

## RESEARCH REPORT

## The pharmacokinetics of *Hemiscorpius lepturus* scorpion venom and Razi antivenom following intramuscular administration in rat

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Received 05 December 2011; Revised 25 February 2012; Accepted 02 March 2012; Published 24 April 2012

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### ABSTRACT

*Hemiscorpius lepturus* (*H. lepturus*) is one of the most dangerous scorpions in Iran. Intramuscular administration (IM) of available Razi antivenom to *H. lepturus* venom is used by many of Iranian clinicians. The purpose of the current study was to investigate the efficiency of IM route for treatment of envenomed patients by *H. lepturus*. We compared the pharmacokinetics parameters of venom and antivenom via subcutaneous (SC) and IM administration, respectively. The blood samples were taken at various predetermined time intervals, *i.e.*, 10, 40, 60, 180, 210, 360 and 400min following 5 $\mu$ g <sup>131</sup>I-labeled venom and 5, 10, 40, 120 and 360min following 0.2ml of <sup>131</sup>I-labeled antivenom administration. The radio-iodination was carried out using the chloramin-T method. The results showed that pharmacokinetic parameters of the venom were  $T_{\text{elimination half-life}} = 103.25\text{min}$ ;  $V_d/F$  (apparent volume of distribution) = 14.9ml/kg;  $Cl/F$  (total blood clearance) = 0.04ml/kg/min; mean resident residual time (MRT) = 244.3min, and for the antivenom  $T_{1/2} = 628.59\text{min}$ ,  $V_d = 666.66\text{ml/kg}$ ,  $Cl = 0.13\text{ml/kg/min}$  and  $MRT = 1292\text{min}$ . A comparison of pharmacokinetic profiles indicated that the intramuscular administration was helpful in the referral less than 2hr to clinical centers but not those exceeding 3hr. Overall, the data showed that immunotherapy against *H. lepturus* stings was likely to be more effective through intravenous administration.

**KEYWORDS:** Iranian scorpion, *Hemiscorpius lepturus*, Razi polyvalent, Intramuscular administration

### INTRODUCTION

Antivenom is specific treatment for the majority of medically important scorpion stings (Bawaskar and Bawaskar, 2011). The main aim of antivenom therapy is to achieve a group of antibodies able to neutralize the toxins proteins of scorpion venom belonging to the dangerous species in a defined geographical area. These toxins have high specificity towards a wide range of ion channels, receptors and transporters.

The pharmacokinetic study of the behavior of dangerous scorpion venoms and antivenoms is essential. It is recognized that antivenom should contain suitable pharmacokinetic parameters and be distributed rapidly to the tissues to neutralize distributed toxins. The effectiveness in serum therapy also depends on potency of the antivenom, route of administration, elapsed-time between sting and antivenom administration times and prescribed dosage (Ismail and Abd-El salam, 1998; Vazquez et al, 2005). These understandings

may lead to a precise definition of the antivenom quality and therapy applicable to different types of scorpion envenoming (Riviere et al, 1997). Furthermore, recently the multiple improvements for an effective antivenom therapy were reported with the use of smaller or recombinant antibody fragments (Ben-Abderrazek et al, 2009, Hmila et al, 2010; Ben-Abderrazek et al, 2011; Ezzine et al, 2012).

Experimental pharmacokinetic studies has been performed on *Leiurus quinquestriatus*, *Buthotus judaicus*, *Androctonus mauretanicus* maurtonicus, *Buthus occitanus tunetanus* and *Androctonus crassicauda* in rabbit, guinea pigs or rats (Ismail et al, 1980; Ismail and Abd-Elsalam, 1988; Ismail et al, 1994; Revelo et al, 1996; Krifi et al, 2001; Hafny et al, 2002). The pharmacokinetics studies were performed by using labeled venom (Ismail et al, 1974; Ismail et al, 1983; Ismail and Abd-Elsalam, 1988; Ismail et al, 1994; Calderon-Aranda et al, 1999), or by measuring the concentration of toxin with ELISA (Revelo et al, 1996; Santana et al, 1996; Krifi et al, 2001; Hafny et al, 2002). The results of blood radioactivity level show two or three compartment model concerning to scorpion species and prescribed method.

