ANESTHESIOLOGY

Reversing Rivaroxaban Anticoagulation as Part of a Multimodal Hemostatic Intervention in a Polytrauma Animal Model

Farahnaz Rayatdoost, M.Sc., Till Braunschweig, M.D., Benjamin Maron, M.D., Herbert Schöchl, M.D., Necib Akman, M.D., Rolf Rossaint, M.D., Eva Herzog, Ph.D., Stefan Heitmeier, Ph.D., Oliver Grottke, M.D., Ph.D., M.Sc.

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Significant bleeding can occur after trauma, especially with preexisting anticoagulation. Despite multiple therapeutic approaches, optimal management remains to be determined.

What This Article Tells Us That Is New

- In an animal model of rivaroxaban-treated pigs that underwent complex traumatic injury, prothrombin complex concentrates alone and in combination with tranexamic acid and fibrinogen concentrate effectively reduced blood loss, restored hemostasis, and improved thrombin generation.

ABSTRACT

Background: Life-threatening bleeding requires prompt reversal of the anticoagulant effects of factor Xa inhibitors. This study investigated the effectiveness of four-factor prothrombin complex concentrate in treating trauma-related hemorrhage with rivaroxaban-anticoagulation in a pig polytrauma model. This study also tested the hypothesis that the combined use of a low dose of prothrombin complex concentrate plus tranexamic acid and fibrinogen concentrate could improve its subtherapeutic effects.

Methods: Trauma (blunt liver injury and bilateral femur fractures) was induced in 48 anesthetized male pigs after 30 min of rivaroxaban infusion (1 mg/kg). Animals in the first part of the study received prothrombin complex concentrate (12.5, 25, and 50 U/kg). In the second part, animals were treated with 12.5 U/kg prothrombin complex concentrate plus tranexamic acid or plus tranexamic acid and fibrinogen concentrate. The primary endpoint was total blood loss postinjury. The secondary endpoints (panel of coagulation parameters and thrombin generation) were monitored for 240 min posttrauma or until death.

Results: The first part of the study showed that blood loss was significantly lower in the 25 U/kg prothrombin complex concentrate (1,541 ± 269 ml) and 50 U/kg prothrombin complex concentrate (1,464 ± 108 ml) compared with control (3,313 ± 634 ml), and 12.5 U/kg prothrombin complex concentrate (2,671 ± 334 ml, all $P < 0.0001$). In the second part of the study, blood loss was significantly less in the 12.5 U/kg prothrombin complex concentrate plus tranexamic acid and fibrinogen concentrate (1,836 ± 556 ml, $P < 0.001$) compared with 12.5 U/kg prothrombin complex concentrate plus tranexamic acid (2,910 ± 856 ml), and there were no early deaths in the 25 U/kg prothrombin complex concentrate, 50 U/kg prothrombin complex concentrate, and 12.5 U/kg prothrombin complex concentrate plus tranexamic acid and fibrinogen concentrate groups. Histopathologic analyses postmortem showed no adverse events.

Conclusions: Prothrombin complex concentrate effectively reduced blood loss, restored hemostasis, and balanced thrombin generation. A multimodal hemostatic approach using tranexamic acid plus fibrinogen concentrate enhanced the effect of low doses of prothrombin complex concentrate, potentially reducing the prothrombin complex concentrate doses required for effective bleeding control.

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Factor Xa inhibitors have demonstrated superior or equivalent safety and efficacy compared with vitamin K antagonists. They offer practical advantages, such as no requirement for routine coagulation monitoring.[1,2] Many patients taking anticoagulation therapy are elderly and are therefore at risk of falling and sustaining injuries, including traumatic brain injury. The risk of life-threatening hemorrhage and mortality after an injury is increased by anticoagulants, potentially necessitating hemostatic therapy and anticoagulation reversal.[3]

In addition to trauma, emergency surgical intervention may trigger the need for a reversal of anticoagulation. Prothrombin complex concentrates (PCCs) are currently being used for reversing the anticoagulant effects of factor Xa inhibitors and are recommended according to several guidelines.[4] PCCs contain coagulation factors, including

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factors FII, FVII, FIX, and FX, and anticoagulant factors protein C and protein S.

Despite the growing evidence for PCC use in this setting, its efficacy and dosing to reverse impaired thrombin generation are still debatable.[5–8] High doses of PCC might be associated with an increased risk of thromboembolic complications in trauma. However, clinical data indicate that the risk is no higher with PCC than with therapeutic plasma in the reversal of vitamin K antagonists.[9] Additionally, thrombin generation is elevated for 4 days postoperatively after PCC substitution, which may put patients at an increased risk of thromboembolic complications.[10]

Andexanet alfa is a specific reversal agent that neutralizes direct and indirect FXa inhibitors' anticoagulant effects. The Andexanet Alfa, a Novel Antidote to the Anticoagulation Effects of FXa Inhibitors (ANNEXA-4) study has shown mainly the efficiency to neutralize anti-FXa activity in patients with predominantly intracerebral hemorrhage and gastrointestinal bleeding.[11] The short half-life of andexanet alfa requires a bolus plus infusion regimen. Andexanet is not widely available, and it is not licensed for patients requiring urgent surgery.[4]

In massive bleeding with complex coagulopathy, factor Xa inhibitors' reversal may not be sufficient to achieve hemostasis. Instead, multimodal treatment with different hemostatic agents may be required.[4,12] In central European countries, coagulation management is commonly based on coagulation factor concentrates (*e.g.*, fibrinogen concentrate), administered according to point-of-care coagulation monitoring.[13] In other countries such as the United States, there is more reliance upon treatment with allogeneic blood products (*e.g.*, cryoprecipitate and fresh frozen plasma), preferably using a fixed-ratio approach. There is also international recognition that tranexamic acid can reduce mortality by preventing fibrinolysis.[14] However, a recent study, including 927 patients who received tranexamic acid in the prehospital setting, did not have a significantly lower rate of 30-day mortality.[15] The role of tranexamic acid in the trauma system is still unclear, and its effect is highly dependent on transfusion strategies.

Using a lethal polytrauma model of coagulopathy, we investigated the effectiveness of three doses of a four-factor PCC administered to rivaroxaban-anticoagulated pigs. We hypothesized that the subtherapeutic effects of the lowest dose of PCC (12.5 U/kg) in our study might be improved by using a multimodal approach with tranexamic acid and fibrinogen concentrate. The primary outcome was the reduction of blood loss posttrauma. The secondary outcomes were the impact of the study regimens on the several coagulation parameters and the occurrence of thromboembolic complications.

Materials and Methods

The methods for this study were similar to those of several previous studies.[16–18] An overview of the study stages is presented in figure 1.

Ethics and Anesthesia

This study was performed at the RWTH Aachen University Hospital, Aachen, Germany, following German legislation governing animal studies following the Guide for the Care and Use of Laboratory Animals.[19] The government office approved the protocol for animal care and use (Landesamt für Natur, Umwelt und Verbraucherschutz, Recklinghausen, Germany). Before surgery, the pigs were housed in ventilated rooms and allowed to acclimatize to their surroundings for a minimum of 7 days. Examination by a veterinarian ensured the good health of all animals.

