Evaluation of Prothrombin Complex Concentrate and Recombinant Activated Factor VII to Reverse Rivaroxaban in a Rabbit Model

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ABSTRACT

Background: As a potent anticoagulant agent, rivaroxaban exposes a risk of bleeding. An effective way to reverse its effects is needed. Objectives were to study efficacy and safety

What We Already Know about This Topic

- Rivaroxaban is an oral anticoagulant used to prevent or treat venous thromboembolism and prevent stroke
- There is no antidote or reversal agent for the anticoagulant effects of rivaroxaban

What This Article Tells Us That Is New

- In a rabbit model of bleeding and arterial thrombosis, both recombinant activated factor VII and prothrombin complex concentrate partially improved in vitro assessment of coagulation and hemostasis
- Neither recombinant activated factor VII nor prothrombin complex concentrate was able to reduce rivaroxaban-induced bleeding

A dosage ranging study assessed the minimal rivaroxaban dose that increased bleeding. Then, 48 anesthetized and ventilated rabbits were randomized into four groups: control (saline), rivaroxaban (rivaroxaban and saline), rFVIIa (rivaroxaban and recombinant activated factor VII (rFVIIa)), and PCC to reverse the anticoagulant effect of an overdose of rivaroxaban in a rabbit model of bleeding and thrombosis.

Methods: First, a dose-ranging study assessed the minimal rivaroxaban dose that increased bleeding. Then, 48 anesthetized and ventilated rabbits were randomized into four groups: control (saline), rivaroxaban (rivaroxaban and saline), rFVIIa (rivaroxaban and recombinant activated factor VII (rFVIIa)), and PCC to reverse the anticoagulant effect of an overdose of rivaroxaban in a rabbit model of bleeding and thrombosis.

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oxaban and rFVIIa), and PCC (rivaroxaban and PCC). The Folts model was applied: a stenosis and an injury were carried out on the carotid artery, inducing thrombosis, detected as cyclic flow reductions, which were recorded over 20 min. Then the following were measured: ear immersion bleeding time, clotting times, anti-Xa activity, thrombelastometric parameters, and thrombin generation test. Ultimately, a heparinised section was performed and the total amount of blood loss after 15 min was evaluated as primary endpoint.

**Results:** Rivaroxaban increased blood loss (17 g [8–32] vs. 7 g [5–18] for control (median [range]), $P = 0.0004$), ear bleeding time, clotting times, thrombelastographic clotting time, and decreased thrombin generation. In contrast, rFVIIa decreased ear immersion bleeding time (92 s [65–115] vs. 140 s [75–190], $P < 0.02$), but without efficacy on blood loss. PCC and rFVIIa decreased activated partial thromboplastin time as well as thrombelasto-graphic clotting time. Regarding safety, neither rFVIIa nor PCC increased cyclic flow reductions.

**Conclusion:** rFVIIa and PCC partially improved laboratory parameters, but did not reverse rivaroxaban induced-bleeding.

**Rivaroxaban (Xarelto®; Bayer Schering Pharma AG, Leverkusen, Germany),** an oral oxazolidinone-based anticoagulant, is a potent direct factor Xa (FXa) inhibitor that is used in the prevention of venous thromboembolism in adult patients after total hip replacement or total knee replacement surgery. It is also effective for the treatment of symptomatic venous thromboembolism and for preventing stroke in patients with nonvalvular atrial fibrillation. Rivaroxaban is also being evaluated for secondary prevention after acute coronary syndromes.

The potential drawback of any anticoagulant agent is the risk of bleeding complications, especially following trauma, overdose, or in case of urgent surgery. Rapid reversal of anticoagulation is essential, particularly in the presence of life-threatening bleeding. Specific antidotes are available for older anticoagulants: protamine sulfate reverses the effects of unfractionated heparin and neutralizes partially low molecular weight heparins, whereas vitamin K and prothrombin complex concentrate (PCC) are antidotes for vitamin K antagonists. In contrast, rivaroxaban lacks an effective antidote or even a reversal agent, as most recently developed anticoagulants.

