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Pharmacokinetic evaluation of recombinant, activated factor VII in patients with inherited factor VII deficiency

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Background and Objectives. Recombinant factor VIIa (rFVIIa) has been widely used in the treatment of bleedings occurring in hemophiliacs with inhibitors. Very few reports exist on the use of rFVIIa in patients with inherited FVII deficiency. Pharmacokinetic studies on rFVIIa have been performed exclusively in hemophiliacs, patients with cirrhosis or volunteers pretreated with acenocoumarol. The aim of this study was to evaluate the kinetics of rFVIIa in patients naturally deficient of FVII.

Design and Methods. A single dose kinetic study with rFVIIa was performed in 5 patients affected by severe congenital deficiency of factor VII in order to evaluate the true kinetic parameters of rFVIIa without the interference of FVII. Two dosages, 15 and 30 μg/kg, were used in a crossover schedule. FVII:C and FVIIa concentration/time curves were analyzed by a model–independent method. Antithrombin (AT), prothombin fragment 1+2 (F1+2) and tissue factor pathway inhibitor (TFPI) were assayed.

Results. No differences emerged between the dosages with respect to dose-independent parameters [total body clearance (CL), volume of distribution area (VdArea), mean residence time (MRT)]. No significant changes of AT, TFPI, and F1+2 were observed. Comparing the results with those of other studies performed in adult hemophiliacs, in patients affected by cirrhosis or in volunteers on oral anticoagulant therapy (OAT), CL and VdArea of rFVIIa were definitely higher and *in vivo* recovery was lower.

Interpretation and Conclusions. These findings suggest that the kinetics of rFVIIa are not dose-dependent. In the absence of FVII, the changes of VdArea and CL may be in agreement with a mechanism of competition between FVII and rFVIIa for tissue factor binding.

Key words: factor VII, activated recombinant factor VII, pharmacokinetics, factor VII congenital deficiency

Human factor VII (FVII) is a vitamin K-dependent glycoprotein with a molecular weight of 50 kD that circulates in normal plasma at a concentration of 0.5 μg/mL. FVII zymogen initiates the reactions of the extrinsic pathway of blood coagulation by forming a high-affinity complex with its receptor, the cell-bound tissue factor (TF).[1] After conversion to activated FVII (FVIIa), it cleaves factor X and factor IX, eventually leading to thrombin formation.[2] Hereditary FVII deficiency is a rare congenital coagulation disorder with an estimated incidence of 1/100,000 to 1/500,000 cases/population and a bleeding tendency of variable severity, which is currently treated with replacement therapy using concentrates containing FVII.[3-7] The half-life of infused, plasma-derived FVII has been shown to be relatively short (200-400 min) in comparison with that of other clotting factors,[8-10] and therefore, repeated administrations may be required to treat bleeding episodes.

A recombinant form of FVIIa (rFVIIa) has been developed recently as a by-passing agent to treat bleeding in patients with hemophilia A or B who have natural inhibitors to factor VIII or to factor IX.[11–13] In this setting, several clinical trials have demonstrated that the drug, at recommended dose regimens of 60-120 μg/kg every 2 to 6 hours, provides effective and safe hemostasis for spontaneous bleeding and prevents excessive bleeding in surgical interventions. Recombinant FVIIa has also been successfully used to treat various bleeding conditions, including bleeding occurring in FVII congenital deficiencies,[13,14] liver failure,[15] oral anticoagulant overdose,[16] thrombocytopenia[17] and von Willebrand's disease.[18] The mechanism(s) underlying the hemostatic efficacy of the drug in such a heterogeneous array of defects is not well understood, but may be related to rFVIIa binding to platelets and to the subsequent local, platelet-mediated delivery of high concentrations of FVIIa to sites of vascular injury,[19] or to platelet activation.

