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Pharmacokinetics, thrombogenicity and safety of a double viral inactivated factor IX concentrate compared with a prothrombin complex concentrate

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Summary. Therapeutic options for developing countries have to assure an optimum safety and efficacy and low-cost antihaemophilic concentrates. A single blind randomized crossover study was carried out in 12 previously treated HB patients, comparing the pharmacokinetics (PK), thrombogenicity (TG) and safety of two plasma-derived double-inactivated (solvent/detergent heating at 100°C, 30 min) factor IX (FIX) concentrates, UMAN COMPLEX DI (product A) [plasma-derived prothrombin concentrates (PCC)] and a high purity FIX concentrate AIMAFIX DI (product B, HPFIX). In a non-bleeding state, they received one single intravenous dose 50 IU FIX kg^{-1} of PCC or HPFIX, and after a wash-out period of 14 days, the other product. We evaluated acute tolerance and determined PK parameters based on FIX levels measured over a 50 h postinfusion period. We studied fibrinogen, platelets, antithrombin, F1 + 2, TAT, D-dimer, over a 360 min postinfusion period. Ten cases remained in on-demand treatment for 6 months, five with PCC and five with HPFIX. PK and anti-FIX inhibitors were repeated at 3 and 6 months. No inhibitors were detected. PK values (PCC vs. HPFIX): clearance (CL; mL h^{-1} kg^{-1}) 5.2 ± 1.4 vs. 6.5 ± 1.4; the volume of distribution at steady state (mL kg^{-1}) 154.9 ± 54.9 vs. 197.5 ± 72.5; mean residence time (h) 29.7 ± 8.1 vs. 30.7 ± 9.2; $T_{1/2}$ (h) 22.3 ± 7 vs. 23.5 ± 12.3; incremental recovery (IR; U dL^{-1} U^{-1} kg^{-1}) 0.96 ± 0.17 vs. 0.76 ± 0.13. HPFIX showed significant lower IR and higher CL. There were no differences in PK at 3 and 6 months. In TG, significant increments in TAT and F1 + 2 at 30 min and 6 h were found with PCC. Product B PK results agrees with reported results for other HPFIX preparations. Use of PCC product A has to consider its thrombogenic activity.

Keywords: factor IX concentrate, haemophilia B, Plasma-derived prothrombin concentrates, pharmacokinetics, thrombogenicity, treatment

Introduction

There is a broad spectrum of treatment possibilities for haemophilia B patients, including new highly purified plasma-derived factor IX (FIX) concentrates and products manufactured using recombinant DNA technology [1,2]. Plasma-derived prothrombin concentrates (PCC) are highly effective, but occasional thrombotic complications associated with their use have been observed among haemophiliacs, this being generally attributed to the accumulation of activated vitamin K-dependent factors [3]; further purification steps seem to have eliminated those complications [4,5]. Therapeutic options for developing countries are limited so we have to assure an optimum safety and efficacy and low costs for the antihaemophilic concentrates.

The aim of the present study was to compare the pharmacokinetics (PK) and safety of two double-inactivated FIX containing products, a high-purity FIX concentrate (AIMAFIX DI) and a PCC (UMAN

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COMPLEX DI), titrated for its FIX content after intravenous administration to haemophilia B patients.

Patients, materials and methods

FIX concentrates

The UMAN COMPLEX DI (product A) contained 500 IU FIX, 500 IU factor II (FII) and 400 IU factor X (FX) per vial. Antithrombin III (ATIII) and heparin was added before lyophilization. Two different batches were used in this study. Viral inactivation was performed with solvent/detergent and heating of the lyophilized product at 100°C for 30 min.

The AIMAFIX DI (product B) contained 500 IU FIX per vial and was prepared from PCC by affinity chromatography on immobilized heparin. Three different batches were used in the study. Viral inactivation methods as described in product A. Both products are prepared by Kedrion S.p.A (Castelvecchio Pascoli, Italy).

Patients and study design

The study was carried out in one center (Banco Municipal de Sangre, DC, Caracas, Venezuela) according to a single blind randomized crossover design. Written informed consent was obtained from all patients. The study was reviewed and approved by the Ethics Committee of the Banco Municipal de Sangre, DC. Thirteen volunteers previously treated haemophilia B patients (FIX < 2 U dL^{-1}), aged 12–36 years and with a mean weight of 61.4 ± 14.2 kg were enrolled in this study. None of the patients had a history of anti-FIX inhibitors. All were anti-human immunodeficiency virus seronegative; antibodies for hepatitis C virus were detected in six patients, although none of them showed a serum ALT more than 2.5 times the upper limit of normal. Patients who did not use FIX concentrates or plasma during the 7 days prior to infusion were randomized to receive a single dose of either product A (seven patients) or product B (six patients) while in a non-bleeding state. After a wash-out period of 14 days all patients were shifted to receive a single dose of the other product. After the crossover study, 10 cases remained on demand treatment for the following 6 months, using the last product received (five received product A and five product B) as their sole replacement therapy. Inhibitor detection by Bethesda method [6] and pharmacokinetics studies were evaluated in each case at 3 and 6 months of follow-up.

