Pharmacokinetics of a $\text{F(ab')}_2$ scorpion antivenom administered intramuscularly in healthy human volunteers

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Abstract

This paper presents the first study of $\text{F(ab')}_2$ scorpion antivenom pharmacokinetics in humans after intramuscular (im) administration. The specific anti-$\text{Centruroides}$ scorpion antivenom was used in 6 human healthy volunteers. The fabotherapeutic was administered as a 47.5 mg im bolus. Blood samples were drawn at 0, 5, 15, 30, 45, 60, 90, 120, and 180 min, 6 h and at 1, 2, 3, 4, 10 and 21 days after antivenom administration. We measured antivenom concentrations in serum using a specific high sensitivity ELISA method for $\text{F(ab')}_2$. Antivenom concentration in serum was fitted to a 3 compartment model (inoculation site, plasma and extravascular extracellular space), it was assumed that the venom may also be irreversibly removed from plasma. Calculated time course of antivenom content shows that at any time no more than 16.6 (5.3, 31.9)% (median and 95% confidence interval) of the antivenom bolus is present in plasma. The time to peak plasma $[\text{F(ab')}_2]$ was 45 (33, 74) h. The most significant antivenom pharmacokinetic parameters determined were: $\text{AUC}_{0\infty}=803 (605, 1463)$ mg · h · L$^{-1}$; $V_c=8.8 (2.8, 23.6)$ L; $V_{im}=55 (47, 64)$ L; $\text{MRT}_{im}=776(326, 1335)$ h; $CL_{im}=3.7 (0.6, 1.9)$ mL · min$^{-1}$; $f_{im}=0.300 (0.153, 0.466)$. Comparing these parameters with the ones obtained intravenously by Vázquez et al. [2], the parameters were more disperse between subjects, determined with more uncertainty in each individual subject, and the peak $\text{F(ab')}_2$ in plasma occurred with considerable delay; all indicating that the IM route should not be used to administer the antivenom, with the possible exception of cases occurring very far from hospitals, as an extreme means to provide some protection before the IV route becomes available.

1. Introduction

We will use the term fabothereapeutic, similar to the Spanish ‘faboterapético’ [1] in reference to the antivenom used, meaning an antitoxin made of $\text{F(ab')}$ or $\text{F(ab')}_2$. Fabothereapeutics are used as specific antidotes in envenomations from scorpion stings, the bites of spiders and different species of snakes. Fabothereapeutics have a lower mean distribution and elimination times, as well as a larger distribution volume than pure immunoglobulin preparations [2]. With recent $\text{F(ab')}_2$ purification techniques the risks of allergies, anaphylactic shock and serum sickness are reduced.

The preferred way to administer antivenoms is intravenous (IV). This preference derives from the large molecular weight of antivenoms which may hinder absorption, and from the need to quickly neutralize the venom’s effects; IV administration eliminates the restraint produced by absorption from other parenteral routes of administration. Still, a question often posed by laypersons, paramedics and clinicians is: What to do if the use of the IV route is not available due to the lack of skill, distance from hospitals or situations such as in infants? Several recent publications suggest that when the intramuscular (im) route is used, antivenoms are poorly effective [3–8]. There is information on $\text{F(ab')}_2$ antiscorpion pharmacokinetics in humans after a bolus of antivenom is given [9], but there is no information on antivenoms used in humans by any other route. We evaluated here the intramuscular $\text{F(ab')}_2$’s pharmacokinetics in healthy human volunteers.

2. Materials and methods

2.1. Characteristics of the antivenom

The specific anti-$\text{Centruroides}$ scorpions antivenom Alacramyn®/Anascorp© (A/A) (Alacramyn is its registered name in Mexico; Anascorp is its registered name in the USA) was used. The antivenom was made from hyper-immune horse plasma. Equine plasma was digested with

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doi:10.1016/j.intimp.2010.08.018
pepsin, and F(\text{ab}′)_2 fragments were separated by fractioned precipitation. Low molecular weight components were separated and discarded, and the F(\text{ab}′)_2 fragments were collected and concentrated on an Amicon membrane (retaining peptides of $>30$ kDa). One ampule of the product is able to neutralize 150 mouse LD_{50} (lethal dose 50%; i.e., the dose of venom which kills half of the mice receiving this dose) of *Centruroides limpidus limpidus* scorpion venom; 150 mouse LD_{50} are equivalent to 0.75 mg protein of *C. limpidus limpidus* venom. One ampule of A/A is the dose used clinically in 76.9% of patients, but up to 5 ampules may be used in severe cases (for further reference see [11]). One ampule A/A contains 47.5 mg of antivenom; the antivenom used was all from Lot. BOJ04, having the following composition (determined by triplicate, using FPLC on a Zorbx GF250 4.6 $\times$ 250 mm column): Dimeric (aggregated) F(ab′)_2, 1.9%; F(ab′)_2, 87.7%; F(ab′), 4.8%; low molecular weight compounds: 5.6% and was albumin-free. In this study all volunteers received 1 ampule (47.5 mg) of antivenom intramuscularly.

