

Absorption and Elimination of Viper Venom after Antivenom Administration

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ABSTRACT

The mechanisms by which antivenom neutralizes the venom are still poorly understood. In the present work, we studied the effects of antivenom, constituted with either $F(ab')_2$ or Fab, on the processes of absorption and elimination of *Vipera aspis* venom in experimentally envenomed rabbits. We first concluded from this study that during the few hours after intramuscular injection, the venom rapidly disappeared from the site of injection but did not immediately reach the vascular system, suggesting that it is partly absorbed via the lymphatic circulation. Concerning the elimination process of the venom in the presence of antivenom, we observed that the elimination of

$F(ab')_2$ /venom complexes is slower than that of free venom in the absence of antivenom but faster than that of free $F(ab')_2$, suggesting that $F(ab')_2$ /venom complexes are eliminated by phagocytosis. The Fab/venom complexes, on the other hand, are eliminated more slowly than free Fab. These complexes are not eliminated through the renal route in agreement with their high molecular weight. In addition, we observed that the treatment of envenomed rabbits with antivenom made of Fab, but not $F(ab')_2$, is responsible for an oliguria that could be responsible for clinical problems.

Immunotherapy performed with specific Fab fragments is widely used to reverse digitalis (Smith *et al.*, 1976) or colchicine (Baud *et al.*, 1995) intoxications. These immunoglobulin fragments neutralize the toxicity of the toxic compounds by reducing their volume of distribution due to their ability to form immunocomplexes and by increasing their elimination through the renal route (Butler *et al.*, 1977; Sabouraud *et al.*, 1992). Immunotherapy is also widely used in the case of snake and scorpion envenomations (De Rezende *et al.*, 1995; Thomas *et al.*, 1996). In this case, most of the antivenoms are $F(ab')_2$ prepared from horses hyperimmunized against the concerned venoms (Theakston and Warrell, 1991). However, some authors recommend the use of immunoaffinity purified sheep Fab (Consroe *et al.*, 1995; Rawat *et al.*, 1994; Sjoström *et al.*, 1994), which proved to be responsible for a lower rate of side effects than horse $F(ab')_2$ (Hickey *et al.*, 1991; Smith *et al.*, 1992).

It has been recently shown in clinical and experimental studies (Karlsonstiber *et al.*, 1997; Rivière *et al.*, 1997), that specific Fab induce a more transient neutralizing effect than $F(ab')_2$ on the toxicity of the venom mainly due to their shorter elimination half-life, $t_{1/2} = 4.3$ hr compared with that of $F(ab')_2$, $t_{1/2} = 18$ hr (Meyer *et al.*, 1997). In agreement with the long duration of snake venom envenomations (Audebert

et al., 1994), these results indicated that Fab fragments have to be reinjected after a few hours to maintain their neutralizing action, whereas a single injection of $F(ab')_2$ neutralizes the venom for a much longer time (up to several days). Thus, the efficacy of specific venom Fab in treating envenomation is lower than that of specific colchicine or digitalis Fab in treating intoxications. Moreover, the process of detoxification of the venom with specific Fab seems to be different than when Fab is directed against digitalis or colchicine.

In the present report, we examine the mechanisms by which specific fragments of immunoglobulins [$F(ab')_2$ or Fab] detoxify the venom. We first determined the mechanism of absorption of the venom injected intramuscularly and the effect of the intravenous injection of antivenom on the venom absorption. We then analyzed the effects of antivenom on the elimination process of *Vipera aspis* venom. Finally, we examined the interest of the intramuscular route for the administration of antivenoms made of $F(ab')_2$ or Fab.

Methods

Detection of the venom and of antivenoms. *V. aspis* venom (Latoxan, Rosans, France) was 125 I-iodinated according to the method of Fraker and Speck (1978), as modified by Audebert *et al.* (1994).

Plasma concentrations of free venom were quantified using dou-

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ABBREVIATIONS: AUC, area under the curve; AUMC, area under the first moment curve; Cl_T , total clearance; ELISA, enzyme-linked immunosorbent assay; MRT, mean residence time; T_{max} , time for maximal concentration; Vd_{ss} , volume of distribution at steady state.

ble-sandwich ELISAs as described elsewhere (Rivière *et al.*, 1997). Briefly, specific *V. aspis* venom antibodies from IPSEER Europe antivenom were coated onto a microtiter plates (Nunc, Roskilde, Denmark). After saturation of the wells with phosphate-buffered saline containing 3% bovine serum albumin, samples and scale diluted in nonvenomated rabbit plasma were added to each well. Antibodies conjugated with peroxidase were then added. After incubation, the colored reaction was developed using *o*-phenylenediamine dichloroamide (2 mg·ml⁻¹) mixed with 0.06% of H₂O₂. This procedure permitted to detect only free venom in plasma. Total venom concentrations were determined by counting the TCA-precipitable fraction.