Product administration

Prior to receiving the infusion, each case was clinically evaluated. Dose was calculated according to the weight and the FIX content reported on the pertinent analytical certificate by the manufacturer. Each patient received 50 U of FIX per kg of body weight. The rate of infusion was of 2 mL min^{-1}. The time for infusion for the product A varied from 43 to 80 min (mean: 67.8 ± 14.4 SD) and for product B varied from 15 to 42 min (mean: 30.9 ± 9.9 SD).

Acute tolerance evaluation

Vital signs, namely body temperature, pulse, respiratory rate and supine blood pressure were determined prior to during and after infusion, at the same time clinical observation and adverse events were also recorded.

Blood collection

Blood was collected by clean venipuncture from the arm opposite to the one used for infusion of factor concentrate, in 3.8% trisodium citrate (9:1 v/v) for factor IX determinations and thrombogenicity (TG) studies and in EDTA for platelet count.

Pharmacokinetics evaluation

Samples were collected prior to the start of infusion, immediately after the end of infusion, and at 0.5, 1, 3, 6, 9, 12, 24, 30, 36 and 50 h postinfusion. Aliquots of plasma were kept frozen at −70°C until analysed. FIX activity was analysed by one-step method, using three plasma dilutions, in all plasma samples obtained at the above-mentioned periods. Each dilution was performed in duplicated by the same technician. FIX Immunodepleted Substrate was used (Helena Laboratories, Beaumont, TX, USA). A local reference plasma obtained from 20 health volunteers was calibrated against an International Standard of FIX.

Pharmacokinetic parameters of the model were estimated through a non-linear fitting procedure using the computer program Excel. Non-compartment method for the estimation of pharmacokinetic parameters was used. The following pharmacokinetic parameters were assessed: area under the curve (AUC), computed using the trapezoidal rule; the area under the first moment of the curve (AUMC) that is the product of each point of the AUC for the respective time; incremental recovery (IR): the peak

level of FIX determined at the first hour after infusion; mean residence time (MRT): the rate of FIX decrease after infusion; clearence (CL): the amount of plasma free of the drug/h; the volume of distribution at steady state (V_{dss}); the maximal concentration of FIX and the time when it is achieved (C_{max} and T_{max} respectively); and the terminal half-life ($T_{1/2}$) of the FIX infused.

Thrombogenicity studies

Samples were taken prior to infusion and at 30, 180 and 360 min after. The following parameters were evaluated by standard procedures: Fibrinogen (Clauss method; Diagnostica Stago, Asniers, France), platelets count, Thrombin–antithrombin complex (Enzygnost TAT Micro; Behringwerke AG, Marburg, Germany), prothrombin fragment F1 + 2 (Dade Behring, Marburg, Germany), antithrombin III (Stachhrom; Diagnostica Stago), D-dimer (Asserachrom D-Di; Diagnostica Stago), as markers of the activation of the haemostatic system. Results were compared with the reference values obtained in our laboratory from 20 healthy control.

Statistical analysis

Pharmacokinetics data were calculated according to the ISTH recommendations [7–9]. All pharmacokinetic parameters were summarized with mean, standard deviation and 95% confidence interval of mean values for each combination of time with the different type of preparation. Data analysis was performed using STATA 7.0® (Stata Corporation, College Station, TX, USA) [10]. Paired two-tailed Student's *t*-test was used to compare mean values of two products for each different pharmacokinetic parameters and was used to evaluate the potential TG of products A and B. In the crossover analysis ANOVA model (Fisher's test) was used to compare mean values of concentrations of AUC between the product A and B and to evaluate their bioequivalence. This model considered both 'period effect' and 'treatment effect' and their interaction. In safety analyses all reported *P*-values are two-sided. Significance was assessed with α set at 0.05. Statistically significant results are flagged by their degree ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$) [11,12].

