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Pharmacokinetics of a F(ab′)$_2$ scorpion antivenom in healthy human volunteers

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Abstract

This paper presents the first study of F(ab′)$_2$ scorpion antivenom pharmacokinetics in humans. We have studied the pharmacokinetics of an antiscorpion venom preparation (Alacramyn™) in eight human healthy volunteers. The fabotherapic was administered as a 47.5 mg i.v. bolus. Blood samples were drawn at 0, 5, 15, 30, 45, 60, 90, 120, 180 and 360 min after antivenom administration. Subsequently, the volunteers made seven visits to the hospital. Four of them at 24 h intervals, one at day 10, and one at day 21. We measured antivenom plasmatic concentrations using a specific high sensitivity ELISA method for F(ab′)$_2$. The time course of F(ab′)$_2$ in serum of seven subjects was well described by a lineal combination of three exponential components; a four exponential component model was necessary to fit the eighth subject. The most significant antivenom pharmacokinetic parameters determined were: $AUC_\infty = 596.9$ (369.3, 891.2) mg h l^{-1}; $V_c = 3.1$ (2.3, 4.3) l; $V_{ss} = 15.4$ (12.8, 39.9) l; MRT = 250.0 (218.8, 310.2) h; CL = 96.6 (58.0, 139.2) ml h^{-1}; $t_{1/2,\tau_1}$ (also called $t_{1/2,\alpha}$) = 0.25 (0.13, 0.37) h; $t_{1/2,\tau_z}$ (corresponding to the slowest component) = 161.3 (141.0, 212.0) h.

Keywords: Scorpion; F(ab′)2 antivenom; Pharmacokinetics

1. Introduction

Scorpion envenoming in Mexico is a serious health problem (Dehesa-Davila and Possani, 1994) with an incidence of 213,458 cases in 2004 (Anonymous, 2005). The effectiveness of antivenom treatment depends on the potency of the antivenom, its activity spectrum, the time that elapses from the envenomation to the treatment onset, as well as the antivenom pharmacokinetics (PK). The ideal antivenom must adequately reach the different tissues in which venom produces its toxic effect and, once bound to toxin, the complex must be rapidly eliminated (Ismail and Abd-Elsalam, 1998; Boyer et al., 1999; Seifert and Boyer, 2001). Another important aspect is the difference in safety of antivenoms constituted by whole IgG, on one hand, and, on the other hand, F(ab′)$_2$ and F(ab) antivenoms. Adverse events associated with the use of antivenoms are related to their purity and constitution (Theakston et al., 2003).

Fabotherapics are used as a specific antidotes in envenomation from scorpion stings, the bites of spiders and different species of snakes. Fabotherapics have a lower

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mean distribution and elimination times, as well as a larger distribution volume than pure immunoglobulin preparations. With recent $F(ab')_2$ purification techniques the risks of allergies, anaphylactic shock or serum sickness are reduced.

There is no information on $F(ab')_2$ antiscorpion pharmacokinetics in humans. This, in spite of a wealth of information on snake and scorpion antivenin PK in laboratory animals (Ismail et al., 1980, 1983, 1996; Audebert et al., 1994; Pépin et al., 1995; Pepin-Covata et al, 1996a,b; Santana et al., 1996; Rivière et al., 1997; 1998; Ismail and Abd-Elsalam, 1998; Calderón-Aranda et al., 1999; Krifi et al., 2001; Sevcik et al., 2004). Yet, information exists in humans $F(ab')_2$ on snake antivenom PK (Than et al., 1985; Ho et al., 1990; Mayer et al., 1997; Bazin-Redureau et al., 1998; Ariaratnam et al., 1999; Seifert and Boyer, 2001). Here, we studied the PK of Alacramyn™ (Bioclón, México) an anti-*Centruroide*s scorpion venom fabotherapic, in healthy human volunteers in absence of envenoming.

2. Methods

Eight clinically healthy volunteers from the Antiscorpion Center of the Mexican Red Cross, León, branch, Guanajuato, Mexico, were enrolled. The administration route was intravenous. Before joining the study, all the participants signed a valid informed consent form. The specific scorpion antivenom Alacramyn™ was used. One ampoule of the product is able to neutralize 150 mice lethal doses 50% equivalent to 0.75 mg protein of *Centruroides limpidus limpidus* scorpion venom. One ampule of Alacramyn™ is the dose used clinically in 76.9% of patients (Oasnaya-Romero et al., 2001). The Alacramyn™ used was all from Lot. BOJ04, and had the following composition determined by triplicate using FPLC on a Zorbax GF250 column (4.6×250 mm): Dimeric (aggregated) $F(ab')_2$, 1.9%; $F(ab')_2$, 87.7%; $F(ab')$, 4.8%; low molecular weight compounds: 5.6%.