Rivaroxaban binds directly to the catalytic site of the serine protease FXa, independently of antithrombin, and inhibits both free FXa and FXa within the prothrombinase complex. It could be hypothesized that thrombin formation might be restored by either FX administration or FII administration, or both, thanks to PCC, or by recombinant activated factor VII (rFVIIa), which can accelerate FXa formation. Thus, the use of rFVIIa as well as PCC has been proposed. However, it is poorly supported by few unpublished studies and efficacy assessment is only based on laboratory tests. We hypothesized that these two prohaemostatic agents could be effective to reverse rivaroxaban in case of overdose. The aim of the present study was to investigate the effects of rFVIIa and PCC for the reversion of the anticoagulant effect of rivaroxaban in a rabbit model of microvascular bleeding and arterial thrombosis. The primary efficacy endpoint was hepatosplenic blood loss, to assess different prothrombotic and antithrombotic drugs. Secondary endpoints were ear immersion bleeding time and hematocrit. The other part of the model measured thrombotic events, which are the main side effects of rFVIIa and PCC.

We designed a randomized controlled study to investigate the efficacy and safety of rFVIIa and PCC to reverse rivaroxaban in rabbits. In addition, the impact of these agents on laboratory assays was assessed.

**Materials and Methods**

**Animals**

Animals were treated in accordance with the ethical rules of the Institut National de la Santé et de la Recherche Médicale (INSERM), the Institut National de la Recherche Agronomique (INRA), and the Comité Régional d’Ethique en matière d’Expérimentation Animale (CREEA). Male New Zealand rabbits, 12–14 weeks old, and of the same blood group were obtained from the Cegav Breeding Colony (Les Hautes Noës, St Mars d’Egremnies, France), were housed one per cage, and were provided with tap water and food *ad libitum*. Animals were used in experiments after an acclimatisation period of at least 1 week.

**Anaesthesia, Ventilation and Monitoring**

All steps were carried out after rabbits received general anaesthetic. A 22-gauge catheter was introduced into the marginal vein of the ear (BD Insyte Autoguard, Franklin Lakes, NJ). Anaesthesia was induced by 15 mg/kg ketamine (Ketamine 1000®; VIRBAC Santé Animale, Carros, France) and 0.5 mg/kg xylazine (Rompun®; Bayer, Leverkusen, Germany) and maintained by a continuous ketamine infusion (50 mg·kg$^{-1}$· h$^{-1}$). Maintenance of slight corneal reflex was tested using saline drops.

A median neck incision was made and tracheotomy was performed to ensure mechanical ventilation (Harvard Apparatus, Kent, United Kingdom), with a respiratory rate of 40 cycles/min and a tidal volume of 5 ml/kg, with 1 l/min of oxygen-enriched air (Air Liquide®, Paris, France). After dissection of the groin, a 20-gauge catheter was introduced into the freed femoral artery (Infu-Surg 1000 ml; Ethox® Corp., Buffalo, NY) and connected to a calibrated blood pressure monitor (BIOPAC® MP30, BIOPAC Systems Inc., Goleta, CA) for continuous monitoring. Heart rate was recorded from the blood pressure wave. The monitor was connected to an Apple MacBook (Apple®, Cupertino, CA). The software application was BIOPAC Student Lab Pro 3.7.1.1 for Mac OS 10.4. Body temperature was continuously recorded, monitored by a rectal probe (Homeothermic Blankets Control Unit®, Kent, United Kingdom). Body temperature was maintained in the range of 38° and 39°C using a heated table.
Thrombosis Model

The arterial thrombosis protocol was derived from the Folts model of coronary thrombosis to study interactions between platelets, intima, and media. It was performed to assess the thrombotic risk of rFVIIa and PCC.