### 2.2. Serum collection and storage

The first day, before administration of the drug, the volunteers were put on a 0.9% NaCl drip and blood samples were obtained for routine hematology and blood chemistry. A sample of urine was obtained for urinalysis, prior to and 24 h after administering A/A.

Blood samples were drawn at 0, 5, 15, 30, 45, 60, 90, 120, and 180 min, 6 h and at 1, 2, 3, 4, 10 and 21 days. During each of the visits, the patients were questioned about the presence of signs and symptoms of type I or III immune reaction such as rash, itchy skin, fever or chills, joint pain, muscle pain, vomiting or diarrhea and difficulty in breathing.

Venous blood was drawn into vacuum tubes and allowed to stand for one hour at room temperature to clot. Then, serum was centrifuged and transferred to sterile glass tubes; tubes were closed with rubber caps, labelled and kept at $-18^\circ$C until used.

### 2.3. Polyclonal antibodies specific for horse F(\text{ab}′)_2 fragments

Three chickens were hyperimmunized with eight weekly injections of 100 $\mu$g whole horse IgG (Sigma) emulsified with Incomplete Freund Adjuvant. Whole IgYs were purified with EGGstract® IgY Purification System (Promega). Specific anti-horse F(ab′)_2 antibodies were purified by affinity chromatography on a Sepharose 4B CNBr-activated column to which 7 mg/mL of A/A was coupled. Crude IgYs in 50 mM Tris/HCl pH 8, were applied on to the column. The bound fraction was eluted with acetic acid 0.1 M and collected into tubes containing 500 $\mu$L Tris/HCl 1 M pH 8. In order to remove antibodies that cross-react with human or goat immunoglobulins, the affinity purified chicken antibodies were rendered specific bysuccessive absorption with human or goat IgGs coupled to Sepharose 4B.

### 2.4. Antivenom measurement in serum

A highly specific sandwich immunoassay was for measuring horse F(\text{ab}′)_2 fragments in human serum was used as described previously [9]. Yet, due to the low serum [F(\text{ab}′)_2] after an IM bolus, in most cases, the values from the low dilutions (1:2) were used to quantitate antivenom concentration in serum samples.

### 2.5. Statistical procedures

The data were processed using nonparametric statistical procedures. Data are presented as medians and their 95% confidence interval calculated with the procedure of Hodges and Lehmann. Statistical significance of differences was decided with Mann–Whitney (Wilcoxon) test. Linear regressions were carried out with the Theil procedure. Multiple comparisons were done with the nonparametric Kruskall–Wallis analysis of variance. The statistical analysis of ratios between random variables was done using nonparametric Moses statistics. See Hollander and Wolfe [12] for all details of nonparametric methods used. Differences between treatments were considered significant if the probability that the null hypothesis was true was $<0.05$ ($P<0.05$, two tails).

### 3. Results

#### 3.1. Selection of volunteers

Six healthy volunteers from the Scorpion Envenomation Center of the Mexican Red Cross, León Branch, Guanajuato, Mexico, were enrolled. Before joining the study, all the participants signed a valid informed consent form. The demographic characteristics of the volunteers are presented in Table 1.

#### 3.2. Determining pharmacokinetic constants for antivenom

In this work, we will express the amount of drug in each component of the pharmacokinetic models, as mass; the word mass is used here in as physics, meaning the quantity of matter as determined from its weight. Fig. 1 presents three fits between the model represented by Eq. (1) and the antivenom concentration of the volunteers’ serum; the plots were selected to show the variability between cases, as well as the good fit between data (--) and the model (solid lines). Table 2 presents the kinetic constants obtained when the compartmental analysis represented by Eq. (1) was fitted to data of the 6 volunteers as indicated in the Pharmacokinetics appendix. In addition to the experimental estimates of the kinetic constants defining Eq. (1), Table 2 presents the venom distribution volumes in plasma ($V_{pl}$) and in the extra vascular extracellular space ($V_{ev}$), the time to reach half of the maximum F(\text{ab}′)_2 plasma concentration ($t_{1/2}$), the half time to decay from the maximum F(\text{ab}′)_2 concentration in plasma ($t_{1/2}$), the clearance from plasma due to F(\text{ab}′)_2 elimination from the body ($CL_{t}$) and the clearance from plasma due to diffusion to the extravascular space ($CL_{d}$). Table 3 present a second set of pharmacokinetic parameters calculated without compartmental assumptions as described by Eqs. (10)–(22).