2.1. Obtaining samples

The first day, before applying the drug, the volunteers were put on a 0.9% NaCl drip and blood samples were obtained for haematic biometry (BH) and blood chemistry (BC). A sample of urine was obtained for general urine tests (GUT), at baseline and 24 h after the administration of Alacramyn™.

Initially, blood samples were drawn at 0, 5, 15, 30, 45, 60, 90, 120, 180 and 360 min after administering the antivenom. Subsequently, the volunteers made seven visits to the hospital. Four of them at 24 h (±3 h) intervals, one at day 10 (±3 h), and one at day 21 (±3 h) to undergo the same first day procedure. During each of the visits, the patients were questioned about the presence of signs and symptoms such as rash, itchy skin, fever or chills, joint pain, muscle pain, vomiting or diarrhea and difficulty in breathing; in the event of any reaction the patient would have been treated by a physician.

2.2. Serum obtention and storage.

Venous blood from volunteers was drawn into vacuum tubes (Vacutainer Serum Tube, Becton Dickinson, Rutherford, NJ, USA) and allowed to stand for 1 h 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 °C until used.

2.3. Polyclonal antibodies specific for horse $F(ab')_2$ fragments.

Three hens were hyperimmunized with eight weekly injections of 100 μg 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 Alacramyn™ 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 μl Tris/HCl 1 M pH 8. In order to remove antibodies that cross-reacted with human or goat immunoglobulins, the affinity purified chicken antibodies were rendered specific by successive absorption with human or goat IgGs coupled to Sepharose 4B.

2.4. Antivenom measurement in serum.

A highly specific sandwich immunoassay was developed for measuring horse $F(ab')_2$ fragments in human serum. The immunoassay uses the specific chicken antibodies prepared as above as the capture antibody, and a commercial conjugate to detect Alacramyn™. Polystyrene micro plates (Maxisorp, Nunc, Inc., USA) were coated overnight at 4 °C with 100 μl/well of 5 μg/ml immunopurified chicken IgY anti-Alacramyn™ in 100 mM carbonate/bicarbonate buffer, pH 9.5. The plates were then washed four times with washing buffer (50 mM Tris/HCl pH 8, 150 mM NaCl and 0.05% Tween 20). The remaining binding sites were blocked with Tris/HCl pH 8, containing 0.5% gelatin and 0.05% Tween 20 for at least 2 h at 37 °C. The plates were then washed four times with washing buffer. Alacramyn™ standards were prepared in 10% of a pool human sera from healthy donors in vehicle buffer (50 mM Tris/HCl pH 8, 500 mM NaCl and 0.1% gelatin, 0.05% Tween 20); serum samples were also diluted in the same buffer. Samples and antivenom standards (100 μl/well) were added to the plates and incubated 1 h at 37 °C. Plates were washed four times with washing buffer and then 100 μl/well of affinity purified

goat anti-horse F(ab′)$_2$ conjugated to peroxidase (Rockland, Gilbertsville, PA) were added and incubated 1 h at 37 °C. After washing, 100 μl/well ABTS solution (Roche) were added and incubated 15 min at 25 °C; after this time the reaction was stopped with 25 μl/well of 20% sodium dodecyl sulfate. Absorbances were read at 405 nm in a Microplate Reader Model 550 (BIO-RAD). To measure the concentration of antivenom, serum samples were diluted 1:10 and 1:50 and incubated by triplicate. Alacramyn™ calibration curves were run in triplicate on each plate using a range of antivenom concentrations from 1.5 to 30,000 ng/ml, as determined by a Coomassie Protein Assay Kit (Pierce). Sigmoidal curves were generated using a non-linear regression program (Prism, GraphPad). The useful range was found to be between 15 and 1100 ng/ml. High sample dilutions (1:50) were used to quantitate antivenom concentration in samples obtained within 6 h after antivenom administration whereas a lower dilution (1:10) was used for samples collected after 24 h.

