Recombinant activated factor VII and prothrombin complex concentrates have different effects on bleeding and arterial thrombosis in the haemodiluted rabbit

F. Le Saché¹ ², B. Le Bonniec¹ ², P. Gaussem¹ ² ³, B. Dizier¹ ², M. Tagzirt¹ ², A. Godier¹ ² ⁴, J. Emmerich¹ ² and C.-M. Samama¹ ² ⁴ *

¹ INSERM UMRS 765, ² Université Paris Descartes, ³ Department of Biological Haematology, Georges Pompidou University Hospital, and ⁴ Department of Anaesthesiology and Intensive Care Medicine, Hôtel-Dieu University Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France

* Corresponding author. E-mail: marc.samama@htd.aphp.fr

Editor’s key points

- Recombinant activated factor VII (rFVIIa) and prothrombin complex concentrates (PCCs) are often used for treating major haemorrhage.
- Evidence for their efficacy and safety is lacking.
- The effects of rFVIIa and PCC were investigated in a rabbit model.
- Neither agent reduced hepatosplenic blood loss.
- Both controlled bleeding time without affecting thrombosis risk.

Background. Recombinant activated factor VII (rFVIIa) is indicated in bleeding patients when a life-threatening haemorrhage occurs. Prothrombin complex concentrates (PCCs) are also used for this indication in several countries, without any evidence-based rationale. Our objective was to compare the efficacy and safety of PCC and rFVIIa in a model of bleeding and thrombosis in haemodiluted rabbits.

Methods. Forty-eight rabbits were randomly allocated into four groups: a control group and three treatment groups, in which animals were haemodiluted with hydroxyethyl starch 130/0.4 then administered either placebo, 160 µg kg⁻¹ rFVIIa, or 25 IU FIX kg⁻¹ PCC. The primary endpoint was hepatosplenic (HS) blood loss. Secondary endpoints were: (i) ear immersion bleeding time (IBT); (ii) thrombosis risk assessed by cyclic flow reductions (CFRs) of the carotid artery; and (iii) activated partial thromboplastin time (aPTT), and progress of thrombin activity.

Results. Haemodilution increased HS blood loss by 80% from 8 g (5–16) (control group) to 14 g (8–45) (placebo group) (P < 0.01). HS blood loss was not different in animals receiving either rFVIIa [10 g (7–22)] or PCC [15 g (4–33)] (P < 0.05) compared with the placebo group. Ear IBT was reduced with both rFVIIa and PCC. CFRs disappeared after haemodilution and were not restored with any treatment. Although PCC nearly doubled the total amount of thrombin generated, no significant change in the total amount of thrombin was seen in animals treated with rFVIIa.

Conclusions. Neither rVIIa nor PCC reduced HS blood loss, whereas they both controlled the bleeding time, without increasing the thrombosis risk.

Keywords: arterial thrombosis; bleeding; haemodilution; prothrombin complex concentrates; recombinant activated factor VII

Accepted for publication: 15 November 2011

Haemorrhagic shock is a life-threatening condition that may lead to circulatory collapse. Its management involves the specific treatment of the cause of bleeding (surgery, interventional radiology, and therapeutic haemostatic agents) and symptomatic treatment to limit the impact of shock on vital organs.¹ ³ ⁴

Recombinant activated factor VII (rFVIIa) was primarily developed for the treatment of haemophilic patients with inhibitory allo-antibodies against factor VIII or IX but is currently also used to treat severe haemorrhagic shock after surgery, obstetrical procedures, or trauma.⁵ ⁹ However, rare cases of arterial and venous thrombotic complications have been reported.¹⁰ ¹¹ Prothrombin complex concentrates (PCCs) contain the vitamin K-dependent clotting factors (FII, FVII, FIX, FX), and the coagulation inhibitors proteins C and S.¹² The concentration of factor VII in PCC is 300-fold lower than in rFVIIa (8000 IU ml⁻¹). Currently, PCC is recommended for the rapid control of haemostasis in patients suffering from a vitamin K antagonist (VKA) overdose or presenting an active bleeding with VKA therapy, the only indication in which its haemostatic efficacy has been assessed.¹³ ¹⁴ Nonetheless, PCC is widely used for the treatment of bleeding in trauma or surgical patients in several European countries with no evidence-based rationale.⁵ ¹⁵ In addition, very few reports are currently available on the thrombotic events induced by PCC in these indications.¹⁶ ¹⁷