#### 3.3. Using the model to make inferences

The parameters determined by fitting Eq. (1) to experimental data enable us to predict the mass of F(\text{ab}′)_2 as function of time at the inoculation site (the muscle in our case) and in the extracellular extravascular space. Fig. 2A presents a prediction of the mass of antivenom (AV) in each experimental compartment using Eq. (2), here the model’s rate constants are the medians of the parameters in Table 2. Fig. 2B is a rescaled version of Fig. 2A. In the figure the amount of antivenom is expressed as percentage of the initial bolus which is present in the compartments as function of time. The solid line is AV at the injection site (muscle), dash dot lines (---) are AV in the plasma, the dashed line (---) is AV mass in tissues, and finely dashed

<table>
<thead>
<tr>
<th>Character</th>
<th>Subject</th>
<th>Median (95% CI)</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>21-22</td>
<td>23.5 (20.5, 27.5)</td>
<td>years</td>
</tr>
<tr>
<td>Gender</td>
<td>M</td>
<td>73.0 (62.0, 83.8)</td>
<td>kg</td>
</tr>
<tr>
<td>Weight</td>
<td>69.83</td>
<td>1.64 (1.57, 1.70)</td>
<td>mts</td>
</tr>
<tr>
<td>Height</td>
<td>1.61</td>
<td>26.75 (24.93, 29.49)</td>
<td>kg · mts⁻²</td>
</tr>
</tbody>
</table>

| BMI       | 26.62  | 32.18 | kg · mts⁻² |

BMI = body mass index; F = female; M = male; CI = confidence interval of the median.
observations. Rather than fractions of hours, Abscissa is time in minutes, data to Eq. (1). Although data in panels A and C have some resemblance, the concentration in panel C takes more time to reach a maximum, and then decays faster than in the other two subjects. Since the calculus with non fractional numbers is more accurate, the absissa was introduced in minutes (the first points are separated by a few minutes) rather than fractions of hours. Abscissa is time in minutes, ordinate is serum antivenom concentration (μg/mL), □ represents experimental observations.

**Table 2**

<table>
<thead>
<tr>
<th>Character</th>
<th>Median (95% CI)</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>( f_{\text{im}} )</td>
<td>8.8 (2.8, 23.6)</td>
<td>L</td>
</tr>
<tr>
<td>( f_{\text{im}}/BW )</td>
<td>0.127 (0.040, 0.342)</td>
<td>L · kg(^{-1})</td>
</tr>
<tr>
<td>( V_{\text{im}} )</td>
<td>31.8 (17.9, 46.7)</td>
<td>L</td>
</tr>
<tr>
<td>( V_{\text{im}}/BW )</td>
<td>0.465 (0.260, 0.606)</td>
<td>L · kg(^{-1})</td>
</tr>
<tr>
<td>( k_a )</td>
<td>7.52 (2.92, 15.6)</td>
<td>10(^{-4}) min(^{-1})</td>
</tr>
<tr>
<td>( k_{\text{ol}} )</td>
<td>4.53 (2.57, 373.2)</td>
<td>10(^{-4}) min(^{-1})</td>
</tr>
<tr>
<td>( k_{\text{eol}} )</td>
<td>1.62 (0.30, 58.1)</td>
<td>10(^{-2}) min(^{-1})</td>
</tr>
<tr>
<td>( k_{\text{ep}} )</td>
<td>3.84 (1.05, 8.44)</td>
<td>10(^{-3}) min(^{-1})</td>
</tr>
<tr>
<td>( \lambda_1 )</td>
<td>1.77 (0.60, 59.6)</td>
<td>10(^{-2}) min(^{-1})</td>
</tr>
<tr>
<td>( \lambda_2 )</td>
<td>6.06 (4.17, 49.7)</td>
<td>10(^{-5}) min(^{-1})</td>
</tr>
<tr>
<td>( \tau_1 )</td>
<td>3.19 (0.66, 6.50)</td>
<td>h</td>
</tr>
<tr>
<td>( \tau_2 )</td>
<td>317.7 (167.8, 427.5)</td>
<td>h</td>
</tr>
<tr>
<td>( \tau_1/2 )</td>
<td>2.21 (0.46, 4.51)</td>
<td>h</td>
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<tr>
<td>( \tau_2/2 )</td>
<td>220.3 (116.3, 296.3)</td>
<td>h</td>
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<tr>
<td>( C_L )</td>
<td>3.7 (0.6, 1.9)</td>
<td>mL · min(^{-1})</td>
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<tr>
<td>( C_L/BW )</td>
<td>46.9 (25.1, 84.6)</td>
<td>L · min(^{-1}) · kg(^{-1})</td>
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<tr>
<td>( C_L_{\text{im}} )</td>
<td>89.8 (38.3, 155.8)</td>
<td>L · min(^{-1})</td>
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<tr>
<td>( C_L_{\text{im}}/BW )</td>
<td>130.1 (55.5, 225.7)</td>
<td>L · min(^{-1}) · kg(^{-1})</td>
</tr>
</tbody>
</table>

