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 of recombinant activated factor VII (rFVIIa) and prothrombin complex concentrate (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 (rivar-

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

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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 hepatosplenic 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 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 thrombelastographic 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.[1] It is also effective for the treatment of symptomatic venous thromboembolism[2] and for preventing stroke in patients with nonvalvular atrial fibrillation.[3] 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,[4] whereas vitamin K and prothrombin complex concentrate (PCC) are antidotes for vitamin K antagonists.[4] 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[5–7] 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 prothombotic and antithrombotic drugs.[8–10] 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'Egrenne, France), were housed one per cage, and were provided with tap water and food *ad libidum*. Animals were used in experiments after an acclimation period of at least 1 week.

Anaesthesia, Ventilation and Monitoring

All steps were carried out after rabbits received general anesthetic. A 22-gauge catheter was introduced into the marginal vein of the ear (BD Insyte Autoguard, Franklin Lakes, NJ). Anesthesia 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 $mg \cdot kg^{-1} \cdot h^{-1}$). 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

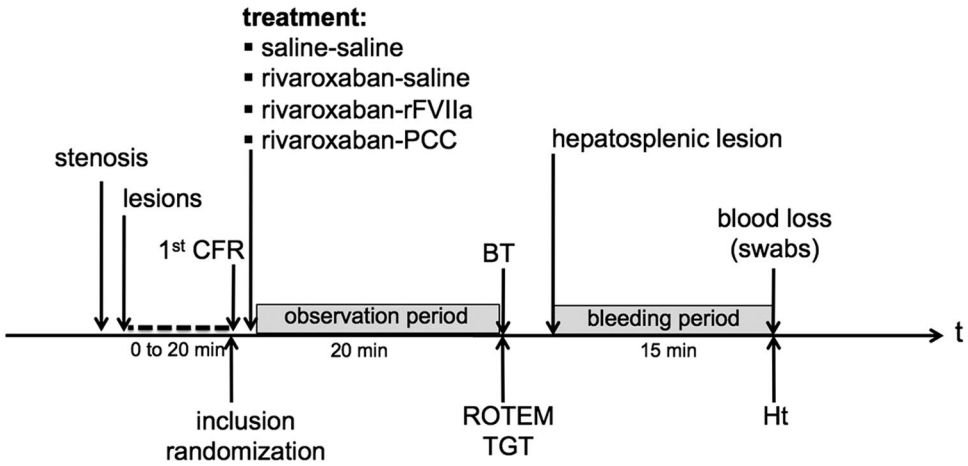

Fig. 1. Protocol design. BT = bleeding time; CFR = cyclic flow reductions; Ht = hematocrit; PCC = prothrombin complex concentrate; rFVIIa = recombinant activated factor VII; TGT = thrombin generation test.

(Animal Heated Table AH 50®; Scientific Research Instruments, Kent, United Kingdom).

Thrombosis Model

The arterial thrombosis protocol was derived from the Folts model of coronary thrombosis to study interactions between platelets, intima, and media.[11] 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 blindly 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,[10,12] 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.[12] 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/H_2O/glycerol (996 g/100 g/60 g).[13] As hepatosplenic blood loss was the primary efficacy outcome of the study, the rivaroxaban dose was selected after the completion 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 hepatosplenic 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.[10] PCC (Kaskadil®; LFB, Les Ullis, France) was supplied 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 FIX, 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.[14] We chose the dose of 40 U/ml, as this dose was previously used in this rabbit model and was effective to reverse fondaparinux.[12]

Laboratory Parameters

Blood samples were collected through the femoral artery catheter at the end of the observation period, except for hematocrit, which was measured at the end of the bleeding period. The samples were anticoagulated with 0.129 M trisodium citrate tubes (9NC; BD Vacutainer, Plymouth, United Kingdom). Hematocrit was measured by centrifugation in heparinized capillary tubes (Hirschmann Laborgeräte, Eberstadt, Germany) after hepatosplenic bleeding. Prothrombin time (Neoplastine CI; Diagnostica Stago, Asnieres, France), activated partial thromboplastin time (aPTT triniclot; Trinity Biotech, Wicklow, Ireland), and plasma fibrinogen level (Thrombin reagent; Siemens Healthcare Diagnostics, Marburg, Germany) were determined on platelet-poor plasma (centrifugation at 4,000 revolutions per minute for 10 min, twice) on a mechanical device automatically (STA-R Evolution®; Diagnostica Stago). Antifactor Xa chromogenic assay was used for the quantitative determination of rivaroxaban, as previously reported.[15] The Diagnostica Stago Rotachrom® Heparin assay (Diagnostica Stago), on the STA-R analyzer was calibrated with a standard curve generated with serial dilution of rivaroxaban in a pool of rabbit platelet-poor plasma. Plasma concentrations of rivaroxaban were expressed as μg/ml.

The modified rotation thrombelastogram analyzer (ROTEM®; Tem Innovations, Munich, Germany) was used to examine whole blood coagulation. Methods and parameters for thrombelastography have been previously described in detail.[16] In brief, thrombelastography measures the firmness of a blood clot during clot formation and subsequent fibrinolysis. The ROTEM generates a reaction curve in real time and provides several numerical measures calculated from the shape of the reaction curve. Four assays were performed according to the manufacturer's instructions using their recommended reagents: the INTEM assay explores the intrinsic pathway using ellagic acid as activator; the HEPTEM assay is the same, except it is performed in the presence of heparinase; the EXTEM assay explores the extrinsic pathway using tissue factor as an activator; and the FIBTEM assay explores the fibrin formation using tissue factor as an activator and cytochalasin D to inhibit platelet function. For each assay, five parameters were recorded: the clotting time, which represents the time required to reach a recognizable clot; the clot formation time, which describes the subsequent period until an amplitude of 20 mm is reached; the maximum clot firmness, which the clot achieves during the measurement; and the α angle, given by the angle between the center line and a tangent to the curve through the 2 mm amplitude point.