Experimental Methods

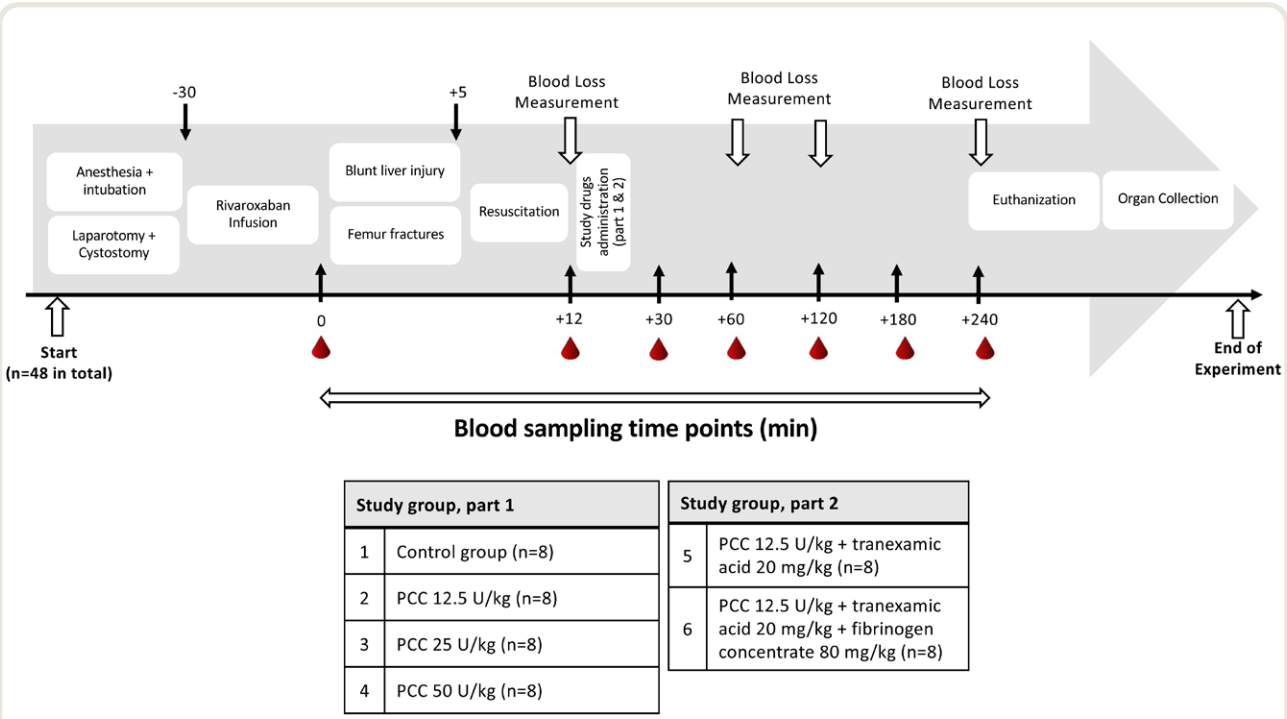
Forty-eight (n = 48) male pigs were anesthetized, prepared, and monitored as described (see Supplemental Digital Content, http://links.lww.com/ALN/C667, for additional details of animal preparation and anesthesia).[20] Concealment of allocation was carried out as the animal entered the trial. After line placement, a midline laparotomy with cystostomy was performed. Subsequently, a 30-min infusion of rivaroxaban (1 mg/kg; Bayer Healthcare, Germany) was started. After this infusion of 30 min, trauma was induced. Therapy was started 12 min after the infliction of injury. Animals (n = 8/group) were allocated to receive normal Ringer's solution (control) or 12.5, 25, or 50 U/kg PCC (Beriplex P/N; CSL Behring, Germany; U.S. brand name Kcentra). In the second part of this study, the animals were allocated to receive tranexamic acid (20 mg/kg; Cyclocapron, Pfizer, USA) plus PCC (12.5 U/kg) or tranexamic acid (20 mg/kg) plus PCC (12.5 U/kg) plus fibrinogen concentrate (80 mg/kg; Haemocomplettan P, CSL Behring).

Injury Phase and PCC Application

After localizing the femur with a needle, a captive bolt gun (Karl Schermer and Co., Germany) was used to fracture both femurs and create a soft tissue injury midshaft. Next, a reproducible blunt liver injury was induced using a custom-made instrument described elsewhere.[21] Five minutes after injury and after the onset of hemorrhagic shock, the animals were resuscitated with Ringer's solution (1 l over 5 min). Twelve minutes after injury, blood loss was measured by suctioning intraperitoneal blood. Study treatments were then administered. Animals surviving for the 240-min observation period after injury were euthanized with pentobarbital. Immediately after death, total postinjury blood loss was determined, and the internal organs (heart, lungs, liver, and kidneys) were examined macroscopically and histologically by a blinded pathologist for the occurrence of thromboembolic events. The tissue sections were embedded in paraffin and stained by hematoxylin and eosin based on a standard protocol.[22,23]

Blood Sampling and Analytical Methods

The blood samples were obtained before infusion of rivaroxaban; after a 30-min infusion of rivaroxaban; and 12, 30,



Study group, part 1	
1	Control group (n=8)
2	PCC 12.5 U/kg (n=8)
3	PCC 25 U/kg (n=8)
4	PCC 50 U/kg (n=8)

Study group, part 2	
5	PCC 12.5 U/kg + tranexamic acid 20 mg/kg (n=8)
6	PCC 12.5 U/kg + tranexamic acid 20 mg/kg + fibrinogen concentrate 80 mg/kg (n=8)

Fig. 1. Schematic overview of animal experiments. The blood samples were collected before trauma induction and 12, 30, 60, 120, 180, and 240 min postinjury. Treatments included control group, 12.5 U/kg prothrombin complex concentrate (PCC), 25 U/kg PCC, 50 U/kg PCC, 12.5 U/kg PCC plus tranexamic acid, and 12.5 U/kg PCC plus tranexamic acid and fibrinogen concentrate.

60, 120, 180, and 240 min after injury. For animals dying before 240 min postinjury, the last sample was obtained immediately after death. Details of the tests performed on the blood samples are provided in the Supplemental Digital Content (http://links.lww.com/ALN/C667).

Statistical Analysis

In total, 48 animals were included in this study, and each group (groups 1 to 6) consisted of 8 animals. The sample size was based on previous experiments performed by our group with a similar animal model that investigated PCC monotherapy for dabigatran reversal.[16] Therefore, no *a priori* statistical power calculation was conducted. One-way ANOVA was used to assess the differences in total blood loss between groups, which was the primary endpoint of the study. *Post hoc* comparisons of all means were performed using a Tukey–Kramer test. A repeated-measures ANOVA (mixed model with restricted maximum likelihood) was used to evaluate the effects of treatments on coagulation parameters, blood cell count, and hemodynamic variables, representing the secondary endpoints. The model included intervention as a group factor and time as a repeated factor. The Sidak method was applied for α error correction of multiple comparisons in this case. A Shapiro–Wilk test was performed to test for normality. Survival was analyzed by applying the pairwise log-rank tests. All data are reported as the means ± SD, the significance was set at $P < 0.05$, and early animal death before the end of the experiment time was the only reason for missing data. In the control group, one animal died before 60 min; thus, the number of animals was seven at 120 min and five at 180 min, and it was reduced to one animal at 240 min posttrauma. In the 12.5 U/kg PCC plus tranexamic acid group, there were seven animals at 120 min and five at 240 min after trauma induction. Two-tailed statistical analyses were performed using SPSS Statistics software (version 25.0, SPSS, USA), and GraphPad Prism (version 8.2.1, GraphPad Software, Inc., USA) was used for graphical purposes.