**Table 3**

<table>
<thead>
<tr>
<th>Character</th>
<th>Median (95% CI)</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \theta_{\text{max}} )</td>
<td>45 (33.7, 74)</td>
<td>h</td>
</tr>
<tr>
<td>( \theta_1 )</td>
<td>3.8 (1.8, 7.1)</td>
<td>h</td>
</tr>
<tr>
<td>( \theta_2 )</td>
<td>329 (250, 438)</td>
<td>h</td>
</tr>
<tr>
<td>( C_{\text{max}} )</td>
<td>0.79 (0.61, 1.02)</td>
<td>ng · L(^{-1})</td>
</tr>
<tr>
<td>( M_{\text{max}} )</td>
<td>7.9 (2.5, 15.2)</td>
<td>mg</td>
</tr>
<tr>
<td>100 · ( M_{\text{max}}/D )</td>
<td>16.8 (3.3, 31.9)</td>
<td>%</td>
</tr>
<tr>
<td>( AUC_{\text{cr}} )</td>
<td>803 (305, 1463)</td>
<td>mg · h · L(^{-1})</td>
</tr>
<tr>
<td>( AUMC_{\text{cr}} )</td>
<td>1.13(0.102, 2.23) · 10(^5)</td>
<td>mg · h(^2) · L(^{-1})</td>
</tr>
<tr>
<td>( MRT_{\text{cr}} )</td>
<td>776 (126, 1335)</td>
<td>h</td>
</tr>
<tr>
<td>( MRT_{\text{cr}} )</td>
<td>279 (42, 1166)</td>
<td>h</td>
</tr>
<tr>
<td>( V_{\text{cr}, \text{im}} )</td>
<td>54.9 (47.1, 64.2)</td>
<td>L</td>
</tr>
<tr>
<td>( V_{\text{cr}, \text{im}}/BW )</td>
<td>0.80 (0.68, 0.93)</td>
<td>L · kg(^{-1})</td>
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<tr>
<td>( V_{\text{cr}, \text{im}} )</td>
<td>51.3 (43.3, 63.6)</td>
<td>L</td>
</tr>
<tr>
<td>( V_{\text{cr}, \text{im}}/BW )</td>
<td>0.74 (0.6, 0.9)</td>
<td>L · kg(^{-1})</td>
</tr>
<tr>
<td>( C_{\text{cr}, \text{im}} )</td>
<td>1.43 (0.67, 3.61)</td>
<td>mL · min(^{-1})</td>
</tr>
<tr>
<td>( C_{\text{cr}, \text{im}}/BW )</td>
<td>29.1 (15.7, 56.8)</td>
<td>L · min(^{-1}) · kg(^{-1})</td>
</tr>
<tr>
<td>100 · ( f_{\text{m,v}} )</td>
<td>30.0 (15.3, 46.5)</td>
<td>%</td>
</tr>
</tbody>
</table>

**Fig. 1.** Examples of plots of measured antivenom plasma concentration vs time in 3 healthy human volunteers, and model prediction for the data. The figures were plotted directly by Adapt II the PK environment used to build and solve the model. The first points are reached AV mass in tissues is relatively high (60.1% of the bolus), but this occurs after 53 h.

**lines (---) are eliminated AV. In agreement with the experimental observations, the maximum mass of AV in plasma predicted by the model, equivalent to 14.3% of the bolus, occurs after 50 h. The maximum AV mass in tissues is relatively high (60.1% of the bolus), but this occurs after 53 h.**
The pharmacology of antivenoms is determined by two facts. First, the affinity and avidity of a good antivenom for its target are so high that the drug–receptor interaction in this case can be seen as irreversible. This is of course not exactly true, but it is a good approximation since the dissociation rate constant of the venom–antivenom complex is much smaller than the opposite process; once the venom–antivenom complex is formed it remains until it is eliminated from the body. Second, antivenom therapy is aimed at eliminating venom which is administered by a poisonous creature in very variable (and usually unknown to the clinician) amount which is independent of any patient characteristic such as age or body mass. The best guess on the amount of venom to neutralize is, commonly, based only on the severity of symptoms: the severity of the envenomation. In such instance there is no effective concentration but an effective dose, meaning the amount of antivenom needed to neutralize all the venom as fast and as completely as possible, since venom’s effects are, or quickly become, irreversible. The effective dose is largely based on clinical experience, sometimes assisted by paraclinical tests. Unlike most drugs, the dose of antivenom does not depend on any patient characteristic, but mostly in the amount of venom to be neutralized. The dosing is thus the same for all patients and does not depend on weight or body surface area. The priority is speed: early treatment and fast diffusion to any body compartment that may contain venom.