2.5. Pharmacokinetic analysis

The serum concentrations of Alacramyn™ versus time were adjusted to a z exponential equation such as

$$C(t) = \sum_{i=1}^{z} C_i \, \mathrm{e}^{-t/\tau_i} \tag{1}$$

using a simplex algorithm (Nelder and Mead, 1965) to minimise the absolute value of the differences between observed and predicted values. Here, C_i is the drug concentration in component i at $t=0$ and τ_i is the time constant of decay. Since there is no uniformly accepted way to write symbols for multi-exponential curves we decided to follow the notation of Blode et al. described in http://www.agah-web.de/PK-glossary.pdf. A constrain is used to force all the predicted C_i and τ_i values to assume positive values. The algorithm minimises the absolute value of the deviations between observations and the predicted values, such as the expression

$$\varepsilon_m = \sum_{k=1}^{n} |y_k - C(t_k)|$$

reaches a minimum (n is the number of data points and y_k is the observed or experimental value at t_k). The values of ε for the eight fits were 8.04922 (3.87568, 12.514) ranging from 2.73426 to 20.7383; the best and worst cases were JTM and RLS, respectively. The iterations of the simplex algorithm stop at the $m+1$ iteration (one count when each unknown parameter was modified) if

$$\frac{|\varepsilon_{m+1} - \varepsilon_m|}{\varepsilon_m} \leq 10^{-5}$$

For the eight fittings, m was 15088 (10412, 20442). The algorithm was coded in C++ and is available through the Internet (ftp://toxico.ivic.ve/pub/programs/3exp.zip) may be compiled using the GCC C++ compiler (freely available from www.gcc.org). For reasons of convenience, the author compiles it with the C++BuilderX development environment available for both Linux and MS Windows from www.borland.com. The modification of the source code provided to fit a number of exponentials $\neq 3$ is straightforward and only implies modifications of the 3exp.cpp program. The simplex algorithm demands that a set of initial values of the searched parameters is provided but, if the initial parameters are too far away from the parameters at the minimum the minimisation may fail. The algorithm also needs an initial multiplier to modify the parameter values, 0.25 is good. Still the requirements of the initial values when dealing with exponentials are usually very lax, and can readily be guessed by the experimenter. The initial values used here were $\tau_1=0.25$, $\tau_2=4$, $\tau_3=200$, $C_1=10$, $C_2=4$ and $C_3=1$. The half lives were calculated from the time constants as $t_{1/2,\tau_i} = \tau_i \ln(2)$. From Eq. (1) is evident that the initial drug concentration in serum is

$$C_{p_0} = \lim_{t\to 0} C(t) = \sum_{i=1}^{z} C_i \tag{2}$$

and the area under the curve at t$=\infty$ is

$$\mathrm{AUC}_\infty = \int_0^\infty C(t)\mathrm{d}t = \sum_{i=1}^{z} [\tau_i C_i] \tag{3}$$

The initial plasmatic distribution volume (also called central compartment volume) may be estimated as

$$V_\mathrm{C} = \frac{D}{\sum_{i=1}^{z} C_i}. \tag{4}$$

The apparent volume of distribution during the slowest component was calculated as

$$V_z = \frac{D\tau_z}{\mathrm{AUC}_\infty} \tag{5}$$

The systemic clearance of the drug is

$$\mathrm{CL} = \frac{D}{\mathrm{AUC}_\infty} \tag{6}$$

Another parameter of interest is the area under the mean curve

$$\mathrm{AUMC}_\infty = \int_0^\infty tC(t)\mathrm{d}t = \sum_{i=1}^{m} [\tau_i^2 C_i] \tag{7}$$

The mean residence time (mean time a F(ab′)$_2$ molecule stays in the body) is defined as

$$\mathrm{MRT} = \frac{\mathrm{AUMC}_\infty}{\mathrm{AUC}_\infty} \tag{8}$$

finally the, so-called, steady-state distribution volume was determined as

$$V_{ss} = \frac{D\,\mathrm{AUMC}_\infty}{\mathrm{AUC}_\infty^2} \quad (9)$$

Body mass index is defined (Anonymous, 1998) as

$$\mathrm{BMI} = \frac{W}{H^2}$$

where W is the body weight in kg and H the height in meters.

2.6. Statistical procedures

Since the sample size was small and the distribution of data unknown, we used 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. See Hollander and Wolfe (1973) for all details of nonparametric methods used. Differences between frequencies of events were compared with the χ^2 test with Yates correction for continuity (Fleiss, 1973). Differences between treatments were considered significant if the probability that the null hypothesis was true was ≤ 0.05 ($P \leq 0.05$, two tails).