Results

Forty-eight pigs were included in this study. Until the time of death, complete data were available for all animals for all study variables. Baseline laboratory and hemodynamic parameters were comparable between the groups before the injury.

Plasma Levels of Rivaroxaban and Resulting Anticoagulation before the Infliction of Injury

After the 30-min infusion of rivaroxaban, the overall mean plasma rivaroxaban concentration (all animals) was 693 ±

91 ng/ml (mean ± SD), and both prothrombin time and extrinsic thromboelastometry clotting time were prolonged (table 1; fig. 2, A and B). Plasma concentration of rivaroxaban 12 min after trauma induction and before the administration of the study treatments was 368 ± 98 ng/ml. Rivaroxaban did not affect hemoglobin, platelet count, nor fibrinogen concentration (table 1; fig. 3, A and B). A further effect of rivaroxaban was measured in the reduction of thrombin generation (fig. 4). Four hours after rivaroxaban administration, the plasma concentrations of rivaroxaban were reduced to less than 50 ng/ml in all treatment groups (table 1).

Table 1. Rivaroxaban Concentration, Prothrombin Time, Hemoglobin, and Platelet Count

Time Point	Study Group	Rivaroxaban (ng/ml)	Prothrombin Time (s)	Hemoglobin (g/dl)	Platelets (×10³/μl)
Baseline	Control		10 ± 0.5	10.2 ± 0.5	379 ± 66
	12.5 U/kg PCC		10.8 ± 0.7	10.1 ± 0.3	308 ± 59
	25 U/kg PCC		10 ± 0.6	10.2 ± 0.5	359 ± 60
	50 U/kg PCC		10 ± 0.5	10.3 ± 0.6	358 ± 78
	12.5 U/kg PCC + tranexamic acid		10.8 ± 1	10 ± 0.9	298 ± 55
	12.5 U/kg PCC + tranexamic acid + fibrinogen		10.6 ± 0.8	9.6 ± 0.7	375 ± 66
After rivaroxaban infusion	Control	668 ± 102	75 ± 18	9.7 ± 0.4	326 ± 50
	12.5 U/kg PCC	784 ± 146	72 ± 19	9.5 ± 0.5	290 ± 51
	25 U/kg PCC	656 ± 183	68 ± 9	9.6 ± 0.4	311 ± 54
	50 U/kg PCC	649 ± 111	77 ± 18	9.5 ± 0.5	311 ± 72
	12.5 U/kg PCC + tranexamic acid	766 ± 105	77 ± 9	8.9 ± 0.9	247 ± 33
	12.5 U/kg PCC + tranexamic acid + fibrinogen	807 ± 133	77 ± 18	8.6 ± 0.6	337 ± 61
12 min posttrauma	Control	402 ± 128	54 ± 20	6.5 ± 0.7	259 ± 49
	12.5 U/kg PCC	373 ± 112	49 ± 12	6.7 ± 0.6	217 ± 31
	25 U/kg PCC	351 ± 76	45 ± 6	6.8 ± 0.5	260 ± 36
	50 U/kg PCC	337 ± 99	48 ± 11	7.0 ± 0.7	273 ± 59
	12.5 U/kg PCC + tranexamic acid	346 ± 68	50 ± 7	6.2 ± 0.4	190 ± 31
	12.5 U/kg PCC + tranexamic acid + fibrinogen	398 ± 103	52 ± 12	6.3 ± 0.8	260 ± 42
30 min posttrauma	Control	244 ± 102	45 ± 14*	4.8 ± 0.9	231 ± 63
	12.5 U/kg PCC	214 ± 87	30 ± 8	5.6 ± 0.6	198 ± 40
	25 U/kg PCC	209 ± 63	22 ± 3	5.8 ± 0.7∞	219 ± 33
	50 U/kg PCC	201 ± 77	19 ± 3	5.5 ± 0.6	229 ± 60
	12.5 U/kg PCC + tranexamic acid	207 ± 47	30 ± 5	5.5 ± 0.9	173 ± 42
	12.5 U/kg PCC + tranexamic acid + fibrinogen	235 ± 87	30 ± 7	4.7 ± 0.8	230 ± 55
60 min posttrauma	Control	142 ± 60	41 ± 14*	3.5 ± 1.1*	186 ± 63
	12.5 U/kg PCC	137 ± 45	28 ± 7	4.7 ± 0.4	170 ± 30
	25 U/kg PCC	144 ± 34	21 ± 3	5.1 ± 0.9	205 ± 31
	50 U/kg PCC	128 ± 52	16 ± 2¶	5.3 ± 0.6	209 ± 54
	12.5 U/kg PCC + tranexamic acid	139 ± 41	28 ± 6	4.5 ± 0.7	153 ± 42
	12.5 U/kg PCC + tranexamic acid + fibrinogen	174 ± 73	27 ± 6	4.6 ± 0.9	232 ± 48§
120 min posttrauma	Control	76 ± 50	36 ± 14	3.1 ± 0.5*	142 ± 46
	12.5 U/kg PCC	85 ± 42	26 ± 7	4.4 ± 0.3	128 ± 56
	25 U/kg PCC	76 ± 27	18 ± 3†	5.1 ± 0.5§	202 ± 45
	50 U/kg PCC	73 ± 39	15 ± 2†	5.2 ± 0.6§	213 ± 59†¶
	12.5 U/kg PCC + tranexamic acid	72 ± 25	25 ± 7	4.0 ± 0.7	138 ± 47
	12.5 U/kg PCC + tranexamic acid + fibrinogen	105 ± 52	23 ± 6	4.4 ± 0.7	216 ± 37†¶§
180 min posttrauma	Control	55 ± 44	46 ± 13*	2.2 ± 0.6	105 ± 26
	12.5 U/kg PCC	45 ± 24	25 ± 7	3.7 ± 0.7†	128 ± 31
	25 U/kg PCC	38 ± 12	16 ± 3	4.9 ± 0.7†¶	182 ± 36†
	50 U/kg PCC	33 ± 22	14 ± 2	5.1 ± 0.5†¶	203 ± 56†§
	12.5 U/kg PCC + tranexamic acid	56 ± 18	24 ± 9	3.4 ± 1.1	110 ± 57
	12.5 U/kg PCC + tranexamic acid + fibrinogen	74 ± 46	21 ± 6	4.2 ± 0.9†	204 ± 37†§
240 min posttrauma	Control			1.9	65
	12.5 U/kg PCC	22 ± 10	25 ± 6	3.3 ± 1.0	104 ± 29
	25 U/kg PCC	17 ± 11	15 ± 3	5.0 ± 0.9¶	175 ± 35
	50 U/kg PCC	18 ± 15	13 ± 2¶	5.2 ± 0.5¶	176 ± 46
	12.5 U/kg PCC + tranexamic acid	36 ± 20	23 ± 8	4.2 ± 1.2	145 ± 56
	12.5 U/kg PCC + tranexamic acid + fibrinogen	50 ± 36	19 ± 5	4.4 ± 1.0¶	204 ± 39¶

The data are shown as means ± SD. Initially, n = 8/group; in the control group, n = 7 at 120 min, n = 5 at 180 min, and n = 1 at 240 min after trauma. In the 12.5 U/kg prothrombin complex concentrate (PCC) plus tranexamic acid group, n = 7 at 120 min, and n = 5 at 240 min after trauma. The tranexamic acid dose was 20 mg/kg, and the fibrinogen dose was 80 mg/kg.

