Age effects on the pharmacokinetics of tityustoxin from *Tityus serrulatus* scorpion venom in rats

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Abstract

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The pharmacokinetics of scorpion venom and its toxins has been investigated in experimental models using adult animals, although, severe scorpion accidents are associated more frequently with children. We compared the effect of age on the pharmacokinetics of tityustoxin, one of the most active principles of *Tityus serrulatus* venom, in young male/female rats (21-22 days old, N = 5-8) and in adult male rats (150-160 days old, N = 5-8). Tityustoxin (6 μg) labeled with ^{99m}Technetium was administered subcutaneously to young and adult rats. The plasma concentration *vs* time data were subjected to non-compartmental pharmacokinetic analysis to obtain estimates of various pharmacokinetic parameters such as total body clearance (CL/F), distribution volume (Vd/F), area under the curve (AUC), and mean residence time. The data were analyzed with and without considering body weight. The data without correction for body weight showed a higher C_{max} (62.30 ± 7.07 *vs* 12.71 ± 2.11 ng/ml, P < 0.05) and AUC (296.49 ± 21.09 *vs* 55.96 ± 5.41 ng h^{-1} ml^{-1}, P < 0.05) and lower T_{max} (0.64 ± 0.19 *vs* 2.44 ± 0.49 h, P < 0.05) in young rats. Furthermore, Vd/F (0.15 *vs* 0.42 l/kg) and CL/F (0.02 ± 0.001 *vs* 0.11 ± 0.01 l h^{-1} kg^{-1}, P < 0.05) were lower in young rats. However, when the data were reanalyzed taking body weight into consideration, the C_{max} (40.43 ± 3.25 *vs* 78.21 ± 11.23 ng kg^{-1} ml^{-1}, P < 0.05) and AUC (182.27 ± 11.74 *vs* 344.62 ± 32.11 ng h^{-1} ml^{-1}, P < 0.05) were lower in young rats. The clearance (0.03 ± 0.002 *vs* 0.02 ± 0.002 l h^{-1} kg^{-1}, P < 0.05) and Vd/F (0.210 *vs* 0.067 l/kg) were higher in young rats. The raw data (not adjusted for body weight) strongly suggest that age plays a pivotal role in the disposition of tityustoxin. Furthermore, our results also indicate that the differences in the severity of symptoms observed in children and adults after scorpion envenomation can be explained in part by differences in the pharmacokinetics of the toxin.

Introduction

Tityus serrulatus is one of most venomous scorpions. The venom of this scorpion is composed of a variety of water-soluble and -insoluble proteins among which tityustoxin (TsTX) is the most toxic component (1-3). The majority of toxins present in the venom are highly neurotoxic. The major site of action of the toxins is the sodium ion channel (4) where they modulate the release of neurotransmitters (5,6). This leads to a variety of adverse effects which include respiratory failure (7), lung edema (8,9), arrhythmias, tachycardia followed by bradycardia (10), skeletal muscle stimulation, lacrimation, convulsions, and enlarged pupils (11), among others (12,13).

A number of studies have investigated the effect of age on the toxicity of *T. serrulatus* scorpion venom and other scorpion venoms (2,7,8,13,14). Clot-Faybesse et al. (15) showed that 3-7-day-old mice were more susceptible to scorpion neurotoxin than 10-week-old adult mice. Similar study from our laboratory has shown that 21-22-day-old rats were more susceptible to a venom water extract administered subcutaneously (*sc*) than adult rats (150-160 days old) (16). These differences in toxicity have been underscored in a variety of clinical studies which have clearly shown that children exhibit a higher degree of envenomation symptoms associated with the cardiovascular and central nervous systems (17-19).

Pharmacokinetic studies of the venom of several scorpions have been reported in the literature (10,11,20-24). Santana et al. (23) have explained the disposition of scorpion venom in adult animals. The venom exhibits a rapid absorption, extensive distribution from blood to tissue and slow elimination from the organism (23). However, the influence of age and body weight on the disposition of the various toxins of scorpion venom has not been studied in detail. The present investigation was undertaken in an attempt to compare the effect of age on the disposition of TsTX in young (21-22 days old) and adult (150-160 days old) male rats.

