

## Blood Coagulation, Fibrinolysis and Cellular Haemostasis

# Pharmacokinetics of Beriplex P/N prothrombin complex concentrate in healthy volunteers

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### Summary

Prothrombin complex concentrates (PCCs) are widely administered for emergency oral anticoagulation reversal and for coagulation defects in liver disease. Pharmacokinetic data may help to optimize treatment. The objective of this study was to characterize the pharmacokinetics of a PCC (Beriplex P/N) containing coagulation factors II (FII), VII (FVII), IX (FIX) and X (FX) and anticoagulant proteins C and S. Fifteen healthy volunteers received a single rapid 50 IU/kg infusion of PCC and underwent frequent blood sampling until 144 hours (h) after infusion. Coagulation factors and anticoagulant protein pharmacokinetic parameters were estimated by non-linear regression. The mean infusion rate of PCC was 7.9 ml/min, equivalent to 196.4 IU/min. By the earliest post-infusion sampling point at 5 minutes (min),

plasma FIX concentration increased by a median of 73%. Median increases in FII, FVII and FX at 5 min were 122%, 62% and 158%, respectively. Proteins C and S also increased rapidly. The median terminal half-life of FIX was 16.7 h, FII 59.7 h, FVII 4.2 h and FX 30.7 h. The median in-vivo recovery of FIX was 1.57 %/IU/kg and that of the other three coagulation factors > 2 %/IU/kg. Plasma concentration of thrombogenicity marker D-dimer did not increase, and there was no clinical evidence of thrombosis. Through up to 12 weeks follow-up there were no laboratory findings indicating PCC-related viral exposure. Rapid PCC infusion produced prompt sustained increases in coagulation factors and anticoagulant proteins with no clinical evidence of thrombosis or viral transmission.

### Keywords

Prothrombin complex concentrates, pharmacokinetics, safety, blood coagulation factors, anticoagulation reversal

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### Introduction

Plasma-derived prothrombin complex concentrates (PCCs) contain the four vitamin K-dependent coagulation factors II (FII), VII (FVII), IX (FIX), and X (FX) produced by the liver. In addition to these four factors, PCCs can also contain the vitamin K-dependent anticoagulant proteins C and S, which are also produced by the liver. Originally developed in the late 1950s for the replacement of FIX in haemophilia B patients, PCCs are primarily used in clinical practice today for reversal of coumarin anticoagulation and management of coagulation defects in patients with liver disease. Coumarin oral anticoagulants, the most widely used of which are warfarin, phenprocoumon and acenocoumarol, act by inhibiting the synthesis of functional vitamin

K-dependent coagulation factor molecules (1). Because the factors are concentrated, PCCs in small volumes can rapidly reverse coumarin effects (2–5) and are recommended for emergency situations (6–11).

A challenge in reversing anticoagulation in these high-risk patient populations is to prevent life-threatening bleeding or to allow emergency surgery without heightening the risk of thromboembolic events (12, 13). The counterbalancing properties of the coagulation factor and anticoagulant protein components of PCCs are thought to help achieve this objective. Knowledge of PCC pharmacokinetics (PK) could be of value in determining dosing. Surprisingly, despite the long clinical experience with PCCs, a rigorous PK study of coagulation factors and anticoagulant proteins following PCC administration has yet to be reported.

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The PK of FIX have thus far been better delineated than those of the other coagulation factors, probably in major part due to the role of PCCs in the treatment of haemophilia B and their subsequent use as a standard of comparison in the development of highly purified FIX concentrates (14–18). Although the intravascular persistence of FVII is known to be shorter than that of FIX, FII (prothrombin) and FX, very few PK data are currently at hand pertaining to FII, FVII, FX and the anticoagulant proteins contained in PCC preparations. PK profiles are increasingly being used to individualize coagulation factor treatment in haemophilia A and B (19) and explore variability in patient response. For PCC therapy it has not been feasible to capitalize fully on PK due to insufficient data.

Reliable analysis of PK depends upon sampling frequency and length. Sampling over a sufficiently long time period is especially important when the proteins of interest exhibit long half-lives and slow clearance, as in the case of FII and FX (16). The present study was designed to provide the first detailed PK characterization of all major PCC components through frequent sampling up to 144 hours (h).

## Materials and methods

This prospective PK study was conducted from June to September 2005 at the Clinical Pharmacology Research Unit Germany of Parexel International GmbH, Berlin, Germany. Study participants rendered their informed written consent. The study protocol was approved by the ethics committees of the Ärztekammer Berlin and the Berlin Landesamt für Gesundheit und Soziales.