*$P < 0.05$ *versus* all other groups; †$P < 0.05$ *versus* control; ¶$P < 0.05$ *versus* 12.5 U/kg PCC; §$P < 0.05$ *versus* 12.5 U/kg PCC plus tranexamic acid; ∞$P < 0.05$ *versus* 12.5 U/kg PCC plus tranexamic acid and fibrinogen.

Blood Loss and Survival

In the control group (rivaroxaban plus placebo), postinjury blood loss was 3,313 ± 634 ml compared with 1,541 ± 269 ml and 1,464 ± 108 ml in the 25 and 50 U/kg PCC groups, respectively ($P < 0.0001$ *vs.* 25 and 50 U/kg PCC; fig. 5). The mortality rate was 100% in the control group, and the mean survival time was 131 min (range, 50 to 200 min). All animals that received 25 U/kg PCC or 50 U/kg PCC survived the 240-min observation period ($P < 0.01$ *vs.* control). Hemodynamic variables and lactate levels were stabilized (see table, Supplemental Digital Content, http://links.lww.com/ALN/C667, hemodynamic variables).

Treatment with 12.5 U/kg PCC (blood loss, 2,671 ± 334 ml; $P = 0.934$ *vs.* control) and 12.5 U/kg PCC plus tranexamic acid (blood loss, 2,910 ± 856 ml; $P = 0.931$ *vs.* control) did not significantly reduce blood loss compared with control, whereas the mean survival time was 234 min (range, 195 to 240 min) and 186 min (range, 46 to 240 min), respectively. The mortality rates were 88 and 50% in the 12.5 U/kg PCC and 12.5 U/kg PCC plus tranexamic acid groups, respectively.

All animals in the 12.5 U/kg PCC plus tranexamic acid and fibrinogen concentrate group survived, and the total postinjury blood loss was reduced to 1,836 ± 556 ml (all $P < 0.001$ *vs.* control, 12.5 U/kg PCC, and 12.5 U/kg PCC plus tranexamic acid groups; fig. 5). Stabilization of hemodynamic variables and lactate levels was also observed in this group (see table S1, Supplemental Digital Content, http://links.lww.com/ALN/C667, hemodynamic variables).

Measurements after Standardized Polytrauma and Coagulation Analysis

Control Animals. Despite fluid resuscitation, control animals developed severe shock after trauma, with low mean arterial pressure and cardiac output attributable to ongoing blood loss (see table S1, Supplemental Digital Content, http://links.lww.com/ALN/C667, hemodynamic variables). Control animals also had the lowest platelet counts at 120 min posttrauma compared to 12.5 U/kg PCC plus tranexamic acid and fibrinogen concentrate and 50 U/kg PCC groups (all $P < 0.001$) and the lowest hemoglobin levels of all treatment groups at 60 min after trauma induction (table 1). Furthermore, these animals developed severe coagulopathy with deterioration in all coagulation parameters over time (fig. 3 and 4; table 1). Extrinsic thromboelastometry maximum clot firmness was significantly lower in this group at 2 h posttrauma than the 12.5 U/kg PCC ($P = 0.014$ *vs.* control), 25 U/kg PCC, 50 U/kg PCC, and 12.5 U/kg PCC plus tranexamic acid and fibrinogen concentrate groups (all $P < 0.0001$) and at 3 h posttrauma compared with the PCC 12.5, PCC 25, PCC 50, 12.5 U/kg PCC plus tranexamic acid, and 12.5 U/kg PCC plus tranexamic acid and fibrinogen concentrate groups (all $P < 0.0001$ *vs.* control; fig. 2, C and D). Additionally, the plasma fibrinogen concentrations were significantly lower than in the 12.5 U/kg PCC plus tranexamic acid and fibrinogen concentrate group at 30 min posttrauma ($P < 0.0001$ *vs.* control; fig. 3B). The impairment of thrombin generation caused by rivaroxaban was sustained among control animals throughout the observation period (fig. 4).

Effects of 12.5, 25, and 50 U/kg PCC. After PCC therapy, prothrombin time was reduced dose-dependently in these three groups at 60 min after trauma (all $P < 0.01$ *vs.* control; table 1). Thrombin generation increased dose-dependently immediately after PCC administration. At 30 min after trauma, endogenous thrombin potential was 419 ± 60 nM min in the 12.5 U/kg PCC group, 655 ± 89 nM min in the 25 U/kg PCC group, and 981 ± 159 nM min in the 50 U/kg PCC group, whereas the peak height was 28 ± 6, 31 ± 7, and 49 ± 11 nM, respectively (fig. 4). Endogenous thrombin potential remained significantly greater than baseline throughout the remainder of the observation period in both the 25 and 50 U/kg PCC groups (all $P < 0.0001$ *vs.* control). Peak height was below the baseline value in all PCC groups and subsequently increased over time while maintaining baseline value 240 min posttrauma. Additionally, peak height in the 50 U/kg PCC group of treated animals was significantly higher 30 min after trauma and over the observation time compared to all other groups (all $P < 0.0001$; fig. 4, C and D).

Corresponding with significant blood loss reductions, hemodynamic parameters remained stable in the 25 and 50 U/kg PCC groups after fluid resuscitation and trauma (see table S1, Supplemental Digital Content, http://links.lww.com/ALN/C667, hemodynamic variables). Due to ongoing blood loss, endogenous thrombin potential in the 12.5 U/kg PCC group decreased slightly over time, and platelets were significantly lower than the 50 U/kg PCC group at 120 min posttrauma ($P = 0.008$; fig. 4A; table 1). The reduction in hemoglobin and platelet counts continued until the end of the observation period compared to the 25 and 50 U/kg PCC groups (table 1).

Substitution with 12.5 U/kg PCC plus Tranexamic Acid and 12.5 U/kg PCC plus Tranexamic Acid and Fibrinogen Concentrate

Animals in the 12.5 U/kg PCC or 12.5 U/kg PCC plus tranexamic acid groups developed severe shock after injury, with low mean arterial pressure attributable to ongoing blood loss 2 h after trauma induction (See table S1, Supplemental Digital Content, http://links.lww.com/ALN/C667, Hemodynamic variables). Animals in the 12.5 U/kg PCC or 12.5 U/kg PCC plus tranexamic acid groups also had lower platelet counts than animals in the 12.5 U/kg PCC plus tranexamic acid and fibrinogen concentrate group (table 1). Endogenous thrombin potential in these animals was restored above baseline immediately after PCC administration but decreased slightly over time (fig. 4B).