Results

Thirteen patients were enrolled and 12 patients finished the study. Clinical and laboratory assessments are summarized in Table 1. No adverse events were observed with high purity FIX; one patient during PCC infusion experienced a mild bronchial asthma crisis treated with an aerosol inhaler.

Pharmacokinetics

In Table 2 we can observe the results of the analysed pharmacokinetic parameters. The crossover study showed significant differences between the pharmacokinetic behaviour of the two study products as the mean of the values of the following PK parameters: AUC, AUMC, IR and CL, resulted statistically significant when comparing the pooled data for patients treated with product A vs. B. Subjects

Table 1. Clinical and laboratory characteristics of patients.

Patient	Age (years)	Factor IX (U dL^{-1})	Weight (kg)	HAV Ab	HBsAg	HCV Ab	HIV 1 + 2 Ab	Platelet (10^9 L^{-1})	Hgb (g dL^{-1})	Hct	WBC (10^9 L^{-1})	ALT (U L^{-1})	AST (U L^{-1})
1	32	0.4	79.7	Positive	Negative	Negative	Negative	222	13.3	0.39	7.7	68	45
2	20	1	75	Negative	Negative	Positive	Negative	236	13	0.39	6	57	61
3	15	1	48.5	Negative	Negative	Positive	Negative	353	11.5	0.34	7.2	15	27
4	19	1	55.5	Negative	Negative	Positive	Negative	186	13.1	0.40	5.8	32	27
5	15	1.4	58.8	Positive	Negative	Negative	Negative	222	13.5	0.43	3.8	15	29
6	14	1.4	58	Positive	Negative	Positive	Negative	267	12.7	0.40	4.4	30	18
7	14	1.2	61.7	Negative	Negative	Negative	Negative	282	14.7	0.43	5.4	12	25
8	17	0.8	45.5	Negative	Negative	Negative	Negative	221	12.9	0.40	5.1	30	26
9	12	0.9	36.5	Negative	Negative	Negative	Negative	262	13.3	0.42	6.4	18	29
10	36	1	85	Positive	Negative	Positive	Negative	338	11.9	0.36	6	33	37
11	27	1	73	Positive	Negative	Negative	Negative	220	14.7	0.44	6.7	24	10
12	21	<1	69	Positive	Negative	Positive	Negative	172	14.1	0.42	9.2	32	24
13	18	0.7	53	Negative	Negative	Negative	Negative	207	15.6	0.47	7.7	30	24
Reference values		62–138						130–400	13.5–17	40–54	4–11	<38	<40

HAV, Hepatitis A virus; HCV, Hepatitis C virus; Ab, Antibodies; Ag, Antigen.

Table 2. Pharmacokinetic variables after bolus administration of UMAN COMPLEX DI (product A) and AIMAFIX DI (product B) at crossover study and after 3 and 6 months of treatment follow-up.

Product/PK parameter	Crossover study	3 months	6 months
UMAN COMPLEX DI	$n = 13$	$n = 5$	$n = 5$
AUC [0–T_{max}] (U h^{-1} mL^{-1})	812.2 ± 195.3*	1059.90 ± 143.2	994.8 ± 127.9
AUMC (v h^{-2} mL^{-1})	13404.60* ± 3364.80	17810.90 ± 2544.10	16002.30 ± 3160.20
Incremental recovery (U dL^{-1} U^{-1} kg^{-1})	0.96 ± 0.17**	1.11 ± 0.2	1.08 ± 0.24
MRT (h)	29.7 ± 8.1	28.7 ± 5.1	22.2 ± 3.2
Clearance (mL h^{-1} kg^{-1})	5.2 ± 1.4*	3.9 ± 0.4	4.5 ± 0.6
V_{dss} (mL kg^{-1})	154.9 ± 54.9	112.2 ± 27.6	99.2 ± 13.3
C_{max} (U mL^{-1})	38.8 ± 8.7	56.5 ± 10.1	55.1 ± 11.4
T_{max} (h)	0.6 ± 0.8	0.2 ± 0.3	0.3 ± 0.5
$T_{1/2}$ terminal (h)	22.3 ± 7	20.5 ± 6.6	14.5 ± 2.4
AIMAFIX DI	$n = 12$	$n = 5$	$n = 5$
AUC [0–T_{max}] (U h^{-1} mL^{-1})	644 ± 132.8	702.9 ± 264.9	850.4 ± 216.4
AUMC (v h^{-2} mL^{-1})	10807.40 ± 2740.10	11 193.20 ± 5400.50	13 976.70 ± 3811.70
Incremental recovery (U dL^{-1} U^{-1} kg^{-1})	0.76 ± 0.13	0.78 ± 0.29	0.9 ± 0.3
MRT (h)	30.7 ± 9.2	27.7 ± 12.9	25.3 ± 3.1
Clearance (mL h^{-1} kg^{-1})	6.5 ± 1.4	6.8 ± 2.8	5.3 ± 1.6
V_{dss} (mL kg^{-1})	197.5 ± 72.5	164.6 ± 56.2	131.1 ± 25.6
C_{max} (U mL^{-1})	38.9 ± 6.2	40.1 ± 14.4	46.2 ± 13
T_{max} (h)	0.4 ± 0.4	0.5 ± 0.4	0.4 ± 0.5
$T_{1/2}$ terminal (h)	23.5 ± 12.3	21.6 ± 12.2	17.6 ± 3.5