One of the common carotid arteries was isolated and cleared of the surrounding fascia. A Doppler flowprobe (R-series; Transonic Systems Inc., Ithaca, NY) was placed around it and connected to a flowmeter for instantaneous blood flow measurements (TS420; Transonic Systems Inc.). Mean and phasic flows were recorded continuously (BIOPAC). Once flow was stable again, a silicone vascular clamp (Harvard Apparatus) was placed on the artery to induce a circumferential stenosis. An anticipated 75% stenosis was achieved by reducing mean basal carotid artery blood flow by 10%.

Once the reduced flow was stabilized, an arterial injury of the carotid with deendothelialization was induced by cross-clamping the middle of the exposed segment of the artery, three consecutive times within an elapsed period of 3 s. This was accomplished with a Mayo-Hegar needle holder forceps (Harvard Apparatus) closed at the first ratchet. The clamp was then positioned over the injured segments. Carotid blood flow was recorded over a period of 20 min with one of two outcomes:

1. Mean and phasic flow might decline gradually until embolus formation. Indeed, blood flow decreases as thrombus size increases in the injured vascular segment, until the pressure gradient is such that the thrombus is released and local arterial blood flow is suddenly restored. This is known as a cyclic flow reduction (CFR). A rabbit was included in the study only as soon as a spontaneous CFR occurred during this 20-min period.

2. If no CFR was recorded, an adjacent carotid segment was injured, and recording was resumed for 20 min. In case of lack of CFR, the controlateral carotid was injured.

Treatment Protocol

Immediately after the first CFR occurred, rabbits were assigned into one of five groups using a randomization table: control (saline followed by saline), rivaroxaban (rivaroxaban, saline), rFVIIa (rivaroxaban, rFVIIa), or PCC (rivaroxaban, PCC). They blindedly received intravenously 1 ml of either rivaroxaban solution or saline. One minute later, saline, rFVIIa, or PCC was injected in an unblinded way. Any CFR occurring during the 20 min following intravenous injection was recorded (observation period P) (fig. 1). For CFR analysis, the readers were blinded to the treatment group.

Bleeding

Ear immersion bleeding time was measured on the rabbit ear. A 5-mm long, 1-mm deep incision was made with an automated blade (Surgicutt; ITC, Edison, NJ) on the external surface of the ear. The ear was then immersed in a beaker containing saline warmed around 38.5°C. Bleeding time was defined as previously reported, as the time between the incision and the complete arrest of bleeding. Bleeding time was measured at the end of the observation period P. Hepatosplenic blood loss was measured at the end of the experiment, immediately after P, through a xyphopubic laparotomy. The spleen and liver were isolated and incised in a standardized fashion. The spleen was transected at its free border from the lower pole to the mid level (3 to 4 cm). For the liver, 10 1-cm sections were made between the right and left lobes. Three swabs were placed close to the spleen and the liver before the transection. The total amount of blood loss (spleen and liver bleeding) was measured 15 min after the lesions by weighing these swabs. At the end of this period, after blood sampling, rabbits were sacrificed by injection of a lethal dose of pentobarbital (60 mg/kg, Nembutal; Abbott, Abbott Park, IL).

Dose Selection

As no intravenous formulation for rivaroxaban was available, raw material was dissolved in a solution of polyethylene gly-
col 400/H₂O/glycerol (996 g/100 g/60 g). As hepar- 
splenic blood loss was the primary efficacy outcome of the 
study, the rivaroxaban dose was selected after the comple- 
tion of a dose-ranging study (control, vehicle, 3 mg/kg; 5 mg/kg; 
10 mg/kg) including 35 rabbits and designed to assess the 
minimal dose that increased heparosplenic blood loss. This 
was the preliminary condition in order to evaluate a potential 
reversal agent. A single bolus of rFVIIa was given as 150 
µg/kg, as this dose was previously used in our laboratory and 
was effective to correct the coagulopathy in the same rabbit 
model. PCC (Kaskadil®; LFB, Les Ullis, France) was sup- 
plied as a lyophilized powder. It contains different amounts 
of clotting factors: 37 U/ml of FII, 25 U/ml of FVII, 25 U/ml of 
FVIII, and 40 U/ml of FX; 4.8 U/ml of protein C, 10.3 
U/ml of protein S, and less than 5 U/ml of unfractionated 
heparin. We chose the dose of 40 U/ml, as this dose was 
previously used in this rabbit model and was effective to 
reverse fondaparinux.