The available polyvalent antivenom is produced by the Razi Vaccine and Serum Production and Research Institute against the 6 medically important scorpions: *Odonthobuthus doriae*, *Mesobuthus eupeus*, *Androctonus crassicauda*, *Buthotus (Hottentota)Saulcyi*, *Buthotus sach* and *H. lepturus* (Latifi and Tabatabai, 1979). This product contains a dilution of the F(ab')<sub>2</sub> fraction of equine immunoglobulins achieved after double saline precipitation and pepsin digestion (Table 1).

At present, there is no definite study to guide Iranian clinicians on the choice of an appropriate route of administration. Therefore, this study was performed to assess the efficacy of intramuscular administration against one of the most dangerous scorpions in Iran, *H. lepturus* (Jalali et al, 2010a) and further realization of the available treatment protocol in parallel with the performed study on other medically important scorpion, *Odonthobuthus doriae* (Jalali et al, 2010b).

## MATERIALS AND METHODS

### Animals

Male rats weighing 250-300gm were prepared from Razi Institute (Karaj, Tehran). The rats were housed in groups of three in PVC cages, and had free access to tap water and hard food pellets. The animals were kept at 23 ±2°C, and maintained at 12 hourly light/dark cycle, starting at 7am to 7pm.

**Table 1.** Some properties of the available scorpion antivenom (each 5ml/amp).

Tests	Mean ±SD
pH	6.4 ±0.1
Protein N (mg/ml)	10.3 ±3.0
Total Protein (g.%)	6.5 ±1.8
Total solid (g.%)	9.8 ±2.6
Albumin	non

All pharmacokinetic experiments were conducted in accordance with principles and guidelines of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. The Ethic Committee of the Jundishapur University, Ahvaz approved the design of the experiments.

### Materials

The CNBr-activated Sepharose and Sephadex G<sub>50</sub> were prepared from Pharmacia (Uppsala, Sweden). CM-Sepharose was from Sigma (St Louis, MO, USA). Sodium dodecyl phosphate, Hydrogen peroxide, potassium phosphate buffer, sulfuric acid, sodium sulfate, phenylenediamine and Tris-buffer were from Merck (Darmstadt, Germany). *H. lepturus* lyophilized venom and antivenom were presented by Razi institute. Venom was collected by electrical stimulation, extracted with water, freeze-dried and stored at -20°C until further use (Miranda et al, 1970).

### Radioiodination of the venom and antivenom

Radioiodination of venom and antivenom were carried out using the chloramin-T method. This method specifically iodinated tyrosine residues in proteins forming a stable covalent protein-<sup>131</sup>I bond. The method is generally accepted to be mild enough so as not to affect the activity of the protein being labeled (Hunter and Greenwood, 1962; Greenwood et al, 1963). Briefly, 0.3mCi (300µl) of <sup>131</sup>I was added to 30µl of deionized H<sub>2</sub>O. Then the following solutions were added in this order: 3.5mg of venom in 300µl of 0.5M phosphate buffer, pH 7.2-7.4, 100µl of 6mg/ml chloramin-T; and 100µl of 6mg/ml sodium metabisulfite. Buffers were used to control the pH of solution for optimum efficiency of the proteins. To separate unincorporated <sup>131</sup>I from the iodinated venom, a column packed with Sephadex G<sub>50</sub> (Penefsky, 1979) collected the labeled venom in 1ml fractions.

### Biologic activity of radiolabelled venom

To identify the activity of the venom toxins being labeled, LD<sub>50</sub>, representing toxicity was assessed before and after radiolabelling in mice (18-20gm). Reed and Muenesh method was used to determine LD<sub>50</sub> (Reed and Muench, 1938). The radiolabelled solutions were made up at the rate of 1mg per ml. LD<sub>50</sub> test was conducted by administration various amounts of radiolabelled venom in constant volume (0.2ml of saline solution).