We hypothesized that PCC might control bleeding with less thrombotic risk than rFVIIa. We therefore compared

---

© The Author [2012]. Published by Oxford University Press on behalf of the British Journal of Anaesthesia. All rights reserved. For Permissions, please email: journals.permissions@oup.com
the efficacy and safety of PCC and rFVIIa in a randomized placebo-controlled experiment in rabbits in which bleeding was amplified by haemodilution. We used the Folts canine model of arterial stenosis, which has been adapted to the rabbit and coupled in our laboratory to a bleeding model.18–22

**Methods**

**Animals**

Animals were treated in accordance with the ethical rules of the Institut National de la Recherche Médicale (INSERM), the Institut National de la Recherche Agronomique (INRA), and the Comité Régional d’Ethique en matière d’Expérimentation Animale. The study started was approved by the regional ethics committee (agreement number: CREEA-P2.MS.027.07).

Male, 12–14-week-old, New Zealand rabbits of the same blood group were obtained from the Cegav Breeding Colony (Les Hautes Noèes, St Mars d’Egrenne, France) and allowed to acclimatize for 7 days in an animal facility.

Once randomized, all rabbits entered the clinical protocol. Exclusion criteria were: severe bleeding related to arterial catheterization, anatomical abnormalities, occurrence of less than three cyclic flow reduction (CFR) episodes at P1 catheterization, and anaesthesia-induced mortality.

**Anaesthesia, ventilation, and monitoring**

A 22 G catheter was introduced into the marginal vein of the ear (BD Insyte Autoguard, Le Pont-de-Claix, France). All the next steps were carried out under general anaesthesia. Anaesthesia was induced by 15 mg kg$^{-1}$ ketamine (Ketamine 1000®; VIRBAC Santé Animale, Carros, France) and 0.5 mg kg$^{-1}$ xylazine (Rompun®; Bayer, Leverkusen, Germany) and maintained by a continuous ketamine infusion (50 mg kg$^{-1}$ h$^{-1}$) and by xylazine boluses (0.25 mg kg$^{-1}$). The absence of the corneal reflex was tested with saline drops.

After vertical cold-knife incision and dissection of the pre-tracheal fascia, a tracheostomy was performed and a Y-shaped metal stent was introduced to initiate mechanical ventilation (Harvard Apparatus, Kent, UK) with oxygen-enriched air (1 litre min$^{-1}$) (Air Liquide Santé®, Châtillon, France) at a volume of 5 ml kg$^{-1}$ and frequency of 40 min$^{-1}$.

After dissection of the groin, a 20 G catheter was introduced into the freed femoral artery (Infu-Surg 1000 ml, Ethox® Corp, Buffalo, NY, USA) and connected to a calibrated arterial pressure monitor (BIOPAC®, MP30, Goleta, CA, USA) for continuous monitoring. Heart rate was recorded from the arterial pressure wave. The monitor was connected to an Apple MacBook computer (Apple®, Cupertino, CA, USA). The software application was using BIOPAC Student Lab Pro 3.7.1.1 for Mac OS 10.4.

Body temperature was measured with a rectal probe (Homeothermic Blankets Control Unit®, Kent, UK). A normal core temperature was maintained between 38 and 39°C using a heated table (Animal Heated Table AH 50®, Scientific Research Instruments, Kent, UK).

**Measurement of carotid artery blood flow**

The carotid artery was dissected free from surrounding paratracheal tissues. A Doppler probe (R-series®, Transonic Systems Inc., Ithaca, NY, USA) was then placed around the artery for instantaneous blood flow measurements (TS420, Transonic Systems Inc.). Blood flow was monitored continuously (BIOPAC®) and analysed using a mean flow curve. Once the flow was stable, a silicone vascular clamp (Harvard Apparatus, Holliston, MA, USA) was placed on the artery to induce circumferential stenosis. The extent of stenosis was controlled through air injection to achieve a 15–20% reduction in mean basal blood flow (75% stenosis).