**Laboratory Parameters**

Blood samples were collected through the femoral artery 
catheter at the end of the observation period, except for he- 
matocrit, which was measured at the end of the bleeding 
period. The samples were anticoagulated with 0.129 M triso-
dium citrate tubes (9NC; BD Vacutainer, Plymouth, United 
Kingdom). Hematocrit was measured by centrifugation in 
heparinized capillary tubes (Hirschmann Laborgeräte, Eber-
stadt, Germany) after heparosplenic bleeding. Prothrombin 
time (Neoplastine Cl; Diagnostica Stago, Asnieres, France), 
activated partial thromboplastin time (aPTT trinicol; Trinity 
Biotech, Wicklow, Ireland), and plasma fibrinogen level 
(Thrombin reagent; Siemens Healthcare Diagnostics, Mar-
burg, Germany) were determined on platelet-poor plasma 
(Timation of the null hypothesis.

**Statistics**

Statistical analysis was performed with StatEL® software (V 2.2; 
AdScience, Paris, France). Data are expressed as mean ± SD, 
except for discrete variables (CFRs) and variables with non-
Gaussian distribution (bleeding time, spleen and liver bleeding), 
which are expressed as medians with ranges.

Sample size was calculated assuming that tested agents 
would decrease bleeding by 40%. We have based our calcula-
tion on an estimated rivaroxaban-induced heparosplenic 
bleeding of 17 ± 5 g; this value was the fondaparinux-
induced heparosplenic blood loss measured in our previous 
study. Eleven rabbits per group appeared to be appropriate 
to have a 20% β risk and a 5% α risk.

Probability values α < 0.05 were required to reject the 
null hypothesis in a two-tailed test. The more conservative 
nonparametric tests were used for comparisons: data were 
compared using the Kruskall–Wallis test for independent 
measures, followed, when significant, by a Mann–Whitney 
U test with Bonferroni correction of the criterion for rejec-
tion of the null hypothesis.

**Results**

**Dose-ranging and Baseline Characteristics**

Eighty-three rabbits were included in this study. Thirty-five 
rabbits were included in the dose-ranging study (table 1).
**Table 1. Dose-ranging Study**

<table>
<thead>
<tr>
<th>Bleeding Parameters</th>
<th>Control (n = 8)</th>
<th>PEG (n = 3)</th>
<th>Rivaroxaban 3 mg/kg (n = 9)</th>
<th>Rivaroxaban 5 mg/kg (n = 7)</th>
<th>Rivaroxaban 10 mg/kg (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood loss (g)</td>
<td>11.5 (5–20)</td>
<td>9.3 (7–11)</td>
<td>11.8 (4–22)</td>
<td>25.1 (17–36)*</td>
<td>20.5 (10–29)*</td>
</tr>
<tr>
<td>Ear immersion bleeding time (s)</td>
<td>75.5 (40–113)</td>
<td>65 (65–70)</td>
<td>96 (61–120)</td>
<td>100 (83–110)</td>
<td>126 (80–230)*</td>
</tr>
</tbody>
</table>

Values are median (range).
* $P < 0.05$ vs. control.
PEG = polyethylene glycol 400/H₂O/glycerol.

The 3 mg/kg dose of rivaroxaban had no detectable effect, but doses of 5 and 10 mg/kg both increased hepatosplenic blood loss; 5 mg/kg was the selected dose for the current study.

A total of 48 rabbits were included in the protocol. No significant difference between groups was observed from baseline characteristics (table 2). Values were comparable to those previously published.12

**Hepatosplenic Blood Loss and Bleeding Time**

In accordance to the preliminary dose-ranging study, values for blood loss and bleeding time were significantly increased in the rivaroxaban group, as compared with the control group (table 3). Neither rFVIIa nor PCC reduced blood loss in rivaroxaban-treated rabbits ($P = 0.54$ and $P = 0.93$, respectively). rFVIIa only decreased bleeding time (92 s [65 to 115] vs. 140 s [75 to 190], $P = 0.02$), as compared with the rivaroxaban-treated rabbits.

