pm standard deviation) are shown for the six animals in each group. *Significantly different from time 0. There was significant difference at $p < 0.05$ according to paired Student t-test. ** Significantly different between venom and remaining two (SAV0, SAV60) groups. There was significant difference at $p < 0.05$ according to Tukey's test ($n = 6$ in each group).

Effects of *Me* venom and immunotherapy on Neutrophils and WBC serum. In the first group of animals, *Me* venom inoculation induced a significant increase in neutrophil value at 30, 60 and 180 min when compared to the baseline. Venom injection also caused a significant increment in WBC values when compared to the baseline. Simultaneous injection of venom and antivenom in the 2nd group rabbits prevented any alteration in neutrophil and WBC levels. In the third group, delayed immunotherapy reversed

increased neutrophil and WBC serum level related to the *Me* venom injection within 3h (Table 2).

Table 2. Effects of *Mesobuthus eupeus* scorpion venom and immunotherapy (SAV0 and SAV60) on neutrophils and WBC serum levels).

Variable	Mean \pm SD			
	0	0.5 h	1 h	3 h
Neutrophils				
venom	4.4 \pm 0.24	6.2 \pm 0.37*	9.4 \pm 1.32*	17.1 \pm 3.13*
SAV0	4.2 \pm 0.27	4.6 \pm 0.12**	4.1 \pm 0.51**	5.2 \pm 0.68**
SAV60	4.1 \pm 0.31	6.8 \pm 0.39	10.2 \pm 1.9	8.1 \pm 0.9**
Control	4.7 \pm 0.46	4.2 \pm 0.61	4.4 \pm 0.53	4.9 \pm 0.37
WBC				
venom	13.8 \pm 1.04	15.6 \pm 2.1*	17.9 \pm 2.9*	19.8 \pm 2.89*
SAV0	13.7 \pm 1.31	13.5 \pm 1.12 **	13 \pm 1.9 **	14.3 \pm 2.01**
SAV60	13.9 \pm 1.43	15.8 \pm 1.7	17.4 \pm 2.7	16.1 \pm 2.34**
Control	13.3 \pm 1.55	14.1 \pm 1.62	13.4 \pm 1.78	13.7 \pm 1.53

(Mean $\times 10^6$ cells/mL) \pm (standard deviation) are shown for the six animals in each group. *Significantly different from time 0. There was significant difference at $p < 0.05$ according to paired Student t-test. ** Significantly different between venom and remaining two (SAV0, SAV60) groups. There was significant difference at $p < 0.05$ according to Tukey's test ($n = 6$ in each group).

DISCUSSION

This is the first study to analyze neutralization effects of produced commercial polyvalent F(ab')₂ antivenom against inflammatory response induced by *Me* scorpion envenomation. Severe scorpion envenomation induces a systemic inflammatory response syndrome (SIRS). This syndrome may cause cardio-respiratory complications and shock. Production of pro-inflammatory mediators such as TNF- α , IL1 and IL6 contribute to the promotion of the inflammation in vital organs such as lung and heart (Pastor *et al* 2003). In the study described here, *Me* venom injection induces an early increase in serum TNF- α and IL1 values at 30 min following envenomation. The similar effects previously were reported in mice envenomated by *Androctonus australis hector* scorpion venom (Adi-Bessalema *et al* 2008). TNF- α and IL1 are the main