### Blood Sampling

21 male Wistar rats (250-300gm) have been divided to 7 groups (n=3) and 200µl of radiolabelled venom injected subcutaneously. For injections, the lower dorsum of rat, under ketamine anaesthesia, was wet shaved by a surgical blade and cloth dried. These groups were sampled at 10, 40, 60, 180, 210, 360 and 400min following SC administration of 5µg venom supplement with trace amounts of <sup>131</sup>I. 18 male rats divided in 6 groups (n=3) were sampled at 5, 10, 40, 60, 120 and 360min following IM administration of 0.2ml labeled antivenom. The time course of venom and antivenom concentration in the plasma was followed by radioactivity. Samples of whole blood were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant (concentration 0.05M), immediately before

and at regular time intervals after the end of the administrations. The concentration of scorpion  $^{131}\text{I}$ -labeled venom in plasma was determined following trichloroacetic acid (TCA) precipitation. Plasma samples (50 $\mu\text{l}$ ) were added to 450 or 400 $\mu\text{l}$  of antivenom and then precipitated with 500 $\mu\text{l}$  of 20% (v/v) TCA. After a 30-60min incubation period, mixtures were centrifuged for 15min, and the radioactivity was determined in the pellet in a  $\mu$ -counter (Pharmacia, Uppsala, Sweden). The results are presented as percent injected dose/ml blood. The percent radioactivity data were presented after conversion to ng/ml.

### Determination of pharmacokinetic parameters

The plasma concentration vs time data was subjected to a non-compartmental pharmacokinetic analysis to obtain an estimate of various pharmacokinetic parameters, such as total blood clearance (CL/F), distribution volume (Vd/F), area under the curve (AUC), and mean residence time (MRT). The area under the plasma concentration versus the time curve (AUC) was calculated using the linear trapezoidal rule with extrapolation to infinity. CL/F, MRT and  $V_{ss/F}$  were calculated using the following non-compartmental equations (Krifi et al, 2005):

$$\text{MRT} = \text{AUMC}/\text{AUC}$$

$$\text{CL}/\text{F} = \text{Dose}/\text{AUC}$$

$$V_{ss} = \text{CL}/\text{F} \times \text{MRT}$$

AUMC (area under the first moment curve) is the area under the C plotted against  $t$  from time 0 to infinitive.

### Statistical analysis

All values were presented as mean  $\pm$  standard deviation. The means were calculated from at least three separate experiments. The significance of the data was analyzed by the two-tailed unpaired or paired Students' test. The level of significance was considered at  $P < 0.05$ .

## RESULTS

### Biologic activity of radiolabelled venom

The  $\text{LD}_{50}$  of radiolabelled venom was estimated 136  $\pm$  24.9 $\mu\text{g}/\text{mice}$  (6.8mg/kg). This value is not significant than determined  $\text{LD}_{50}$  following non radiolabelled venom test (5.81mg/kg or 116.2  $\pm$  27 $\mu\text{g}/\text{mice}$ ) (Latifi and Tabatabai, 1979).

### Pharmacokinetics of the venom

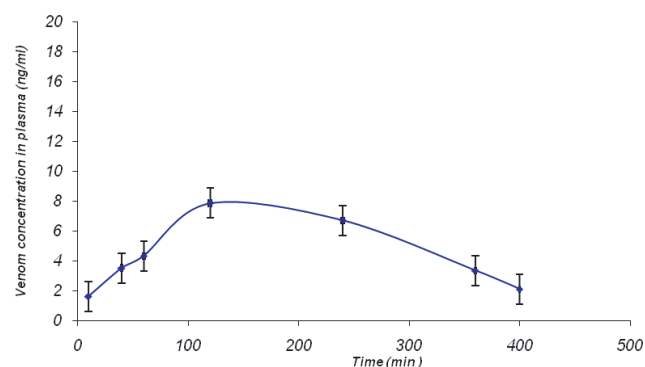
Figure 1 shows mean concentration-time curve and the corresponding pharmacokinetic parameters are noted in Table 2. The time to reach the maximal venom concentration in the blood,  $T_{\text{max}}$ , was about 2hr and the apparent terminal half-life was 103min. The pharmacokinetics of the venom showed that after a relatively slow ascending phase, indicating a slow absorption and gradual distribution of scorpion toxins from central compartment, the toxin concentrations in plasma reached a steady-state plateau and a maximal value ( $C_{\text{max}}$ ) after  $T_{\text{max}}$  of 120min. Then the curve followed a rather slow bi-phasic decline, followed by a slow rate descending phase as seen in ascending phase (Figure 1).