The need for quick venom neutralization is a must in cases of Centruroides scorpion poisoning, which is able to kill an infant in as little as 30 min and is life threatening to adults in a few hours [16,17] or Tityus and other scorpions where fatal complications such as respiratory distress syndrome may occur after a few hours of untreated poisoning [18–20]. Intravenous injection of an AV bolus is able to produce a maximum plasma concentration in <5 min, or a couple of blood circulation times [21]; this is close to the resolution of the method, since sampling at <2 min intervals is difficult. In humans, after an IV bolus injection of $F(ab')_2$ the plasma concentration decays in a triexponential fashion, with a first component which decays to one half in 0.25 (0.13, 0.37) h, and the variability of all the parameters is low [9]. Similar results were obtained in rams [22] and mice [23].

In spite of anecdotal references to successful treatment of poisoning using antivenoms via IM injection, there is a wealth of clinical evidence suggesting that this route is unreliable [3–8]. Still, there were no quantitative pharmacokinetic data on IM $F(ab')_2$. Here we provide two data sets, one derived from an implicitly compartmental model represented by Eq. (1), from which it is possible to obtain the parameters defined by Eqs. (3)–(9); and another data set, where no assumptions are made on the model, and the parameters are obtained by numerically integrating the concentration vs time curve [Eqs. (10)–(22)].

Although parameters obtained from the two approaches may not have exactly the same meaning, some are roughly equivalent; this is the case for example of $V_s$, $V_a$ and $V_{av} + V_s$ or $C_{LD}$ and $C_{tim}$, which were found to be quite similar considering method uncertainties and different meanings of $V_s$ and $V_{av}$ (for example see [(23), Eq. A.10]). The similar values mean that our pharmacokinetic parameter estimates are largely model independent. Table 3 presents a second set of pharmacokinetic parameters calculated without compartmental assumptions as described by Eqs. (10)–(22). Several of these parameters deserve particular attention. One is $t_{max}$ the time it takes to reach $C_{max}$ [45 (33, 74) h]; after an IV bolus the plasma antivenom concentration reaches a maximum in <5 min [9,22], thus $t_{max}$ says that it takes at least 540 (396, 888) times longer after an IM bolus to reach $C_{max}$ Also important is the mean time a molecule of a drug stays in the body (MRT). For an IM bolus $MRT_{im}$ is 776 (326, 1335) h, 2.2 (1.2, 6.1) times 250 (219, 310) h the MRT of an IV bolus [19, Table 3]. This prolonged $MRT_{im}$ is not necessarily an indication of prolonged protection by the IM AV. Since, as it may be calculated with Eq. (18) and the data on $F(ab')_2$ IV pharmacokinetics [9], the mean absorption time of $F(ab')_2$ from muscle ($MAT_{im}$) is 279 (42, 1166) h [Eq. (18)]; thus the AV stays a long time where its protective effect is of little use: at the injection site. Particularly important is that $C_{max}$, the maximum plasma antivenom concentration, of 0.79 (0.61, 1.02) mg · L$^{-1}$ occurs when there is $M_{max}$ of $F(ab')_2$ in blood [the maximum mass of antivenom in blood, 7.9 (2.5, 15.2) mg]; given that the IM bolus (D) was 47.5 mg in all cases, this means that when the IM route is used to administer $F(ab')_2$, there is never more than 16.6 (5.3, 32.0)% of D in blood.

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**Fig. 2.** Time course of antivenom mass distribution in healthy human volunteers. Panel A: Time courses predicted with Eq. (2) with the median kinetic constants presented in Table 2 assuming that 100 μg of antivenom was administered at time = 0. The solid line is antivenom at the injection site (muscle), dash dot lines (-----) are antivenom in the plasma, the dashed line (——) is antivenom mass in tissues, and finely dashed lines (-----) are eliminated antivenom. Panel B: Same as panel A but rescaled to show the events occurring in the first 10 min. Abscissa is the amount of venom in each compartment expressed as percentage of the bolus injected at time = 0. Other details are in the text of the communication.

