ANESTHESIOLOGY

Prothrombin Complex Concentrate-induced Disseminated Intravascular Coagulation Can Be Prevented by Coadministering Antithrombin in a Porcine Trauma Model

Oliver Grottke, M.D., Ph.D., Markus Honickel, M.D., Till Braunschweig, M.D., Anne Reichel, M.D., Herbert Schöchl, M.D., Rolf Rossaint, M.D.

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Prothrombin complex concentrates are increasingly used as part of bleeding management algorithms in surgery and trauma
- There are potential risks of thromboembolic complications and disseminated intravascular coagulopathy with prothrombin complex concentrate in this setting, despite the low risks in warfarin reversal

What this Article Tells Us That Is New

- In this animal polytrauma model, 50 IU/kg prothrombin complex concentrate is associated with a risk of disseminated intravascular coagulopathy and thromboembolism
- The addition of antithrombin appears to balance the procoagulant effects of prothrombin complex concentrate, consequently reducing the risk of complications without impairing efficacy

ABSTRACT

Background: The risk of thromboembolic complications with prothrombin complex concentrates (PCCs) appears low when used for reversal of vitamin K antagonists but might be different in other indications (*e.g.*, trauma). A difference in risk could arise from the plasma ratio of pro- *versus* anticoagulant proteins. This study used a porcine trauma model to investigate combined treatment with PCC and antithrombin. The hypothesis was that antithrombin can modulate prothrombotic effects and prevent adverse events of PCC.

Methods: Nine treatment groups (n = 7 per group) were included: control (placebo), PCC (50 IU/kg), PCC plus antithrombin (three groups, with antithrombin doses of 12.5, 25, or 50 IU/kg), fibrinogen concentrate (100 mg/kg) plus PCC, fibrinogen concentrate plus PCC plus antithrombin dose of 50 IU/kg, tranexamic acid (15 mg/kg) plus fibrinogen concentrate plus PCC, and tranexamic acid plus fibrinogen concentrate plus PCC plus antithrombin dose of 50 IU/kg. In each group, bilateral femur fractures and thorax contusion were followed 60 min later by blunt liver injury. Study treatment was then administered, and animals were subsequently observed for 210 min.

Results: Total blood loss (mean ± SD) was statistically significantly lower in all three PCC plus antithrombin groups (PCC plus antithrombin dose of 50 IU/kg, 672 ± 63 ml; PCC plus antithrombin dose of 25 IU/kg, 535 ± 72 ml; and PCC plus antithrombin dose of 12.5 IU/kg, 538 ± 50 ml) than in the PCC group (907 ± 132 ml), which in turn had statistically significantly reduced bleeding *versus* the control group (1,671 ± 409 ml). Signs of disseminated intravascular coagulation were apparent with PCC monotherapy, and early deaths occurred with fibrinogen concentrate plus PCC, attributable to pulmonary emboli. Antithrombin was protective against both of these effects: signs of disseminated intravascular coagulation were absent from the PCC plus antithrombin groups, and there were no early deaths in the group with fibrinogen concentrate plus PCC plus antithrombin dose of 50 IU/kg.

Conclusions: According to this trauma model, 50 IU/kg PCC increases the risk of disseminated intravascular coagulation and other thromboembolic complications, most notably when coadministered with fibrinogen concentrate. The addition of antithrombin appears to reduce this risk.

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Prothrombin complex concentrates (PCCs) are plasma-derived products, historically used as a source of coagulation factor IX for treatment of hemophilia B. They have been superseded in that setting but continue to be used in patients requiring supplementation of the vitamin K-dependent coagulation factors (II, VII, IX, and X).[1,2] PCCs are available with either three or four coagulation factors (II, IX, and X, with or without factor VII), and these may be formulated in their activated or unactivated forms. Differences between PCCs in relation to both efficacy and safety have been reported.[3,4]

Today's PCCs are approved for urgent reversal of vitamin K antagonists, based on strong evidence from large clinical trials.[5–7] In European and some additional countries, PCCs

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are also labeled for "treatment and prophylaxis of bleeding in acquired deficiency of the prothrombin complex coagulation factors," meaning they may be used to treat bleeding unrelated to anticoagulation (*e.g.*, trauma-related or perioperative bleeding).[8] There is some evidence for using PCC in these settings, but it is relatively weak, and to date no phase II or III trials are available.[9–13] Further investigations of PCC are therefore warranted, and at least one clinical study of PCC as early treatment in trauma is ongoing.[14]