Immunoglobulin fragments [F(ab')₂ or Fab] were detected using double-sandwich enzyme-linked immunosorbent assay as described previously (Rivière *et al.*, 1997). Rabbit anti-horse immunoglobulin antibodies were purchased from Biosys (Compiègne, France). This test was set up to detect free or complexed immunoglobulin fragments.

Preparation of Fab fragments. Fab fragments were prepared as described previously (Rivière *et al.*, 1997). Control experiments indicated that the proteolytic treatment does not modify the affinity of the fragments for the venom components. In particular, first, the protective effects of F(ab')₂ and Fab, determined by premixing with venom and testing the residual toxicity by lethality assay, were identical. Second, an ELISA performed under equilibrium conditions indicated that the venom complexation was identical for F(ab')₂ and Fab and that dissociation does not occur after a 1-hr incubation. Because of the respective molecular weight of F(ab')₂ and Fab, 100 and 50 kDa, respectively, the administration of the same quantity of F(ab')₂ or Fab leads to the injection of the same number of binding sites. The doses of Fab and F(ab')₂ were therefore adjusted according to their protein concentration.

Purification of specific anti-*Vipera* venom F(ab')₂ and Fab. Specific anti-*Vipera* venom F(ab')₂ or Fab were purified from IPSEER Europe horse serum (Pasteur Mérieux Sérums et Vaccins, Lyon, France) by immunoaffinity chromatography on immunosorbent columns coupled with a mixture of *V. aspis*, *V. berus* and *V. ammodytes* venoms (Latoxan, Rosans, France), in equal proportions, after the method of Avrameas and Ternynck (1969). Specific fragments were eluted with 0.1 M HCl-glycine buffer, pH 3.

Detection of the venom at the site of injection. The leg muscles were dissected immediately after the death of the rabbit and stored at 4°C until used. Muscles were homogenized in phosphate-buffered saline using successively a Virtis instrument and a Potter homogenizer. After filtration (cutoff, 100 μm), radioactivity was counted using liquid scintillation spectrometry (LS-6000-SC, Beckman).

Protein content was quantified using the procedure of Folin-Lowry modified by Markwell *et al.* (1978). Briefly, a 200-μl sample was mixed with a solution containing: 2% sodium bicarbonate, 0.4% sodium hydroxide, 0.16% sodium tartrate and 0.004% copper sulfate (w/v). This mixture was incubated for 10 min at room temperature, before addition of 60 μl of a 2-fold diluted solution of Folin and Ciocalteu's Phenol Reagent (Sigma Chemical, St Louis, MO). Absorbance was measured at 660 nm after 45 min. Bovine serum albumin was used as a standard.

Pharmacokinetics. All pharmacokinetic experiments were conducted in accordance with the "Principles of Laboratory Animal Care," as described previously (Rivière *et al.*, 1997). Briefly, New Zealand rabbits weighing 2.75 to 3 kg (CEGAV, St Mars-d'Egrenne, France) were placed in metabolism cages that allow the collection of urine and feces. Food and water were provided *ad libitum*. Intravenous injections of the venom were administered in the marginal vein of the ear over an 8-min period in a volume of 5 ml of 0.15 M NaCl using a syringe pump (Bioblock, Illkirch, France). We tested the effects of a high dose of antivenom (125 mg) injected *via* the intravenous route 5 hr after an intravenous injection of venom to study the effects of antivenom on the process of elimination of the venom.

Intramuscular injections of the venom were performed in the leg

in a final volume of 500 μl of 0.15 M NaCl. Determination of the pharmacokinetic parameters of Fab injected intramuscularly was done after an injection performed in the leg in a final volume of 1 ml. Blood was collected in heparinized tubes and centrifuged at 1500 × *g* for 15 min to obtain plasma.