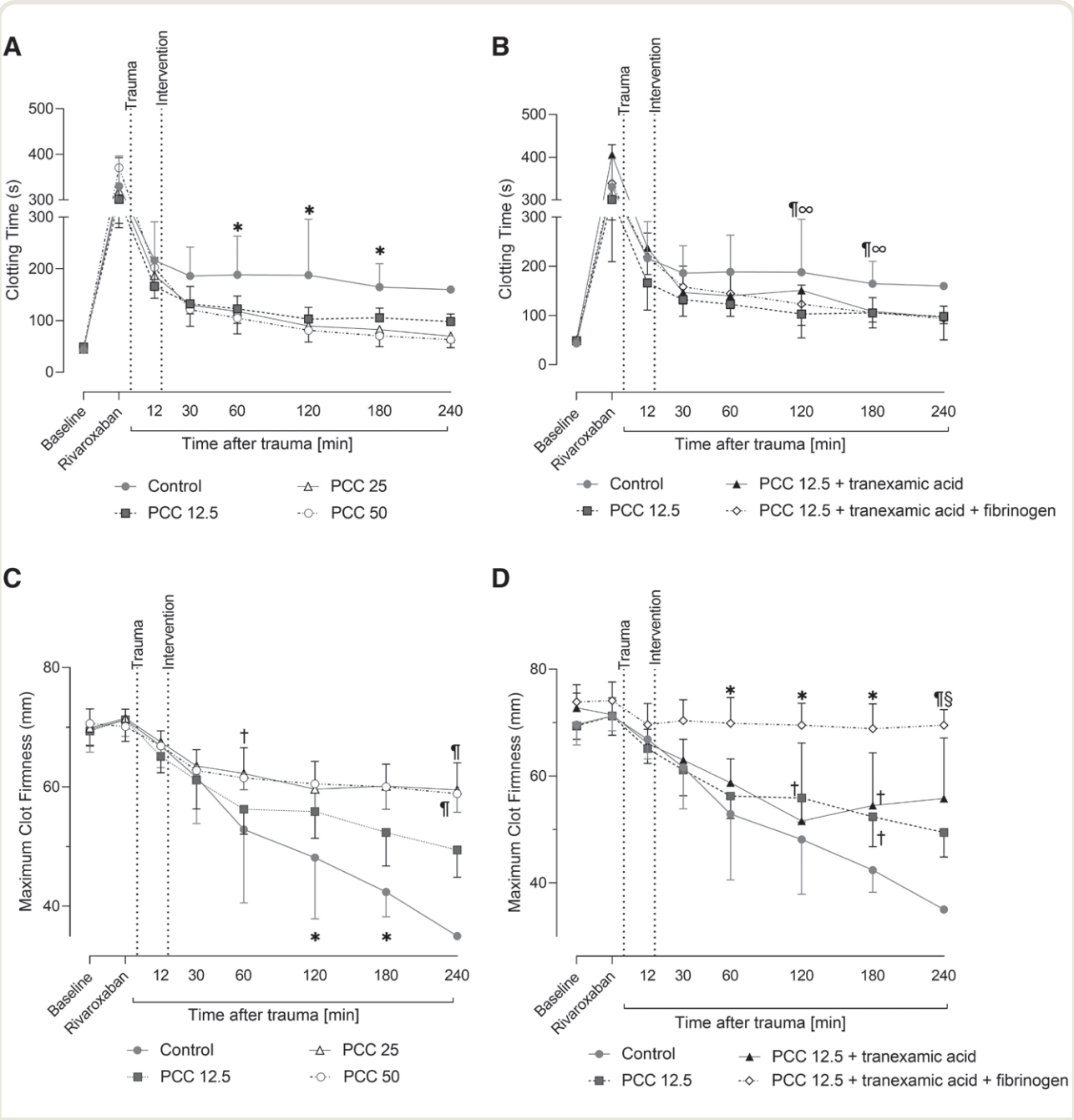


Fig. 2. Thromboelastometry of clotting time (*A*, *B*) and maximum clot firmness (*C*, *D*). (*A*, *C*) Study part 1: control, 12.5 U/kg prothrombin complex concentrate (PCC), 25 U/kg PCC, and 50 U/kg PCC. (*B*, *D*) Study part 2: control, 12.5 U/kg PCC, 12.5 U/kg PCC plus tranexamic acid, and 12.5 U/kg PCC plus tranexamic acid and fibrinogen concentrate. The data are presented as means ± SD, considering study group × observation time interaction. Initially, n = 8/group; in the control group, n = 7 at 120 min, n = 5 at 180 min, and n = 1 at 240 min after trauma; in the 12.5 U/kg PCC plus tranexamic acid group, n = 7 at 120 min and n = 5 at 240 min after trauma. **P* < 0.05 *versus* all other groups; †*P* < 0.05 *versus* control; ¶*P* < 0.05 *versus* 12.5 U/kg PCC; §*P* < 0.05 *versus* 12.5 U/kg PCC plus tranexamic acid; ∞*P* < 0.05 *versus* 12.5 U/kg PCC plus tranexamic acid and fibrinogen concentrate.

After administering fibrinogen concentrate (80 mg/kg) in the 12.5 U/kg PCC plus tranexamic acid and fibrinogen concentrate group, plasma fibrinogen concentrations were restored to baseline and remained significantly higher than in all other groups up to 240 min postinjury (all $P < 0.0001$; fig. 3B). Similarly, extrinsic thromboelastometry maximum clot firmness was restored close to the baseline (fig. 2D). Although