The risk of thromboembolic complications with PCC therapy appears low in patients needing reversal of vitamin K antagonists.[15] In contrast, preclinical studies in trauma have shown a possible risk of thromboembolic complications and disseminated intravascular coagulation (DIC).[16,17] There may be a difference in the safety profile of PCCs, depending on whether they are used for reversal of vitamin K antagonists or to treat bleeding unrelated to anticoagulation.[2] Theoretical models suggest levels of coagulation inhibitors could be key to this difference.[18,19] Patients receiving anticoagulation therapy have normal levels of antithrombin (previously known as antithrombin III), and by supplementing levels of coagulation factors II, VII, IX, and X, PCC rebalances the coagulation system.[19,20] Patients with bleeding unrelated to anticoagulation therapy may have decreased levels of coagulation inhibitors (*e.g.*, antithrombin), meaning that PCC can cause an imbalance in the coagulation status.[2,16,19,21–23] Moreover, physiologic responses to trauma may include increased thrombin generation potential, increasing the risk of excessive thrombin generation.[24] A type of DIC (fibrinolytic phenotype) can arise as a component of trauma-induced coagulopathy.[25,26]

Coagulation management algorithms based on coagulation factor concentrates, including PCCs, have been published for bleeding related to cardiovascular surgery and trauma.[27–31] However, PCCs are not recommended as first-line intervention in these algorithms. After antifibrinolytic medication, fibrinogen supplementation is usually recommended as first-line hemostatic treatment, provided that there is evidence of hypofibrinogenemia. If the patient continues to bleed despite sufficient fibrinogen levels, PCC administration may be triggered by viscoelastic test results: prolonged clotting time (rotational thromboelastometry) or prolonged reaction time (R-time; thromboelastogram).

Antithrombin has been identified as a key determinant of the activity of procoagulant therapy.[18,32] To mitigate the risk of excessively high thrombin generation potential when using PCC to treat bleeding unrelated to anticoagulation therapy, coadministration of antithrombin has been suggested.[2,33] However, this approach has never been studied. Therefore, we used a porcine trauma model to investigate the effects of combined treatment with PCC and antithrombin on blood loss and markers of DIC. Our hypothesis was that antithrombin, when coadministered with PCC, can modulate prothrombotic effects (*e.g.*, elevated thrombin generation) and prevent adverse events (*e.g.*, DIC).

Materials and Methods

The Principles of Laboratory Animal Care[34] were adhered to in the design of this study, and the protocol was approved by the governmental animal care and use office. Sixty-three male German land race pigs from a disease-free breeding facility (mean body weight ± SD, 44.6 ± 3.8 kg; age range, 3 to 4 months) were included in the study. The animals were fasted during the night before surgery and given unlimited access to water. All of the study experiments were performed at the Department of Laboratory and Animal Science of Aachen University (Aachen, Germany). A time schedule for the study is provided in the Supplemental Digital Content (http://links.lww.com/ALN/B960).

Before surgery, azaperone (4 mg/kg) and atropine (0.1 mg/kg) were administered by intramuscular injection, followed by intravenous propofol (3 mg/kg). The animals were ventilated at 20 to 22 breaths/min with tidal volume 8 ml/kg (Cato ventilator; Dräger, Germany). Anesthesia was maintained with isoflurane (end-tidal concentration, 1.2 to 1.4%) and fentanyl (constant infusion, 3 μg/kg/h).

Crystalloid solution (2 ml/kg/h) was administered as initial fluid therapy. An AS/3 monitor (Datex Ohmeda, Finland) was used to assess blood temperature; arterial, central venous, and pulmonary arterial pressure; tail pulse oximetry; and electrocardiography.

The first injury phase comprised bilateral femur fractures and a unilateral thorax contusion, both induced with captive bolt guns. Sixty percent of each animal's estimated blood volume was withdrawn (rate: 100 ml/min). Crystalloid solution was infused for 20 min to maintain mean arterial pressure of more than 30 mmHg, then at 5 ml/kg/min for 5 min, followed by 40 ml/kg/h. The second injury phase, comprising grade III blunt liver injury, was performed 60 min after the first injury as described previously.[35]

After the second trauma, the animals were randomized using a computer-generated list and sealed envelopes to receive one of the following treatments:

1. Placebo (control group; n = 7)
2. PCC 50 IU/kg (n = 7)
3. PCC 50 IU/kg plus antithrombin 50 IU/kg (n = 7)
4. PCC 50 IU/kg plus antithrombin 25 IU/kg (n = 7)
5. PCC 50 IU/kg plus antithrombin 12.5 IU/kg (n = 7)
6. Fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg (n = 7)
7. Fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg (n = 7)
8. Tranexamic acid 15 mg/kg plus fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg (n = 7)
9. Tranexamic acid 15 mg/kg plus fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg (n = 7)