3. Results

3.1. Demographic characteristics

The sample of volunteers used in this study was determined by the people responding to a call made at the Red Cross Hospital in León, Guanajuato, Mexico; all the people responding were enrolled, thus it is a non selected sample of the hospital employees. Eight volunteers were studied, their gender, weight, age, height and BMI are presented in Table 1. All the volunteers denied previous exposure to scorpion stings, having received Alacramyn™ or suffering from allergies. As it may be observed only 25% were females. We calculated the probability (P) of drawing two instead of four of the subjects with a certain character in a random sample of size 8, drawn from a universe with population proportion of 50%, and found that $P = 0.303$ (χ^2 test). Thus, the proportions of genders in our study could be considered the result of random sampling ($P > 0.05$). Using the criteria of the National Heart, Lung and Blood Institute of the National Institute of Health (NIH, USA; Anonymous, 1998) one of the volunteers was marginally overweight (BMI = 26.9 kg mt^{-2}), one was overweight (BMI = 29.1 kg mt^{-2}) and another very overweight (BMI = 47.9 kg mt^{-2}). The BMI of the remaining volunteers fell in the ideal weight range (19.1–25.8 kg mt^{-2}, for women; 20.7–26.4 kg mt^{-2}, for men).

3.2. Pharmacokinetic analysis

The PK parameters were determined from the multiexponential equation (1) as indicated in methods; the serum concentration versus time curves of seven patients were very well fitted letting $z = 3$. In one of these patients (JLP in Table 2) two of the time constants obtained were so similar (0.63 and 0.68 h) that their mean is presented in Table 2 as τ_4; also C_4 for JLP in the table, is the sum of the original C_i's corresponding to the averaged τ's. In the eighth subject (VVE in Table 2) it was necessary to let $z = 4$ to obtain a good fit. Fig. 1 is representative of the quality the eight fits. The data of volunteer RLM is presented in Fig. 1; the top of the figure shows the fit over all the experimental period, in the bottom the abscissa was magnified to show the very fast initial phase of serum concentration decrease characteristic of all patients studied. The PK constants calculated with the parameters in Table 2 are presented in Table 3.

3.3. Adverse events

The laboratory examinations (QS, PFH, EGO, and BHC) of each volunteer were studied and no abnormal values were found. Alacramyn™ was well tolerated in all the volunteers in this study and no early or delayed allergic reactions to the dose used were observed.

4. Discussion

This paper presents the first study of F(ab′)$_2$ scorpion antivenom PK in humans. We measured antivenom plasmatic concentrations using a specially designed high sensitivity ELISA method for unlabeled F(ab′)$_2$.

Table 1
Demographic characteristics of the volunteers

Parameter	Volunteers								
	BER	CML	JLP	JTM	MMS	RLM	RLS	VVE	Median (95% CI)
Age (years)	25	21	20	26	17	25	24	23	23 (21, 25)
Gender	F	M	M	M	F	M	M	M	
Height (m)	1.63	1.72	1.68	1.77	1.56	1.76	1.73	1.7	1.7 (1.66, 1.74)
Weight (kg)	55	85	76	150	48	81	67	73	74 (64, 102.5)
BMI (kg mt^{-2})	20.7	29.1	26.9	47.9	19.7	26.2	22.4	25.3	25.3 (22.9, 34.3)

BMI: body mass index; F: Female; M: Male.

Table 2
Parameters of the multi-exponential fit for each volunteer

	Volunteers								Median (95% CI)
	BER	CML	JLP[a]	JTM	MMS	RLM	RLS	VVE	
τ_1	0.07	0.73	0.65	0.51	0.41	0.1	0.16	0.26	0.36 (0.18, 0.53)
τ_2	3.58	2.27			5.76	3.16	7.98	5.97	4.7 (3.2, 6.9)
τ_3				15.5				25.5	20.5 (15.5, 25.5)[b]
τ_4	267.8	228.8	433.5	213.1	277.8	166	178.1	236.6	232.7 (203.4, 305.8)
C_1	0.44	2.31	14.1	3.04	9.08	10.11	24.95	9.69	8.6 (5.1, 14.0)
C_2	3.94	6.9			3.52	7.87	13.93	2.67	5.7 (3.5, 9.4)
C_3				7.56				5.95	6.8 (6.0, 7.6)[b]
C_4	3.01	4.89	0.64	1.2	3.68	4.27	1.2	0.84	2.4 (1.2, 3.7)

The parameters are used here as in Eq. (1) of the text, C_i is the drug concentration in component i at $t=0$ and τ_i is the time constant of decay. All C_i are in μg/ml, all τ_i are time constants in hours.
[a] The fit process was carried out with three exponentials but two were too similar (0.63 and 0.68 h) thus their mean is presented.
[b] Range.