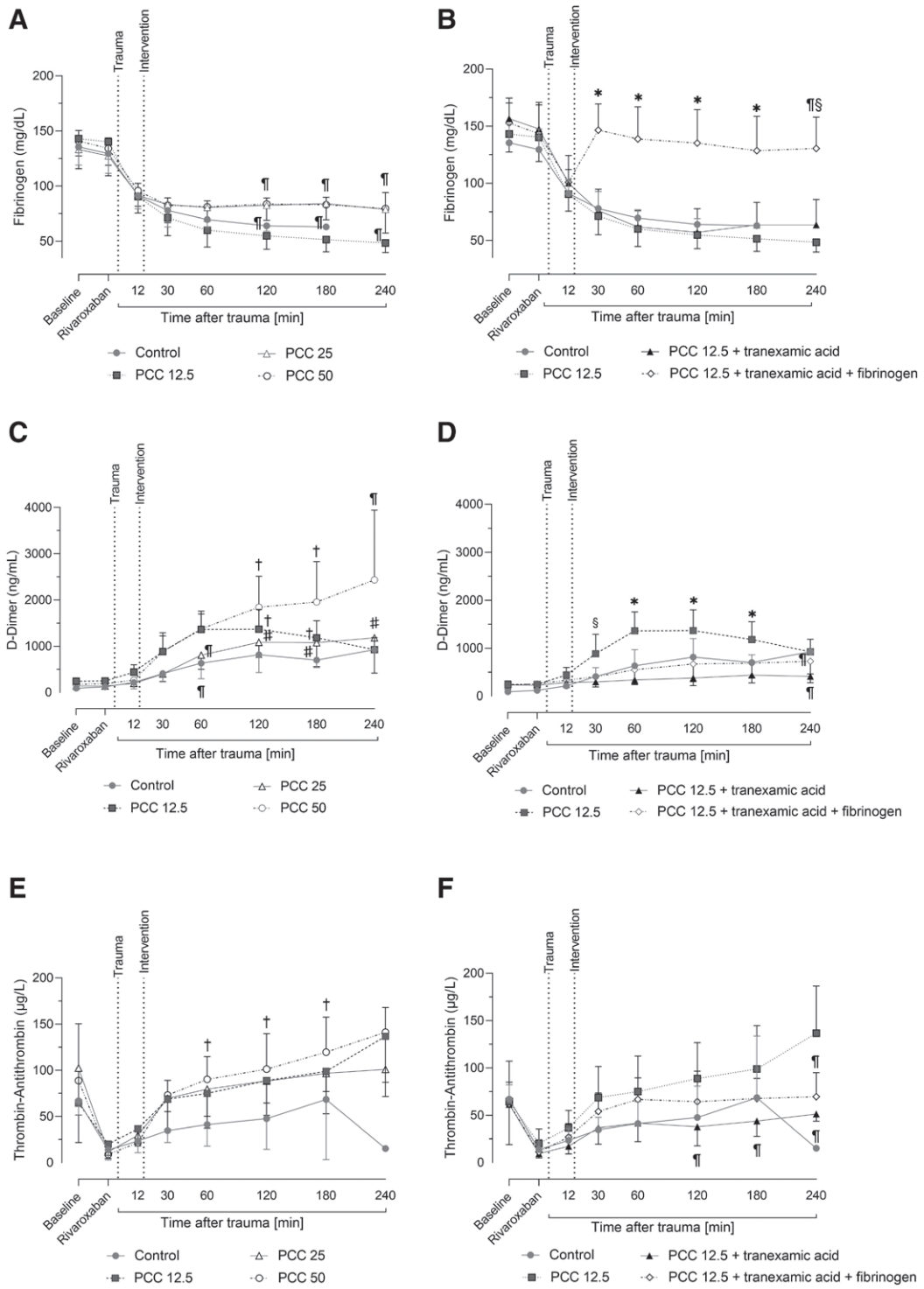


Fig. 3. Fibrinogen (*A*, *B*), D-dimer (*C*, *D*), and thrombin–antithrombin complexes (*E*, *F*). (*A*, *C*, *E*) Study part 1: control, 12.5 U/kg prothrombin complex concentrate (PCC), 25 U/kg PCC, and 50 U/kg PCC. (*B*, *D*, *F*) Study part 2: control, 12.5 U/kg PCC, 12.5 U/kg PCC plus tranexamic acid, and 12.5 U/kg PCC plus tranexamic acid and fibrinogen concentrate. The data are presented as means ± SD, considering study group × observation time interaction. Initially, n = 8/group; in the control group, n = 7 at 120 min, n = 5 at 180 min, and n = 1 at 240 min after trauma. The fibrinogen level of the control animal at 240 min posttrauma was not measurable. In the 12.5 U/kg PCC plus tranexamic acid group n = 7 at 120 min and n = 5 at 240 min after trauma. **P* < 0.05 *versus* all other groups; †*P* < 0.05 *versus* control; ¶*P* < 0.05 *versus* 12.5 U/kg PCC; §*P* < 0.05 *versus* 12.5 U/kg PCC plus tranexamic acid; #*P* < 0.05 *versus* 50 U/kg PCC.

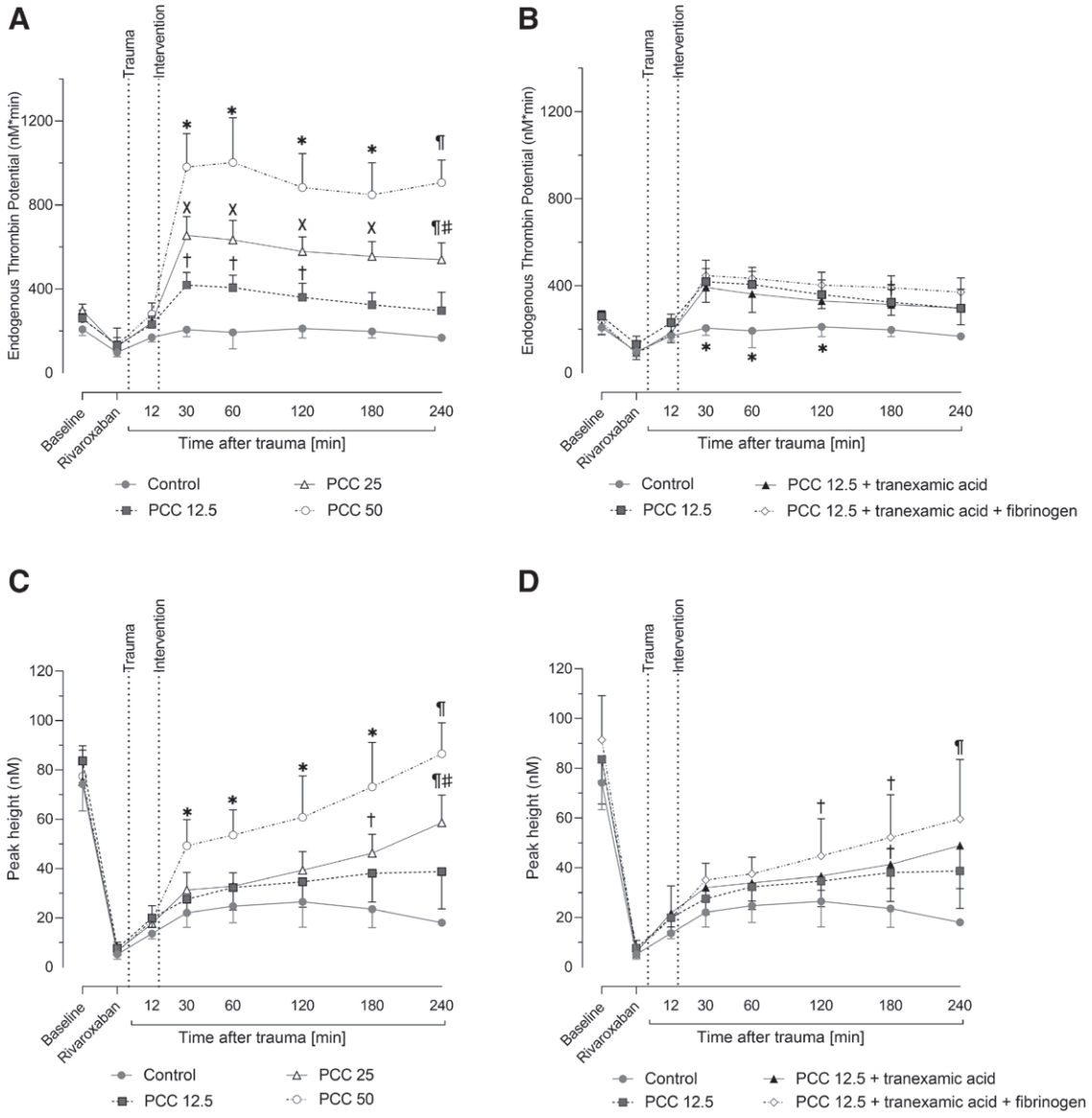


Fig. 4. Thrombin generation: Endogenous thrombin potential (*A*, study part one: control, prothrombin complex concentrate [PCC] 12.5, PCC 25, and 50 U/kg PCC, and *B*, study part two: control, 12.5 U/kg PCC, 12.5 U/kg PCC plus tranexamic acid, 12.5 U/kg PCC plus tranexamic acid and fibrinogen concentrate). Peak height (*C*: study part one, and *D*: study part two). The data are presented as means ± SD, considering study group × observation time interaction. Initially, n = 8/group; in the control group, n = 7 at 120 min, n = 5 at 180 min, and n = 1 at 240 min after trauma. In the 12.5 U/kg PCC plus tranexamic acid group, n = 7 at 120 min and n = 5 at 240 min after trauma. **P* < 0.05 *versus* all other groups; †*P* < 0.05 *versus* control; ¶*P* < 0.05 *versus* 12.5 U/kg PCC; X*P* < 0.05 *versus* control and 12.5 U/kg PCC; #*P* < 0.05 *versus* 50 U/kg PCC.