**Induction of arterial injuries and thrombosis procedure**

After recording the carotid blood flow for 5 min, to ensure that the flow rate was stable, an arterial injury of the carotid with de-endothelialization was induced by cross-clamping the middle of the exposed segment of the artery three consecutive times within an elapsed period of 5 s. This was accomplished with a Mayo-Hegar needle holder forceps (Harvard Apparatus, Les Ulis, France) with three ratchet clicks closed. The clamp was then positioned over the injured segments in order to provide CFRs induced by the association of de-endothelialization and carotid stenosis. Carotid blood flow was recorded over three periods of 20 min on the same arterial injury.

During period 1 (P1), one of the three outcomes was observed:

(i) Mean and phasic flow declined gradually until embolus formation. Indeed, blood flow decreased as thrombus size increased in the injured vascular segment until the pressure gradient was such that the thrombus was released and local arterial blood flow was suddenly restored. This is known as a CFR. A rabbit was included in the study only if it underwent at least three spontaneous CFRs during P1.

(ii) If there were fewer than three CFRs, an adjacent carotid segment was injured and recording was resumed for 20 min. If this proved unsuccessful, the contralateral carotid was injured. However, if no CFR was recorded after two series of three injuries of the opposite carotid, the animal was killed.

(iii) In the event of persistent thrombus, the artery was shaken just once to dislodge thrombus and restore blood flow. If thrombus was not dislodged, injury was reproduced on the contralateral artery.

**Bleeding measurements**

The same physician performed measurements throughout. Ear immersion bleeding time (IBT) was measured after a 5 mm long, 1 mm deep incision (Surgicutt®, ITC, Edison, NJ, USA) of the external ear surface. The ear was immersed in a beaker containing saline at 39°C. IBT, defined as the time between incision and complete cessation of bleeding, was measured at the end of each period.
Hepatosplenic (HS) blood loss was measured on two standardized 1.5 cm long, 2 mm wide lesions made at the free edge of the liver and of the spleen by xyphopubic laparotomy. Blood was collected using five swabs placed for 10 min around the liver, spleen, right and left paracolic gutters, and abdominal wall. Blood loss was estimated by weighing the swabs.

Animals were then killed by injection of 20 mg kg\(^{-1}\) xylazine.

**Study protocol**

The study protocol is summarized in Figures 1 and 2. CFRs were monitored under normal haemostatic conditions for 20 min (P1). Rabbits that had three CFRs or more at the end of P1 were randomly assigned to one of the four groups: a control group and three other groups in which rabbits were haemodiluted and then received either placebo, rFVIIa, or PCC.

Haemodilution was achieved by replacing 15 ml kg\(^{-1}\) of blood by 15 ml kg\(^{-1}\) of hydroxyethyl starch 130/0.4 (Voluven\(^{\circledR}\), Fresenius Kabi France, Sèvres, France). The target was around 40% haemodilution to induce disorders mimicking the consumption/dilution of clotting factors in severe haemorrhagic shock. After haemodilution, CFRs were monitored over a second 20 min period (period 2, P2). Treatment was initiated at the end of P2. Ten minutes later either placebo, rFVIIa, or PCC.

Arterial femoral catheter Infusion 4 ml kg\(^{-1}\) h\(^{-1}\)

Catheter 22 G
Marginal ear vein

Anaesthesia
Ketamine–Xylazine

Tracheotomy
Vt = 5 ml kg\(^{-1}\) x 40 min\(^{-1}\)

Arterial femoral catheter

Endothelial injury

Haemodilution
~15 ml kg\(^{-1}\) Blood + 15 ml kg\(^{-1}\) HES 130 (Voluven\(^{\circledR}\))

Surgical procedure

Carotid dissection
Doppler probe

Laparotomy
Spleen incision and liver incision

Haematocrit

IBT, TA, aPTT, fibrinogen

IBT, TA, aPTT, fibrinogen

IBT, TA, aPTT, fibrinogen

Fig 1 Study protocol. VT, Tidal volume; IBT, ear immersion bleeding time; TA, thrombin activity; aPTT, activated partial thromboplastin time; P1, period 1; P2, period 2; P3, period 3.