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4. Discussion

For over a century [13–15], aside from some efforts based on ethnopharmacology, the pharmacology of antivenoms has been little more than the pharmacology of antibodies. Central to the pharmacology of antivenoms is the fact that, unlike most drugs, antivenoms are not aimed at a patient’s tissue receptor, neither against a receptor in an infecting organism, nor against a receptor in some malignant invading neoplastic cell. In all these instances, the drug effect is in an infecting organism, nor against a receptor in some malignant invading neoplastic cell. In all these instances, the drug effect is...
When comparing pharmacokinetic IM and IV parameters [9], the differences are large, with only two exceptions. The first exception is the equivalent time constants \( t_1 \) \([9], 232.7 (203.4, 305.8) \) h, Table 2) and \( t_2 = 317.7 (162, 427.5) \) h, in Table 2 in this communication. The second exception is that the two estimates of total clearance from the body after an IM bolus, \( C_{\text{LIM}} \) (Table 2) and \( C_{\text{LIV}} \) (Table 3) are not only statistically not different, but that they are not different from \( CL \) (the IV systemic clearance) in Table 3 of [9]. The similar values of this constants suggests that the catabolic processes in the elimination phase are similar in humans who receive \( F(ab')_2 \) either IM or IV, and that variable IM absorption explains the poor performance of the IM route.

\( F(ab')_2 \) applied IM has a long mean absorption time (Median MAT = 279 h), reaches a maximum concentration in plasma slowly and very variably [time to maximum plasma concentration, 45 (33, 74) h] and has a low and scattered bioavailability [30.0 (15.3, 46.6)%]. Venom pharmacokinetics in humans is a vast unknown; Tityus scorpion venom pharmacokinetics is perhaps the best known of any venom and the evidence was obtained in rams [22]. Tityus venom mass after an s.q. bolus decays exponentially, and after 5 h ≈ 5% of the venom still remains at the site of injection; the concentration in plasma reaches a maximum in ≈ 2 h and then decays. After 5 h the venom is difficult to detect in plasma, but then > 85% of Tityus venom is in the tissues, precisely where it hurts most [22]. When an IM bolus is given, the rate of antivenom concentration change with time in all compartments is very slow (Fig. 2B), and ≈ 15% of the antivenom has reached the tissue compartment after 5 h; these data means that ≈ 7 times more AV is needed to neutralize the venom within the first 5 h of envenoming when the IM instead of the IV route is used. In practice, this would take several IM injections, since 7 ampules of AV amount (approximate) would be ordered by volume. In practice, this large dosing will not be a solution for fast acting venoms such as most spider, scorpion or snake venoms, the IM route could be useful when dealing with venom and the evidence was obtained in rams [22].

When comparing pharmacokinetic IM and IV parameters [9], the second exception is that the two estimates of total clearance from the body after an IM bolus, \( C_{\text{LIM}} \) (Table 2) and \( C_{\text{LIV}} \) (Table 3) are not only statistically not different, but that they are not different from \( CL \) (the IV systemic clearance) in Table 3 of [9]. The similar values of this constants suggests that the catabolic processes in the elimination phase are similar in humans who receive \( F(ab')_2 \) either IM or IV, and that variable IM absorption explains the poor performance of the IM route.

\( F(ab')_2 \) applied IM has a long mean absorption time (Median MAT = 279 h), reaches a maximum concentration in plasma slowly and very variably [time to maximum plasma concentration, 45 (33, 74) h] and has a low and scattered bioavailability [30.0 (15.3, 46.6)%]. Venom pharmacokinetics in humans is a vast unknown; Tityus scorpion venom pharmacokinetics is perhaps the best known of any venom and the evidence was obtained in rams [22]. Tityus venom mass after an s.q. bolus decays exponentially, and after 5 h ≈ 5% of the venom still remains at the site of injection; the concentration in plasma reaches a maximum in ≈ 2 h and then decays. After 5 h the venom is difficult to detect in plasma, but then > 85% of Tityus venom is in the tissues, precisely where it hurts most [22]. When an IM bolus is given, the rate of antivenom concentration change with time in all compartments is very slow (Fig. 2B), and ≈ 15% of the antivenom has reached the tissue compartment after 5 h; these data means that ≈ 7 times more AV is needed to neutralize the venom within the first 5 h of envenoming when the IM instead of the IV route is used. In practice, this would take several IM injections, since 7 ampules of AV amount (approximate) would be ordered by volume. In practice, this large dosing will not be a solution for fast acting venoms such as most spider, scorpion or snake venoms, the IM route could be useful when dealing with venom and the evidence was obtained in rams [22].

The pharmacokinetics of an immunoglobulin or its fractions is not determined by its antigen recognition epitopes. Our work is therefore interested in these situations given the depot nature of IM immunotherapy shown here.