A vs. B: * $P < 0.05$; ** $P < 0.01$.

treated with product B ($n = 12$) showed a lower AUC, AUMC and IR while the same subjects treated with product A showed a lower CL. V_{dss} resulted higher in the product B group, although the difference did not reach statistical significance.

In the patients ($n = 10$) who completed the 6-month follow-up period a further analysis was performed. The comparison of the results obtained after the first infusion and at the end of the crossover period continued showing statistically significant differences between the mean of AUC ($P = 0.001$), AUMC ($P = 0.0008$), CL ($P = 0.004$) and IR ($P = 0.005$) calculated for product A vs. product B. Furthermore, the V_{dss} difference also became significant ($P = 0.026$).

The same parameters were analysed in the five cases treated with product A and B at 3 and 6 months of treatment. When their values were compared with their respective initial results, no statistical differences were observed at the 3- and 6-month periods.

Crossover analysis

ANOVA model was applied to AUC to evaluate the period effect, the treatment effect and their interaction and so the bioequivalence of preparations A and B. In Table 3 we can see the ANOVA results (Fisher's test) for all the 40 observations we had and for the 24 initial data of our cases finished the crossover study. As the results of the initial studies when the 24 initial data were analysed the effect of treat on time (treatment*period) was marginally significant, ANOVA test for bioequivalence was applied and the probability for bioequivalence was 5%. When all 40 data were analysed the effect of treat on time (treatment*period) was no significant and the probability for bioequivalence was 18%.

Table 3. Crossover study: area under the curve (ANOVA test).

	No. observations	Treatment effect	Period effect	Interaction
Visit 1–visit 2–visit 3 months–visit 6 months				
F	40	13.81	3.89	2.3
P		0.0008	0.0177	0.096
Visit 1 + visit 2				
F	24	4.2	0.65	4.2
P		0.053	0.43	0.054

Evaluation of prothrombotic activity

In Table 4 we can observe the results of the different plasma studies performed at preinfusion and 30 min and 6 h postinfusion in patients when receiving product A and product B. Thirty minutes after infusion a significant increment in F1 + 2 and TAT was observed with Product A and increased F1 + 2 persists 6 h later. After infusion of product B we observed a marginally significant increment of TAT, that disappears at 6 h. Platelets increase at 6 h postinfusion in both groups. The mean values of

D-D, ATIII and fibrinogen did not show a significant change during the observation period.

Follow-up period

During this 6-month period, patients received a mean of 16 925 UI ± 5927 of product A and 21 220 UI ± 4761 of product B. The majority of the bleeding episodes were controlled with one infusion and all the patients experience excellent response to FIX. No anti-FIX inhibitor was detected during follow-up period.

Discussion

The *in vivo* recovery of FIX activity shows a wide variability between individuals. Values between 25% and 90% have been reported, and it is in general lower than the expected for FVIII. The precise mechanism for this lower recovery remains unclear. There have been implicated some patients' specific factors, and also the higher extravascular distribution of FIX. It has been suggested that FIX pharmacokinetics could be influenced by proteolysis or by binding to unknown inhibitory molecules or reversible binding of FIX to vascular endothelium [13–15].