### Volunteer selection

Healthy male and female volunteers 18–65 years of age with 18–29 kg/m<sup>2</sup> body mass index were eligible for inclusion. Exclusion criteria consisted of: treatment with any investigational medication within 30 days before study entry; blood donation within the preceding three months; known hypersensitivity to human plasma proteins; inhibitors to FII, FVII, FIX or FX; exposure to PCC concentrates or other blood-derived products over the preceding four weeks; a history of deep vein thrombosis, pulmonary embolism or heparin-induced thrombocytopenia type II; protein C deficiency; smoking; detectable hepatitis B surface antigen (HBsAg) or antibodies directed against hepatitis C virus (HCV) or human immunodeficiency virus 1 (HIV-1) or 2 (HIV-2); and urinary alcohol or history of alcohol or drug abuse within the preceding two years. In addition, female candidates were excluded on the basis of contraceptive use, pregnancy and current or planned breast feeding.

The screening process included complete physical examination, measurement of vital signs, blood and urine sampling and, in females, a pregnancy test. With the collected blood samples haematological and biochemical tests, drug determinations, and HBsAg, anti-HCV and anti-HIV-1 and -2 assays were performed. The measured drugs were: alcohol, barbiturates, opiates, amphetamines, cocaine, cannabis and benzodiazepines. Urinalysis was also carried out.

**Table 1: Composition of Beriplex P/N<sup>†</sup>.**

Constituent	Concentration (IU/ml)
	Mean (SD), n = 3
Factor II	31.0 (3.4)
Factor VII	16.2 (1.9)
Factor IX	28.9 (2.2)
Factor X	40.5 (3.3)
Protein C	34.8 (1.8)
Protein S	25.4 (0.4)
Protein Z <sup>‡</sup>	36.2 (3.5)
Antithrombin III <sup>‡</sup>	0.59 (0.02)
Heparin <sup>‡</sup>	0.52 (0.07)

<sup>†</sup>Based on determinations in three consecutive batches. Data on file, CSL Behring. <sup>‡</sup>Not investigated in present study.