Haemodilution was achieved by replacing 15 ml kg\(^{-1}\) of blood by 15 ml kg\(^{-1}\) of hydroxyethyl starch 130/0.4 (Voluven\(^{\circledR}\), Fresenius Kabi France, Sèvres, France). The target was around 40% haemodilution to induce disorders mimicking the consumption/dilution of clotting factors in severe haemorrhagic shock. After haemodilution, CFRs were monitored over a second 20 min period (period 2, P2). Treatment was initiated at the end of P2. Ten minutes later either placebo, 160 \(\mu\)g kg\(^{-1}\) rFVIIa (NovoSeven\(^{\circledR}\), Novo Nordisk, Bagsvaerd, Denmark), or 25 IU FIX kg\(^{-1}\) PCC (solution with 40 IU FII ml\(^{-1}\), 25 IU FVII ml\(^{-1}\), 25 IU FIX ml\(^{-1}\), 40 IU FX ml\(^{-1}\), 1.5 IU ml\(^{-1}\) protein C, and 0.6 IU ml\(^{-1}\) protein S, heparin 5 IU ml\(^{-1}\), Kaskadil\(^{\circledR}\), LFB, Les Ulis, France) was administered. CFRs were monitored again for a third 20 min period (period 3, P3). A laparotomy was performed at the end of P3 to measure HS blood loss (primary endpoint). At the end of each period, an arterial blood sample was obtained from the femoral artery catheter to measure the blood coagulation parameters.

**Biological parameters**

Arterial blood was collected through a syringe following a 5 ml purge at the end of each period, and transferred in 0.129 M trisodium citrate tubes (9/1 v/v). Platelet-poor plasma was obtained by double centrifugation at 3000 g for 12 min at 24°C and frozen in aliquots until used.
Activated partial thromboplastin time [Automated APTT (Organon, Durham, NC, USA)] to explore the extrinsic coagulation pathway and fibrinogen (Diagnostica Stago, Asnières, France) were measured using an STA compact analyser (Diagnostica Stago). Haematocrit was measured by centrifugation in heparinized capillary tubes.

### Progress of thrombin activity

Thrombin activity was monitored in platelet-poor plasma rendered non-clottable by adding 8 mM Gly-Pro-Arg-Pro-amide to prevent fibrin polymerization, as previously described. Briefly, clotting was triggered by adding to plasma 5 pM human recombinant tissue factor (Innovin®, Dade-Behring, Marburg, Germany), 15 μl APTT reagent, and 20 mM CaCl2. A 4 μl aliquot was removed every 30 s and immediately added to 76 μl of 50 mM Tris pH 8.0 containing 0.15 M NaCl, 10 mM EDTA, and 100 mM benzamidine to stop thrombin production and irreversible inhibition. Quenched reactions were further diluted (1/20; v/v) in Tris 50 mM pH 7.4 containing 0.15 M NaCl and 0.2% polyethylene glycol (6000) and the amount of thrombin was evaluated from the rate of H-D-Phe-Pip-Arg-pNA (S2238; Biogenic, Maurin, France) hydrolysis. Total thrombin activity was quantified by measuring the area under curve up to 90 s and up to 13 min (end of measure).

### Statistics

Results are given as means (SD) for continuous variables, and as medians with ranges for discontinuous or non-Gaussian variables (CFR, bleeding time, HS blood loss). Non-parametric tests (Kruskal–Wallis, Wilcoxon, and Mann–Whitney U-test) were used for comparisons. A P-value of <0.05 was considered significant. For a 50% reduction in HS bleeding in treated rabbits compared with those receiving placebo, we estimated that, for a 5% α-risk and 20% β-risk, we needed to include 12 rabbits per group.

### Results

A total of 55 rabbits entered the protocol during the 4 month study; six were excluded because less than three CFRs were recorded during P1, another one because of anaesthesia-induced mortality. Baseline characteristics for the 48 included rabbits are given in Table 1, with no significant difference between the groups. Baseline values of the biological parameters were comparable with those previously published.

### Blood loss and ear IBT

Values obtained for HS blood loss (primary endpoint) and IBT are summarized in Table 2. Blood loss increased significantly after haemodilution in the placebo group when compared with the control group with no haemodilution (P<0.01). HS blood loss after rFVIIa administration was not different compared with the control group. HS blood loss after PCC was increased compared with the control group (P<0.01). A graphical representation of HS blood loss is reported in Figure 3. No statistical differences in HS blood loss were observed when comparing rFVIIa with placebo (P=0.56), PCC with placebo (P=1), and rVIIa with PCC (P=0.53).