Pharmacokinetic analysis of *V. aspis* venom injected intravenously was done using the MK-Model software (Biosoft, Cambridge, England). The best-fit line was achieved with the least-squares method using weighted function (1/C²_{obs}). We did not insert the 8-min period of infusion in the analysis because of the lack of experimental points. The choice of a two- or three-compartment model was decided according to the Schwarz criterion. The total body clearance (Cl_T) was determined as equal to D/AUC, with D being the injected dose, and AUC being the area under the time *vs.* concentration curve, from injection to infinity. The volume of distribution at steady state (Vd_{ss}) was equal to D*AUMC/AUC² and the mean residence time was defined as AUMC/AUC, AUMC being the area under the first moment *vs.* time curve.

The pharmacokinetic parameters of the F(ab')₂ or Fab complexes were determined as follows. Immunopurified antivenoms (1 mg) were injected intravenously 5 hr after intravenous injection of 250 μg·kg⁻¹ of *V. aspis* venom. The delay of 5 hr was chosen to allow complete distribution of the venom based on the pharmacokinetic parameters obtained using ELISA quantification. Immunoglobulin fragments concentrations were determined using ELISA as described above. The noncompartmental method was used to analyze the pharmacokinetics of the complexes of F(ab')₂ or Fab with venom components. The AUC of the total venom from injection of antivenom to infinity (AUC_{5→∞}) was calculated using the log-trapezoidal rule from time zero to the last experimental point and from the last experimental point to infinity by extrapolation using C/β, C being the concentration measured at the last experimental point and β being the terminal slope.

Statistics. All measurements are expressed as mean ± S.E.M. The mean values were calculated from at least three independent experiments. The significance of the data was analyzed by the two-tailed unpaired or paired Student's *t* test. When multiple comparisons were done, we used one-way analysis of variance followed by Dunnett's or Bonferroni's procedure. The level of significance was set at P < .05.

Results

Pharmacokinetics of *V. aspis* venom. We first determined the pharmacokinetic parameters of ¹²⁵I radiolabeled *V. aspis* venom after intravenous injection in rabbits. For this purpose, we quantified the concentrations of plasma venom by two methods: ELISA and radioactivity (fig. 1). With ELISA, the decrease in venom concentration could be fitted with a biexponential decline, indicating that the venom was distributed into two compartments with a terminal half-life of 14.2 hr, in agreement with the value determined by Audebert *et al.* (1994) with the same method. However, when the venom concentrations were determined by counting the radioactivity, we observed a triexponential decline, with a higher terminal half-life of 27.2 hr, significantly different from that determined by ELISA (P < .05). In fact, all pharmacokinetic parameters (terminal half-life, Vd_{ss} and Cl_T) differed statistically between the two methods of venom quantification (table 1). Such a difference, which has already been reported in a study where the venom was injected *via* the intramuscular route (Rivière *et al.*, 1997), suggests a differential detection of some venom proteins by ELISA and by radioactivity. In fact, Audebert *et al.* (1994) have shown that all the venom proteins are not ¹²⁵I-iodinated with the same specific radioactivity and that they do not respond to

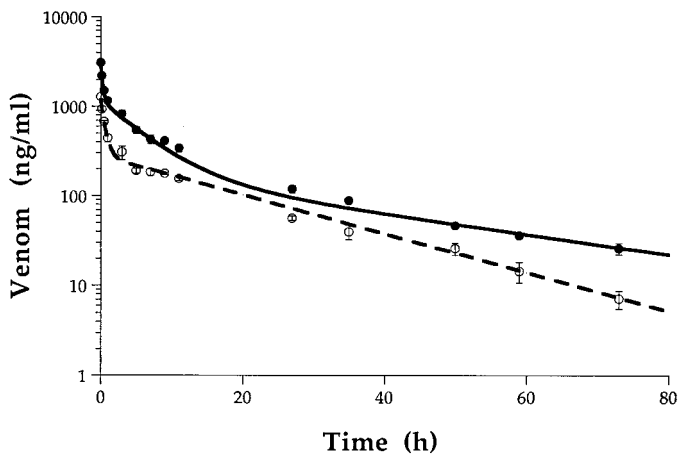


Fig. 1. Pharmacokinetics of $250 \mu\text{g}\cdot\text{kg}^{-1}$ of radioactive *V. aspis* venom after an i.v. injection. Concentrations of plasma venom were quantified either by ELISA (—●—) or radioactivity (---○---). Curves were drawn using pharmacokinetic parameters obtained by model-dependent analysis. Experimental values are the means \pm S.E.M. of 5 independent experiments.