Thrombin Generation Test

In accordance with the method of Hemker *et al.*,[17] thrombin generation test was performed using a calibrated automated thrombogram and analyzed with the Thrombinoscope™ software (Thrombinoscope; Maastricht, The Netherlands). This software estimates the following parameters: lag time, peak height, and endogenous thrombin potential (measured as the area under the thrombin-time integral). Thrombin generation was triggered by the addition of 20 μl platelet-poor plasma reagent containing 5 pM tissue factor (Stago) and 100 μM solution of fluorogenic substrate (Fluca-Kit; Stago) to 80 μl rabbit platelet-poor plasma in each well of a micro-plate. For the thrombin generation test, as well as other laboratory assays performed with platelet-poor plasma, the investigators were blinded to group status.

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 calculation on an estimated rivaroxaban-induced hepatosplenic bleeding of 17 ± 5 g; this value was the fondaparinux-induced hepatosplenic blood loss measured in our previous study.[12] Eleven rabbits per group appeared to be appropriate to have a 20% β risk and a 5% α risk.

Probability values $\alpha < 0.05$ 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 rejection 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

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

Values are median (range).
* $P < 0.05$ *vs.* control.
PEG = polyethylene glycol 400/H_2O/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 thrombelastographic 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 HEPTEM 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 HEPTEM 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

Table 2. Baseline Characteristics

Parameters	Control (n = 13)	Rivaroxaban (n = 12)	R + rFVIIa (n = 11)	R + PCC (n = 12)
Weight (g)	2,728 ± 435	2,664 ± 446	2,752 ± 369	2,868 ± 464
SAP (mmHg)	136 ± 25	138 ± 15	134 ± 17	146 ± 31
Rectal temperature (°C)	36.0 ± 2.3	36.9 ± 1.6	36.4 ± 1.5	36.4 ± 1.6

Values are means ± SD.
PCC = prothrombin complex concentrate; R = rivaroxaban; rFVIIa = recombinant activated factor VII; SAP = systolic arterial pressure.

Table 3. Bleeding

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

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.

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 evocated as potential candidate. 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 anticoagulants[19,20] 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 vivo* normal volunteer[23] and *ex vivo* data[24,25] 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[5]: 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,[5] whereas a dose of 50 U/kg was effective in both rats[5] 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 4. Conventional Coagulation Tests

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

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

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

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,[10,12,33] the lack of efficacy should be interpreted with caution. Rivaroxaban dose might be too high to be fully reversed, but several arguments against this hypothesis can be raised. This dose was selected after a dose-ranging study, and the lower dose (3 mg/kg) did not increase hepatosplenic bleeding. Rivaroxaban plasma levels were close to those found with therapeutic doses (0.32 ± 0.02 μg/ml with 20 mg bid doses[5]). Last, whereas only high doses of anticoagulant decrease CFR,[34] CFR were unchanged after rivaroxaban administration compared with control. This leads to conclude to the inefficacy of prohaemostatic agents rather than to massive rivaroxaban overdose. Moreover, extrapolation of these rabbit results to humans requires caution.

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.[5,7,23,32] 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.[35] 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[36] and PCC.[37,38] 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

Parameters	Control	Rivaroxaban	R + rFVIIa	R + PCC
ETP (nM × min)	290 ± 123	50 ± 63*	113 ± 121*	91 ± 205*
Peak (nM)	54.2 ± 41.6	5.7 ± 10.2*	8.3 ± 7.3*	5.7 ± 9.3*
Lag time (min)	2 ± 1.8	5.9 ± 5.3	1.2 ± 2.2	9 ± 18.6*†

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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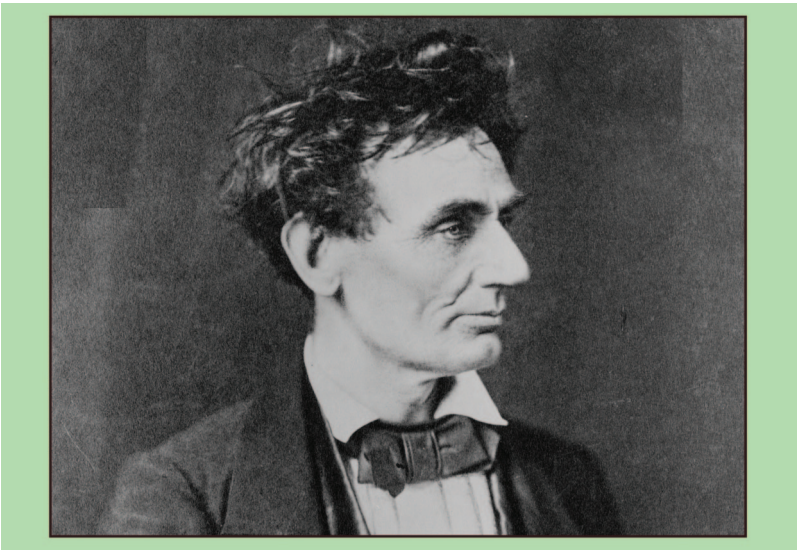
ANESTHESIOLOGY REFLECTIONS

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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