The pharmacokinetics of rFVIIa have previously been investigated in animals,[20] in patients with hemophilia with or without inhibitors,[21] and in healthy volunteers receiving oral anticoagulant treatment.[22] In these studies, rFVIIa was consistently shown to have a shorter half-life (100-160 min) than that reported for the plasma-derived zymogen (120-300 min),[23] however, this kinetic estimate, in the presence of normal synthesis of autologous FVII, may be affected by various factors including the patient's own FVII activation by the infused serine protease.

The aim of the present study was to evaluate the pharmacokinetics of rFVIIa in patients with severe congenital deficiency in a non-bleeding state. The administration of high doses of rFVIIa to the otherwise normal FVII congenital deficient patients also gave us the opportunity to gain further information on the activation of the coagulation cascade following the administration of rFVIIa, which may be relevant with regards to safety.

Design and Methods

Patients

Five patients with severe FVII deficiency (clotting activity <1%) were considered for the study in three Italian hemophilia centers. The patients had no sign of atherosclerotic disease, severe hepatic failure and were not pregnant, and had not had bleeding episodes and/or treatment with FVII-containing products in the previous month. A FVII antigen (FVIIAg) assay showed that two patients had antigen levels below 2% of normal (FVIIAg negative), while the other three patients had FVIIAg levels of 19, 80 and 95% of normal (FVIIAg positive). All the patients were fully informed of the purpose of the study and gave their written consent to participation. The demographic data of the patients are shown in Table 1.

Table 1. Demographic and laboratory data of patients.

Age(years) and sex	*CRM*	*FVII:C (U/dL)*
20 Male	Neg	<1
20 Male	Pos	<1
33 Female	Pos	<1
36 Male	Neg	<1
43 Female	Pos	<1

CRM, cross-reacting material; FVII:C, FVII clotting activity.

Study design, drug administration, and sample collection

In this open, non-controlled study, each patient received, in a random sequence, two doses of rFVIIa (Novoseven™, Novo Nordisk A/S, Bagsvaerd, Denmark), 15 and 30 µg/kg, with a washout period between the two doses of at least 3 days. Vials containing 1.2 mg of rFVIIa were reconstituted in 2 mL sterile water immediately before use and the rFVIIa was administered as a single intravenous bolus injection within 2 minutes. Blood samples were collected before the infusion and 10, 15 and 30 minutes, 1, 2, 4, 6, 8, 10 and 24 hours after each bolus. Blood samples (10 mL) were drawn from the arm not used for rFVIIa infusion into tubes containing sodium citrate 0.13 mol/L in the proportion of 9 parts blood to 1 part sodium citrate, and centrifuged at 2,000 × g for 20 minutes no more than 45 minutes after blood collection. Plasma aliquots (0.25 mL) were frozen immediately to -30°C or below.

Laboratory assessments

Samples were dispatched and analyzed at a single central laboratory where the following assays were performed: FVII clotting activity (FVII:C), by a one-stage coagulation method using an immune-depleted FVII-deficient plasma (Dade-Behring, Milan, Italy) and a recombinant tissue factor preparation (Dade-Behring, Milan, Italy). Activated FVII (FVII:Ca) was assayed using a diagnostic kit (Staclot VIIa-rTF, Diagnostica Stago, Asnières France) as was antithrombin amidolytic activity (Ortho Diagnostics, Milan, Italy). F1+2 levels were measured with an immune-enzyme assay kit (Dade-Behring, Milan, Italy). Plasma levels of TFPI were measured by solid-phase chromogenic assay as previously described.[24] Normal ranges of both F1+2 and TFPI were determined in 25 (15 male and 10 female) blood donors.