ELISA with the same intensity. On the other hand, it has been shown that iodination of low-molecular-weight proteins (<80 kDa) could alter their pharmacokinetics (Bauer *et al.*, 1996; Kuo *et al.*, 1997). These differences, however, have no consequence on the further interpretation of the results obtained in this investigation because the two methods have been used independently to measure the extent of redistribution or the pharmacokinetics of immune complexes in different conditions. Moreover, when these data sets are considered together, they yield a useful and complementary picture of the concentration-time curves.

Influence of specific $F(ab')_2$ or Fab antibodies on the pharmacokinetics of *V. aspis* venom. We first tested the effects of 125 mg of immunoglobulin fragments [$F(ab')_2$ or Fab] injected intravenously 5 hr after an intravenous injection of $250 \mu\text{g}\cdot\text{kg}^{-1}$ of *V. aspis* venom on the pharmacokinetic of the venom. In the case of $F(ab')_2$, no free venom was detectable by ELISA up to 72 hr after venom administration (fig. 2A). This indicates that all the venom antigens were immunocomplexed. Moreover, the AUC value, determined for radioactive (free and immunocomplexed) venom from the time of antivenom injection to infinity ($AUC_{5-\infty}$), was considerably higher than that calculated in the absence of antivenom ($14,600 \pm 450$ compared with $3300 \pm 300 \text{ ng}\cdot\text{hr}^{-1}\cdot\text{ml}^{-1}\cdot\text{kg}^{-1}$, respectively, $P < .05$). In contrast to this experiment performed with $F(ab')_2$, free venom could be detected after an injection of 125 mg of Fab under the same conditions (fig. 2B): the concentration of free venom measured by ELISA immediately after immunotherapy with Fab was $\sim 10 \text{ ng}\cdot\text{ml}^{-1}$. The $AUC_{5-\infty}$ value was higher than that without antivenom (5000 ± 450 compared with $3300 \pm 300 \text{ ng}\cdot\text{hr}^{-1}\cdot\text{ml}^{-1}\cdot\text{kg}^{-1}$, respectively, $P < .05$) but significantly ($P < .05$) lower than that after the injection of $F(ab')_2$ (5000 ± 450 and $14,600 \pm 650 \text{ ng}\cdot\text{hr}^{-1}\cdot\text{ml}^{-1}\cdot\text{kg}^{-1}$, respectively).

When *V. aspis* venom is injected *via* the intramuscular route, the venom components undergo a slow resorption for up to 72 hr, with an apparent terminal half-life of ~ 30 hr (Audebert *et al.*, 1994), and we have shown (Rivière *et al.*, 1997) that an intravenous injection of antivenom [$F(ab')_2$ or Fab] induced a substantial redistribution of the venom

from the extravascular compartment to the vascular space. This phenomenon did not occur or was very modest when the venom was injected intravenously (fig. 2). This could be explained by the fact that the Vd_{ss} was higher when the venom was injected intramuscularly ($Vd_{ss} = 2 \text{ liter}\cdot\text{kg}^{-1}$) than when it was intravenously injected (table 1). Thus, we investigated the origin of the venom that appears in the vascular space during the redistribution process observed after immunotherapy in the case of an intramuscular injection of the venom. When we assayed the venom at the site of injection immediately after an intramuscular injection of $750 \mu\text{g}\cdot\text{kg}^{-1}$ of *V. aspis* venom, we detected 70 ng of venom/mg of total muscle protein; this value decreased to 2.9 and $1 \text{ ng}\cdot\text{mg}^{-1}$ after 7 and 30 hr, respectively. When the intramuscular injection of the venom was followed 7 hr later by an intravenous injection of 125 mg of IPSEER Europe antivenom, the venom concentration observed at the site of injection after 30 hr was not significantly different ($0.75 \text{ ng}\cdot\text{mg}^{-1}$).