5. Pharmacokinetic appendix

5.1. Compartmental pharmacokinetic model

The following simultaneous differential equations were adjusted to data of \( F(ab')_2 \) concentrations in serum vs time in the volunteers. They express rates of change of drug amount in muscle, plasma and extravascular space, as derivatives with respect to time assuming first order diffusion kinetics. The equations were programmed in Fortran 95 and fit to data using the ADAPT II Pharmacokinetic/Pharmacodynamic Analysis Software [25] to obtain empirical values of the kinetic constants:

\[
\begin{align*}
\frac{dM_{\text{MOC}}}{dt} &= -k_a \cdot M_{\text{MOC}} \\
\frac{dM_p}{dt} &= k_a \cdot M_{\text{MOC}} - (k_{\text{pe}} + k_{\text{el}}) \cdot C_p \cdot V_p \\
\frac{dM_e}{dt} &= k_{\text{pe}} \cdot M_p - k_{\text{ep}} \cdot M_{\text{ev}}
\end{align*}
\]

The symbols are: \( k_a \), rate of diffusion of antivenom from the injection site to plasma; \( k_{\text{pe}} \), rate of elimination of antivenom from plasma; \( k_{\text{ep}} \), diffusion rate constant of antivenom from the extravascular compartment to plasma; \( M_{\text{MOC}} \), mass of antivenom in the inoculation site; \( M_p \), mass of antivenom in plasma; \( M_{\text{ev}} \), mass of antivenom in the extravascular space; \( C_p \), concentration of venom in plasma; \( V_p \), distribution volume of venom in plasma. In this work, we will express the amount of drug in each component of the pharmacokinetic models, as mass; the word mass is used here as in physics, meaning the quantity of matter as determined from its weight.

In equation set (1) we have that \( M_p = V_p \cdot C_p \); it is trivial to transform this set into the following system in terms of masses only:

\[
\begin{align*}
\frac{dM_{\text{MOC}}}{dt} &= -k_a \cdot M_{\text{MOC}} \\
\frac{dM_p}{dt} &= k_a \cdot M_{\text{MOC}} - (k_{\text{pe}} + k_{\text{el}}) \cdot M_p \\
\frac{dM_e}{dt} &= k_{\text{pe}} \cdot M_p - k_{\text{ep}} \cdot M_{\text{ev}}
\end{align*}
\]

This is useful to use the model parameters to predict the amounts of antivenom in the inoculation site (muscle), in plasma and in the extravascular extravascular compartment as function of time. These masses are otherwise not directly available by empirical means.

The Adapt II environment was chosen because it is a time-proved freeware (for research) package which simplifies writing the model equations as Fortran templates, and saves a lot of programming time. The package implements the simplex algorithm to fit model to data [26], the LSODA method to solve general differential equations [27,28] and a matricial exponential method to solve linear differential equations [29]. The package also permits plotting the model to data [26], fit model to data [25], as follows [25]. The total clearance

\[
CL_p = k_a \cdot V_p.
\]

the clearance from plasma due to diffusion into tissues

\[
CL_d = k_{\text{pe}} \cdot V_p.
\]

the distribution volume of the extra vascular space

\[
V_{\text{ev}} = \frac{V_p \cdot k_{\text{pe}}}{k_{\text{ep}}},
\]

5.2. Determining pharmacokinetic parameters from the compartmental model

Some PK parameters were directly calculated using the values of the kinetic constants produced by the Adapt II program used to solve Eq. (1), as follows [25]. The total clearance

\[
CL_p = k_a \cdot V_p.
\]

the clearance from plasma due to diffusion into tissues

\[
CL_d = k_{\text{pe}} \cdot V_p.
\]

the distribution volume of the extra vascular space

\[
V_{\text{ev}} = \frac{V_p \cdot k_{\text{pe}}}{k_{\text{ep}}},
\]
the rate constants
\[
\lambda_1 = \frac{\left(k_{el} + k_{pe} + k_{ep}\right) + \sqrt{\left(k_{el} + k_{pe} + k_{ep}\right)^2 - 4k_{el}k_{ep}}}{2}
\]
(6)
\[
\lambda_2 = \frac{\left(k_{el} + k_{pe} + k_{ep}\right) - \sqrt{\left(k_{el} + k_{pe} + k_{ep}\right)^2 - 4k_{el}k_{ep}}}{2}
\]
(7)
of rise and decay, respectively, and the time constants \((\tau_1)\) and half times \((t_{1/2})\):
\[
t_{1/2} = \ln(2) \cdot \left(\lambda_1^{-1}\right)
\]
(8)
\[
t_{1/2} = \ln(2) \cdot \left(\lambda_2^{-1}\right)
\]
(9)
of rise and decay, respectively.

