RESEARCH REPORT

The pharmacokinetics of Iranian scorpion *Odonthubuthus doriae* venom and the available antivenom

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Received: 07 August 2010; Revised: 11 September 2010; Accepted: 27 September 2010; Published online: 15 October 2010

J Venom Res, 2010, Vol 1, 48-53

ABSTRACT

In Iran intramuscular (IM) administration of antivenoms is used for the treatment of human scorpion envenoming of six medically dangerous scorpion species, including *Odonthubuthus doriae* (*O. doriae*). The purpose of the current study is to investigate the efficiency of the intramuscular route and the delay of injection on the neutralizing effect of the available polyvalent antivenom. We compared the pharmacokinetics parameters of *O. doriae* venom and its antivenom. 5 µg ^{131}I-labeled venom and 0.2 µl of antivenom were administered via subcutaneous (SC) or IM into rats. Blood samples were taken at various predetermined time intervals during a 24 hr period for the venom and a 360 min period for the antivenom. The radio-iodination was carried out using the chloramin-T method. The results showed that pharmacokinetic parameters of the venom were $T_{1/2}$ = 496.53 min; V_d = 1522 ml/kg; Cl = 2.12 ml/kg/min; mean resident residual time (MRT) = 555.77 min, and for the antivenom $T_{1/2}$ = 902.13 min, V_d = 666.66 ml/kg , Cl = 0.512 ml/kg/min and MRT = 1292 min. The total body clearance of the venom is relatively low in agreement with a high mean residence time. Higher AUC and C_{max} values for the antivenom as well as its longer residence time indicate that the venom and antivenom are expected to have enough opportunity to interact in the tissue compartments. Over, this study suggests that the intramuscular administration of a single dose of antivenom (2 vials each of 5 ml) based on current protocol in Iran is a suitable route for the treatment of envenomation with *O. doriae*. Prudently, further clinical studies with similar aims need to be carried out to confirm these findings in human victims.

KEYWORDS: Iranian scorpion, *Odonthubuthus doriae*, Razi polyvalent antivenom, pharmacokinetic parameters, venom

INTRODUCTION

Scorpion envenomation has lead to the study of biochemical and pharmacological actions of the toxic components of scorpion venom. This has resulted in a better understanding of the envenomation syndrome, and to improve the treatment. Antivenom immunotherapy remains the only specific treatment for scorpion envenomations. The optimization of toxin neutralization by specific antibodies depends on the pharmacokinetics of both the antibody and the toxin molecules, and the capacity of the specific antibodies to sequester the toxin. Thus, the improvement in immunotherapeutic envenomations requires a better understanding of scorpion venom pharmacokinetics (Krifi et al, 2005).

Experimental toxicokinetic studies of *Androctonus amoreuxi*, *Tityus serrulatus*, *Buthus occitatanus tunetanus*, *Leiurus quinquestriatus*, *Buthotus judaicus*, *A. mauretanicus mauretanicus* and *A. crassicauda* radiolabeled venom have been performed after intravenous or subcutaneous injections in rabbit, guinea-pig

and rat models (Ismail et al, 1980; Ismail et al, 1992; Ismail et al, 1994; Revelo et al, 1996; Ismail et al, 1998; Krifi et al, 2001; El Hafny et al, 2002). Most of these studies used employed measurement of radioactivity levels in blood, which showed bi- or tri-compartmental pharmacokinetics depending upon the scorpion species and the injection route.

The Iranian polyvalent antivenom is produced by the Razi Vaccine and Serum Production and Research Institute through the immunization of horses against the 6 medically important scorpion species in Iran: *O. doriae, Mesobuthus eupeus, Androctonus crassicauda, Buthotus (Hottentota) Saulcyi, Buthotus sach* and *Hemiscorpius lepturus* (Latifi and Tabatabai, 1979). The antivenom contains a dilution of the $F(ab')_2$ fraction of equine immunoglobulins obtained after double saline precipitation and pepsin digestion.