PK was studied in eight non-envenomed human volunteers which have not previously received $F(ab')_2$. The information is interesting per se, and also represents patients which receive antivenom after a dry (non-envenoming) scorpion stings. In Table 6 of Oasnaya-Romero et al. (2001), the authors indicate that 9.6% of children stung by *Centruroides* seeking assistance at the Cuernavaca, México, Hospital del Niño Morelense with only mild symptoms (defined by the authors as limited to local or no pain at arrival, or erythema and paresthesia at the sting area) received 1 dose of $F(ab')_2$. This patients are very likely dry stings. Fifty-eight *Tityus* sting patients seeking help at the Victorino Santaella Hospital in Los Teques, Venezuela, with only local pain received 1 ampoule of $F(ab')_2$ antivenin. The venom blood level was latter determined in all these patients by ELISA (D'Suze et al., 2003); nine out of these patients (15.5%) tested within $\leq$2 h following the sting (when venenemia is likely to be at maximum values, Sevcik et al., 2004) had blood venom values $\leq$2 ng/ml, within the 95% confidence interval of the control (non envenomed patients) median (G. D'Suze, unpublished). These patients are also likely to be dry stings. We do not know which of the remaining patients were also dry stings, since venenemia gets low after 2 h and thus none of these patients with venenemia in the control range was counted; the 15.5% estimate may thus be an underestimation for *Tityus* victims. If use the data of Oasnaya-Romero et al. (2001) and apply them to 44.7% (mild envenomings, Oasnaya-Romero et al., 2001) of 213,458 cases documented in Mexico in 2004 (Anonymous, 2005), as many as 9160 healthy subjects might have received antivenom after a dry sting, only in 2004 in Mexico. Dry snake bites are also well documented, and may occur in as many as 50% of the cases occurring in Central America (Russell et al., 1997). We do not have data on how many of them receive antivenom, but the percentage is likely to be high, since snake bitten patients usually ask for quick antivenom therapy.

Alacramyn™, the pharmaceutical formulation of $F(ab')_2$ utilized in this study, is administered to over 200,000 cases of scorpionism per year in Mexico, and is currently under evaluation by the FDA for its use in the USA. The ample Mexican clinical experience with Alacramyn™ indicates that severe anaphylactic reactions do not occur even in patients stung by scorpions and treated in more that one occasion. In < 5% of patients a form of mild cutaneous rash is observed; this rash subsides uneventfully untreated or at most with some corticosteroid or antihistamine treatment. The lack of adverse effects in our sample is, thus, not surprising.

Our results show that $F(ab')_2$ kinetics in serum is a complex multi-exponential process. Since we have no evidence on $F(ab')_2$ catabolism, the interpretation of the different exponentials is complex. Still in all eight volunteers there was an initial rapid phase of serum $F(ab')_2$ decay phase with a $t_{1/2,\tau_1} = 0.25(0.13, 0.37)$ h [15.0 (7.8, 22.2) min]. In a recent study in rams, Sevcik et al. (2004) compared the PK of *Tityus discrepans* scorpion venom and of its $F(ab')_2$ antivenin, and found a fast initial decay of pure fluorescently labeled $F(ab')_2$ with a $t_{1/2,\tau_1} = 14.2(9.8, 24.8)$ min. Sevcik et al. (2004) showed that this fast component in rams is about 4-fold faster that venom extravasation. Concluding that the faster antivenom outflow can only be explained in terms of the existence of some kind of active mechanism of extrusion of $F(ab')_2$ through the vascular endothelium (Sevcik et al., 2004). Our PK calculations indicate that this mechanism of active $F(ab')_2$ extravasation also seems to be present in humans. This very rapid extravasation mechanism seems to be absent from some results in the literature where the fastest component $t_{1/2,\alpha}$ is close to 2 h (see Table II of Gutiérrez et al. (2003) for a convenient summary). In some instances this is due to the experimental protocol chosen which did not include enough points at short times; this is the case, for example, of Pépin et al. (1995), Pepin-Covata et al. (1996a) and Rivière et al. (1997). Ismail et al. (1998) measured PK of ^{125}I-anti snake venom $F(ab')_2$; in their experimental situation a three component kinetics was identified. They called $t_{1/2\pi}$, $t_{1/2,\alpha}$ and $t_{1/2,\beta}$ to our $t_{1/2,\tau_1}$, $t_{1/2,\tau_2}$ and $t_{1/2,\tau_3}$,