pro-inflammatory cytokines in the acute phase of inflammatory response. TNF- α and IL1 elevation induces production of the prostaglandins, platelet activating factor, leukoterienes and nitric oxide which in turn, by their hematopoietic stimulatory impacts and activation of leukocyte formation. Increase of these pro-inflammatory factors contributes to appearance of systemic inflammatory response syndrome in scorpionism (Meki & Mohey El-Dean 1998, D'Suze *et al* 2003). In the present investigation, release of inflammatory cells into the circulation was observed early following *Me* envenomation. Serum neutrophils were increased early 30 min after *Me* venom injection. Neutrophils are usually predominant cells in an immediate inflammatory response and the first type of cells to migrate damaged tissues. Cytokine elevation related to the scorpion envenomation may cause to increase neutrophil release into the circulation (Boujoukos *et al* 1993). In our study, leukocytosis was observed following venom injection. However, leukocytosis and neutrophils may contribute to injury in remote organs such as lung and heart (Boujoukos *et al* 1993, Eum *et al* 2003, Fukuhara *et al* 2003). Severity of scorpion envenoming may related to leukocytosis. Activated leukocytes release superoxide anions products which are injurious oxygen free radical molecules. These molecules may disturb the oxygen delivery by their effects on the circulation which can cause multiple organ failure (MOF) (Deitch 1992). In previous investigations, multiple organ failure was observed in scorpion envenomated cases (D'Suze *et al* 1999, Meki *et al* 2003b). According to the (Novoa *et al* 2003), systemic inflammatory response syndrome related to the scorpion envenoming may cause multiple organ failure. *Me* Scorpion venom induces a severe inflammatory syndrome which may cause pathohistological complications including severe alveolar edema, hemorrhage, thrombus formation, congestion and interstitial inflammation in lungs as well as myocytolysis, coagulation necrosis, myocardial edema and hemorrhage in heart (Zayerzadeh *et al* 2012). Serum cytokines elevation is an important factor

in the pathogenesis of multiple organ failure (Lefkowitz et al 2002, Novoa et al 2003). In the present study, *Me* venom induced hyperglycemia in treated animals (unpublished data). Probably, autonomic storm and inhibition of insulin release following envenomation and serotonin content of the venom caused hyperglycemia (Blanco et al 1999, D'Suze et al 2003). Simultaneous injection of antivenom with venom inoculation prevented TNF- α and IL1 alterations throughout the experiment. Additionally, immunotherapy prevented neutrophils and WBC changes. Furthermore, hyperglycemia did not observe in this group of rabbits following immunotherapy. Therefore, according to the findings obtained in our study, composition and dosage of antivenom were sufficient to entirely neutralize circulating venom. Delayed immunotherapy neutralized and reversed TNF- α and IL1 elevation back to normal in experimented animals. In this group, antivenom administration also inhibited neutrophils and WBC increment and reversed back to normal up to the end of the experiment. Delayed immunotherapy also neutralized and reversed glucose elevation back to normal in experimented rabbits (unpublished data). Therefore, this antivenom seemed to have a potent efficacy on neutralization of inflammatory response following *Me* scorpion envenomation. Previous studies have shown Razi antivenom provided a potential treatment for two Iranian dangerous scorpions including *O. doriae*, and *H. lepturus* due to its fast absorption, appropriate distribution into the extracellular space and its prolonged mean residence time (MRT) (Jalali et al 2010, 2012). Previous study has demonstrated potent effects of immunotherapy on total leukocytes, monocytes and neutrophils after *Androctonus australis hector* venom injection in experimented animals (Sami-Merah et al 2008). Another clinical study has emphasized immunotherapy decreased serum leukocytosis related to the *T.serrulatus* envenomation in humans (Rezende et al 1998). In another investigation, neutralizing effects of intravenous administration of antivenom in children

severely envenomed by *Androctonus australis* and *B. occitanus tunetanus* scorpions was considered (Krifi et al 1999). The time delay of immunotherapy following scorpion envenomation is a very important issue that affects clinical efficacy of treatment by antivenom, due to the pharmacokinetic characteristics of venom and antivenom (Ismail & Abd-Elsalem 1998). The results of the present study confirmed the importance of administration time of polyvalent antivenom to envenomated victims. In conclusion, our observations indicate *M.eupeus* scorpion envenomation induces severe systemic inflammatory response which can be ameliorated with razi polyvalent antivenom immunotherapy at golden time.

Ethics

All animals were kept in compliance with the recommendation of the animal care committee of the Tehran University based on the 'Guide for Care and Use of Laboratory Animals' (NIH US publication 86-23, revised 1985).

Conflict of Interest

The authors have no conflict of interest.

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