the total blood loss was lower significantly in the 12.5 U/kg PCC plus tranexamic acid and fibrinogen concentrate group compared with the other groups in the second part of the study ($P < 0.0001$ *vs.* control, $P = 0.001$ *vs.* 12.5 U/kg PCC, $P < 0.001$ *vs.* 12.5 U/kg PCC plus tranexamic acid; fig. 5), endogenous thrombin potential and peak height were comparable with the 12.5 U/kg PCC or 12.5 U/kg PCC plus tranexamic acid groups over time. However, peak height was significantly lower at 240 min after trauma in animals treated with 12.5 U/kg PCC compared with those in the 12.5 U/kg PCC plus tranexamic acid and fibrinogen concentrate ($P = 0.007$; fig. 4, B and D).

Histopathologic Analysis

The histopathologic examination of injured liver sections revealed homogenous tissue damage and comparable lacerations in all animals. No thromboembolic or

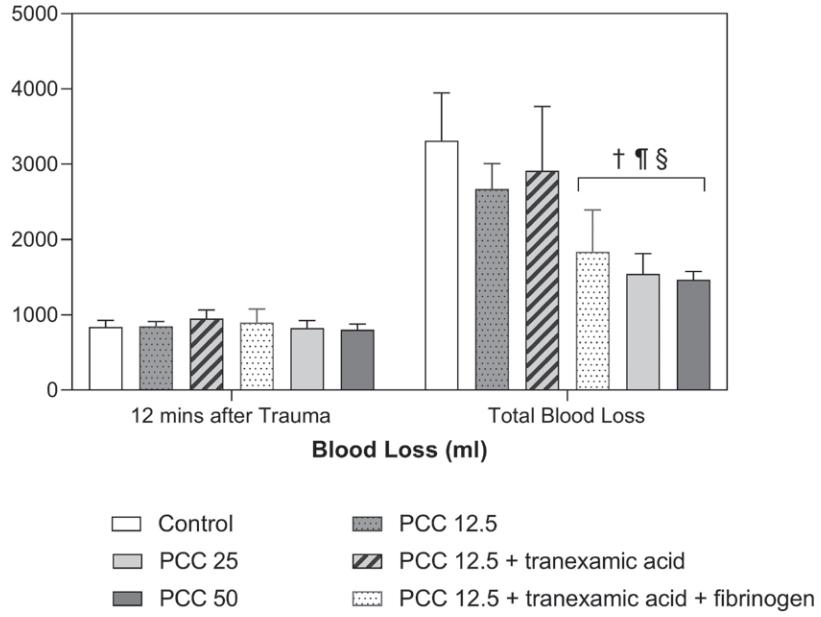


Fig. 5. Blood loss 12 and 240 min postinjury. The data are presented as means ± SD. Initially, n = 8/group; in the control group, n = 1 at 240 min after trauma. In the 12.5 U/kg prothrombin complex concentrate (PCC) plus tranexamic acid group, n = 5 at 240 min after trauma. †$P < 0.05$ *versus* control; ¶$P < 0.05$ *versus* 12.5 U/kg PCC; §$P < 0.05$ *versus* 12.5 U/kg PCC plus tranexamic acid.

other remarkable pathologic changes were present in kidneys, lungs, heart, or nontraumatized liver tissue.

Discussion

This study shows that PCC at 25 and 50 U/kg can effectively control bleeding and restore hemostasis under rivaroxaban anticoagulation in a clinically relevant experimental polytrauma animal model. Administration of PCC at a lower dose of 12.5 U/kg, even though it reduced the blood loss postinjury compared with control animals, was not effective in reversing the anticoagulant effects of rivaroxaban either as monotherapy (12.5 U/kg) or in combination with tranexamic acid. These results imply that a threshold level of coagulation factors or restoration of thrombin generation is needed to overcome rivaroxaban-induced thrombin inhibition. This level depends on the concentration of rivaroxaban and PCC. The addition of fibrinogen plus tranexamic acid to a low dose (12.5 U/kg) of PCC could compensate for this lack of efficacy.

PCC, at appropriate doses (25 or 50 U/kg), was effective in treating coagulopathy without excessive activation of coagulation at the level of anticoagulation used in this model. This is in agreement with several previous animal studies, as well as limited experience in humans.[5,24–26] In trauma-associated bleeding with rivaroxaban anticoagulation, PCC-mediated increase in factor X and factor II levels are increasing to the point that thrombin generation should, theoretically, overcome the inhibitory effects of rivaroxaban. This observation is supported by the inefficacy of the lower dose of PCC (12.5 U/kg) to overcome the rivaroxaban-induced coagulopathy in our porcine model. However, the exact mechanism whereby PCC may improve hemostasis under factor Xa inhibition is not fully understood. The mechanism of action can probably not be explained by a simple stoichiometric reaction. On a molar basis, the concentration of factor X in PCC is not sufficient to overcome the antagonizing effect of the factor Xa inhibitor. Instead, PCCs may restore hemostasis in patients on direct oral anticoagulants because they increase not only factor X levels but also factor II, VII, and IX concentrations at the site of injury and may exert a compensatory prohemostatic effect with increased thrombin generation potential. However, PCC's efficacy to restore hemostasis is also dependent on different factors such as clinical status, the hemostatic system of the species under investigation, trauma severity, blood loss, and coagulopathy status.

PCC's benefit for managing factor Xa inhibitor–associated bleeding was explored in animal models of acute hemorrhage using different injury approaches and spiking experiments in blood from human volunteers.[25–33] These studies have also shown a dose-dependent effect of PCC on the reversal of factor Xa inhibitors. The observational multicenter cohort study Unactivated Prothrombin complex concentrates for the Reversal of Anti-factor Ten inhibitors (UPRATE), which was performed in Sweden and Canada,

investigated the efficacy of a four-factor PCC at a median dose of 25 U/kg to restore hemostasis in rivaroxaban- or apixaban-anticoagulated patients with major acute bleeding (mainly intracerebral hemorrhage and gastrointestinal bleeding) defined according to the International Society on Thrombosis and Haemostasis.[34,35] In both cohorts, 68 and 69% of patients showed "effective" hemostasis after four-factor PCC therapy. However, due to the lack of a control group and the lack of factor Xa activity measurements, it is difficult to draw definitive conclusions about PCC therapy's efficacy and appropriate PCC dosing.