Material and Methods

Labeling of TsTX

TsTX was obtained from *T. serrulatus* scorpion venom by gel filtration and ion exchange chromatography (1). The toxin concentration was estimated by the formula: protein (mg/ml) = absorbance at 280 nm/ 3.584. Absorbance measurements were made with a Hitachi spectrophotometer, model U-2001 (Kyoto, Japan). TsTX (200-250 µg) was labeled with 37 MBq sodium pertechnetate as described in Ref. 25. Silica gel ascending and Whatman paper descending chromatography (26) was used to monitor the labeling efficacy. $^{99m}TcO_2$ not incorporated into the protein was retained on the Sephadex G-10 (Sigma, St. Louis, MO, USA) gel filtration column (4.0 x 1.5 cm) equilibrated with 0.9% (w/v) NaCl. Fractions 3-6 were pooled and injected *ip* into mice (16 µg/mouse) weighing 20-25 g (N = 4) to observe the characteristic signs of intoxication.

Blood samples for ^{99m}Tc-TsTX determination

Holtzman adult rats (150-160 days old) weighing 353 ± 33 g and young rats (21-22 days old) weighing 39 ± 6 g were used. The animals received water and food *ad libitum*. Groups of animals were injected *sc* with 6 µg ^{99m}Tc-TsTX and sacrificed at 0.08, 0.5, 1, 2, 3, 6, 8 and 12 h. For sedation, all rats were injected *ip* with urethane (140 mg/100 g) 30 min before sacrifice by decapitation. Blood samples were collected into a tube containing ethylenediaminetetraacetic acid (EDTA) anticoagulant at a final concentration of 0.05 M. Blood aliquots (200 µl) were counted with an automatic scintillation counter (ANSR, Abbott, Chicago, IL, USA) and the

results are reported as percent injected dose/ml blood. ^{99m}Tc-TsTX (6 µg) was used as a positive control. The percent radioactivity data were reported after conversion to ng/ml blood. Animal studies were performed in accordance with the guidelines of the United Kingdom Biological Council on the use of living animals in scientific investigations.

Pharmacokinetic analysis

Groups of rats consisting of a minimum of five and a maximum of eight animals were sacrificed at 0.08, 0.5, 1, 2, 3, 6, and 12 h after *sc* administration of 6 µg TsTX. Due to the limitations imposed by the size of the young rats (21-22 days old), only one blood sample was collected per animal. This methodology of sample collection (one sample per animal) hampers the use of traditional methods of pharmacokinetic data analysis because the calculation of parameters like total area under the curve ($AUC_{0-\infty}$), maximum concentration (C_{max}) and elimination rate constant (K) requires the time-dependent collection of blood samples to obtain intra-animal concentration profiles. Consequently, an alternate method of pharmacokinetic and statistical data analysis was required which would help calculate and statistically compare the pharmacokinetic parameters obtained from "one sample per animal" data.

To overcome this problem, a bootstrapping procedure was developed using the Visual Basic feature of Excel. Bootstrap resampling is a very robust statistical method for estimating the accuracy of parameters obtained from experimental data (27). The resampling procedure generates data sets after random iterations. Resampling techniques such as bootstrapping have been shown to provide robust estimators of pharmacokinetic and pharmacodynamic parameters (28).

The program was designed to randomly select one value from a pool of concentration values at a given time point. This proce-

dure was iterated for all the other time points to define individual concentration time profiles. The resampled data from adult and young rats were then analyzed using noncompartment analysis to obtain the mean and standard deviation of different pharmacokinetic parameters such as K using the last points from the blood concentration *vs* time curve, body elimination half-life ($T_{1/2}K$), AUC_{∞}, C_{max}, time to reach C_{max} (T_{max}), mean residence time (MRT), and clearance (CL/F). The distribution volume (Vd/F) was determined to be equal to $[D/(AUC_{\infty} \times K)]$, where D = 6 µg/animal, using the WINNOLIN computer program for non-compartmental analysis.

Results are reported as means ± SD or means ± SEM. The pharmacokinetic parameters obtained were analyzed by the two-tailed unpaired Student *t*-test, with the level of significance set at $P < 0.05$.

Results

The yield of radioactivity obtained after labeling the toxin was 75 to 85%. TsTX activity after labeling was intact, as determined by the observation of characteristic signs of acute scorpion intoxication in mice (piloerection, salivation, tachycardia, dyspnea, muscle contraction, and convulsion) after *ip* injection of the labeled toxin (16 µg/mouse).