### Pharmacokinetics of the antivenom

Pharmacokinetic parameters of antivenom determined after an IM administration of antivenom by measurement of radioactivity method are shown in Table 3. AUC last and  $C_{\text{max}}$  were about 314 times higher and equivalent, respectively, in antivenom pharmacokinetic if compared with venom values. The  $T_{\text{max}}$  was reached earlier in antivenom ( $T_{\text{max}} = 25$  vs 120min). Vd and MRT of antivenom were very higher than venom in the pharmacokinetic parameters obtained. The time course of antivenom concentration in plasma, determined by radioactivity measurements, showed a bi-exponential decline (Figure 2), indicating that the antivenom was distributed into peripheral compartments with a terminal half-life ( $T_{\text{elimination half-life}}$ ) of 628.59min.

## DISCUSSION

Currently, the scorpion sting victims in Iran, including those of *H. lepturus* sting, are treated in the most clinical



**Figure 1.** Plasma concentration time profile of venom after SC (subcutaneous) administration of 5 $\mu\text{g}$  of *H. lepturus* venom supplemented with trace amount of radiolabeled  $^{131}\text{I}$  to rat. Data are reported as means  $\pm$  SEM (N = 3).

**Table 2.** Pharmacokinetic parameters of *H. lepturus* venom determined after SC injection of 5 $\mu\text{g}$  venom in rat.

Toxicokinetic parameters of venom	Value (mean $\pm$ SEM)	CV%
$T_{\text{max}}$ (min) *	120 $\pm$ 6	5%
$C_{\text{max}}$ (ng/ml) **	7.86 $\pm$ 2.1	26%
$\text{AUC}_{\text{last}}^{***}$ (ng/min/ml)	2004.9 $\pm$ 354	17%
$T_{\text{Half life}}^a$ (min)	103.25 $\pm$ 18	17%
$\text{AUC}_{\text{INF}}$ (ng/min/ml)	2476.66 $\pm$ 87	3.5%
Vd/F $^b$ (ml/kg)	14.9 $\pm$ 2.1	14%
Cl/F $^c$ (ml/kg/min)	0.04 $\pm$ 0.001	2.5%
MRT (min)	<b>244.3</b>	

\*The amount of time that venom is present at the maximum concentration in serum.

\*\* $C_{\text{max}}$  in **pharmacokinetics** refers to the maximum (or peak) concentration of venom in serum

\*\*\*AUC is the **area under** a plot of venom concentration-time profile

$^a$  Elimination half-life

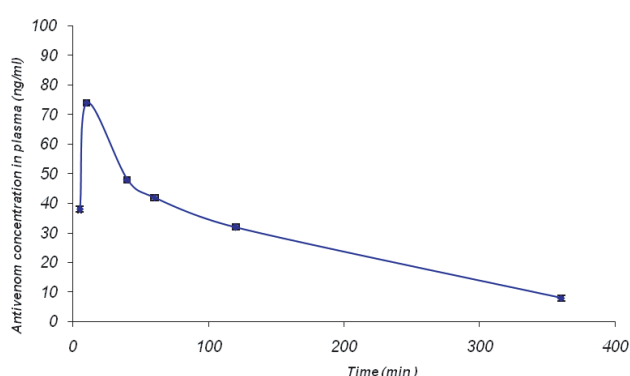
$^b$  Apparent volume of distribution

$^c$  Total (apparent, in case of venom) systemic clearance

MRT: mean resident residual time

**Table 3.** Pharmacokinetic parameters of antivenom determined after IM administration of 0.2 ml of Razi polyvalent antivenom in rat.