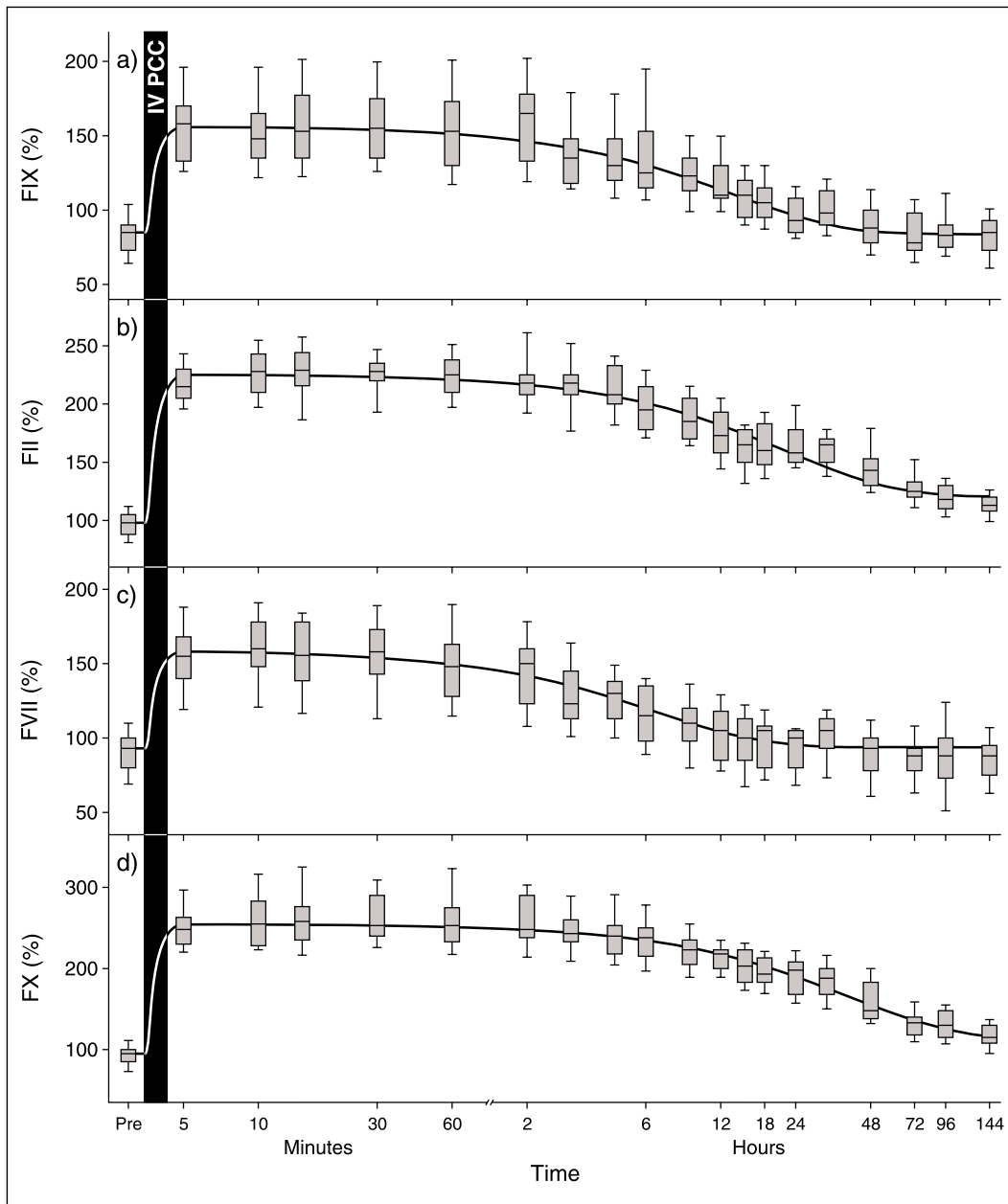
### Infusion

Volunteers reported to the study center on the evening before infusion and remained for 24 h after infusion, returning periodically thereafter for further planned evaluations, including blood sampling. Concomitant medications were discouraged, and alcohol, stimulants and oral contraceptives prohibited during the study. Medications considered necessary for the welfare of the volunteer and unlikely to interfere with the activity of the test PCC were allowed at the discretion of the investigator.

A single 50 IU/kg dose of PCC (Beriplex<sup>®</sup> P/N, CSL Behring GmbH, Marburg, Germany) based on FIX content was infused intravenously (IV) at a maximum rate of 210 IU/min under perfusor control. The major components of Beriplex P/N (Table 1) are the coagulations factors (FII, FVII, FIX and FX) and anticoagulant proteins C and S that are depleted by coumarin therapy.

### Assessments

Single 5 ml citrated blood samples were drawn for assays of PCC coagulation factors and anticoagulant proteins prior to infusion and at intervals of 5, 10, 15 and 30 minutes (min) and 1, 2, 3, 4.5, 6, 9, 12, 15, 18, 24, 32, 48, 72, 96 and 144 h after the end of infusion. Thrombogenicity was evaluated by measurements of prothrombin fragment 1+2 (F<sub>1+2</sub>) and D-dimer before infusion and at 15 min and 3 and 24 h afterward. Before infusion a battery of viral assays was performed for: antibodies against hepatitis A virus (HAV); anti-HCV; anti-HIV-1 and -2; antibodies to parvovirus B19 (B19V); and HBsAg. In addition, reverse transcriptase polymerase chain reaction (RT-PCR) was used to measure HAV, HCV, hepatitis B virus (HBV), HIV-1 and B19V. Measurements of B19V by RT-PCR were repeated at 7–10 days after infusion; anti-B19V and anti-HAV at 28–31 days; and anti-HCV, anti-HIV-1 and -2, anti-HAV and HBsAg at approximately 12 weeks. Adverse events occurring during the study through 7–10 days after infusion were recorded.



**Figure 1: Plasma concentrations of a) FIX, b) FII, c) FVII and d) FX in healthy volunteers receiving a single PCC infusion.** Lower and upper box boundaries show 25th and 75th percentiles, respectively, lower and upper error bars 10th and 90th percentiles, respectively, and horizontal line within bars the median. Smooth curves show best least squares fit exponential decay functions. At 15 min  $n = 14$ ; at all other time points  $n = 15$ .

### Pharmacokinetics

Analysis of FII, FVII, FIX, FX, protein C and protein S PK was conducted by non-linear regression modeling using WinNonLin Professional 3.2 computer software (Pharsight Corp., Mountain View, CA, USA) and incorporating endogenous baseline concentrations. Either a one- or two-compartment model was selected based on goodness of fit to the empirical individual subject data. Estimated PK parameters were half-life ( $t_{1/2}$ ), area under the concentration-time curve (AUC), clearance, mean residence time (MRT) and volume of distribution at steady state ( $V_{d,ss}$ ). In-vivo recovery (IVR) was calculated as the maximum percent increase in plasma concentration within 3 h of infusion divided by dose in IU/kg.