Pharmacokinetics of $F(ab')_2$ or Fab/venom complexes. To study the elimination process of immune complexes formed between antivenom and venom, we immunopurified immunoglobulin fragments [$F(ab')_2$ or Fab]. It is known that most antivenoms contain both venom-specific immunoglobulins (or fragments of immunoglobulins) and nonspecific immunoglobulins. In the case of IPSEER Europe antivenom, 20% of the $F(ab')_2$ of the antivenom preparation is able to bind venom antigens (data not shown). Thus, the use of commercial antivenom to determine the pharmacokinetic parameters of the $F(ab')_2$ or Fab/venom complexes is inappropriate because the nonspecific immunoglobulin fragments will not be complexed with the venom antigens. We therefore immunopurified Fab or $F(ab')_2$, as described in Methods, to perform these studies. Rabbits were injected intravenously with $250 \mu\text{g}\cdot\text{kg}^{-1}$ *V. aspis* venom and 5 hr later with 1 mg of purified fragments [Fab or $F(ab')_2$] (rabbit fig. 3). In these experimental conditions, the venom is in excess, as shown by the presence of free venom detected throughout the experiment, and all the immunoglobulin fragments [$F(ab')_2$ or Fab] are complexed with venom components, as indicated by the absence of free specific fragments [$F(ab')_2$ and Fab], so a specific ELISA directed to horse $F(ab')_2$ or Fab will quantify only immune complexes. Fab/venom and $F(ab')_2$ /venom complexes are eliminated with similar elimination half-lives, but the mean residence time of Fab/venom complexes is shorter than that of $F(ab')_2$ /venom complexes (table 2). On the other hand, the injection of 1 mg of Fab, but not of $F(ab')_2$, in envenomed rabbits greatly reduced the volume of urine collected during the first 24 hr of the experiment: 5 ± 5 ml of urine instead of 64 ± 24 ml in the case of rabbits that received venom but not antivenom ($P < .05$). At subsequent periods, the volumes of collected urine were not statistically different with or without antivenom. It is important to remember that this phenomenon did not occur with venom-specific $F(ab')_2$.

Pharmacokinetic of Fab fragments injected intramuscularly. When 10 mg of Fab was injected into nonenvenomed rabbits *via* the intramuscular route, the time-dependent change in plasma concentration of Fab showed two phases (fig. 4): an absorption phase followed by an elimination phase characterized by an apparent terminal half-life of 13.2 ± 0.3 hr. The elimination of intramuscu-

TABLE 1

Pharmacokinetic parameters of *Vipera aspis* venom after intravenous injection of $250 \mu\text{g} \cdot \text{kg}^{-1}$ Venom concentrations were determined by ELISA or radioactivity. Results are expressed as mean \pm S.E.M. of five independent experiments.

	Distribution phase(s)		Elimination phase		$V_{d_{ss}}$	Cl_T
	hr		hr		$\text{ml} \cdot \text{kg}^{-1}$	$\text{ml} \cdot \text{h}^{-1} \cdot \text{kg}^{-1}$
ELISA	0.53 ± 0.14		14.2 ± 1.2		700 ± 50	40 ± 1
Radioactivity	0.25 ± 0.06	4.8 ± 1	27 ± 2^a		400 ± 30^b	18 ± 1.7^b

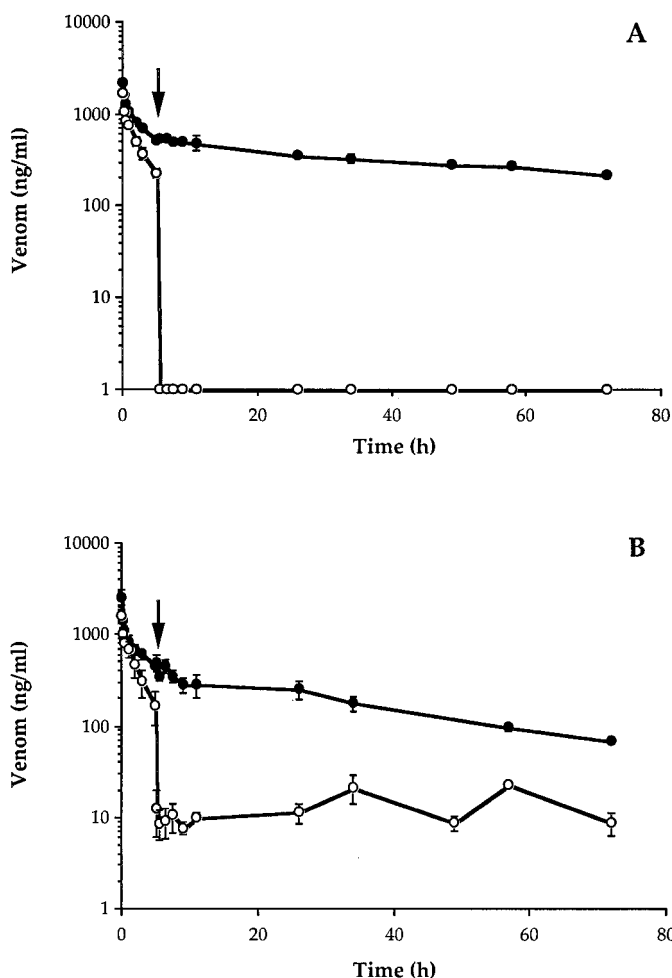
^a $P < .05$ compared with ELISA values.^b $P < .01$ compared with ELISA values.