Figure 1A and B shows the concentration-time profiles of ^{99m}Tc-TsTX in adult and young male rats, respectively, with and without correction for body weight. When data were not corrected for body weight (Figure 1A), ^{99m}Tc-TsTX showed a significantly higher blood concentration in young animals. C_{max} was reached faster by young rats while the elimination phase was apparently slower. Figure 1B, with data corrected for body weight, shows that the ^{99m}Tc-TsTX concentration in blood at 5 and 30 min was higher in young rats, but the elimination was similar to that observed without correction

for body weight.

Table 1 shows the parameters calculated from data without correction for body weight. All comparisons of parameters between the adult and young groups were significantly different. C_{max} was five times higher for young rats and occurred earlier than in adult

rats ($T_{max} = 0.64 \pm 0.19$ *vs* 2.44 ± 0.49 h). There was a five-fold difference in $AUC_{0-\infty}$ between the young and adult groups (296.49 ± 21.09 *vs* 55.96 ± 5.41 ng h^{-1} ml^{-1}), indicating a five-fold higher systemic exposure to the toxin in young rats. $T_{1/2}K$ and MRT were longer in young rats ($T_{1/2}K = 5.00 \pm 1.96$ *vs* 2.70 ± 0.48 h, MRT = 6.77 ± 1.66 *vs* 4.36 ± 0.59 h). CL/F and Vd/F were higher in adult than in young rats.

Table 2 shows the values of the pharmacokinetic parameters obtained after correcting for body weight. AUC and C_{max} were about two times lower in young than in adult rats, but C_{max} was again reached earlier by young animals ($T_{max} = 0.84 \pm 0.23$ *vs* 2.47 ± 0.51 h). The correction for body weight did not change $T_{1/2}K$, MRT or K values (Tables 1 and 2). CL/F and Vd/F, however, were higher in young animals after correction for body weight.

Discussion

A number of studies have been performed to investigate the disposition of injected TsTX. However, most of these studies have been performed on adult rats or mice. To the best of our knowledge, very few studies have investigated the disposition of TsTX in 21-22-day-old young rats (29).

In the present study, 6 µg TsTX was administered *sc* to young and adult rats. The dose was not adjusted for differences in body weight because, as the result of a scorpion bite, the same amount of toxin is injected, irrespective of body weight. The blood concentration versus time data were used to calculate and statistically compare the pharmacokinetic parameters. The data were analyzed with and without taking the effect of body weight into consideration. However, the pharmacokinetic parameters obtained on the basis of data not corrected for body weight should have significantly higher physiological relevance.

After the *sc* administration of the same

Figure 1. Blood concentration-time profile of adult (circles) and young (squares) male rats after subcutaneous injection of 6 µg ^{99m}Tc-TsTX. Data are reported as means ± SEM (N = 5-8) without correction for body weight (A) and corrected for body weight (B). *P < 0.05 compared to adult rats (Student *t*-test).
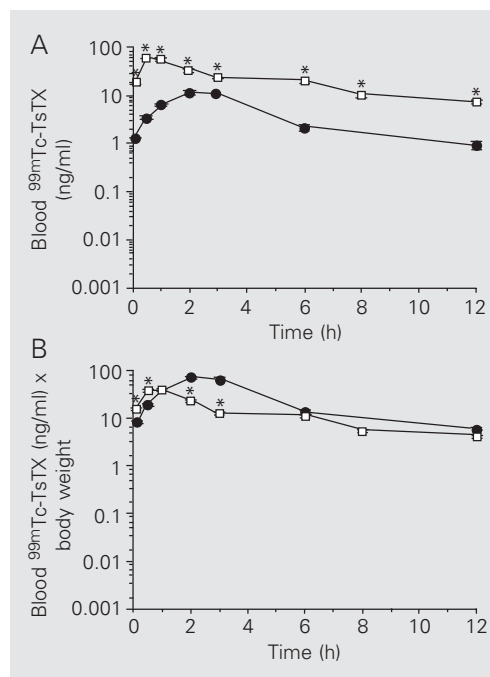

Table 1. Pharmacokinetic parameters of subcutaneous injection of 6 µg ^{99m}Tc-TsTX into adult (150-160 days old) and young (21-22 days old) male rats, without correction for body weight.