### Laboratory tests

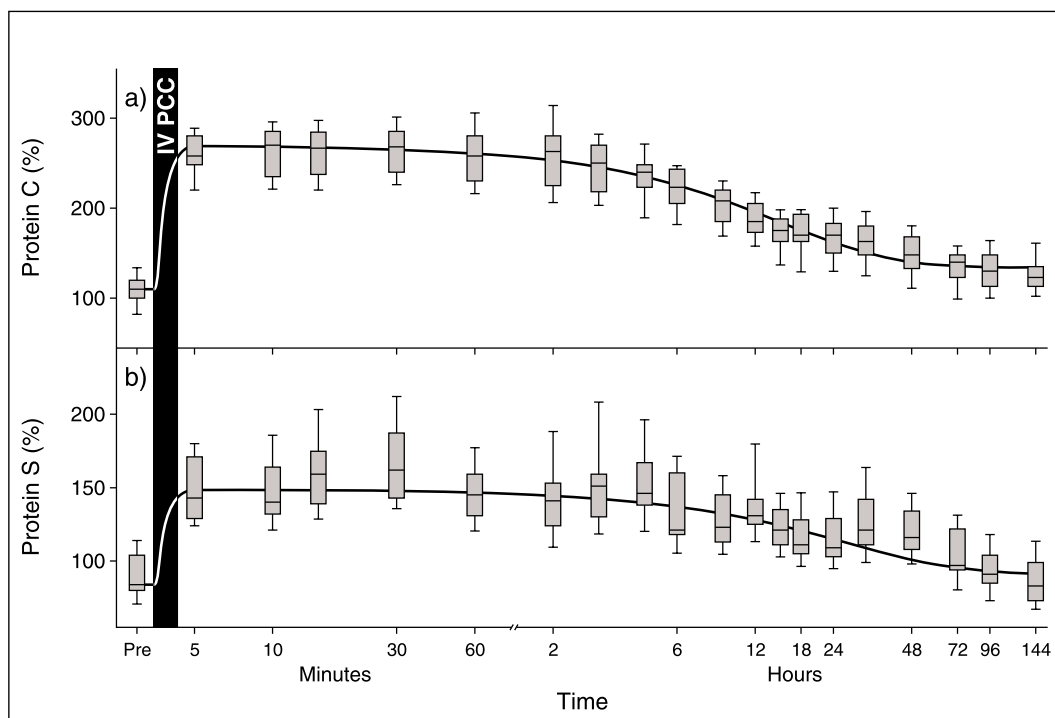
All laboratory tests were performed at the following central laboratories: the Gesellschaft für Labortechnologie in Wissenschaft und Technik, Berlin, Germany; the Laboratory of the Prof. Gisela Enders Partnership, Stuttgart, Germany; Parexel International GmbH, Berlin, Germany; and CSL Behring GmbH, Marburg, Germany.

FII, FVII, FIX and FX were determined by one-stage assay and protein C by chromogenic assay, all using the BCT<sup>®</sup> Analyzer (Dade Behring, Marburg, Germany), and protein S (Kordia, Leiden, the Netherlands) and thrombogenic marker  $F_{1+2}$  by enzyme immunoassay on the BEP<sup>®</sup> III Processor (Dade Behring). D-dimer was measured by latex-enhanced immunoturbidometric test on the BCT<sup>®</sup> Analyzer. The intra-assay coefficients of variation for FII, FVII, FIX, FX and proteins C and S were 2.0, 1.4,

**Table 2: PCC coagulation factor pharmacokinetics.**

Parameter	Median (IQR), n = 15			
	FIX	FII	FVII	FX
Terminal half-life (h)	16.7 (14.2–67.7)	59.7 (45.5–65.9)	4.2 (3.9–6.6)	30.7 (23.7–41.4)
IVR (%/IU/kg)	1.57 (1.38–1.90)	2.11 (1.95–2.45)	2.43 (2.33–2.77)	2.08 (1.94–2.39)
AUC (IU/dl-h)	1490 (1153–2376)	6577 (5870–7912)	424 (331–742)	6707 (5234–8577)
Clearance (ml/kg-h)	3.63 (2.27–4.68)	0.97 (0.81–1.09)	7.06 (4.04–9.05)	1.25 (0.98–1.60)
MRT (h)	21.6 (17.1–83.8)	81.7 (62.0–87.6)	6.1 (5.6–9.5)	44.3 (34.2–59.8)
V <sub>d<sub>ss</sub></sub> (ml/kg)	92.4 (76.2–182.2)	71.0 (61.2–78.9)	41.8 (39.3–52.5)	56.1 (52.9–60.1)

AUC, area under the concentration-time curve; IQR, interquartile range; IVR, in vivo recovery; MRT, mean residence time; V<sub>d<sub>ss</sub></sub>, volume of distribution at steady state.



**Figure 2: Plasma concentrations of a) protein C and b) protein S in healthy volunteers receiving a single PCC infusion.** At 15 min n = 14; at all other time points n = 15. Graphic conventions as in Figure 1.

0.3, 2.1, 2.4 and 4.0%, respectively. Corresponding inter-assay coefficients of variation were 4.1, 1.3, 0.9, 4.7, 4.0 and 8.3%.