However, to-date no information of pharmacokinetic parameters of the Iranian scorpion venom and Razi Institute polyvalent antivenom is available. Currently, the scorpion sting victims in Iran, including those of *O. doriae* sting, are treated with intramuscular administration of antivenom. The scope of the present study is to determine the pharmacokinetics of the *O. doriae* venom in experimentally envenomed rats as well as the pharmacokinetics of the available antivenom to evaluate, or optimize, the available therapies and routes of administration.

MATERIALS AND METHODS

Animals

The male rats weighing 250-300 gm were from Razi Institute (Karaj, Tehran). 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 and the Guiding Principles in the Use of Animals in Toxicology" which were adopted by the Society of Toxicology in July1989 and revised in March 1999.

Materials

The CNBr-activated Sepharose and Sephadex G_{50} were purchased from Pharmacia (Uppsala, Sweden). CM-Sepharose was from Sigma (St Louis, MO, USA). Sodium dodecyl phosphate, Hydrogen peroxide, potassium phosphate buffer, sulforic acid, sodium sulfate, phenylenediamine and Tris-buffer were from Merck (Darmastadt, Germany). The Agar gel and immunoglobulin fragments against scorpion from horse were purchased from Razi institute (Karaj, Iran). *O. doriae* lyophilized venom and antivenom were prepared in 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

Radio-iodination of venom and antivenom were carried out using the chloramin-T method. This method specifically iodinates tyrosine residues in proteins forming a stable covalent protein-^{131}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, 0.3 mCi (300 µl) of ^{131}I was added to 30 µl of deionized H_2O. Then the following solutions were added in sequence: 3.5 mg of venom in 300 µl of 0.5 M phosphate buffer, pH 7.2-7.4, 100 µl of 6 mg/ml chloramine-T; and 100 µl of 6 mg/ml sodium metabisulfite. Buffers were used to control the pH of solution for optimum efficiency of the proteins. To separate unincorporated ^{131}I from the iodinated venom, a column packed with Sephadex G50 (Penefsky, 1979) collected the labeled venom in 1 ml fractions.

Blood Sampling

Six groups, consisting of three rats each, were sacrificed at 10, 20, 40, 60, 120, 360 and 1440 min following SC administration of 5 µg venom supplement with trace amounts of ^{131}I. The same groups of rats were sacrificed at 5, 10, 40, 60, 120 and 360 min following IM administration of 0.2 ml available antivenom supplement with trace amount of ^{131}I. The rats received water and food *ad libitum*. The time course of venom concentration in the plasma was followed by radioactivity. Samples of whole blood were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant (final concentration 0.05 M), immediately before and at regular time intervals after the end of the administrations. The concentration of scorpion ^{131}I-labeled *O. doriae* venom in plasma was determined after trichlroacetic acid (TCA) precipitation. Plasma samples (50-100 µl) were added to 450 or 400 µl of immunoglobulin from horses and then precipitated with 500 µl of 20% (v/v) TCA. After a 30-60 min incubation period, mixtures were centrifuged for 15 min, and the radioactivity was measured in the pellet in a γ-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 body clearance (CL/F), distribution volume (V_d/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):

$$MRT = AUMC/AUC$$

$$Cl/F = Dose/AUC$$

$$V_{ss} = Cl/F \times MRT$$

Whereas, AUMC (area under the first moment curve) is the area under the Cxt plotted against *t* from time 0 to infinitive.

Statistical analysis

All values were presented as mean ± 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 set at $P < 0.05$.

RESULTS

Pharmacokinetics of the *O. doriae* venom

The result of the pharmacokinetic analysis, performed in three groups of rats after SC injection of the 5 µg venom dose, are shown in Figure 1, and the corresponding pharmacokinetic parameters are noted in Table 1. The time to reach the maximal venom concentration in the blood, Tmax, was virtually brief (about 2 hr) and the apparent terminal half-life was 496 min.

The pharmacokinetics of the venom showed that after a rapid ascending phase, which means a fast absorption of scorpion toxins, the toxin concentrations in plasma reached a maximal value (C_{max}) after T_{max} of 120 min. Then the curve followed a rather slow bi-phasic decline, followed by a slow and a more slowly declining phase (Figure 1).