Parameters	Adult rats	Young rats
K (h^{-1})	0.27 ± 0.05	0.16 ± 0.04
$T_{1/2}K$ (h)	2.70 ± 0.48	5.00 ± 1.96
AUC_∞ (ng h^{-1} ml^{-1})	55.96 ± 5.41	296.49 ± 21.09
MRT (h)	4.36 ± 0.59	6.77 ± 1.66
C_{max} (ng/ml)	12.71 ± 2.11	62.30 ± 7.07
T_{max} (h)	2.44 ± 0.49	0.64 ± 0.19
CL/F (l h^{-1} kg^{-1})	0.11 ± 0.01	0.02 ± 0.001
Vd/F (l/kg)	0.42	0.15

AUC_∞ = area under the curve; CL/F = total body clearance; C_{max} = maximum blood concentration; K = elimination rate constant; MRT = mean residence time; $T_{1/2}K$ = elimination half-life; T_{max} = time between drug administration and achievement of the maximum blood concentration; Vd/F = body distribution volume. Data are reported as means ± SD (N = 5-8). All parameters for young rats were statistically different from those for adult rats (P < 0.05, Student *t*-test). Vd/F was not statistically analyzed because the SD value for this parameter was not available from the computer program used.

amount of ^{99m}Tc-TsTX (6 µg) the young rats showed higher C_{max}, higher $AUC_{0-\infty}$ and an earlier T_{max}, indicating a faster and higher uptake of the toxin. The increased uptake of the toxin can also be explained on the basis of a higher absorption rate for the toxin in young rats, also explaining the lower value found for T_{max}. As expected, the Vd/F for the uncorrected data was higher in adult rats than in young rats.

When the blood concentration versus time data were corrected for body weight, Vd/F and CL/F were increased in young rats and decreased in adult rats, inverting the relationship observed for noncorrected data. However, these parameters are strongly influenced by body surface area (30). After correcting for body weight, the Vd/F of young animals was twice that of adult animals, indicating a possible increased distribution in young rats.

The pharmacokinetic profile of TsTX found in *sc* injected adult rats was similar to that reported with the use of crude venom (23). The present data indicate higher and faster absorption and distribution in young rats. In spite of the limitation of the experimental protocol due to the number of points at the end of the curve, it can be said that in young rats there is a slower elimination of the toxin. This statement is based on the fact that the observed K for adult rats, which is consistent with the literature (23), was higher than for young animals.

The results indicate that pharmacokinetics can explain in part the differences in toxicity observed between children and adults after scorpion envenomation. Due to these differences in disposition of the toxin between children and adults, pharmacological interventions in children after scorpion en-

venomation need to be further investigated. These data are important considering that in experimental models with adult animals the pharmacokinetic data for scorpion antivenom are different from those for scorpion venom (23,31,32). Antivenom absorption is lower, as also is its distribution from blood to tissues compared to venom. Since in young rats the disposition of the toxin is modified compared to adult rats, these alterations should be investigated in terms of treatment of scorpion envenomation, especially immunotherapy.

Acknowledgments

Table 2. Pharmacokinetic parameters determined after subcutaneous injection of 6 µg ^{99m}Tc-TsTX into adult (150-160 days old) and young (21-22 days old) male rats, with correction for body weight.

Parameters	Adult rats	Young rats
K (h^{-1})	0.26 ± 0.04	0.15 ± 0.04
$T_{1/2}K$ (h)	2.73 ± 0.42	5.08 ± 1.84
AUC_∞ (ng h^{-1} ml^{-1})	344.62 ± 32.11	182.27 ± 11.745
MRT (h)	4.39 ± 0.49	6.58 ± 1.58
C_{max} (ng kg^{-1} ml^{-1})	78.21 ± 11.23	40.43 ± 3.25
T_{max} (h)	2.47 ± 0.51	0.84 ± 0.23
CL/F (l h^{-1} kg^{-1})	0.02 ± 0.002	0.03 ± 0.002
Vd/F (l/kg)	0.067	0.210

For abbreviations, see legend to Table 1. Data are reported as means ± SD (N = 5-8). All parameters for young rats were statistically different from those for adult rats (P < 0.05, Student *t*-test). Vd/F was not statistically analyzed because the SD value for this parameter was not available from the computer program used.

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