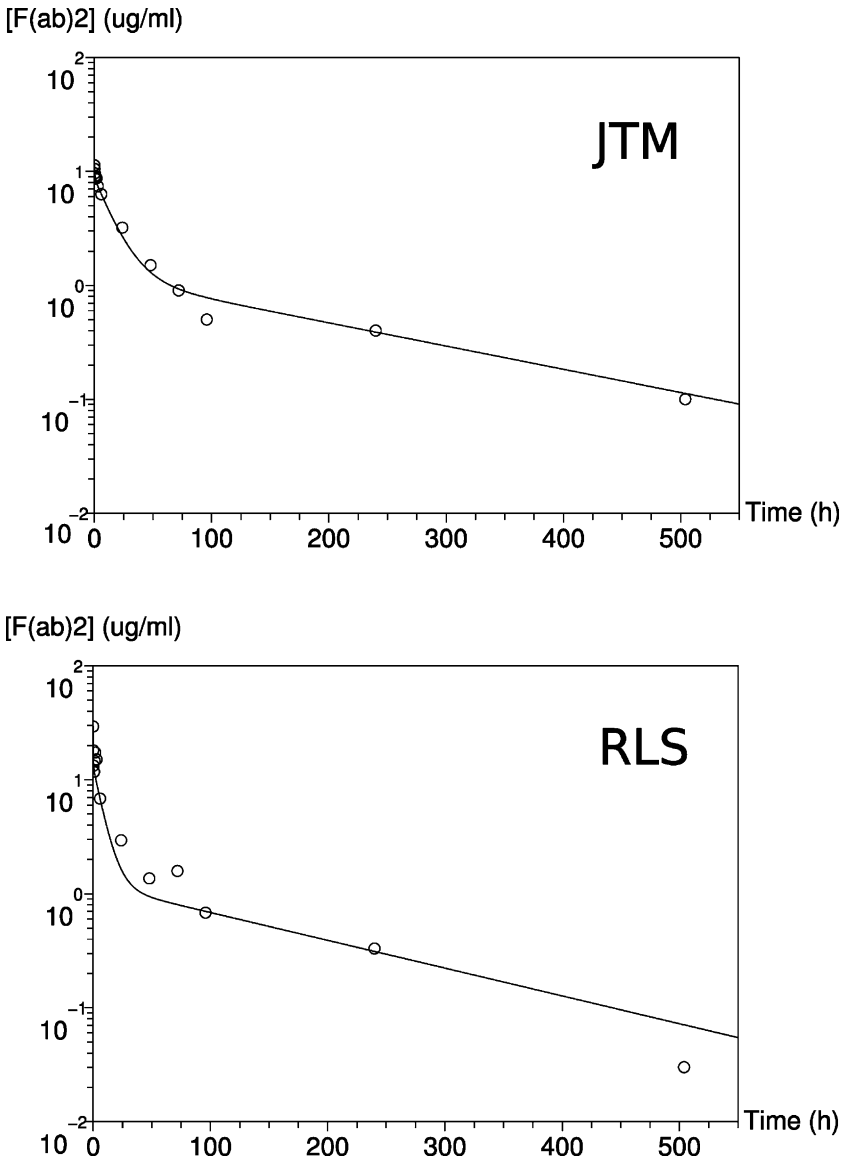

Fig. 1. Examples of the best and worse fits of data to Eq. (1). The upper half of the figure is the best fit between data and Eq. (1) which was obtained for JTM, the lower half of the figure is the worst data fit in this paper obtained for RLS. The abscissa is time in minutes. The ordinate for both halves is $F(ab')_2$ serum concentration (μg/ml) in logarithmic scale. Best and worst are defined here in terms of the final value of ε obtained, see Methods for details.

respectively. Their values are in remarkably good agreement with ours.

The slowest component observed (τ_4) is perhaps due to the slow catabolism of the antivenin, but a precise explanation of the processes related to τ_2 and τ_3 is not possible at the present. In regard to these it is good to point out that Alacramyn™ is mostly $F(ab')_2$ but it contains a small amount of $F(ab')$. The possibility exists that some intermediate exponential process (represented by either τ_2 or τ_3) may be due to the faster elimination of $F(ab')$.

Several PK parameters have been used to estimate the volume where a drug distributes after a prolonged time, all these parameters imply assumptions and simplifications, and some have been subject of controversy (see, for example, Gobburu and Holford, 2001). For compatibility with the literature, we included in Table 3 parameters such as V_c (sometimes called V_α in bi-exponential situations), V_z (sometimes called V_β in bi-exponential situations) and V_{ss} customarily called volume in 'steady state' (in spite of the fact that in a bolus situation the system is never in steady state!). These parameters are also presented normalized by