**Arterial Thrombosis**

The number of CFR was similar among the four groups: 1 (0–4) CFR in the control group versus 1 (0–2) in the rivaroxaban-saline group, 0 (0–3) in the rivaroxaban-rFVIIa group, and 0 (0 to 1) in the rivaroxaban-PCC group.

**Laboratory Tests**

Compared with control, rivaroxaban treatment increased prothrombin time threefold, activated partial thromboplastin time 1.7-fold, and rivaroxaban concentrations evaluated with chromogenic assay up to 0.5 ± 0.2 μg/ml (table 4). In rivaroxaban-treated rabbits, rFVIIa and PCC normalized activated partial thromboplastin time and partially corrected prothrombin time. Plasma fibrinogen concentration was similar in all groups.

**Rotational Thrombelastography**

Presence of rivaroxaban was easily detected by thrombelasto- graphic analysis, through the increase of clotting time and clot formation time values, both in the INTEM and EXTEM tests (table 5). In contrast, maximum clot firmness was essentially unchanged, albeit it was moderately reduced in the FIBTEM analysis. Among the rivaroxaban-treated rabbits and compared with saline, injection of rFVIIa decreased clot formation time value in the INTEM test, the clotting time value in the EXTEM test, and increased maximum clot firmness in INTEM as well as moderately in EXTEM ($P = 0.056$). PCC injection normalized the clot formation time value in the INTEM test and reduced the clot formation time as well as clotting time values in the EXTEM test. Small amount of heparin contained in PCC had no detectable influence on the clotting time, clot formation time, maximum clot firmness, and α angle since values in HEPEM were comparable to those in INTEM (data not shown). Finally, addition of heparinase did not modify the values of clotting time, clot formation time, and α angle obtained in control and rivaroxaban-treated rabbits both in the INTEM and HEPEM tests (data not shown).

**Thrombin Generation Test**

Presence of rivaroxaban was also easily detected by thrombogram analysis, clearly inhibiting thrombin generation. Compared with control, lag time increased whereas endogenous thrombin potential and peak height decreased (table 6). rFVIIa injection somewhat improves endogenous thrombin potential and peak height. In fact, rFVIIa was the only agent to correct the lag time. PCC injection moderately improved endogenous thrombin potential without correcting peak height.

**Discussion**

In this randomized controlled animal study, neither rFVIIa nor PCC fully reversed the bleeding induced by rivaroxaban...
overdose. They only partially corrected some clinical or laboratory parameters.

As every anticoagulant, new anticoagulants are associated with a bleeding risk, and therefore antidotes are highly required. Although new oral compounds undoubtedly have an improved benefit/risk ratio, bleeding threat still exists. There is no known antidote to rivaroxaban, yet it is already marketed and prescribed. A recombinant and inactive factor Xa has been proposed as a potential antidote, but available data are limited to one abstract.18 Thus there is an urgent need to find a safe and effective alternative to manage potential overdose or uncontrolled hemorrhage. Agents known to reverse hemostatic defects and enhance wound-localized thrombin generation have promptly been evoked as potential candidates. To date, however, few clinical data on their use are available specifically for patients receiving rivaroxaban.