Toxicokinetic parameters of antivenom	Value (mean±SEM)	CV%
T <sub>max</sub> (min)	25.34±2.758	10.88%
C <sub>max</sub> (ng/ml)	8.1±0.16	1.97%
AUC <sub>last</sub> (ng/min/ml)	629860±46609	7.40%
T <sub>Half life</sub> (min)	628.59±56.55	9%
AUC <sub>INF</sub> (ng/min/ml)	9779.1581±462.83	4.7%
Vd/F (ml/kg)	666.66±85	12%
Cl/F (ml/kg/min)	0.13±0.00973	7.4%
MRT (min)	<b>1292.87</b>	----



**Figure 2.** Plasma concentration-time profile of antivenom F(ab)<sup>2</sup> after IM (intramuscular) administration of 0.2ml of antivenom supplemented with trace amount of radiolabeled <sup>131</sup>I to rat. Data are reported as means ±SEM (N=3).

centers with intramuscular administration of available antivenom instead of intravenous administration to avoid presumed effects of serum sickness. Therefore, the aim of this study was to determine the pharmacokinetics of *H. lepturus* venom and the available polyvalent antivenom to evaluate, or optimize the treatment protocols through intramuscular administration. In the present study, 5µg venom and 0.2ml antivenom were used. The adjusted dose of venom, as the result of a scorpion sting, was the same as the amount that was injected. Also, 0.2ml of the antivenom injection was adjusted to the rats, since in the current Iranian protocol, 2 vials of 5ml antivenom are recommended for an adult.

The data presented in Tables 2 and 3 show slow absorption and gradual distribution from blood into tissue and slow removing of venom. So it is likely that the release of venom from the site of injection was gradual. However, available antivenom had suitable speed in entering into the blood circulation and long domain of elimination. The CL<sub>total</sub> of venom was 0/04ml/kg/min indicating the slow elimination of venom. This slow elimination is indicative of slow clearance of *H. lepturus* toxins in the performed experiment. This may have due to the experimental protocol designed which did not contain sufficient points at the end of experiments.

Considering T<sub>max</sub> value, it is likely that venom absorption was slower than antivenom. So the maximal concentration of venom reached 1.5hr after antivenom. The main reason may be that SC administration route leads to slower absorption to reach central compartment (blood). In Borchani et al study a 33kDa *H. lepturus* venom protein endowed with a sphingomyelinase D was isolated and identified. This toxin has hemolytic and dermonecrotic activities and exceeds 6-fold the size of the scorpion venom toxins (Borchani et al, 2011). Therefore, it may be concluded that at least a fraction of this venom failed to diffuse and distribute readily from the vascular compartment to the various tissues (Hmila et al, 2010).

Antivenom has higher rate of entry into blood and more extended half. Furthermore, IM administration of the antivenom showed higher C<sub>max</sub>, higher AUC<sub>last</sub> and an earlier T<sub>max</sub>. These parameters are indicative of a relatively faster uptake of the antivenom than venom. As predicted, the Vd/F value of antivenom was more than venom. So the antivenom possesses pharmacokinetic characteristics enabling it to distribute sufficiently to neutralize the distributed toxins to tissues. However, the highly significant difference between Vd/F and AUC values of antivenom than venom indicated that the venom and antivenom did not have sufficient possibility of interacting in tissues. This possibility is essential to alleviate the occurrence of common severe clinical signs following a *H. lepturus* sting with delay manifestations (Pipelzadeh et al, 2007; Jalali et al, 2011a). This difference can be considered a reason for the lack of neutralizing activity observed *in vivo* (Jalali et al, 2011b).