Table 3
Pharmacokinetic parameters

Parameter	Median (95% CI) ($n=8$)	Units
AUC_∞	596.9 (369.3, 891.2)	mg h l^{-1}
$AUMC_\infty$	135.1 (79.6, 202.4)	g h^2 l^{-1}
C_{p_0}	16.3 (13.3, 24.9)	μg ml^{-1}
V_c	3.1 (2.3, 4.3)	L
V_c/BW	38.2 (28.9, 71.6)	ml kg^{-1}
V_z	20.6 (13.1, 41.3)	L
V_z/BW	274.6 (196.8, 538.8)	ml kg^{-1}
V_{ss}	15.4 (12.8, 39.9)	L
V_{ss}/BW	204.2 (183.1, 518.9)	ml kg^{-1}
CL	96.6 (58.0, 139.2)	ml h^{-1}
CL/BW	1.304 (0.846, 1.657)	ml h^{-1} kg^{-1}
MRT	250.0 (218.8, 310.2)	h
$t_{1/2,\tau_1}$	0.25 (0.13, 0.37)	h
$t_{1/2,\tau_2}$	3.3 (2.2, 4.8)	h
$t_{1/2,\tau_3}$	14.2 (10.7, 17.7)[a]	h
$t_{1/2,\tau_4}$	161.3 (141.0, 212.0)	h

AUC_∞, area under the curve at time $t=\infty$; $AUMC_\infty$, area under the mean curve; BW, body weight; C_{p_o} = Concentration at the central (plasma) compartment at $t=0$; CL = systemic clearance; MRT, mean time of residence; $t_{1/2,\tau_1}$, $t_{1/2,\tau_2}$, $t_{1/2,\tau_3}$ and $t_{1/2,\tau_4}$ half times of decay of the four exponential components; V_c, volume of the central compartment; V_z, volume during the slowest exponential component; V_{ss}, steady-state volume.
[a] Median and range.

body weight. The observed V_c is very close to the expected volume of serum of the subjects (Manery, 1954) as it is to be expected since the fabotherapic is administered as an i.v. bolus. V_{ss} and V_z are rough means to estimate the final distribution volume of the fabotherapic (by definition $V_z > V_{ss}$ if $z > 1$); the experimental values shown are close to the expected value of the extracellular compartment (Manery, 1954) indicating that the fabotherapic distributes into the whole extracellular space. The value of V_{ss} for Alacramyn™ is interestingly larger than the same parameter determined for clinically useful monoclonal antibodies isotypes of IgG1 and IgG2, which are close to the serum volume of the study subjects (Lobo et al., 2004). The values of V_c, V_z and V_{ss} (Table 3) also indicate that in the absence of venom there are no high affinity receptors for $F(ab')_2$ able to alter its distribution volumes. An interesting parameter is MRT, an estimate of the mean time a fabotherapic molecule stays in the body, 10.4 (9.1, 12.9) days for Alacramyn™. The serum half-life of IgG has been estimated as 23 days, it has been proposed that this long half-life involves the Fc chain interacting with FcRn receptors in tissues (Lobo et al., 2004). Yet, the long MRT for $F(ab')_2$ must be due to a different mechanism since it has no Fc chain. The large MRT ensures a long protection to the patient if enough fabotherapic is administered. Still, this study does not give any insight on the catabolism of $F(ab')_2$ molecules after they bind to venom.

Antivenom is administered to bind the venom and change its PK. Unfortunately there are few good PK studies of venom or antivenom, usually what is provided is a measure of free venom in plasma. So when antivenom is given, free venom in plasma drops from high levels to near zero almost instantaneously. However, this does not define the whole PK of venom which will now really be the PK of the antivenom-venom complex. Digoxin-specific F(ab) is eliminated via renal and nonrenal routes, having a volume of distribution slightly exceeding extracellular volume (0.40 L/kg) and an elimination half-life of 16–20 h in presence of digoxin. Patients with renal impairment and end-stage renal disease have elimination half-life values that are prolonged up to 10-fold in magnitude, while volume of distribution is unaffected (Ujhelyi and Robert, 1995).

Many studies of venom kinetics, have clearly shown that antivenom affects venom PK, and in fact the use of F(ab) is based in the hope that it will increase the clearance of venom. For toxins acting at sites outside blood, binding venom in blood effectively neutralizes venom action immediately by changing the disposition of venom (to blood, rather than extravascular tissue), same as for digoxin.

The converse is also likely to be true: that venom affects the PK of antivenom, particularly for IgG or $F(ab')_2$ because these have much longer half-lives unless they bind to venom and then they are eliminated by the body as immune-complexes and are more rapidly eliminated. This makes intuitive sense because the body wants IgG to hang around as long as possible, until it meets its antigen (usually an infective source) after which the body then wants the antigen–antibody complex eliminated.