Fig. 2. Effect of an i.v. injection of 125 mg of $F(ab')_2$ (A) or Fab (B) injected 5 h (arrows) after an i.v. injection of $250 \mu\text{g} \cdot \text{kg}^{-1}$ of *V. aspis* venom. Total venom concentrations (—●—) and free venom concentrations (—○—) were determined up to 72 hr. Experimental values are the means \pm S.E.M. of 5 independent experiments.

larly injected Fab thus occurs faster than that of $F(ab')_2$ ($t_{1/2\beta} = 59.6$ hr; Pépin *et al.*, 1995). Moreover, it has been shown that $F(ab')_2$ is absorbed more slowly than Fab, with an observed T_{max} of 48 hr for $F(ab')_2$ (Pépin *et al.*, 1995) and 12 hr for Fab.

Discussion

The injection of a high dose of antivenom (125 mg), composed of either $F(ab')_2$ or Fab, 5 hr after an intravenous administration of *V. aspis* venom increased the $AUC_{5 \rightarrow \infty}$ of the venom complexed to the antibodies and detected by radioactivity and decreased the plasma concentration of non-complexed venom. The immunocomplexation of the venom in

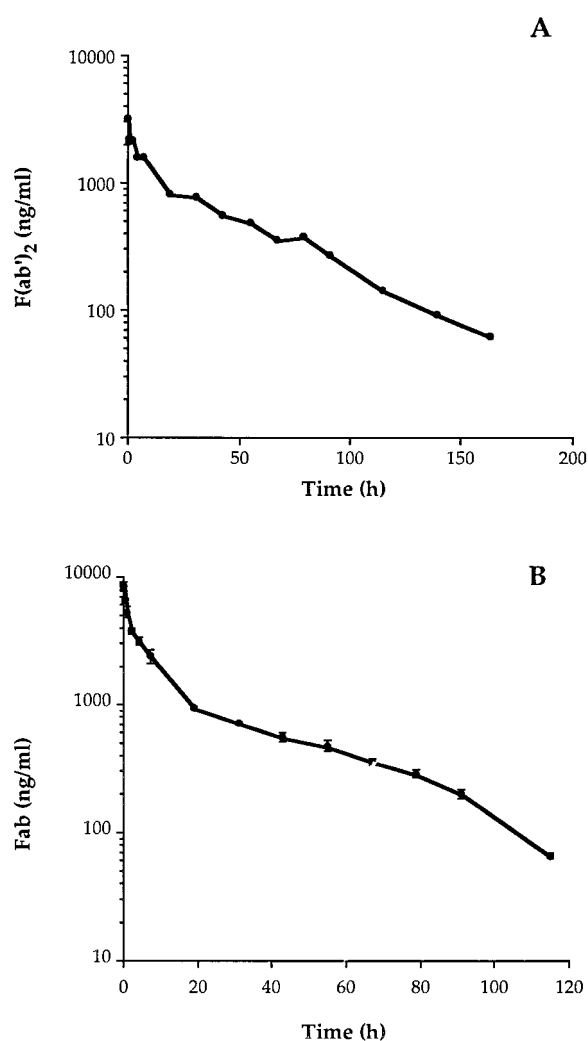


Fig. 3. Pharmacokinetics of $F(ab')_2$ -venom (A) and Fab-venom (B) complexes. One milligram of specific immunoglobulin fragments was injected 5 hr after an i.v. injection of $250 \mu\text{g} \cdot \text{kg}^{-1}$ of *V. aspis* venom. Experimental values are the means \pm S.E.M. of independent experiments.

plasma was complete (free venom was undetectable) with $F(ab')_2$, whereas it was not in the case of Fab. This cannot be attributed to a weaker neutralizing capacity of Fab compared with $F(ab')_2$. On the other hand, the larger $V_{d_{ss}}$ calculated for Fab than for $F(ab')_2$ (Rivière *et al.*, 1997) is related to a lower Fab concentration in plasma responsible for the lower immunocomplexation of the venom. The phenomenon of immunocomplexation of the venom is well understood in the case of intoxications by colchicine (Cano *et al.*, 1995), digitalis (Smith *et al.*, 1976) and phencyclidine (Valentine *et al.*, 1994). The injection of Fab induces a redistribution of the drug from the extravascular compartment (where it exerts its

TABLE 2

Pharmacokinetic parameters of specific immunoglobulin fragments complexed with *Vipera aspis* venom. Results are expressed as mean \pm S.E.M. of five independent experiments.