In order to obtain accurate measurement of FIX activity during a longer period of 72 h, the patients should receive at least a dose of 75 U kg^{-1} [9,16]. In our protocol, designed to compare a high purity FIX concentrate with a PCC, we did not consider convenient to use a dose larger than 50 U kg^{-1} of FIX concentrates. The present study shows that the IR, terminal half-life, MRT, V_{dss} and CL of both products used, are in the range of those reported in the literature for other high-purity FIX concentrates and PCC [16–18]. The terminal half-life for purified FIX concentrate product B of 23.5 ± 12.3 h found in this study, is comparable with the reported values for other high purity concentrates, evaluated in a similar total blood sampling time of ≤50 h, (Mononine 23 ± 8.1 h, Immunine 17 ± 4 h, Nanotiv 19.8 ± 3 h) [17–19] and it is similar to the PCC product A (22.3 ± 7 h) used by us. Incremental recovery of Aimafix (0.76 ± 0.13) is similar to the described for Mononine (0.6 ± 0.14) [17]. Among the four more important parameters: CL, MRT, V_{dss} and IR to consider for bioequivalence [20,21], we have found significant differences between the two preparations in CL and IR. The influence of the time elapsed between infusions of the two study products could not be discarded. On the contrary, the crossover study does not show a significant difference in treatment by period effect, so it seems unlikely that these differences in FIX behaviour between products would be clinically important. The study performed 3 and 6 months later on the same subjects yielded similar pharmacokinetics results.

Table 4. Comparative evaluation of thrombogenicity as measured before a single dose of UMAN COMPLEX DI (product A)/AIMAFIX DI (product B) at predose, and after 30 min and 6 h.

	UMAN COMPLEX DI (product A)			AIMAFIX DI (product B)		
Predose	Mean	SD	*t*-test (*P*-value)	Mean	SD	*t*-test (*P*-value)
Fibrinogen (mg dL^{-1})	343.77	75.47	–	316.53	58.36	–
ATIII (%)	100.92	9.63	–	92.38	27.22	–
TAT (μg L^{-1})	1.02	0.66	–	0.86	0.25	–
D-D (μg L^{-1})	486.69	274.52	–	433.07	305.8	–
F1 + 2 (nm L^{-1})	0.71	0.38	–	0.79	0.39	–
Platelet ($\times 10^9$ L^{-1})	243.77	60.74	–	254.53	66.35	–
30 min postinfusion			Pre vs. 30 min			Pre vs. 30 min
Fibrinogen (mg dL^{-1})	339.08	76.78	0.70	323.84	62.38	0.37
ATIII (%)	100.46	8.91	0.77	98.76	10.14	0.43
TAT (μg L^{-1})	2.40	1.09	0.0001	1.39	0.78	0.049
D-D (μg L^{-1})	511.54	309.32	0.38	414.61	277.27	0.65
F1 + 2 (nm L^{-1})	2.69	1.03	0.0005	0.71	0.3	0.5
Platelet ($\times 10^9$ L^{-1})	252.00	59.68	0.11	244.07	48.86	0.32
6 h postinfusion			Pre vs. 6 h			Pre vs. 6 h
Fibrinogen (mg dL^{-1})	353.92	72.32	0.17	324.76	64.82	0.52
ATIII (%)	99.31	9.38	0.20	104.84	7.76	0.15
TAT (μg L^{-1})	1.38	0.63	0.05	1.01	0.55	0.42
D-D (μg L^{-1})	486.15	263.99	0.92	425.38	227.17	0.88
F1 + 2 (nm L^{-1})	1.32	0.45	0.00004	0.64	0.36	0.06
Platelet ($\times 10^9$ L^{-1})	262.69	57.06	0.0016	273.84	57.38	0.028

In relation to TG, significant differences were found. The increase in F1 + 2 and in TAT, more than twice the baseline level, observed with the PCC (product A) illustrates the coagulation activation properties of this product. It could make it useful in some cases of FVIII or FIX inhibitors. On the contrary, the purified FIX (product B) shows a very low and transitory formation of TAT, which differs significantly from the values found with product A, and speaks in favour of a poor prothrombotic activity of this product. In regard to TAT levels, it has been reported that mean TAT values could increase after infusion of high purity concentrates but less pronounced than with PCC and possibly related to its presence in the preparation [18].

In conclusion, the study showed that both plasma-derived products containing FIX, the high purity concentrate, product B, as well as the prothrombin complex concentrate, product A, were well tolerated, and there was no evidence of inhibitor development during the follow-up period with both products. Both products showed clinical efficacy in controlling bleeding episodes in patients during the 6-month follow-up period. The pharmacokinetic parameters evaluated are in agreement with those previously observed in similar preparations of FIX concentrates. The results in bioequivalence could allow us the use of this PCC in haemophilia B bleedings. In the case of repeated doses or the presence of other thrombogenic risk factors such as liver disease, surgery or prolonged immobilization the potential thrombogenic activity of this PCC concentrate should be considered so as to take additional thromboprophylaxis measures individually in each case or to use purified FIX is available. Similar considerations must be taken into account if this PCC is used for the treatment of FII and FX deficiencies.

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