Where it was used, tranexamic acid (15 mg/kg; Cyklokapron; Pfizer, Germany) was administered intravenously 5 min after

the second trauma. Fibrinogen concentrate (100 mg/kg; Haemocomplettan P; CSL Behring, Germany; lot 31169911A) was given intravenously for more than 5 min, starting 10 min after the second trauma. PCC (50 IU/kg, intravenous infusion; Beriplex PN, CSL Behring; lot C6660111B) was administered from 16 to 26 min after trauma. Antithrombin (12.5, 25, or 50 IU/kg, intravenous bolus; Kybernin, CSL Behring; lot 88667111E) was administered from 26 min after trauma. Thus, the order in which treatments were given is reflected by the term used to describe each study group. All animals received the equivalent infusion volume, with saline administered instead of any missing treatment(s).

Animals were observed until 210 min after completion of study drug administration (*i.e.*, 240 min after the second trauma), at which time they were euthanized with pentobarbital. The abdomen was reopened post mortem, and the vena cava was clamped cranial to the liver. Blood loss was measured by collecting intraperitoneal blood (an abdominal incision provided access, and blood was suctioned out).

Blood samples were collected at baseline, 10 min after the first injury (hemorrhagic shock), after completion of study drug administration (*i.e.*, 30 min after the second trauma), and at 30, 90, 150, and 210 min after completion of study drug administration. For animals dying before 210 min, the last blood sample was taken immediately before death. Each blood sample was assessed using a blood gas analyzer (ABL825; Radiometer GmbH, Germany), with measurement of pH, partial pressures of oxygen and carbon dioxide, base excess, and lactate. A hematology analyzer (MEK-6108; Nihon Kohden, Japan) was used to assess platelet count and hemoglobin concentration. Prothrombin time (PT), activated partial thromboplastin time, and fibrinogen concentration were determined using Dade Behring tests (Siemens, Germany) on a steel-ball coagulometer (MC 4 plus; Merlin Medical, Germany). Using a Sysmex coagulation analyzer (Siemens) and the appropriate test from Siemens, D-dimers were measured. An enzyme-linked immunosorbent assay (Enzygnost; Siemens) was used to quantify the thrombin–antithrombin complex. A rotational thromboelastometry device (Tem Innovations GmbH, Germany) was used to perform the whole-blood EXTEM and NATEM assays. In the EXTEM assay, coagulation is activated extrinsically using tissue factor, whereas the NATEM assay is performed without a coagulation activator to enable assessment of "native" coagulation. The following parameters were measured in both assays: clotting time (seconds), clot formation time (seconds), and maximum clot firmness (mm). A calibrated automated thrombogram (Thrombinoscope BV, The Netherlands; tissue factor concentration, 5 pM) was used to assess plasma thrombin generation. Antithrombin was measured on an ACL TOP 550 device (Werfen, Germany) using the standard reagent (antithrombin liquid). The presence or absence of DIC was determined in each animal according to clinical judgement and criteria published by the International Society on Thrombosis and Haemostasis.[36] Four measurements are specified by the International Society on Thrombosis and Haemostasis for determining whether DIC is present or absent: platelet count, levels of fibrin-related markers, PT, and levels of fibrinogen. Clinical judgement was based on thrombin generation parameters, the occurrence of intravascular coagulation (thrombosis in small- to medium-sized vessels), and ongoing bleeding.

After death, the heart, lungs, liver, and kidneys were fixed in 10% buffered formalin. Injured parts of the liver were cut into 3-mm-thick slices, and the degree of injury was determined through macroscopic and microscopic examination. Samples from all four organs (including both lungs) were examined for thromboembolic events. Sections were paraffinized and stained, both by hematoxylin/eosin and by a standard elastica van Gieson protocol. Samples from regions of the lungs known to be susceptible to thrombus formation were investigated for the presence of fibrinogen (antibody and detection kit from Dako, Denmark).[16,37]

The objective of this study was to investigate the effects of treatment with PCC, with *versus* without antithrombin. Combinations of human fibrinogen concentrate and PCC with/without antithrombin were investigated to reflect interventions recommended in treatment algorithms and used in clinical practice.[29,38] Also, because of coagulation management approaches in some regions (*e.g.*, parts of Europe), the effects of first-line tranexamic acid were assessed.[28,29] These considerations explain why we included nine different groups.