rFVIIa was one of the first drugs anticipated to reverse the effect of several anticoagulants19,20 as a potent hemostatic bypassing agent. Whereas rivaroxaban inhibits both free and prothrombinase-bound FXa,13 rFVIIa can generate Xa on the surface of activated platelets, promote thrombin generation,21 and improve fibrin quality.22 In vitro normal volunteer23 and ex vivo data24,25 suggest that rFVIIa antagonizes the anticoagulant effect of a variety of agents, but its potential to reverse the adverse effect of rivaroxaban had not been demonstrated. Nevertheless, preliminary studies, reported as abstract only, mention the use of rFVIIa in animal models.6,26 They suggest that it partially reduces rivaroxaban-induced prolongation of bleeding time and prothrombin time. Doses of rFVIIa used were higher than in our study: 400 μg/kg in rats and 210 μg/kg in primates. We found that rFVIIa normalized activated partial thromboplastin time and corrected several thrombelastographic parameters. Regarding thrombogram analysis, rFVIIa decreased lag time, as previously demonstrated in vitro with fondaparinux, whereas peak height and endogenous thrombin potential were not significantly modified.27 Thus laboratory analysis corroborates that rFVIIa partially corrected hemostasis.

Prothrombin complex concentrate is approved for the reversal of vitamin K antagonists,28 but is increasingly prescribed for bleeding patients without preexisting coagulopathy in several European countries.29–31 It could be interesting for rivaroxaban reversal. One supportive hypothesis is that PCC contains factor X and therefore could exhaust rivaroxaban, as increasing FX concentration (thus FXa production) would overcome rivaroxaban effect. Moreover, factor II administration is likely to contribute to thrombin generation. At least two studies support the beneficial effect of PCC. In rats, PCC (Beriplex®; CSL Behring, Marburg, Germany; 50 U/kg) reversed the effect of rivaroxaban; PCC nearly completely normalized the mesenteric bleeding time and partially reversed prothrombin time prolongation. Furthermore, the same dose of PCC improved prothrombin time and the thrombogram in six healthy subjects.32 In our model, PCC corrected several laboratory parameters, including activated partial thromboplastin time, but failed to reduce hepatosplenic bleeding or bleeding time. Among several hypotheses, 40 U/kg PCC dose could be insufficient, although it was potent enough to reduce fondaparinux-induced bleeding.12 Indeed, injection of 25 U/kg PCC had no effect on rat bleeding time,3 whereas a dose of 50 U/kg was effective in both rats3 and humans.32 The PCC used in this study contains a small amount of heparin, which could interfere with coagulation. The observed increase of the lag time in the thrombogram studies could result from these traces of heparin. In fact, this effect on the thrombogram had been reported previously: PCC prolonged the lag time, whereas addition of protamine sulfate normalized its value.27 Nevertheless, in our study, PCC also reduced activated par-

### Table 3. Bleeding

<table>
<thead>
<tr>
<th>Bleeding Parameters</th>
<th>Control</th>
<th>Rivaroxaban</th>
<th>R + rFVIIa</th>
<th>R + PCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatosplenic blood loss (g)</td>
<td>7 (5–18)</td>
<td>17 (8–32)*</td>
<td>15 (10–25)*</td>
<td>19.5 (4–28)*</td>
</tr>
<tr>
<td>Ear immersion bleeding time (s)</td>
<td>77 (41–101)</td>
<td>140 (75–190)*</td>
<td>92 (65–115)*†</td>
<td>130 (55–165)*‡</td>
</tr>
</tbody>
</table>

Values are median (range).

* $P < 0.05$ vs. control. † $P < 0.02$ vs. rivaroxaban. ‡ $P < 0.006$ vs. rivaroxaban and recombinant activated factor VII.

PCC = prothrombin complex concentrate; R = rivaroxaban; rFVIIa = recombinant activated factor VII.