	$t_{1/2\beta}$	Cl_T	MRT	Vd_{ss}
	hr	$ml \cdot hr^{-1} \cdot kg^{-1}$	hr	$ml \cdot kg^{-1}$
F(ab') ₂ (Rivière <i>et al.</i> , 1997)	55 \pm 9	2.1 \pm 0.01		130 \pm 5
Fab (Rivière <i>et al.</i> , 1997)	8 \pm 0.3	53 \pm 0.5		230 \pm 2
F(ab') ₂ venom complex	31.5 \pm 2.9 ^b	4.8 \pm 0.3 ^b	45.9 \pm 3.8	220 \pm 21 ^b
Fab venom complex	25.6 \pm 1.5 ^b	3.9 \pm 0.1 ^b	31.7 \pm 1.0 ^a	125 \pm 5 ^{a,b}

Analysis were performed using one-way ANOVA followed by Bonferroni's test.

^a P < .05 compared with F(ab')₂.

^b P < .05 compared with uncomplexed fragments.

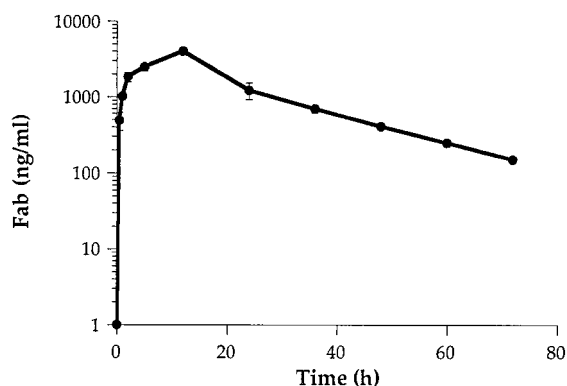


Fig. 4. Pharmacokinetics of 10 mg of Fab injected *via* the intramuscular route. Experimental values are the means \pm S.E.M. of independent experiments.

toxicity) to the vascular space (where it is neutralized). In the case of intravenously injected *V. aspis* venom, the administration of a high dose of specific immunoglobulin fragments did not induce the marked redistribution observed with these low-molecular-weight toxins (colchicine, digitalis, phencyclidine). This might be explained by the fact that the Vd_{ss} values of colchicine, digitalis and phencyclidine are very large, >3 liter \cdot kg⁻¹ (Sabouraud *et al.*, 1992; Timsina and Hewick, 1992; McClurkan *et al.*, 1993), allowing a marked redistribution when brought back in the vascular space by the Fab, whereas the Vd_{ss} value of the venom injected by the intravenous route is rather small (725 or 400 ml \cdot kg⁻¹, using ELISA or radioactivity quantification, respectively) and close to the vascular volume, not allowing the antibodies to cause a significant redistribution of the venom.

Surprisingly, Rivière *et al.* (1997) reported that the intravenous injection of antivenom induces a larger redistribution of the venom when the venom is injected *via* the intramuscular route instead of the intravenous route. This is in good correlation with the Vd_{ss} value of the venom, which is higher when injected *via* the intramuscular route than the intravenous one. Audebert *et al.* (1994) showed that the resorption of intramuscularly injected viper venom is a slow process, developing up to 72 hr, which could suggest that the venom was sequestered at the site of injection during this time. However, we observed that at 7 hr after its intramuscular injection, all the venom had disappeared from the site of injection, whereas only 25% of the venom has reached the vascular space (Audebert *et al.*, 1994). This suggests that during the first hours after the intramuscular envenomation, most of the venom has been absorbed in the lymphatic circulation, but it is not yet significantly released in the vascular compartment. In agreement with this conclusion, it has been

observed that *V. aspis* venom causes an important vascular extravasation and edema to appear at the site of injection during the first hours after snake bite (Sorkine *et al.*, 1995), which might be responsible for a 4- to 9-fold increase in the flow rate of lymph (Ikomi and Schmid-Schonbein, 1996). In this context, the large increase in the venom concentrations observed in the vascular space after intravenous administration is simply explained by the fact that antibodies [F(ab')₂ or Fab] could induce a redistribution of the venom from the lymphatic compartment to the vascular space rather than displacing it from the site of injection. Indeed, these two compartments are closely connected (Garlick and Renkin, 1970). Interestingly, the absorption of the venom *via* the lymphatic system was suggested a long time ago by Barnes and Trueta (1941) in the case of the venom of *Notechis scutatus*.