Pharmacokinetics of the antivenom

Pharmacokinetic parameters of antivenom determined after an IM injection of antivenom by measurement of radioactivity method are shown in Table 2. AUC_{last} and C_{max} were at about 14 x and 3 x higher, respectively, in antivenom pharmacokinetic if compared with venom values. The C_{max} was reached earlier in antivenom (T_{max} = 60 vs 120 min). V_d and MRT of antivenom were lower and higher, respectively, 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 two compartments with a terminal half-life ($t_{1/2}$) of 496.43 min.

DISCUSSION

Despite the development of improved antivenom preparations and the conviction widely shared among clinicians that the antivenom therapy is the most effective treatment of severe cases of scorpion envenomations, the use of such specific treatment is still justified mainly on the basis of empirical observations. The knowledge of the neutralization capacity and kinetics of scorpion venom and antivenom can contribute to the improvement of the treatment strategies. Achieving such a goal is directly dependent upon the efficacy of specific bindings of the circulating toxins to the antivenom, and on the kinetics of both the venom and anitvenom to move from the extravascular compartment to the vascular ones, or possibly both. The antivenom used in serotherapy should possess pharmacokinetic characteristics enabling it to distribute rapidly enough to neutralize the venom fraction distributed to tissues. Pharmacokinetics contributes to the understanding of the relationship between dose and response in the design of new therapies and/or modifications of the current medicinal products.

Most of the reported scorpion venom pharmacokinetic studies were performed using ^{125}I-labeled venom (Ismail and Abd-Elsalam, 1988; Ismail et al, 1994; Revelo et al, 1996; Calderone-Aranda et al, 1999) or by following toxin concentration by ELIZA (Revelo et al, 1996; Santana et al, 1996; Kirifi et al, 2001; El-Hafney et al, 2002). In the current study, venom pharmacokinetics was followed by measuring TCA perceptible radiolabeled toxins in experimentally envenomed rats. Indeed, after the injection, the *in vivo* degradation of radioiodinated venom proteins could yield toxin fragments and/or free radioactive iodine, which might bind non-specifically to the plasma proteins still measured by this method. The free and non-specific plasma radioactivities and/or the degraded radio labeled venom toxins are still being quantified by the radioactive method; while ELIZA could not detect those. The ELISA assay could present limitations and disadvantages, such as the relative insensitivity, the failure to detect the venom antigen by slight modification in the body and the antibody binding sites are mostly different from the pharmacologic active ones, *etc*. These disadvantages could be limited by an accurate standardization of the assay (Krifi et al, 2001).

In the present study, 5 µg *O. doriae* venom and 0.2 ml antivenom were administered via SC and IM, respectively. The adjusted dose of venom, as the result of a scorpion sting, was the same as the amount that was injected. Also, 0.2 ml of the antivenom injection was adjusted to the rats, since in the current Iranian protocol, 2 vials of 5 ml antivenom are recommended for an adult.

As shown in Tables 1 and 2 the volume of distribution of antivenom was 666 ml/kg, which is significantly lower compared to the V_d of venoum determined after SC injection (1522 ml/kg). Considering the T_{max} values, the venom was

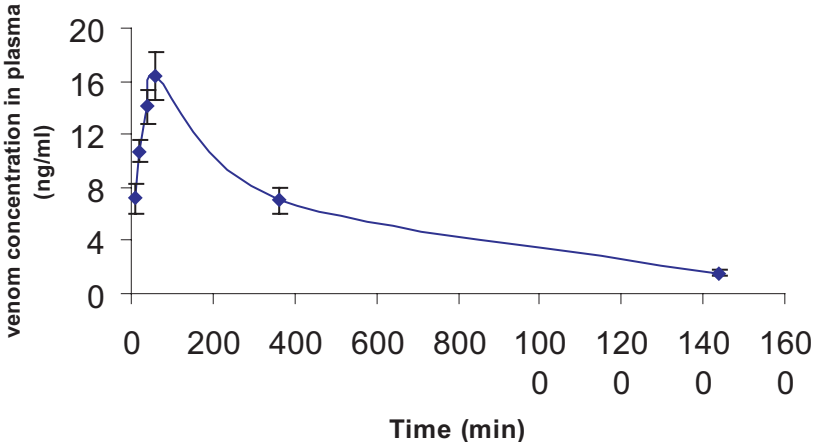

Figure 1. Plasma concentration-time profile of venom after SC administration of 5 µg of *O. doriae* venom supplemented with trace amount of radiolabeled ^{131}I. Data are reported as means ± SEM (N = 3).