### Table 4. Conventional Coagulation Tests

<table>
<thead>
<tr>
<th>Coagulation Tests</th>
<th>References Ranges</th>
<th>Control</th>
<th>Rivaroxaban</th>
<th>R + rFVIIa</th>
<th>R + PCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin time (s)</td>
<td>20–25</td>
<td>22.7 ± 3.9</td>
<td>65.3 ± 24.1*</td>
<td>51 ± 16.9*</td>
<td>51.5 ± 8.9*</td>
</tr>
<tr>
<td>Activated partial thromboplastin time (s)</td>
<td>53–63</td>
<td>58.1 ± 7.4</td>
<td>97.3 ± 28.8*</td>
<td>63.1 ± 9.7†</td>
<td>62.1 ± 14.7‡</td>
</tr>
<tr>
<td>R concentration (μg/ml)</td>
<td>0–0.02</td>
<td>0</td>
<td>0.5 ± 0.3*</td>
<td>0.5 ± 0.2*</td>
<td>0.7 ± 0.3*</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>2.3–2.9</td>
<td>2.8 ± 0.9</td>
<td>2.6 ± 0.7</td>
<td>3.1 ± 1.1†</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42–47</td>
<td>45 ± 2</td>
<td>46 ± 5</td>
<td>49 ± 7</td>
<td>47 ± 5</td>
</tr>
</tbody>
</table>

All values are means ± SD.

* $P < 0.05$ vs. control. † $P < 0.02$ vs. rivaroxaban. ‡ $P < 0.003$ vs. rivaroxaban.

PCC = prothrombin complex concentrate; R = rivaroxaban; rFVIIa = recombinant activated factor VII.
Table 5. Rotational Thrombelastography (ROTEM®) Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Rivaroxaban</th>
<th>R + rFVIIa</th>
<th>R + PCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTET CT (s)</td>
<td>163 ± 28</td>
<td>452 ± 178*</td>
<td>343 ± 78*</td>
<td>340 ± 73*</td>
</tr>
<tr>
<td>CFT (s)</td>
<td>39 ± 6</td>
<td>65 ± 21*</td>
<td>48 ± 9*</td>
<td>44 ± 9*</td>
</tr>
<tr>
<td>MCF (mm)</td>
<td>71 ± 4</td>
<td>73 ± 5</td>
<td>77 ± 5*†</td>
<td>73 ± 3</td>
</tr>
<tr>
<td>EXTEM CT (s)</td>
<td>44 ± 15</td>
<td>452 ± 353*</td>
<td>282 ± 164†</td>
<td>258 ± 103†</td>
</tr>
<tr>
<td>CFT (s)</td>
<td>52 ± 11</td>
<td>661 ± 764*</td>
<td>377 ± 248*</td>
<td>123 ± 71†</td>
</tr>
<tr>
<td>MCF (mm)</td>
<td>67 ± 4</td>
<td>70 ± 16*</td>
<td>77 ± 6*</td>
<td>71 ± 3*</td>
</tr>
<tr>
<td>FIBTEM MCF (mm)</td>
<td>15 ± 3</td>
<td>13 ± 4*</td>
<td>14 ± 6</td>
<td>12 ± 2*</td>
</tr>
</tbody>
</table>

All values are means ± SD. Results were compared using the Kruskall–Wallis test for independent measures and followed, when significant, by a Mann–Whitney U test with Bonferroni correction of the criterion for rejection of the null hypothesis.

* P < 0.05 vs. control. † P < 0.05 vs. rivaroxaban.

CFT = clot formation time; CT = clotting time; MCF = maximum clot firmness; PCC = prothrombin complex concentrate; R = rivaroxaban; rFVIIa = recombinant activated factor VII.

tial thromboplastin time, and comparison of the results obtained using the INTEM and HEPTEM tests suggested that, if any, contribution of heparin was limited. Last, our rivaroxaban dose could be too high, suggesting that PCC is ineffective to reverse rivaroxaban overdose, even if it could partially reverse its effect in standard conditions.

Our study has some limitations, especially on the choice of doses. Only one dose of each prohaemostatic agent was tested. Even if both were selected, because they decreased bleeding in previous rabbit studies, the lack of efficacy of any, contribution of heparin was limited. Last, our rivaroxaban dose could be too high, suggesting that PCC is ineffective to reverse rivaroxaban overdose, even if it could partially reverse its effect in standard conditions.

Conventional laboratory tests are widely used to identify a potential antidote, laboratory assays of doses. Only one dose of each prohaemostatic agent was tested. Even if both were selected, because they decreased bleeding in previous rabbit studies, the lack of efficacy of any, contribution of heparin was limited. Last, our rivaroxaban dose could be too high, suggesting that PCC is ineffective to reverse rivaroxaban overdose, even if it could partially reverse its effect in standard conditions.