Pharmacokinetic analysis

Pharmacokinetic parameters were evaluated according to the recommendations of the *FVIII/IX Scientific and Standardization Committee of the International Society for Thrombosis and Hemostasis* using a model-independent method.[25,26] The following parameters were measured from each curve: area under the curve (AUC), area under the statistical moment curve (AUMC), total body clearance (CL), volume of distribution area (VdArea), volume of distribution at steady state (Vss), mean residence time (MRT) and half-life ($t^1/_2$). The pharmacokinetic parameters are reported as mean ± 1S.D. and the results stratified into four groups: FVII:C and FVII:Ca assays for the two treatment doses (15 and 30 µg/kg). Statistical analysis of results was performed using non-parametric ANOVA (Kruskal-Wallis ANOVA by ranks) and 2-tailed and paired Student's t test, using the complete statistical system (CSS) program provided by StatSoft Inc., Tulsa, OK, USA.

In vivo recovery (IVR)

IVR was evaluated both as a percentage (IVR1), according to Nilsson's formula,[27] and as an incremental recovery (IVR2), according to the Prowse's recommendation.[28]

Nilsson's formula is:

$$\text{IVR (\%):} \quad \frac{[\text{FVII peak (U/dL)} - \text{FVII baseline (U/dL)}] \times \text{Plasma volume (mL)}}{\text{Total dose FVII infused}} \times 100$$

Prowse's formula is:

$$\text{Incremental recovery (U/dL/U/kg)} = \frac{[\text{FVII peak (U/dL)} - \text{FVII baseline (U/dL)}]}{\text{Total dose FVII infused/body weight}}$$

In order to obtain a more precise evaluation of IVR, we did not use a fixed time, single parameter FVII concentration (10 min) but rather the peak value of both FVII:C and FVII:Ca.

Results

Pharmacokinetics

Tables 2 and 3 summarize the FVII:C and FVII:Ca kinetic results, respectively.

Recovery

The FVII:C peak value concentration occurred at 10 minutes in 3 of 5 patients infused with 15 µg/kg and at 15 minutes in patients infused with 30 µg/kg. The FVII:Ca peak value concentration occurred at 10 minutes in all patients infused both with 15 µg/kg and 30 µg/kg.

Table 2. Factor VII:C single-dose kinetics.

		Dosage				
		15 µg/kg		*30 µg/kg*		
		Mean	S.D.	Mean	S.D.	*p* values *
AUC	U.h/L	12084	5380	19840	4810	0.027
AUMC	U.h²/L	36916	13090	77077	35977	0.046
CL	mL/h/kg	70.8	24.1	79.1	17.5	0.74
MRT	h	3.80	0.70	3.75	0.83	0.91
$t_{1/2}$	h	2.82	0.87	3.11	1.10	0.75
VdArea	mL/kg	290	110	340	60	0.60
Vss	mL/kg	280	100	290	50	0.91
IVR 1	%	18.94	3.43	22.2	4.28	0.52
IVR 2	U/dL/U/kg	0.44	0.09	0.51	0.07	0.63

**ANOVA (Kruskal-Wallis ANOVA by ranks). AUC, area under the curve; AUMC, area under the statistical moment curve; CL, total body clearance; MRT, mean residence time; $t_{1/2}$, half life; VdArea, volume of distribution area; Vss, volume of distribution at steady state; IVR1, in vivo recovery according to Nilsson's formula; IVR2, in vivo recovery according to Prowse's formula.*

Table 3. Factor VII:Ca single-dose kinetics.

		Dosage				
		15 µg/kg		*30 µg/kg*		
		Mean	S.D.	Mean	S.D.	p values *
AUC	U.h/L	13390	6703	23699	7228	0.027
AUMC	U.h²/L	43496	31123	81429	24207	0.046
CL	mL/h/kg	64.9	22.7	67.7	17.9	0.75
MRT	h	3.33	0.52	3.46	0.64	0.75
$t_{1/2}$	h	2.49	0.36	2.62	0.63	0.75
VdArea	mL/kg	240	90	260	80	0.75
Vss	mL/kg	210	70	230	70	0.46
IVR 1	%	27.7	10.29	22.6	7.49	0.30
IVR 2	U/dL/U/kg	0.64	0.24	0.53	0.20	0.25

AUC, area under the curve; AUMC, area under the statistical moment curve; CL, total body clearance; MRT, mean residence time; $t_{1/2}$, half life; VdArea, volume of distribution area; Vss, volume of distribution at steady state; IVR1, in vivo recovery according to Nilsson's formula; IVR2, in vivo recovery according to Prowse's formula.