Chemiluminescent microparticle immunoassays (Abbott ARCHITECT ci8200, Abbott Diagnostics, Wiesbaden, Germany) were performed for anti-HCV, anti-HIV-1 and -2, anti-HAV and HBsAg using the ARCHITECT anti-HCV, HIV Ag/Ab Combo, HAVAb-G and HBsAg reagents (Abbott Diagnostics), respectively. Measurement of anti-B19V IgG/IgM was by the Parvovirus B19 IgG and IgM Enzyme Immunoassays (Biotrin, Dublin, Ireland) using the BEP<sup>®</sup> III Processor. LightCycler<sup>®</sup> instrumentation and reagents (Roche Diagnostics GmbH, Mannheim, Germany) were used for RT-PCR determination of HAV and B19V and COBAS AmpliPrep<sup>™</sup> and COBAS TaqMan (Roche Diagnostics) for HCV, HBV and HIV-1.

### Statistical analysis

Descriptive statistics consisted of the mean, median, standard deviation (SD) and interquartile range (IQR). Median differences and

their exact 95% confidence intervals (CI) were determined by Hodges-Lehmann estimation using StatXact 6.3 (Cytel Software Corp., Cambridge, MA, USA) statistical software. Absence of zero from the CI implies a statistically significant difference ( $p < 0.05$ ).

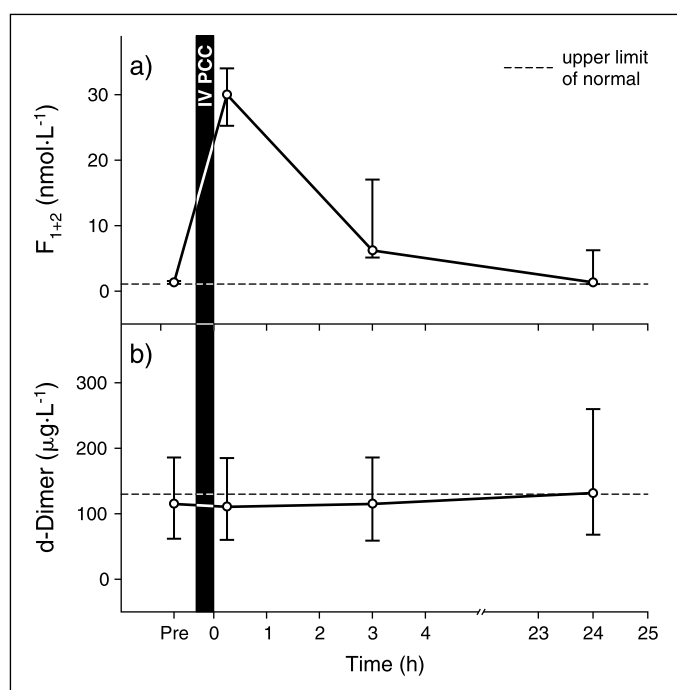
### Results

Fifteen volunteers, seven female (47%) and eight male (53%), were enrolled in the study. All were Caucasian. Their mean (SD) age was 41 years (13 years), body mass index 24.3 kg/m<sup>2</sup> (1.9 kg/m<sup>2</sup>) and body weight 74.5 kg (9.6 kg). All received the single PCC infusion and finished the planned study follow-up. PCC was infused at a mean rate of 7.9 ml/min (SD, 0.5 ml/min, n = 15), equivalent to 196.4 IU/min (SD, 12.2 IU/min, n = 15), over a mean period of 18.9 min (SD, 1.9 min, n = 15). Measurements were not recorded for the 15 min sampling point in one volunteer. Otherwise the data sets for plasma PCC coagulation factor and anticoagulant protein concentrations were complete.

**Table 3: PCC anticoagulant protein pharmacokinetics.**

Parameter	Median (IQR), n = 15	
	Protein C	Protein S
Terminal half-life (h)	47.2 (28.4–65.1)	49.1 (39.0–59.7)
IVR (%/IU/kg)	2.76 (2.59–3.07)	2.02 (1.7–2.17)
AUC (IU/dl·h)	5276 (2847–7092)	3667 (2854–4098)
Clearance (ml/kg·h)	1.10 (0.82–2.04)	1.11 (0.99–1.42)
MRT (h)	57.0 (31.3–85.3)	69.2 (53.3–85.0)
Vd <sub>ss</sub> (ml/kg)	62.9 (50.1–65)	76.6 (69.8–85.8)

AUC, area under the concentration-time curve; IQR, interquartile range; IVR, *in vivo* recovery; MRT, mean residence time; Vd<sub>ss</sub>, volume of distribution at steady state.