Due to the complexity of results from nine groups, a *post hoc* decision was taken to simplify the data. First, we focused only on one dose of antithrombin: the 50 IU/kg dose, which the results suggested would be preferable. Furthermore, the effects of tranexamic acid were modest. Therefore, five groups were considered as "primary": control, PCC, PCC 50 IU/kg plus antithrombin 50 IU/kg, fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg, and fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg. Statistical between-group comparisons were performed only on these groups, and the results section below is focused on the same five groups. Results for the other four groups (PCC 50 IU/kg plus antithrombin 25 IU/kg, PCC 50 IU/kg plus antithrombin 12.5 IU/kg, tranexamic acid 15 mg/kg plus fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg, and tranexamic acid 15 mg/kg plus fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg) are reported in the Supplemental Digital Content (http://links.lww.com/ALN/B960).

Statistics

This was an exploratory study; consequently, the sample size was based on the authors' previous experience with the animal model being used, and no statistical power calculation was performed. A two-way repeated-measures ANOVA was used to compare coagulation and hemodynamic variables across the five key study groups. Q–Q plots were used to

test for normal (Gaussian) distribution. Total blood loss was analyzed using one-way ANOVA on a log-transformed scale. The Sidak method was applied to account for multiple comparisons. Survival was analyzed by pairwise log-rank tests, and none of the observation period data were censored. Statistical significance was determined at the 0.05 level, and tests were performed two-tailed. The software used for statistical analysis was SPSS 23 (SPSS, USA). The data are presented as means ± SD, if not otherwise indicated. There were seven animals per study group, except where animals died before the end of the observation period; early deaths were the only cause of missing data. In the control group, the number of animals was reduced to six at 150 min after study drug administration and five at 210 min. In the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg group, there were six animals at 90 min after study drug administration, four at 150 min, and two at 210 min.

Results

PCC Monotherapy

Total blood loss was statistically significantly lower in the PCC monotherapy group than in control animals (907 ± 132 ml *vs.* 1,671 ± 409 ml; $P < 0.001$). All animals treated with PCC only survived for the entire follow-up period, whereas in the control group two of seven animals (29%) died prematurely (mean survival time, 180 min; fig. 1).

Animals receiving PCC monotherapy showed marked prolongation of PT and activated partial thromboplastin time from 90 min after completion of study drug administration (table 1 and Supplemental Digital Content, http://links.lww.com/ALN/B960). Statistical analysis indicated that differences in PT *versus* the PCC 50 IU/kg plus antithrombin 50 IU/kg, fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg, fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg, and control groups were statistically significant. In addition, plasma fibrinogen levels declined over time. D-Dimer levels increased in response to the first trauma but were then stable over time in the control group (table 1). In contrast, D-dimer levels increased over time in the PCC monotherapy group. Numerically lower platelet counts were observed after study intervention in the PCC group *versus* the control group, although statistically significant differences were not observed.

Thrombin generation potential was increased by PCC, as shown by peak height and endogenous thrombin potential (fig. 2). In contrast, peak height and endogenous thrombin potential changed little in the control group. The administration of PCC had no apparent effect on lag time (Supplemental Digital Content, http://links.lww.com/ALN/B960).

In the control group, little change was seen in clotting time or clot formation time in the EXTEM or the NATEM assay, whereas maximum clot firmness in both assays declined gradually. In comparison, in the PCC-only group, clotting time and clot formation time were prolonged, and maximum clot firmness was reduced in the EXTEM and NATEM assays (Supplemental Digital Content, http://links.lww.com/ALN/B960).

Upon histopathologic analysis, thrombi with diameters of at least 1 mm were present in 29% (4 of 14) of lung specimens from the PCC monotherapy group (fig. 3). No such thrombi were seen in the control group.

Coadministration of Antithrombin with PCC

Total blood loss after the second injury phase in the PCC 50 IU/kg plus antithrombin 50 IU/kg group (672 ± 63 ml)