In our model, the causes of coagulopathy included consumption and dilution of coagulation factors, platelets, and impaired thrombin generation. As reported above, a PCC dose of 25 U/kg was shown to restore hemostasis effectively. Similarly, the combination of tranexamic acid and fibrinogen concentrate plus a low dose of PCC at 12.5 U/kg restored hemostasis. In contrast, tranexamic acid plus a low dose of PCC at 12.5 U/kg had no impact on blood loss. From a mechanistic perspective, administration of fibrinogen concentrate increased the plasma fibrinogen levels to enable fibrin clot formation.[36] At the same time, tranexamic acid inhibits the fibrinolysis pathway and attenuates clot degradation.[36,37]

In major blood loss, fibrinogen is one of the first coagulation factors decreasing below the critical level (100 mg/dl).[38,39] The critical minimum concentration of fibrinogen to maintain hemostasis is a matter of debate. The recent European guidelines recommend threshold concentrations of 150 to 200 mg/dl relating to perioperative bleeding and trauma.[4] Plasma concentrations of fibrinogen after trauma were below this threshold in our trauma model. Therefore, when treating trauma patients who are receiving rivaroxaban therapy, it is possible that the early administration of fibrinogen and antifibrinolytic medication could reduce the dose of PCC that is required for restoring hemostasis.

The propensity of PCCs to increase thrombin generation is the key to their efficacy but may also increase the risk of thromboembolic events. Kinetic parameters of thrombin generation potential (endogenous thrombin potential and peak height) and coagulation (D-dimers) were increased above baseline after PCC therapy. However, no adverse clinical signs or histopathologic signs of prothrombotic changes were observed in the current study. Excessive concentrations of prothrombin (factor II) to antithrombin have been suggested to cause thromboembolic complications.[40] In an experimental animal model of liver injury without anticoagulation, it was reported that 50 U/kg PCC might increase the risk of disseminated intravascular coagulation, attributable to an imbalance of procoagulant and anticoagulant proteins.[22,40] These data are in line with those from an observational, clinical study of four-factor PCC in severely injured patients.[10] In contrast, in experiments in a similar animal model of acute injury, PCC doses as high as 100 U/kg were well tolerated in the presence of dabigatran anticoagulation. These data imply that PCC's impact might be different in clinical situations with and without previous anticoagulation.

Similarly, in eight uncontrolled studies using PCC for factor Xa inhibitor–associated major bleeds, the crude pooled incidence of thromboembolic events was 5%.[41] Therefore, it seems likely that an intrinsic thromboembolic event rate of 4 to 5% in these specific patients has to be accepted when oral anticoagulants are reversed for major bleeding. Conclusively, there is reason to believe that the use of 25 to 50 U/kg PCC for management of factor Xa inhibitor–associated bleeding has a positive effect without significantly increasing the risk of thromboembolic events. The current study suggests a comparable effect and safety profile of either treatment option. Follow-up investigations are needed to determine whether a multimodel therapy may provide an improved safety profile as compared to higher doses of PCC monotherapy during extended follow-up periods.

At present, no preclinical or clinical studies have investigated the efficacy of PCC *versus* andexanet alfa to control severe bleeding and/or restore hemostasis under direct oral anticoagulant therapy in a direct head-to-head comparison. Previous studies in the pig polytrauma model, however, evaluated the effect of andexanet alfa *versus* vehicle control (bolus dose of 1,000 mg, or bolus of 1,000 mg plus infusion of 1,200 mg over 2 h) to reverse apixaban-associated bleeding.[42] It could be shown that andexanet alfa significantly reduced total blood loss posttrauma and achieved a 100% survival rate compared with control animals, similar to the results seen in the current study. A more detailed cross-study comparison to the effects of PCC *versus* andexanet alfa is hindered by key differences in study designs, including oral *versus* systemic administration and anticoagulation *via* apixaban *versus* rivaroxaban. Follow-up studies are therefore required to allow direct statistical comparisons. Due to the high costs of andexanet alfa, limited availability, and improvement of hemostasis not yet being established,[43] clinical studies, such as A Phase 4 Randomized Clinical Trial Of AndexXa (Portola Pharmaceuticals, Inc., USA) In Acute Intracranial Hemorrhage In Patients Receiving An Oral Factor Xa Inhibitor (ANNEXA-I), investigating andexnat alfa *versus* PCC, are urgently needed to guide clinical therapy.

This animal model has its limitations. In this study, we used only males to keep the groups as homogenous as possible at baseline to minimize the SD. The injury was induced in anesthetized healthy animals. To obtain therapeutic plasma concentrations of rivaroxaban, intravenous infusion of rivaroxaban was administered for 30 min. Therefore, the observed results may not be directly applicable in clinical settings. Because our study used a standardized trauma model, it may not show the differences between bleeding sites, patient's physiologic responses, and drug–drug interactions. The absence of thromboembolic events is limited by the observation time of 4 h.

Conclusions

In conclusion, this study investigated effective hemostatic control mediated by either monotherapy of PCC or a

multimodal regimen with low-dose PCC plus additional tranexamic acid and fibrinogen concentrate in the presence of rivaroxaban anticoagulation in a severe bleeding polytrauma setting. The primary outcomes of this study demonstrates that the utility of a high dose of PCC greater than 25 U/kg may not have any additional advantage in significantly reducing the bleeding in trauma complications. Additionally, coadministration of a low dose of 12.5 U/kg PCC with tranexamic acid and fibrinogen concentrate compared with a higher dose of PCC can be considered as an alternative therapeutic option. The findings also confirm that PCC may be more efficient as part of a multimodal therapy than monotherapy in restoring hemostasis and balancing thrombin generation.

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Competing Interests

Dr. Grottke has received research funding from Bayer AG (Wuppertal, Germany), Biotest (Dreieich, Germany), Boehringer Ingelheim (Ingelheim, Germany), CSL Behring (Marburg, Germany), Octapharma (Lachen, Switzerland), Novo Nordisk (Bagsværd, Denmark), Nycomed (Konstanz, Germany), and Werfen (Munich, Germany). He has also received honoraria for lectures and consultancy support from Baxalta (Unterschleißheim, Germany), Bayer AG, Boehringer Ingelheim, Ferring (Kiel, Germany), CSL Behring, Octapharma, Pfizer (Berlin, Germany), Takeda (Zurich, Switzerland), Portola (San Francisco, California), and Sanofi (Berlin, Germany). Dr. Schöchl has received speaker fees from CSL Behring, TEM International (Munich, Germany), Haemonetics (Signy-Centre, Switzerland), and Stago (Asnieres, France). Dr. Rossaint has received honoraria for lectures and consultancy from CSL Behring, Bayer AG, Boehringer Ingelheim, and Novo Nordisk (Mainz, Germany). Dr. Herzog is an employee of CSL Behring (King of Prussia, Pennsylvania). Dr. Heitmeier is an employee of Bayer AG. The other authors declare no competing interests.

Correspondence

Address correspondence to Dr. Grottke: RWTH Aachen University Hospital, Pauwelsstrasse 30, 52074 Aachen, Germany. ogrottke@ukaachen.de.

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