Table 4. Tissue factor pathway inhibitor (U/mL), mean±1 SD.

	Time (min)						
Dose	*0*	*15*	*30*	*120*	*240*	*480*	*Normal range*
15 µg/kg	1.05±0.2	1.02±0.3	1.10±0.3	1.13±0.2	1.15±0.3	1.15±0.3	0.7-1.5 U/mL
30 µg/kg	0.99±0.3	1.03±0.2	1.11±0.3	1.04±0.2	1.09±0.3	1.03±0.3	

Table 5. Prothrombin fragment 1+2 (nmol/L), mean ±1 SD.

	Time (min)						
Dose	*0*	*15*	*30*	*120*	*240*	*480*	*Normal range*
15 µg/kg	0.84±0.3	1.56±0.6	2.03±1.2	2.24±1.1	2.09±1.0	1.59±0.9	0.15-1.07 nmol/L
p value (#)	-	0.023	0.109	0.031	0.025	0.097	
30 µg/kg	0.80±0.3	1.76±0.4	2.20±0.4	2.98±0.6	3.36±1.3	2.17±1.5	
p value (#)	-	0.008	0.001	0.0001	0.008	0.105	

 # 2-tailed and paired Student's t test, with respect to baseline.

AT, TFPI and F1+2 assays

Results and normal ranges are shown in Tables 4 and 5. Antithrombin (data not shown) and TFPI plasma levels did not show significant changes, while F1+2 increased slightly between the first and fourth hour after the infusion (the highest mean level was 3.1 nmol/L). The levels of F1+2 were statistically increased compared with baseline values in both treatments schedules (Table 5). In both regimens, the F1+2 peak level was observed during the 120 to 240 minute period after the end of rFVIIa infusion.

Discussion

The AUC and AUMC are dose-dependent kinetic parameters. The statistically significant differences observed between the two treatments are due, as expected, to the different dosages used in the crossover study. No statistically significant differences between the two dosages were observed with respect to CL, VdArea and MRT parameters,these being parameters that are dose-independent.

Even though it is not strictly correct to compare results from different studies, the CL of Novoseven™ in FVII deficient patients seems to be at least twice that reported in adults with hemophilia A or B,[21] in healthy volunteers on oral anticoagulants[22] and in patients with liver cirrhosis.[15] Small changes (increase of CL and a faster $t^1/_2$) have been reported in hemophiliacs with bleeding compared with the non-bleeding patients.[21] In patients receiving oral anticoagulation therapy, the drug regimen and the value of the international normalized ratio did not affect factor VIIa kinetics[22] and similar kinetic parameters have been reported in cirrhotic patients[15] (Table 6). Similar to our findings, only in young patients with hemophilia were higher values of CL reported.[29] Our patients were aged between 20 and 43 years, therefore age cannot explain the faster CL observed. In addition, the VdArea of Novoseven™ in FVII-deficient patients seems to be very large, at least twice that observed in hemophiliacs.[21] A comparative summary of the pharmacokinetic findings, as derived from a literature review and including the results of our study, is reported in Table 6.

Recently, it has been demonstrated that the presence of FVII zymogen reduces, in vitro, FXa generation by the TF-VIIa complex.[30] According to this report, at low TF concentrations there is competition between FVII zymogen and FVIIa because binding to TF occurs wherever and whenever the blood coagulation mechanism is triggered. Similar findings were reported by Zur[31] in an in vitro bovine system. If this mechanism is relevant in vivo, the hemostatic effect of FVIIa in hemophiliacs occurs only when FVIIa plasma concentrations (about 2 nmol/L) overcome the competitive effect of the patient's own zymogen. In FVII-deficient patients, the binding of infused FVII to TF may be faster because there is no competition with its zymogen. The TF-FVIIa complex is then rapidly cleared from the plasma and/or bound to the TFPI-FXa complex. This mechanism could also explain the very large Vss and low IVR of Novoseven™ observed in FVII-deficient patients.