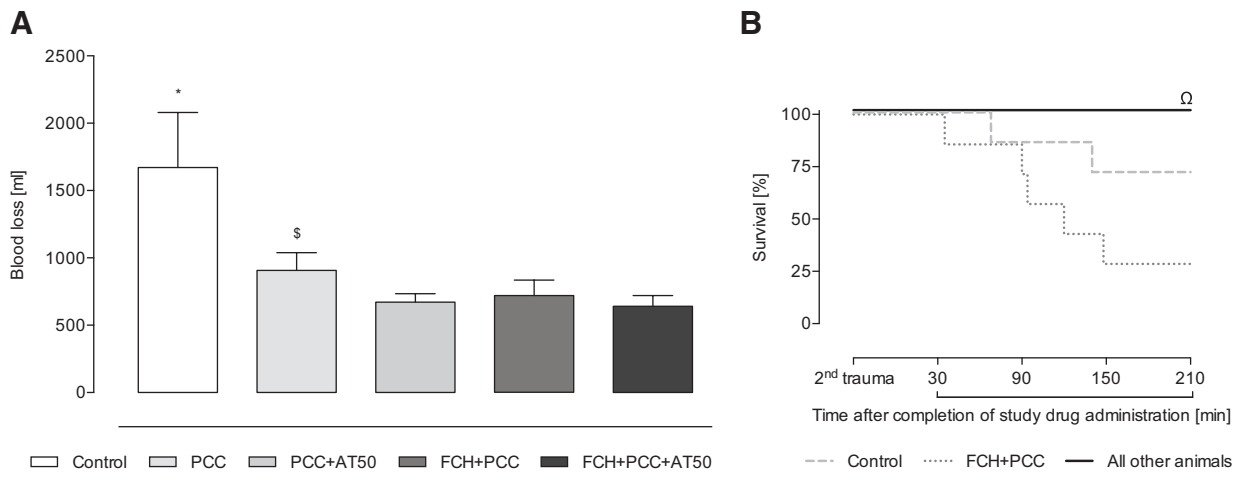

Fig. 1. Total blood loss (*A*) and survival (*B*) after the second trauma; survival data are presented as a Kaplan–Meier curve. *$P < 0.001$, control *versus* all other groups; $^{\$}P < 0.05$, prothrombin complex concentrate (PCC) *versus* all other groups except human fibrinogen concentrate (FCH) + PCC; $^{\Omega}P < 0.05$, control and FCH + PCC *versus* all other groups (median duration of survival). AT50, antithrombin dose of 50 IU/kg.

Table 1. Coagulation Parameters

		DIC-related Parameters*					
Timepoint	**Study Group**	**PT, s**	**Fibrinogen, g/l**	**D-Dimer, mg/l**	**Platelets, ×10³/μl**	**AT, %**	**TAT, μg/l**
Baseline	Control	10 ± 1	1.5 ± 0.2	0.5 ± 0.1	333 ± 34	100 ± 12	26 ± 14
	PCC	10 ± 1	1.6 ± 0.2	0.4 ± 0.2	334 ± 26	101 ± 13	19 ± 12
	PCC+AT50	9 ± 1	1.7 ± 0.2	0.6 ± 0.7	319 ± 44	99 ± 15	27 ± 19
	FCH+PCC	10 ± 1	1.6 ± 0.2	0.7 ± 0.5	291 ± 40	97 ± 7	22 ± 11
	FCH+PCC+AT50	10 ± 1	1.8 ± 0.2	0.4 ± 0.1	315 ± 45	99 ± 10	30 ± 20
Hemorrhagic shock†	Control	12 ± 1	0.6 ± 0.1	3.0 ± 1.6	167 ± 16	52 ± 12	38 ± 30
	PCC	13 ± 1	0.6 ± 0.1	2.0 ± 0.6	175 ± 18	46 ± 6	27 ± 8
	PCC+AT50	13 ± 1	0.6 ± 0.1	1.7 ± 0.5	157 ± 20	49 ± 8	30 ± 9
	FCH+PCC	13 ± 1	0.5 ± 0.1	2.1 ± 1.0	146 ± 25	39 ± 9	28 ± 5
	FCH+PCC+AT50	11 ± 2	0.7 ± 0.1	2.7 ± 0.7	174 ± 27	48 ± 8	30 ± 12
Completion of study drug administration‡	Control	15 ± 2	0.5 ± 0.1	2.5 ± 0.9	145 ± 22	46 ± 10	43 ± 16‖
	PCC	13 ± 2	0.5 ± 0.1	2.0 ± 0.3	133 ± 25	39 ± 2	505 ± 275
	PCC+AT50	13 ± 1	0.5 ± 0.1	1.8 ± 0.4	145 ± 18	145 ± 12+	475 ± 145
	FCH+PCC	8 ± 1	2.0 ± 0.2#	2.4 ± 0.8	136 ± 22	38 ± 8	331 ± 237
	FCH+PCC+AT50	9 ± 0	2.0 ± 0.2#	2.4 ± 1.0	154 ± 20	136 ± 17**	350 ± 171
30 min after study drug administration	Control	15 ± 1	0.4 ± 0.1	2.3 ± 0.8	133 ± 18	45 ± 10	41 ± 20‖
	PCC	16 ± 5	0.4 ± 0.1	13.4 ± 9.7	116 ± 30	41 ± 3	1,319 ± 794
	PCC+AT50	13 ± 1	0.5 ± 0.1	5.5 ± 6.5	140 ± 13	142 ± 11**	737 ± 187
	FCH+PCC	8 ± 1	2.0 ± 0.2#	2.5 ± 0.8	118 ± 28	37 ± 7	767 ± 258
	FCH+PCC+AT50	9 ± 1	2.0 ± 0.2#	6.5 ± 6.1	142 ± 20	125 ± 14**	693 ± 283
90 min after study drug administration	Control	17 ± 2	0.4 ± 0.1	2.0 ± 0.5	114 ± 25	44 ± 10	44 ± 14‖
	PCC	89 ± 70§§	0.2 ± 0.2††	91 ± 61	78 ± 41	38 ± 5	2,060 ± 1,280$
	PCC+AT50	13 ± 1	0.6 ± 0.1	7.6 ± 5.8	133 ± 19‡‡	136 ± 11**	751 ± 186
	FCH+PCC	8 ± 1	1.7 ± 0.2#	87.7 ± 107.1	75 ± 35	37 ± 8	869 ± 407
	FCH+PCC+AT50	9 ± 1	1.7 ± 0.1#	7.1 ± 7.7	131 ± 13‡‡	121 ± 14**	643 ± 228
150 min after study drug administration	Control	18 ± 3	0.4 ± 0.1	1.8 ± 0.6	95 ± 19	42 ± 9	51 ± 28‖
	PCC	107 ± 88$	0.1 ± 0.1††	208.4 ± 129.3‖‖	67 ± 31	34 ± 6	2,010 ± 1,370§§
	PCC+AT50	14 ± 1	0.5 ± 0.1	16 ± 10.1	115 ± 18‡‡	128 ± 10**	755 ± 238
	FCH+PCC	10 ± 1	1.2 ± 0.4#	301.4 ± 226.4‖‖	75 ± 39	40 ± 10	631 ± 271
	FCH+PCC+AT50	10 ± 1	1.6 ± 0.1#	37.1 ± 69.0	124 ± 15‡‡	115 ± 13**	572 ± 174
210 min after study drug administration	Control	18 ± 5	0.4 ± 0.1	2.0 ± 0.8	91 ± 21	41 ± 9	89 ± 75‖
	PCC	149 ± 87§§	0.0 ± 0.1††	248.6 ± 175.7‖‖	58 ± 28	30 ± 10	1,604 ± 1,000§§
	PCC+AT50	14 ± 2	0.5 ± 0.1	27.1 ± 19.9	114 ± 17‡‡	126 ± 11**	729 ± 232
	FCH+PCC	10 ± 0	1.1 ± 0.4#	193 ± 179.6‖‖	75 ± 35	46 ± 1	452 ± 69
	FCH+PCC+AT50	10 ± 1	1.5 ± 0.2#	68.2 ± 79.4	115 ± 11‡‡	117 ± 14**	580 ± 161