5.3. Non-compartmental pharmacokinetic modeling

The model represented by Eq. (2) proved to be very efficient (see Results) to describe the time course of \(\left[F(ab')_2\right]\) in volunteers' blood serum, so, independently of the mechanistic value of the model, it may be used as a curve fitting procedure to estimate PK parameters such as the area under the curve (AUC), the area under the first moment curve (AUMC), and related parameters, with precision advantages over the simple integration of the native data using the trapezoidal rule. We solved numerically Eq. (1) using the Euler method \[30\] in \(\Delta = 36\) ms steps, programmed in C++ using the g++ v.4.3 GNU (www.gnu.org) compiler under Ubuntu Linux v8.10. Eq. (1) was used to calculate \(M_p\) from 0 to 504 h, to determine its numeric integral
\[
I_1 = \sum_{i=0}^{t_i} \frac{C_i \cdot \Delta t_i}{2} + C_i((i + 1)\cdot \Delta t_i - \Delta t_i),
\]
(10)
to estimate the time \((t_{\text{max}})\) when the concentrations of antivenom in plasma \(C_p\) reached a maximum, as well as the \(\left[F(ab')_2\right]\) in serum \(C_s\) at \(t_{\text{max}}\). Here \(C_1 = C_p\) and \(C_i((i + 1)\cdot \Delta t_i - \Delta t_i)\) are the values of \(C_p\) at times \(t\) and \(t + \Delta t\), respectively. Since we found (see Results) that after 2 days the \(\ln[F(ab')_2]\) vs time curve is linear, we fitted the following equation using the nonparametric Theil semilog regression procedure (with \(t \geq 504\) h):
\[
\ln C(t) = \ln A - \frac{t}{\tau},
\]
(11)

This equation is the logarithmic transform of
\[
C(t) = A \cdot e^{-t/\tau},
\]
which integrates
\[
I_2 = \int_{t_i}^{\infty} C(t)dt = A \cdot \tau \cdot e^{-t/\tau},
\]
(13)

\(\text{AUC}_{\text{im,\kappa}}\) for IM antivenom is
\[
\text{AUC}_{\text{im,\kappa}} = I_1 + I_2.
\]
(14)
Since in general \[31\],
\[
\text{AUMC}(t_i - t_{i-1}) = \frac{t_i - t_{i-1}}{6} \cdot \left(C_i(t_i) + 2C(t_{i-1}) + C(t_{i+1})\right)
\]
(15)
if \(\Delta_t = t_{i+1} - t_i\) is constant, it is possible to prove that
\[
\text{AUMC}_{\text{im,\kappa}} = \left(\frac{\Delta_t^2}{6}\right) \sum_{i=0}^{t_i} \left\{3C_i \cdot \Delta t_i + (3i + 1)C((i + 1)\Delta t_i)\right\}
\]
\[
+ \tau(T + \tau)C(0)e^{-T/\tau}.
\]
(16)
From these definitions it is easy to obtain other PK parameters such as the mean residence time, which in general for any non IM \((i/v)\) dosage is
\[
\text{MRT}_{i/v} = \frac{\text{AUMC}_{\text{im,\kappa}}}{\text{AUC}_{\text{i/v}}}.
\]
(17)
and is related to the IV MRT as
\[
\text{MRT}_{i/v} = \text{MRT}_{i/v} + \text{MAT}_{i/v}
\]
(18)
where \(\text{MAT}\) stands for mean absorption time. The apparent volume of distribution at equilibrium after an IM bolus \(\left(D_{i/v}\right)\) is
\[
\frac{V_{i/v}}{D_{i/v}} = \frac{A \cdot \text{AUC}_{\text{im,\kappa}}}{A \cdot \text{AUC}_{\text{i/v}}} = \frac{I_1 + I_2}{I_1 + I_2},
\]
(19)
and after a \(i/v\) bolus is
\[
\frac{V_{i/v}}{D_{i/v}} = \frac{C_{p(v)} - C_{p(i)}}{C_{p(i)}},
\]
(20)
where \(f_{im}\) is the IM bioavailability, which thus may be experimentally determined and is
\[
\bar{f}_{i/v} = \frac{V_{i/v}}{V_{i/v}}.
\]
(21)
Finally, the apparent total clearance of drug in plasma after \(i/v\) administration is:
\[
\text{CL}_{i/v} = \frac{C}{f_{i/v}} = \frac{D_{i/v}}{V_{i/v}},
\]
(22)
All IM PK parameters required for the evaluation of the kinetic variables described by Eqs. (2)–(20), were obtained from the archives of our previous work with human volunteers receiving the antivenom IM \[9\].

Acknowledgements

The technical assistance of Lic. Felipe Olvera is gratefully acknowledged.

References