The comparison between the kinetic parameters of FVII with reference to the circulating forms of the factor (the total FVII mass assayed with the FVII:C and the activated form assayed with the FVII:Ca) did not yield a significant difference. This is most probably related to the fact that in the FVII:C assay, at least

Table 6. Review of factor VIIa pharmacokinetic results in patients with different clinical characteristics, compared with those in factor VII deficient patients.

Patients	*CL (mL/h/kg)*	*Vss (mL/kg)*	*Recovery (%)*	*$t^1/_2$ (h)*	*Reference*
Previous studies					
Hemophilia, non-bleeding state	32.84±11.72	108.86±37.15	47.85±13.32	2.82±0.53	(21)
Hemophilia, bleeding state	36.60±8.66	103.54±25.26	46.56±12.15	2.48±0.52	
Volunteers on oral anticoagulants (<20 µg/kg)	30.9±5.1	80.0±8.4		2.43±0.19	(22)
Volunteers on oral anticoagulants (>20 µg/kg)	34.5±7.0	93.6±14.5		2.45±0.25	
Hemophilia A, children	67.0	130.0		1.32	(29)
Cirrhotic patients	34.9±16.5	Not available	Not available	2.45±0.25	(15)
Patients	CL (mL/h/kg)	Vss (mL/kg)	Recovery (%)	$t^1/_2$ (h)	
Present study					
Factor VII-deficient patients (15 µg/kg)	70.8±24.1	280± 100	18.94±3,43	2.82± 0.87	
Factor VII-deficient patients (30 µg/kg)	79.1±17.5	290± 50	22.2±4.28	3.11±1.10	

CL, total body clearance; Vss, volume of distribution at steady state; $t_{1/2}$, half life.

in the FVII-deficient patients, FVII:Ca is the most represented species. In addition, we were unable to demonstrate a difference in pharmacokinetic parameters between the patients with different phenotypic variants ($FVIIAg^+$ or $FVIIAg^-$), but this may be related to the small number of patients studied.

The slight increase of F1+2 levels may be related to the occurrence of a low-grade *in vivo* thrombin generation in our FVII-deficient patients, a fact also observed by others.[32] This observation supports the conclusions reached in a study comprising numerous treatments of FVII-deficient patients with Novoseven™, in whom low doses of the drug (< 20 µg/kg) are considered sufficient to achieve hemostasis and normalize prothrombin time.[14]

In conclusion, we have found that the major pharmacokinetic difference in the use of rFVIIa in FVII-deficient patients and in those subjects who have normal FVII is the factor's clearance and, to a lesser extent, the recovery and volume of distribution, parameters which can be related to each other. These findings mean that a larger loading and maintenance dosages should be recommended during treatment of FVII-deficient patients. The clinical relevance of these data should, however, be validated in a repeated dose kinetic study.

Contributions and Acknowledgments

MM and MB wrote the paper. MB, MS, AR performed the single-dose kinetic studies in their Hemophilia Centers. GL performed the pharmacokinetic analysis. TDP organized the study protocol and provided the drug. GM revised the final release of the paper.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Vicente Vicente, who acted as an Associate Editor. The final decision to accept this paper was taken jointly by Prof. Vicente and the Editors. Manuscript received February 21, 2001; accepted May 2, 2001.

Potential implications for clinical practice

The definition of pharmacokinetic parameters of rFVIIa can be very useful to individualize the dosages not only in FVII-deficient patients but also in hemophiliacs with inhibitors. Because the drug is very expensive, the sparing effect of tailor-made therapy could be very important.

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