Data are shown as means ± SD.

*Parameters identified by the International Society on Thrombosis and Haemostasis for determining the presence/absence of DIC. †10 min after the first trauma. ‡30 min after the second trauma. ‖$P < 0.05$, control *vs.* all other groups. #$P < 0.05$, FCH groups *vs.* all other groups. **$P < 0.05$, AT groups *vs.* all other groups. ††$P < 0.05$, PCC *vs.* PCC + AT50. ‡‡$P < 0.05$, AT groups *vs.* PCC. §§$P < 0.05$, PCC *vs.* all other groups. |||$P < 0.05$, PCC and FCH + PCC *vs.* all other groups.

AT, antithrombin; AT50, antithrombin dose of 50 IU/kg; DIC, disseminated intravascular coagulation; FCH, human fibrinogen concentrate 100 mg/kg; PT, prothrombin time; TAT, thrombin–antithrombin complex.

was statistically significantly lower than in the PCC monotherapy group ($P = 0.010$; fig. 1). All animals in the PCC 50 IU/kg plus antithrombin 50 IU/kg group survived the complete observational period.

PT and activated partial thromboplastin time remained stable over time in the PCC 50 IU/kg plus antithrombin 50 IU/kg group (table 1). Plasma fibrinogen levels showed only a slight decline over time, such that from 90 min after completion of study drug administration, plasma fibrinogen levels were statistically significantly higher in the PCC 50 IU/kg plus antithrombin 50 IU/kg group *versus* PCC only. Platelet counts in the PCC 50 IU/kg plus antithrombin 50 IU/kg group were also statistically significantly higher than with PCC only from 90 min onwards. D-Dimer levels increased over time to a much greater extent in the PCC monotherapy group than in the PCC 50 IU/kg plus antithrombin 50 IU/kg group, with statistically significant differences between the two groups at 150 and 210 min after completion of study drug administration (table 1).