Table 1. Phamacokinetic parameters of *O. doriae* venom determined after sc injection of 5 µg/ml venom in rat.

Toxicokinetic parameters of venom	Value (mean ± SEM)
T_{max} (min)	120 ± 8
C_{max} (ng/ml)	16.42 ± 3.2
AUC_{last} (ng/min/ml)	11175 ± 652
$T_{Half\ life}$ (min)	496.53 ± 46
AUC_{INF} (ng/min/ml)	503.486 ± 46
V_d/F (ml/kg)	1522 ± 135
Cl/F (ml/kg/min)	2.12 ± 0.2
MRT (min)	555.77

Table 2. Phamacokinetic parameters of antivenom determined after IM injection of 0.2 ml of Razi polyvalent antivenom in rat.

Toxicokinetic parameters of antivenom	Value (mean ± SEM)
T_{max} (Min)	60 ± 6
C_{max} (ng/ml)	48.9 ± 4.6
AUC_{last} (ng/min/ml)	147962.5 ± 24356
$T_{Half\ life}$ (min)	902.13
AUC_{INF} (ng/min/ml)	2500.19 ± 325
$V_{d/F}$ (ml/kg)	666.66 ± 115
Cl/F (ml/kg/min)	0.512 ± 0.08
MRT (min)	1292.87

absorbed slowly compared to the antivenom and its maximal concentration reached 1 hr after the antivenom. This slow absorption may explain the delay onset of the clinical signs after an *O. doriae* sting. The antivenom F(ab′)2 had a higher rate of entry into the systemic circulation and a more extended elimination half-life. Thus, it is anticipated that antivenom would efficiently neutralize the venom following the administration of IM.

Furthermore, ^{131}I-antivenom showed higher C_{max} and AUC_{last} values and an earlier T_{max} following the IM administration, indicating a faster absorption and higher uptake than the venom. The increased uptake of the antivenom can also be explained on the basis of a higher absorption rate for the antivenom, explaining the lower value found for T_{max}. It is noteworthy that the values of the AUC determined after the IM administration of 5 µg venom are close to those presented by Krifi and colleagues study that used the IV route.

With regards to the antivenom, we found an elimination half-life of 15 hr after IM injection, which is significantly longer than the reported values for the intravenously administered *Andrectonus mauretanicus mauretanicus* (El-Hafny et al, 2002), *C. limpidus limpidus* (Calderon-Aranda et al, 1999) and *B. occitanus mardoche* (Pepin-Covatta et al, 1996) antivenoms. Furthermore, the AUC determination of the antivenom is higher than that of the venom, which differs significantly. Therefore, these observations indicate a complete absorption of antivenom (and/or venom) from its injection site.

The V_d indicating a fast distribution of the venom into tissues occurred in a large extravascular compartment. As expected, the V_d/F for the venom was higher indicating a possible increased distribution. The Cl_F of venom was 2.12 ml/kg/min, indicating its rapid elimination. These findings suggest a fast clearance of the scorpion toxins in the experiments performed with radioactive toxins. The rapid absorption of venom may be due to the low molecular weight toxins (4-7 kDa) of this scorpion (Jalali et al, 2005; Abdel-Mottaleb et al, 2006; Abdel-Mottaleb et al, 2008). The fast absorption and distribution of the venom indicate that *O. doriae* envenomation is rapid. Therefore, specific and symptomatic treatments must be started as soon as possible after the sting, or else at least as soon as envenomation symptoms appear.