There is a lot of work done on this with antidigoxin F(ab) (Timsina and Hewick, 1991; Timsina and Hewick, 1992; Ujhelyi et al., 1993; Ujhelyi and Robert, 1995) which clearly shows that administration makes free digoxin drop to zero, slightly rebound and then get excreted approximately the same as the F(ab) itself. Free digoxin concentrations fall rapidly after F(ab) administration and then rebounds upwards within 12–24 h. This rebound in free digoxin concentrations, however, is delayed by 12–130 h in patients with renal dysfunction and end-stage renal disease (Ujhelyi and Robert, 1995). Rebound in free digoxin concentrations occurs during the initial phase of the bi-exponential decline of the serum concentration-time profile for digoxin-specific F(ab) in rabbits (Timsina and Hewick, 1992) and in humans (Ujhelyi and Robert, 1995), suggesting that distribution from the extravascular spaces is the likely cause (Ujhelyi and Robert, 1995). Following the increase, free digoxin concentrations decline in a manner that is dependent on renal and nonrenal routes of elimination. During this time period, it is evident that F(ab) retains it capability of binding digoxin while it resides in plasma (Ujhelyi and Robert, 1995). There is no evidence to support a dissociation between the F(ab)–digoxin complex over extended periods of time (Ujhelyi and Robert, 1995).

The rebounds show that F(ab) is eliminated from the body quicker that digoxin, unless the dose of F(ab) is consistently insufficient to bind all the body digoxin (a possible but unlikely systematic error). The second conclusion is that antidigoxin F(ab) is not able to reach all the compartments where digoxin distributes enabling the resurgence of free drug after F(ab) has been eliminated, at least in part, from the body.

Annoying as they may be, rebounds in free plasmatic digoxin are perhaps less serious that rebounds in crotaline toxicity observed when F(ab) is used in snake poisoning (Seifert and Boyer, 2001; Boyer et al., 2001; Dart et al., 2001). Unless patients are carefully monitored and antivenom administration repeated, recurrence of toxicity in crotaline poisoning treated wit F(ab) results in coagulopathies, central nervous system, cardiac and gastrointestinal effects (Dart et al., 2001). Late, persistent or recurrent coagulopathies occur in as many as 53% of crotaline patients treated with F(ab) (Boyer et al., 1999).

It has been proposed (Seifert and Boyer, 2001) that the high frequency of rebounds of toxicity when F(ab) antivenoms are used, is due to a high depot of unneutralized venom in the tissues which keeps being absorbed for times more prolonged than the clearance of unbound F(ab). If this PK scheme is true, it creates the conditions for recurrent venom antigenemia and its effects (Seifert and Boyer, 2001). IgG and $F(ab')_2$ antivenoms do not seem to be renally eliminated in any detectable amount (at least in absence of a nephrotic syndrome). Rebounds of venom effects after $F(ab')_2$ treatment are possible if antivenom dose is insufficient or barely enough (Sevcik et al., 2004); in these conditions free plasma antivenom is exhausted and unneutralised venom continues entering the circulation. Yet, rebounds of venenemia do not seem to occur if enough $F(ab')_2$ is administered in a single bolus.

The problem with a study of PK of antivenom in normal subjects is how clinically useful is this information, since it is likely to be different to the PK in envenomed patients. Still, to be able to confirm that the premises in the preceding arguments are valid, the antivenom PK in absence of venom is needed to know whether it changes in presence of venom or not. As said, good F(ab) antivenom PK in healthy subjects is scanty, but anti-gastrin F(ab) $t_{1/2}$ of elimination in rats has been reported to be 7.3 ± 0.7 h (Ohning et al., 1994), was 16.3 ± 2.4 h for anti-digitoxin F(ab) also in rats (Pentel et al., 1988) and was 8 ± 0.3 h for anti-*Vipera* F(ab) in rabbits (Rivière et al., 1997); the $t_{1/2}$ of elimination of F(ab) fragments, thus, is more or less independent of species and antigen. In contrast with these results, $t_{1/2,\tau_4}$ for anti-*Centruroides* $F(ab')_2$ determined in humans in this study was 161.3 (141.0, 212.0) h which is 10-fold slower that any of the $t_{1/2}$ values cited above, and MRT was 250.0 (218.8, 310.2) h; its perhaps proper to point out that a 95% confidence interval is equivalent 1.96 standard deviations above and below the mean in Gaussian statistics.

A wealth of literature indicates that IgG, $F(ab')_2$ and F(ab) antibodies are equivalent it their efficacies to neutralise their antigens. Yet, this study explains why $F(ab')_2$ antivenoms are the best choice treatment for their fast extravasation (short $t_{1/2,\tau_1}$) their ample distribution into the extracellular space (V_{ss} and V_z) and their prolonged MRT. This study also indicates that a prolonged neutralising capacity of the antibody is more important that renal clearance of the antigen–antibody complex.

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