Surprisingly, *H. lepturus* venom with a slow rate clearance has significantly less T<sub>half-life</sub> than reported value for the intramuscularly administered *O. doriae* venom (Jalali et al, 2010b). The Vd indicating an extended distribution of the venom into tissues occurred in a large extravascular compartment. The Vd/F for the *H. lepturus* venom was greatly reduced than *O. doriae* indicating a low distribution. This value was approximately 100x more for *O. doriae* (Jalali et al, 2010b). This difference was not seen in the earlier studies (Ismail et al, 1974; Ismail et al, 1983; Ismail et al, 1994; Revelo et al, 1996; Calderon-Aranda et al, 1999; Hafny et al, 2002; Hammoudi-Triki et al, 2007). The slow absorption of venom may be due to the high molecular weight toxins of this scorpion (Borchani et al, 2011). Thus it can be concluded that due to Vd the neutralization capacity of antivenom to react the *H. lepturus* toxins in tissues was less than that for *O. doriae* (Jalali et al, 2010b). The pharmacokinetic parameters of the study conducted on *O. Doriae* showed an indistinguishable absorption rate (T<sub>max</sub> = 120min for both) and a slower rate of distribution for *H. lepturus*. The AUC<sub>last</sub> value (area under the concentration-time curve from zero time to complete removal) of *H. lepturus* venom was significantly less than that of the *O. doriae* value. This may indicate the reason for delay in occurrence of clinical manifestations following *H. lepturus* envenoming, and indicating that immediate treatment following *H. lepturus* sting is not an emergency situation. This delay is consistent with clinical conditions and a late occurrence of severe envenoming (Pipelzadeh et al, 2007). However, the full spectrum of reasons behind this requires further investigation.



**Table 4.** Mouse LD<sub>50</sub> of venom neutralized by 1ml of Razi available antivenom (intravenous administration).

Scorpion species	N. LD <sub>50</sub> *
<i>Androctonus crassicauda</i>	10
<i>Buthotus (Hottentona) saulcyi</i>	22
<i>H. Lepturus</i>	7
<i>Mesobuthus eupeus</i>	10
<i>Odonthubuthus doriae</i>	37
<i>Scorpio maurus</i>	5

\* Results of homologous tests

As presented in Table 4, neutralization capacities of the antivenom tested were expressed as the number of LD<sub>50</sub> neutralization per one ml of serum. This potency was highest for *O. doriae* and *Buthotus saulcyi* venom and moderate for *H. lepturus* venom. The potency of 1ml refined antivenom in term of lethal doses neutralized was 7 mice LD<sub>50</sub> for *H. lepturus* venom (Latifi and Tabatabai, 1979) (Table 4). So 1 to 2 vials of antivenom administration (according to the current protocol), ranging from 5 to 10ml, should suffice to neutralize the total amount of venom contained in the telson of any *H. lepturus* scorpion.

## CONCLUSIONS

Comparison of pharmacokinetic profiles of two Iranian scorpions showed that *O. doriae* venom had a much extended distribution in tissues as compared with the dangerous Iranian scorpion, *H. lepturus*. *O. doriae* study indicated that Razi antivenom offered a potential treatment of choice because of its fast absorption, ample distribution into the extracellular space and its prolonged MRT. In contrast, Vd for *H. lepturus* was 100-fold less and MRT was 244.3min. Immediate intramuscular administration of an adjusted dose of available antivenom had a favorable kinetic profile. If only kinetic profile is considered, this route would be appropriate when administered in referrals under 2hr. Furthermore, intramuscular administration does not appear to be appropriate for neutralization of *H. lepturus* envenomation.

## ACKNOWLEDGMENTS

We would like to thank Professor Simin Dadashzadeh (School of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran) and Professor Ali Hassan Rahmani (Dept of Clinical Toxicology, Razi hospital, Jundishapur University) for their suggestions and guidance during this study. This work was supported in part by funding from the Deputy of Research (Jundishapur University).

## COMPETING INTEREST STATEMENT

None declared.

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