A major concern regarding the relevance of antidote assessment studies is the choice of the primary endpoint. Conventional laboratory tests are widely used to identify a reversal efficacy. Although they are undoubtedly required to evaluate a potential antidote, laboratory assays only describe isolated parts of the hemostatic process, and are not reliably predictive of the clinical effectiveness to reduce bleeding. Both rFVIIa and PCC, corrected activated partial thromboplastin time; rFVIIa increased clot firmness; and PCC reduced clotting time, suggesting a potential efficacy of these products. Unfortunately, these results were not correlated with in vivo hemostasis data, whereas other coagulation parameters were conflicting. Thus, the laboratory assays we used failed to predict in vivo effects of reversal agents, suggesting that more robust endpoints are needed to evaluate antidote. Bleeding time is commonly used, but its relevance has been challenged. Therefore, we have added hepatosplenic sections to assess reversal agents in a double-bleeding model. Our results point out that clinical and biologic data are sometime perplexing and do not always correlate.

In this study, an arterial thrombosis model (modified Folt), was performed for safety reasons. Indeed, thromboembolism is the main adverse effect of prohaemostatic agents, and several thrombotic episodes, some severe, have been reported with both rFVIIa and PCC. In our model, none of the evaluated products increased the thrombotic risk.

### Conclusion

In this rabbit model of bleeding and arterial thrombosis, both rFVIIa and PCC partially improved laboratory parameters, including thromboelastography and thrombin genera-

Table 6. Thrombin Generation Test

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Rivaroxaban</th>
<th>R + rFVIIa</th>
<th>R + PCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETP (nm x min)</td>
<td>290 ± 123</td>
<td>50 ± 63*</td>
<td>113 ± 121*</td>
<td>91 ± 205*</td>
</tr>
<tr>
<td>Peak (nm)</td>
<td>54.2 ± 41.6</td>
<td>5.7 ± 10.2*</td>
<td>8.3 ± 7.3*</td>
<td>5.7 ± 9.3*</td>
</tr>
<tr>
<td>Lag time (min)</td>
<td>2 ± 1.8</td>
<td>5.9 ± 5.3</td>
<td>1.2 ± 2.2</td>
<td>9 ± 18.8†</td>
</tr>
</tbody>
</table>

All values are means ± SD. Results were compared using the Kruskall–Wallis test for independent measures and followed, when significant, by a Mann–Whitney U test with Bonferroni correction of the criterion for rejection of the null hypothesis.

* P < 0.05 vs. control. † P < 0.02 vs. rivaroxaban.

ETP = endogenous thrombin potential; PCC = prothrombin complex concentrate; R = rivaroxaban; rFVIIa = recombinant activated factor VII.
tion assay. However, none of them was clinically effective to reduce rivaroxaban-induced bleeding.

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ANESTHESIOLOGY REFLECTIONS

Lincoln v. Chloroform Insanity

During a dispute over a property boundary in June of 1855, Isaac Wyant was shot by a gun-wielding man named Anson Rusk. According to one witness, after surgical amputation of his arm, Wyant emerged from his chloroform anesthetic “ever after morbidly fearful that Rusk would kill him . . . and complained greatly about his head and exhibited many signs of being unsettled in his intellect.” Wyant would not just return home—he would return fire . . . at the county clerk’s office. Rusk expired from the four gunshot wounds that Wyant inflicted. In 1857 a wild-haired young attorney named Abraham Lincoln (above) assisted in prosecuting the murder case now titled People v. Wyant. Unfortunately for Lincoln, Wyant’s defense attorney would prevail by convincing the jury that surgical chloroform had driven Wyant insane. Wyant would be committed to a mental hospital, and, 3 years later, Lincoln would be committed to running for the presidency of the United States. (Copyright © the American Society of Anesthesiologists, Inc. This image also appears in the Anesthesiology Reflections online collection available at www.anesthesiology.org.)

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