**Figure 3: Median plasma concentrations of a)  $F_{1+2}$  and b) D-dimer in healthy volunteers receiving a single PCC infusion.** Error bars show IQR. At 15 min  $n = 14$ ; at all other time points  $n = 15$ .

### PCC coagulation factors

The single 50 IU/kg PCC IV infusion produced a rapid and sustained rise in plasma concentrations of FIX, FII, FVII and FX (Fig. 1). Maximal levels were attained by the earliest post-infusion sampling point at 5 min. The median increase in plasma FIX concentration at 5 min compared with the pre-infusion level was 73% (CI, 66–80 %). Corresponding median increases were 122% (CI, 114–128 %) for FII, 62% (CI, 56–70 %) for FVII and 158% (CI, 149–167 %) for FX.

The results of PK analysis for PCC coagulation factors are presented in Table 2. Of the four PCC coagulation factors, FII exhibited the longest median terminal  $t_{1/2}$  (59.7 h) and FVII the shortest (4.2 h). The median terminal  $t_{1/2}$  of FIX was 16.7 h. The mean (42.4 h) was larger than the median predominantly because of FIX terminal  $t_{1/2}$  estimates > 100 h in three volunteers. With

exclusion of these three outlying values the mean was 23.7 h. Such differences between the median and mean were not evident for FII, FVII and FX.

The terminal  $t_{1/2}$  of FIX in women was longer by a median of 7.1 h (CI, –8.3 to 92.3 h) versus men and of FII by 15.0 h (CI, –5.9 to 44.4 h), although these differences of 43% and 25%, respectively, were not statistically significant. Corresponding median gender-related differences in terminal  $t_{1/2}$  of FVII (0.6 h; CI, –2.1 to 2.7 h) and FX (–1.5 h; CI, –13.8 to 8.9 h) were relatively minor.

Model-independent measures of intravascular persistence were consistent with the terminal  $t_{1/2}$  data. Thus, clearance was the most rapid and MRT the shortest for FVII, but conversely the slowest and longest, respectively, for FII (Table 2). For FIX, median clearance was 3.63 ml/kg h and median MRT 21.6 h. Outlying FIX clearance and MRT estimates were also obtained in the three volunteers with FIX terminal  $t_{1/2}$  > 100 h. With exclusion of these outliers mean FIX clearance was 4.17 ml/kg h and mean FIX MRT 25.8 h.

The median IVR of FIX was 1.57 %/IU/kg, while that of FII, FVII and FX exceeded 2%/IU/kg, as shown in Table 2. Vd<sub>ss</sub>, a model-independent measure of distribution, was greater for FIX (median, 92.4 ml/kg) than the other three PCC coagulation factors.

### PCC anticoagulant proteins

The temporal patterns of post-infusion change in PCC anticoagulant proteins were generally similar to those of the PCC coagulation factors (Fig. 2). A rapid rise in both plasma proteins C and S to maximal concentrations was observed by 5 min. The median increases from baseline to 5 min were 149% (CI, 140–160%) in protein C and 59% (CI, 54–66%) in protein S. The smaller increase in protein S is likely at least partly attributable to the lower concentration of this anticoagulant protein in Beriplex P/N as compared with that of protein C (Table 1).

PK analysis of changes in protein C and protein S concentration is presented in Table 3. The IVR of both these PCC anticoagulant proteins was greater than 2 %/IU/kg.

### Thrombogenicity markers

By the first post-infusion measurement time at 15 min a transient elevation in plasma  $F_{1+2}$  level was observed, which subsided by 3 h (Fig. 3).  $F_{1+2}$  concentration had returned to the baseline level by 24 h. In contrast, no post-infusion changes were evident in plasma D-dimer concentration (Fig. 3). In none of the volunteers was any clinical evidence of thrombosis observed, and mean platelet count 24 h after infusion ( $247 \times 10^9/l$ ; SD,  $41 \times 10^9/l$ ,  $n = 14$ ) was unchanged compared with baseline ( $246 \times 10^9/l$ ; SD,  $44 \times 10^9/l$ ,  $n = 15$ ).

### Viral markers

Prior to PCC infusion, results of anti-HAV assays were positive in six volunteers and of anti-B19V assays in ten. All baseline assays of anti-HIV-1, anti-HIV-2, HBsAg and anti-HCV were negative as were all RT-PCR measurements of B19V. During periodic post-infusion viral assessments through the 12-week follow-up period, no positive result was obtained in any volunteer with a corresponding negative baseline result.

## Adverse events

At seven days after infusion one female volunteer developed a mild common cold, which was judged to be unrelated to PCC exposure. No other adverse events of any kind were encountered during the study.