IBT was comparable along the three periods in the control group, and, as expected, was 30% longer after haemodilution in the three treatment groups. Both rFVIIa and PCC injection decreased IBT compared with placebo (haemodilution) IBT (P<0.05).

### Cyclic flow reductions

Median numbers of CFRs are given in Table 3. In the control group, a gradual CFR exhaustion was observed along periods as previously reported. Haemodilution significantly reduced the number of CFRs in the three treated groups when compared with the control group. The number of CFRs during P3 did not differ significantly between the

---

### Table 1 Baseline characteristics. Kruskal–Wallis test. AP, arterial pressure; mean (sd)

<table>
<thead>
<tr>
<th></th>
<th>Control (n=12)</th>
<th>Placebo, 0.9% NaCl (n=12)</th>
<th>rFVIIa, 160 μg kg⁻¹ (n=12)</th>
<th>PCC, 25 IU FIX kg⁻¹ (n=12)</th>
<th>P, intergroups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>3263 (200)</td>
<td>3219 (210)</td>
<td>3290 (233)</td>
<td>3004 (139)</td>
<td>0.17</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>38.6 (0.2)</td>
<td>38.6 (0.1)</td>
<td>38.7 (0.4)</td>
<td>38.6 (0.4)</td>
<td>0.81</td>
</tr>
<tr>
<td>Mean AP (mm Hg)</td>
<td>85 (16)</td>
<td>89 (22)</td>
<td>98 (18)</td>
<td>75 (14)</td>
<td>0.36</td>
</tr>
<tr>
<td>Systolic AP (mm Hg)</td>
<td>107 (20)</td>
<td>112 (29)</td>
<td>121 (21)</td>
<td>97 (16)</td>
<td>0.22</td>
</tr>
<tr>
<td>Diastolic AP (mm Hg)</td>
<td>73 (14)</td>
<td>78 (19)</td>
<td>86 (17)</td>
<td>64 (13)</td>
<td>0.20</td>
</tr>
</tbody>
</table>

### Table 2 HS blood loss and ear IBT. HS: Mann–Witney test.

<table>
<thead>
<tr>
<th></th>
<th>Control, 0.9% NaCl (n=12)</th>
<th>rFVIIa, 160 μg kg⁻¹ (n=12)</th>
<th>PCC, 25 IU FIX kg⁻¹ (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS blood loss (g)</td>
<td>(5–16)</td>
<td>(8–45)**</td>
<td>(4–33)*</td>
</tr>
<tr>
<td>IBT (s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>50 (25–60)</td>
<td>43 (30–70)</td>
<td>45 (25–80)</td>
</tr>
<tr>
<td>P2</td>
<td>48 (25–70)</td>
<td>78 (45–120)*</td>
<td>75 (45–105)**</td>
</tr>
<tr>
<td>P3</td>
<td>50 (30–65)</td>
<td>53 (30–85)*</td>
<td>40 (25–70)*</td>
</tr>
</tbody>
</table>

Downloaded from https://academic.oup.com/bja/article-abstract/108/4/586/257194 by guest on 09 March 2019
groups, suggesting that the thrombosis risk was not increased by rFVIIa or PCC treatments.

**Biological parameters**

Haematocrit and fibrinogen levels were used to verify that haemodilution was similar between the groups: haematocrit decreased from values around 40% (P1) to 25% (P2) (mean 35% reduction, \( P<0.05 \), Table 4) and mean fibrinogen level decreased by 60% \( (P<0.05) \). As expected, PCC and rFVIIa had no effects on haematocrit and fibrinogen levels. Haemodilution and placebo infusion did not influence aPTT, which was significantly reduced following rFVIIa or PCC injection (mean 44% shortening, P3 and P2, \( P<0.05 \)).

Progress of thrombin activity was similar in all four groups during P1 (Fig. 4 and Table 5), and did not change in the control group and in the placebo group from P1 to P3. Haemodilution by itself had no significant effect on thrombin activity (placebo group vs control group). Injection of rFVIIa or PCC increased the total thrombin activity up to 90 s between P2 and P3 reflecting a faster kinetic of the reaction in the treated groups, but only PCC increased the total thrombin activity (two-fold on the average) between P2 and P3. These results are in line with the observed shortening of the aPTT (Table 4).