As previously reported, F(ab')₂ and Fab differ in their effects on the pharmacokinetics of *V. aspis* venom (Rivière *et al.*, 1997). Although F(ab')₂ causes a complete and durable neutralization of the venom, the action of Fab is much more transient. It is thus interesting to examine whether this might be due to differences in the kinetics of elimination of F(ab')₂/venom and Fab/venom complexes. In both cases, the pharmacokinetic parameters determined for F(ab')₂ or Fab complexed with venom differed from those obtained respectively for noncomplexed immunoglobulin fragments (Rivière *et al.*, 1997). In the case of F(ab')₂, the terminal half-life of F(ab')₂/venom complexes was shorter than that calculated for free fragments, and Cl_T and Vd_{ss} were larger (table 2), as easily explained considering that these immune complexes are large and undergo phagocytosis. On the other hand, in the case of Fab, the terminal half-life of Fab/venom complexes is significantly longer than that of free Fab, whereas Cl_T and Vd_{ss} are smaller, most probably because the Fab/venom complexes are soluble and therefore cannot be eliminated by phagocytosis. This is also different from the colchicine/Fab complexes, which are eliminated with the same terminal half-life as free Fab (Sabouraud *et al.*, 1992). Thus, the elimination process of the Fab/toxin complexes seems to be different when immunotherapy is performed against high-molecular-weight components, as in the case of viper venom or small drugs like colchicine or digitalis. This is certainly due to the fact that Fab/venom complexes cannot be eliminated by the renal route because of their high molecular weight, whereas individual constituents (free Fab, free venom antigens or drugs) and Fab complexes made with small drugs as colchicine or digitalis have a molecular weight smaller than the filtration threshold of the renal glomeruli and are rapidly eliminated in urine (Sabouraud *et al.*, 1992). On the other hand, although the F(ab')₂/venom and Fab/

venom complexes have similar terminal half-lives (31.5 and 25.6 hr, respectively), it cannot be concluded that they have the same route of elimination.

The renal toxicity of Fab fragments is still controversial. Some authors reported that transient oliguria and increased serum creatinine occurred only after the injection of very high doses of nonspecific human Fab (3–5 g·kg⁻¹) in dogs (Keyler *et al.*, 1991). On the other hand, Moran *et al.* (1994), reported that injection of 2 mg·kg⁻¹ digoxin-specific sheep Fab fragments in rats induced marked renal toxicity (urine volume and creatinine clearance were decreased by 30%). In the case of the Fab fragment from IPSEER Europe antivenom, we did not determine creatinine clearance but observed a substantial reduction in the volume of urine in envenomed rabbits during the 24 hr after immunotherapy with Fab but not with F(ab')₂. The renal toxicity of Fab fragments thus seems to depend on their complexation with the venom component and might vary with their species origin (human, sheep or horse) or with the experimental animal (dog, rat or rabbit). In this context, it has been recently shown in a clinical study (Dart *et al.*, 1997) that injection of specific crotalid ovine Fab (up to 9 g per patient) did not induce renal toxicity.

Specific Fab fragments proved to be very effective in the treatment of colchicine and digitalis intoxications (Smith *et al.*, 1976; Baud *et al.*, 1995). Their use, instead of F(ab')₂, also has been recommended because of their lower incidence of adverse reactions (Hickey *et al.*, 1991; Smith *et al.*, 1992). However, specific Fab appeared much less effective than F(ab')₂ in treating viper envenomations when the immunotherapy is performed by intravenous injection (Rivière *et al.*, 1997; Karlsonstiber *et al.*, 1997). The present study emphasizes this conclusion. Because of their faster resorption, Fab might be of value when intravenous injection of antivenom made of F(ab')₂ is not feasible. However, the renal toxicity that we observed in experimentally envenomed rabbits treated with Fab, but not with F(ab')₂, implies that further investigations must be performed to clarify this point before recommending the use of snake antivenoms made of Fab in humans.

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