## Discussion

More precise understanding of PCC PK may aid in treatment decisions and allow a better appraisal of the balance between their haemostatic efficacy and thrombogenic potential. In routine clinical practice, pretreatment international normalised ratio (INR) values but not individual coagulation factor concentrations are likely to be available for patients under oral anticoagulation or with liver disease requiring PCC treatment. Successful emergency reversal of oral anticoagulation has been demonstrated by selection of initial PCC dose based on pretreatment INR (3). The value of comprehensive PK data for individual PCC components may lie mainly in helping devise an appropriate treatment regimen for patients who need multiple PCC infusions. Knowledge of  $t_{1/2}$  values for PCC components might, for instance, aid in timing successive doses to maintain desired levels of individual coagulation factors and anticoagulant proteins.

This investigation has provided the first detailed PK analysis following a rapidly administered single bolus dose PCC infusion in healthy volunteers. All major coagulation factor and anticoagulant protein PCC components increased promptly after infusion. PK analysis revealed substantial differences among the components with respect to the time course of post-infusion decline in coagulation factors and anticoagulant proteins. As expected, FVII displayed the shortest median terminal  $t_{1/2}$  (4.2 h) and FII the longest (59.7 h). Intermediate values were estimated for FIX (16.7 h) and FX (30.7 h). The median terminal  $t_{1/2}$  of protein C (47.2 h) was similar to that of protein S (49.1 h).

Inadequate total blood sampling time can decrease the accuracy of estimated PK parameters, especially when concentrations decline over a prolonged period of time (16). Specifically, insufficient duration of blood sampling may result in underestimation of  $t_{1/2}$  and overestimation of clearance (20). In this study of normal volunteers, sampling was extended to 144 h, much longer than the 48 or 72 h typical in previous investigations that were primarily focused on the PK of FIX in haemophilia B patients. This relatively long sampling period was intended to avoid underestimation of PK parameters, particularly for those PCC components with lengthy plasma persistence.

Prior research on coagulation factor PK has focused predominantly on FIX and factor VIII because of their importance as replacement therapy in haemophilia B and A, respectively. For accurate PK analysis of FIX a sampling period of at least 56 h has been recommended (16). There have been no previous reports on the terminal  $t_{1/2}$  of the FIX component in Beriplex P/N. There have, however, been observations on the FIX component of other PCCs used in haemophilia patients (14, 15, 17, 18, 21, 22). Sampling ended at 72 h in one of those studies (15) and at 48 h or earlier in all the others (14, 17, 18, 21, 22). The mean terminal  $t_{1/2}$  of FIX in PCCs ranged from 17.3 to 23.8 h (14, 15, 17, 18, 21, 22). The corresponding reported range of means for purified plasma-derived FIX was 18–34 h (20). In a PK study using radiolabeled

FIX in patients with haemophilia B, mean terminal  $t_{1/2}$  was found to be 23 h (23). In the present study involving sampling up to 144 h, the mean after exclusion of three outliers (23.7 h) was comparable in magnitude to these previous findings with other PCCs or FIX. Similar outliers would not have been observed in the prior studies of PCC PK because of their far shorter sampling periods. In an empirical study, individual patient  $t_{1/2}$  estimates more than doubled with increasing sampling time over the range from 24 to 104 h (16).

With exclusion of outliers, a mean of 25.8 h was calculated for FIX MRT, the model-independent PK counterpart of terminal  $t_{1/2}$ . The mean FIX MRT values reported in other PK analyses of PCCs have ranged from 17.3 to 28.4 h (14, 15, 17, 18, 21, 22). Mean FIX clearance after outlier exclusion (4.17 ml/kg-h) and mean  $V_{d_{ss}}$  (114.1 ml/kg) were in line with previously reported estimates for these two PK parameters after PCC administration of 4.8–6.0 ml/kg h and 99.9–190.4 ml/kg, respectively (14, 15, 17, 18, 21, 22). The PK of the FII, FVII and FX components in PCCs have not been evaluated in prior studies.

Protein C, a zymogen, and its cofactor protein S are vitamin K-dependent proteins that decrease during coumarin therapy and in patients with liver disease. In the present study, the median terminal  $t_{1/2}$  of protein C was 47.2 h. That comparatively long value suggests lasting availability of this anticoagulant protein after PCC infusion that may maintain balance versus the coagulation factors and help minimize thrombotic risk. On the other hand, most prior evidence has indicated a substantially shorter terminal  $t_{1/2}$  for protein C. In a study of eight normal volunteers receiving a highly purified preparation of protein C, the estimated terminal  $t_{1/2}$  was 10.9 h (24); however, blood was sampled only for 24 h. In another study restricted to 24 h, terminal  $t_{1/2}$  was 7.9 h for the protein C component of a PCC in patients under phenprocoumon anticoagulation (25). In additional studies involving congenital protein C deficiency the range of protein C terminal  $t_{1/2}$  values was 8–10 h (26–30). The mean terminal  $t_{1/2}$  of protein C in patients beginning warfarin treatment for acute deep vein thrombosis was estimated as 13.9 h (31). In contrast, the estimated terminal  $t_{1/2}$  of protein C was 32 h in healthy volunteers receiving warfarin (32). In light of these disparate findings, further studies are needed to establish more firmly the PK of protein C. In the present study, the mean terminal  $t_{1/2}$  of protein S was 50.4 h, an estimate comparable with the 42.5 h value obtained in a study of patients on warfarin therapy (31).