**Discussion**

In this rabbit bleeding model, 35% haemodilution resulted in an 80% increase in HS blood loss and a 60% decrease in fibrinogen level. rFVIIa and PCC were not effective at reducing HS blood loss (primary endpoint), in haemodiluted rabbits, although both drugs were effective in decreasing IBT. No significant increase in the thrombotic risk in terms of CFR was observed either with rFVIIa or with PCC when compared with the control group. The tested doses of rFVIIa and PCC correspond to those routinely used to correct severe bleeding disorders in patients. In addition, although PCC nearly doubled the total amount of thrombin activity detectable, there was no significant increase with rFVIIa but only a faster kinetic compared with the control group.

PCC is approved for the reversal of VKA treatments. However, it is now extensively used in several European countries as a haemostatic agent in the bleeding patient. The rationale behind this off-label prescription are its immediate availability when compared with fresh-frozen plasma, highly concentrated solutions within small vials, control of viral risk with solvent-detergent techniques, and more than 10 yr experience in a number of central European countries, including Germany and Austria. Such widespread clinical practice dissuaded the development of randomized double-blind studies that would have provided the missing evidence-based data of PCC efficacy, a number of physicians even considering that comparing

---

**Table 4** Control of haemodilution efficacy (haematocrit and fibrinogen) and aPTT. Wilcoxon test. aPTT, activated partial thromboplastin time; mean (SD), \( ^*P<0.05 \) vs control; \( ^\dagger P<0.05 \) vs P1. n, number of rabbit experiencing at least one CFR. Median (range)

<table>
<thead>
<tr>
<th></th>
<th>Control ( (n=12) )</th>
<th>Placebo, 0.9% NaCl ( (n=12) )</th>
<th>rFVIIa, 160 ( \mu )g kg(^{-1} ) ( (n=12) )</th>
<th>PCC, 25 IU FIX kg(^{-1} ) ( (n=12) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematocrit (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>37.9 (2.9)</td>
<td>39.3 (2.7)</td>
<td>40.0 (2.1)</td>
<td>37.8 (3.1)</td>
</tr>
<tr>
<td>P2</td>
<td>37.3 (2.9)</td>
<td>26.1 (2)*</td>
<td>24.9 (1.6)*</td>
<td>24.8 (2.1)*</td>
</tr>
<tr>
<td>P3</td>
<td>36.9 (2.9)</td>
<td>25.2 (1.8)*</td>
<td>25.8 (1.5)*</td>
<td>24.1 (2.4)*</td>
</tr>
<tr>
<td>Fibrinogen (g litre(^{-1} ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>1.37 (0.29)</td>
<td>1.40 (0.35)</td>
<td>1.47 (0.34)</td>
<td>1.33 (0.35)</td>
</tr>
<tr>
<td>P2</td>
<td>1.11 (0.28)</td>
<td>0.57 (0.09)*</td>
<td>0.66 (0.19)*</td>
<td>0.59 (0.17)*</td>
</tr>
<tr>
<td>P3</td>
<td>1.14 (0.29)</td>
<td>0.53 (0.12)*</td>
<td>0.58 (0.14)*</td>
<td>0.55 (0.12)*</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>91 (26)</td>
<td>96 (28)</td>
<td>107 (37)</td>
<td>103 (31)</td>
</tr>
<tr>
<td>P2</td>
<td>96 (28)</td>
<td>100 (24)</td>
<td>109 (30)</td>
<td>110 (30)</td>
</tr>
<tr>
<td>P3</td>
<td>94 (34)</td>
<td>94 (26)</td>
<td>61 (18)*</td>
<td>62 (10)††</td>
</tr>
</tbody>
</table>

---

**Table 3** CFR values. Wilcoxon test. \( ^*P<0.05 \) vs control; \( ^\dagger P<0.05 \) vs P1. n, number of rabbit experiencing at least one CFR. Median (range)