The values for peak height and endogenous thrombin potential showed that coadministration of antithrombin mitigated the thrombin generation increases seen with PCC only (fig. 2). Statistically significant differences were observed between the PCC monotherapy and PCC 50 IU/kg plus antithrombin 50 IU/kg groups in both peak

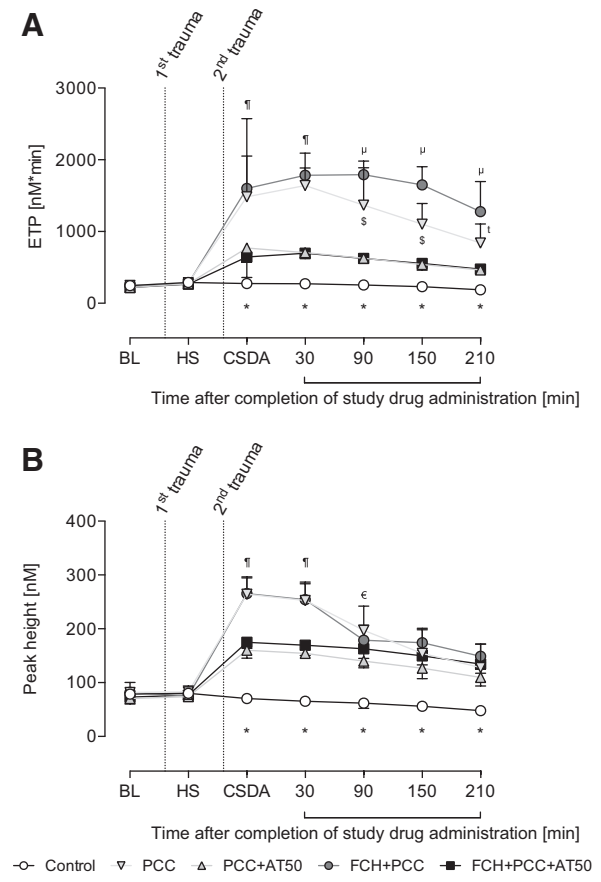

Fig. 2. Thrombin generation parameters: endogenous thrombin potential (ETP; *A*) and peak height (*B*). **P* < 0.05, control *versus* all other groups; ¶*P* < 0.05, prothrombin complex concentrate (PCC) and human fibrinogen concentrate (FCH) + PCC *versus* all other groups; ᵘ*P* < 0.05, FCH + PCC *versus* all other groups; $*P* < 0.05, PCC *versus* all other groups; ᵗ*P* < 0.05, PCC *versus* all groups with AT50; €*P* < 0.05, PCC *versus* PCC + AT50. BL, baseline; CSDA, completion of study drug administration (30 min after the second trauma); HS, hemorrhagic shock (10 min after the first trauma).

height and endogenous thrombin potential. Antithrombin also produced statistically significant reductions in thrombin–antithrombin complex levels *versus* PCC alone, although values remained numerically higher than those in the control group. Consistent with these results, antithrombin reduced the effects of PCC on thromboelastometry variables (clotting time, clot formation time, and maximum clot firmness in the EXTEM and NATEM assays; Supplemental Digital Content, http://links.lww.com/ALN/B960).

None of the lung specimens (0/14) from the PCC 50 IU/kg plus antithrombin 50 IU/kg group contained thrombi with diameters of at least 1 mm. The results with PCC plus lower doses of antithrombin are presented in the Supplemental Digital Content (http://links.lww.com/ALN/B960). Total blood loss was numerically lower in all of the PCC plus antithrombin groups than in the PCC monotherapy group, and there were no premature deaths among any of the PCC pus antithrombin animals. With most variables, the effects of coadministering antithrombin were dose-dependent. Seven percent (1 of 14) and 0% (0 of 14) of lung specimens from the PCC 50 IU/kg plus antithrombin 12.5 IU/kg and PCC 50 IU/kg plus antithrombin 25 IU/kg groups contained thrombi with diameters of at least 1 mm, respectively.

Coadministration of Antithrombin with Fibrinogen Concentrate and PCC

Total blood loss in the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg group (719 ± 115 ml) was numerically higher than in the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg group (640 ± 80 ml; *P* = not significant; fig. 1). Total blood loss in both these groups was lower than with PCC monotherapy (*P* = 0.002 for fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg *vs.* PCC) and statistically significantly lower than in the control group (*P* < 0.001). Five of seven animals (71%) in the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg group died early, whereas all seven animals in the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg group (100%) survived, resulting in a statistically significant difference in mean survival time (130 min *vs.* 210 min, *P* < 0.0001). The early deaths in the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg group were due to pulmonary emboli.