In a randomized controlled trial of scorpion antivenom therapy in Tunisia it was shown that the serotherapy against *Buthus occitanus tunetanus* scorpion was inefficient (Abroug et al, 1999). Although, this investigation was

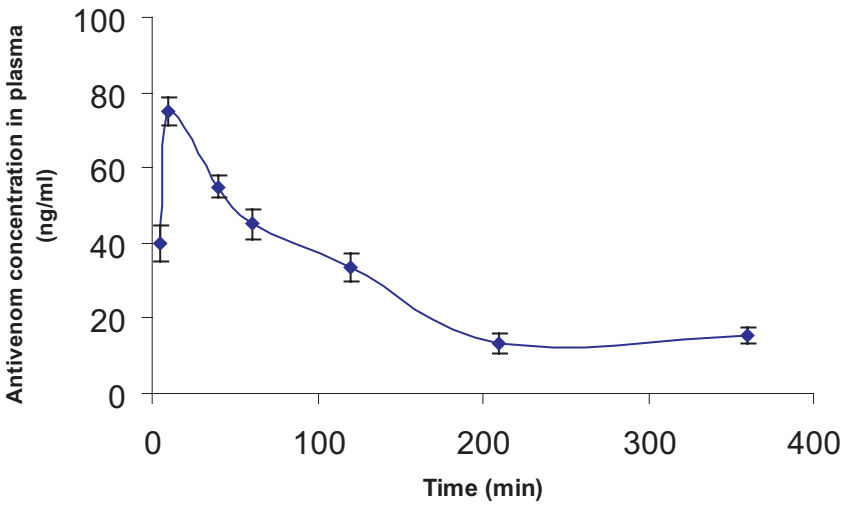

Figure 2. Plasma concentration-time profile of antivenom F(ab′)2 after intramuscular administration of 0.2 ml of antivenom supplemented with trace amount of radiolabeled ^{131}I-*O. doriae* venom to rat. Data are reported as means ± SEM (N = 3).

conducted on a small number of severely envenomed patients, it raises the question whether the dose of antivenom administered was less than the minimal required for neutralizing the toxic manifestations of the venom.

The IM route is still used in immunotherapy of scorpion envenoming. However, it is important to note that it results in a delayed neutralization of toxins that is 10-fold lower efficacy compared to the intravenous route (Ismail and Abd-Elsalam, 1998). Ismail and Abd-Elsalam concluded the IM injection of antivenoms is certainly ineffective in neutralizing the action of venoms, as the venoms and antivenom have no opportunity to meet quickly enough in the central or tissue compartments. But in this current study, the efficacy of used intramuscular route, dose and anticipated delay for antivenom administration indicate efficacious of immunotherapy with Razi antivenom. In support of this, Pe´pin-Covatta et al (1996) reported that less than 40% of the intramuscularly injected antivenom F (ab′)2 are slowly absorbed from the injection site.

Iranian epidemiological data shows that 30% of the patients were taken to medical facilities more than 3 hr after being stung by the scorpion, *H. lepturus* – considered as the most dangerous scorpion in Iran (Pipelzadeh et al, 2007; Jalali et al, 2010). In other studies, a maximum distribution of the toxins and their complete neutralization in plasma were observed when the minimal efficient antivenom dose was injected 4-6 hr after the envenomation. However, it is difficult to predict the clinical results of an antivenom immunotherapy when performed a long time after envenomation. Indeed, it is quite possible that the scorpion toxins may exert their deleterious effects and cause an irreversible damage before the administration of the antivenom.

CONCLUSIONS

- Immediate intramuscular administration of a dose of antivenom (human values corrected in experimental rat) has a favorable profile from kinetic and measured data view.
- The intramuscular route administration of antivenom may be considered appropriate when administered within the gold time of 2 hr.
- IM injection may be suitable route for neutralization of *O. doriae* toxins with the available polyvalent antivenom prepared in the Razi Institute.

ACKNOWLEDGMENTS

We would like to thank Professor Pipelzadeh (School of Medicine, Jundishapur University of Medical Sciences, Ahvaz) for his suggestions and guidance in writing this article. This work was supported in part by funding from the Deputy of Research (Jundishapur University) and the Drug Sciences Research Center (Shahid Beheshti University of Medical Sciences, Tehran).

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