<table>
<thead>
<tr>
<th></th>
<th>Control ( (n=12) )</th>
<th>Placebo, 0.9% NaCl ( (n=12) )</th>
<th>rFVIIa, 160 ( \mu )g kg(^{-1} ) ( (n=12) )</th>
<th>PCC, 25 IU FIX kg(^{-1} ) ( (n=12) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>3 (3–10)</td>
<td>6 (3–9)</td>
<td>5 (3–9)</td>
<td>4 (3–6)</td>
</tr>
<tr>
<td>P2</td>
<td>1 (0–5)†</td>
<td>0 (0–2)*</td>
<td>0 (0–1)*</td>
<td>0 (0–6)*</td>
</tr>
<tr>
<td>P1</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>P2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>0 (0–4)</td>
<td>0 (0–2)</td>
<td>1 (0–5)</td>
<td>0 (0–5)</td>
</tr>
<tr>
<td>P1</td>
<td>5</td>
<td>1</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>
PCC with placebo would be unethical. At first, it could be estimated that a treatment designed to correct a decrease in vitamin K dependent clotting factor may not be best to control bleeding when the most adversely affected parameter is fibrinogen. Nevertheless, several experimental studies in pigs or rabbits and two small retrospective cohort studies suggested that PCC may indeed help to control bleeding.

Pragst and colleagues anaesthetized normothermic rabbits which underwent a haemodilution (50–60%) by blood withdrawal followed by infusion of hydroxyethyl starch and erythrocytes. Animals were randomly assigned to receive either saline placebo, 25 IU kg\(^{-1}\) PCC, or 180 mg kg\(^{-1}\) rFVIIa. In this study, PCC injection improved haemostasis parameters compared with saline and rFVIIa (\(P<0.002\)).

Table 5 Total thrombin activity after 90 s and after 13 min. Wilcoxon test. Means (so). Arbitrary units are used. \(\dagger P<0.05\), \(\dagger\dagger P<0.01\) vs P2

<table>
<thead>
<tr>
<th>Activity</th>
<th>Control (n=12)</th>
<th>Placebo, 0.9% NaCl (n=12)</th>
<th>rFVIIa, 160 μg kg(^{-1}) (n=12)</th>
<th>PCC, 25 UI FIX kg(^{-1}) (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>After 90 s (10(^{-4}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>1.87 (0.68)</td>
<td>1.63 (0.75)</td>
<td>1.50 (0.54)</td>
<td>1.61 (0.51)</td>
</tr>
<tr>
<td>P2</td>
<td>1.84 (0.79)</td>
<td>1.2 (0.48)</td>
<td>1.23 (0.20)</td>
<td>1.56 (0.31)</td>
</tr>
<tr>
<td>P3</td>
<td>2.24 (1.00)</td>
<td>1.92 (0.63)</td>
<td>2.44 (0.60)(\dagger)</td>
<td>2.39 (0.20)(\dagger)</td>
</tr>
<tr>
<td>After 13 min (10(^{-4}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>26.0 (6.0)</td>
<td>24.9 (5.0)</td>
<td>22.2 (5.2)</td>
<td>23.6 (4.2)</td>
</tr>
<tr>
<td>P2</td>
<td>23.4 (5.3)</td>
<td>20.1 (4.4)</td>
<td>19.7 (6.1)</td>
<td>22.7 (5.2)</td>
</tr>
<tr>
<td>P3</td>
<td>23.9 (3.8)</td>
<td>22.6 (5.5)</td>
<td>21.00 (5.7)</td>
<td>52.8 (12.7)(\dagger\dagger)</td>
</tr>
</tbody>
</table>

Fig 4 Progress of thrombin activity after injection of: (a) rFVIIa 160 μg kg\(^{-1}\); (b) PCC 40 IU FIX kg\(^{-1}\); and (c) placebo. Bars are mean (so). HD, haemodilution.
Median time to haemostasis, as defined by the authors, was shorter in the PCC group. PCC reduced blood loss by a median of 43 ml vs saline and 82 ml vs rFVIIa, and augmented peak thrombin generation. Furthermore, using the Wessler stasis model, rFVIIa displayed thrombogenicity, whereas PCC did not. These data confirmed the report from the same group suggesting that PCC was effective in correcting dilutional coagulopathy and bleeding in a pig trauma model where 65–70% of total blood volume was gradually substituted by hydroxyethyl starch and red blood cells.27 In a third study, anaesthetized mildly hypothermic normotensive pigs were haemodiluted by substituting 65–70% of total blood volume in phases with hydroxyethyl starch and red cells.28 Thereafter, animals received isotonic saline placebo, 35 IU kg⁻¹ PCC, or 180 μg kg⁻¹ rFVIIa. Haemodilution markedly prolonged PT and decreased thrombin formation. In PCC recipients, peak thrombin generation was greater by a median of 60.7 nM compared with the rFVIIa group. These results are in line with ours regarding the effects of haemodilution and the increase in total thrombin activity after PCC infusion. However, in these studies, PCC was found to be more effective than rVIIa, which conflicts with our findings, possibly due to species specificity, differences in the bleeding models, or both. In addition, the amount of bleeding was not designed initially to be major in our study to try to conform with what is most often encountered in common clinical practice.