A major concern regarding PCCs is their thrombogenic potential (33). In addition to the four procoagulant factors, Beriplex P/N contains substantial concentrations of the antithrombotic proteins C and S (Table 1). In clinical studies and pharmacovigilance, thrombotic complications during the use of Beriplex P/N to reverse oral anticoagulation and manage critical illness and severe liver disease have been rare (2, 3, 34–37). A prothrombotic state has been defined as a procoagulant shift in the balance between pro- and anticoagulant enzyme activity in the coagulation pathway, but without clinical signs of thrombosis or laboratory evidence of fibrin deposition (38).  $F_{1+2}$  and D-dimer serve as surrogate markers of a prothrombotic state (39). A number of clinical studies have demonstrated elevations in surrogate markers of thrombogenicity following infusion of clinical PCC doses in patients with haemophilia B, while such increases have not been

observed after administration of purified FIX concentrate (14, 15, 40–44).

In the present study, a transient elevation in plasma  $F_{1+2}$  level was observed without change in D-dimer. The elevation was likely the result of infused exogenous  $F_{1+2}$  rather than an endogenous prothrombotic state. The administered PCC contains measurable  $F_{1+2}$  at a mean concentration of 1.00  $\mu\text{M}$  (SD, 0.21  $\mu\text{M}$ ,  $n = 8$ ). It is known that some thrombin generation occurs during the manufacturing process; however, thrombin activity in the final product is undetectable. The expected median peak increase in circulating  $F_{1+2}$  level due to infused exogenous  $F_{1+2}$  was 49.7 nM, as calculated from the known mean  $F_{1+2}$  concentration in the PCC, the volume of administration, and the estimated plasma volume derived from measured individual subject weight, height and haematocrit using separate empirical regression coefficients for normal adult men and women (45). The observed median increase at the 15 min time point was 28.9 nM. Hence, the entirety of the observed elevation can potentially be explained by the  $F_{1+2}$  content of the PCC. Consistent with this interpretation was the rapid  $F_{1+2}$  concentration decline after 15 min, as would be predicted after infusion of a molecule with an estimated circulating half-life of approximately 90 min (40). Other studies have also shown an increase in  $F_{1+2}$  after administration of PCCs (34, 35, 43, 44, 46). It is noteworthy that the healthy volunteers in the present study were exposed to supra-normal coagulation factor levels but nevertheless displayed no clinical evidence of thrombosis.

Another concern with plasma-derived preparations such as PCCs is possible viral transmission. Beriplex P/N is prepared from plasma screened by polymerase chain reaction and sub-

jected to the dual viral elimination procedures of pasteurization and nanofiltration (47–49). There was no evidence of viral exposure in this study. These present thrombogenicity and viral exposure results add further support to the excellent long-term safety record of Beriplex P/N extending from the introduction of this PCC in 1996.

This study in healthy volunteers has laid a foundation for future PK studies of PCCs in patients requiring oral anticoagulation reversal or management of critical illness or severe liver disease. The present findings add to previous empirical data indicating the importance of adequate blood sampling frequency and duration in the conduct of PK studies. One notable characteristic of the time versus concentration curves shown in Figure 1 was the relatively stable levels of the four coagulation factors during the first hour after PCC administration. This observation suggests the feasibility of less frequent early sampling in future studies.

Another prominent feature of the data shown in Figure 1 was the attainment of maximal concentrations within 5 min after infusion. Thus, PCC administration achieved a very rapid rebalancing of the coagulation system, which is essential in situations such as emergency haemorrhage. Another demonstrated advantage of Beriplex P/N was the rate with which it could be infused. The 8 ml/min infusion rate in this study was faster than that reported for other PCCs (46, 50). The combination of high infusion rate and suitability for fast reconstitution at room temperature makes Beriplex P/N useful for undelayed stabilization of patients.

The present results demonstrate prompt sustained increases in coagulation factors and anticoagulant proteins after rapid PCC infusion. These desired effects were produced without clinical evidence of thrombosis or viral transmission.

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