Although PCC injection shortened IBT in our model and dramatically increased thrombin production in vitro, it was unable to reduce HS blood loss. That PCC markedly increased thrombin production most likely resulted from the supplementation of blood with coagulation factors, mainly prothrombin, that did not counterbalance the mechanical decrease resulting from the 35% haemodilution and the concomitant 60% decrease in fibrinogen and antithrombin concentrations.

Recombinant FVIIa decreased IBT in our model, but it did not have a significant effect on HS blood loss, even if, after rFVIIa infusion, HS blood loss was not different from that seen in the control (non-haemodiluted) group. In other words, a non-significant trend was observed in favour of rFVIIa. The 160 μg kg⁻¹ dose was chosen in accordance with the previous studies performed in our laboratory.25 This supports the hypothesis that rFVIIa is involved in an additional pathway of thrombin production and that this pathway is undetected in vitro with platelet-poor plasma as measured with aPTT or progress of thrombin activity tests. Consistent with our data, Viiuf and colleagues31 have shown that blood diluted to 40% using high molecular weight hetastarches responds little to rFVIIa. Current models involve platelets that would allow rFVIIa to activate factor IX even in the absence of tissue factor.32 33 Our data suggest that rFVIIa injection slightly shortened the lag time of clot formation in vitro but had little effect on the total thrombin produced in platelet-poor plasma.

Haemodilution favours bleeding while decreasing the level of coagulation factors, fibrinogen, platelets, and red blood cells. The choice for a 35% haemodilution was based on our previous observation that red blood cell transfusion could restore CFRs for such haemodilution,34 whereas rFVIIa injection failed to restore CFRs for a 50% haemodilution (unpublished observation).

Available PCC solutions are now almost free of activated factors. Reported thromboembolic complications have decreased with the development of new products. This risk depends on the composition of PCC: balance between activators and inhibitors, absence of activated factors, presence of heparin, antithrombin, and protein C and S.12 16 Thromboembolic complications occur in specific populations of haemophiliacs or high-risk patients treated with VKA. No data are available in the setting of massive haemorrhage. Considering that PCC and rFVIIa may promote thrombosis, we used our model to evaluate their safety. As reported by others,35 and in a previous work,32 we observed that CFRs nearly disappeared after haemodilution. The lack of a significant increase in CFRs after PCC and rVIIa infusion is reassuring, suggesting that PCC appears to be as safe as rFVIIa.

Our study has some limitations. Only one dose of PCC and one dose of rFVIIa were tested. Even if fibrinogen concentrates were not part of the design of the study, it could have been interesting to add a fibrinogen arm or to combine fibrinogen with PCC. However, pending the initial hypothesis (primary efficacy endpoint) on the decrease in HS bleeding, it was difficult to include more than four study arms. In addition, rFVIIa and PCC were given after IBT, but before the HS blood loss procedure, as in other animal models.28 29 Therefore, interpreting the lack of efficacy of PCC on HS blood loss should be done with caution. The strengths of our study, on the other hand, are that experiments were performed under controlled conditions (ventilation, pH, temperature, haemodynamics) and that CFRs data were re-analysed blindly a posteriori.

Conclusions

Haemodilution increased bleeding. Injection of rFVIIa or PCC reduced bleeding time, but they did not control HS blood loss, even if a non-significant trend was observed in favour of rFVIIa. Interestingly, none of the treatments tested increased the thrombosis risk. Large randomized double-blind independent academic studies are now mandatory to study the effect of PCC on massively bleeding patients.

Acknowledgements

LFB and NovoNordisk are acknowledged for supplying vials of PCC (Kaskadi®) and rVIIa (NovoSeven®).

Declaration of interest

C.-M.S. has received two unrestricted grants from LFB and from NovoNordisk for the Research, and honorarium for lectures for the LFB.
Funding
Funding was provided by INSERM U765.

References