PT and activated partial thromboplastin time changed little in either the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg or the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg groups, with values remaining similar to controls at all timepoints (table 1 and Supplemental Digital Content, http://links.lww.com/ALN/B960). Plasma fibrinogen concentrations increased after administration of human fibrinogen concentrate to levels a little higher than those seen before the first trauma (table 1). There was a subsequent decrease over time in fibrinogen levels; from 150 min after study drug administration, the decrease was greater with fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg than fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg.

As in the PCC monotherapy group, thrombin generation potential increased in the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg group after study intervention, as shown by peak height and endogenous thrombin potential (fig. 2). The initial increases after study intervention were similar in the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg and PCC monotherapy groups. The subsequent decrease over time in peak height was also similar in the two groups, but the decrease over time in endogenous thrombin potential was greater with PCC than with fibrinogen

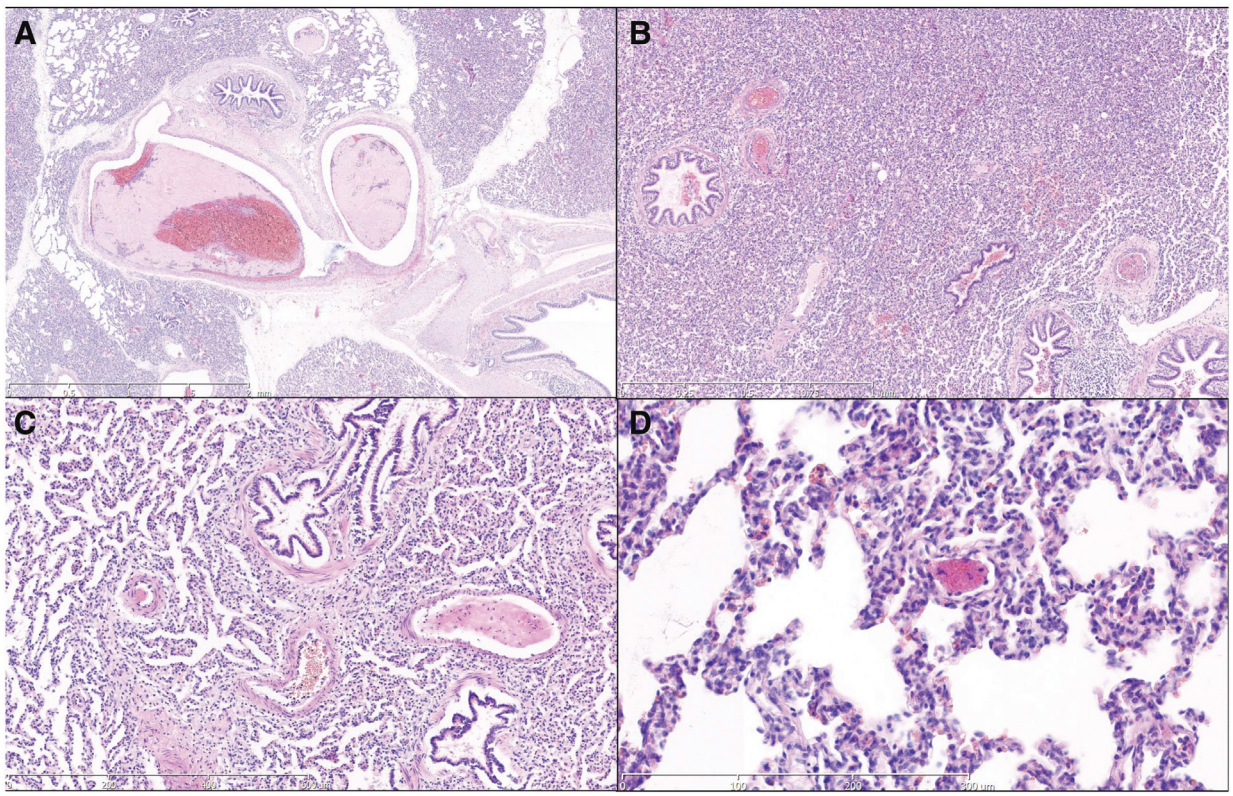

Fig 3. Lung samples from four of the study groups: human fibrinogen concentrate (FCH) + prothrombin complex concentrate (PCC; large thrombi with diameter of at least 1 mm; *A*), PCC monotherapy (large thrombi; *B*), PCC + antithrombin dose of 12.5 IU/kg (reduced number and size of thrombi; *C*), and PCC + antithrombin dose of 25 IU/kg (further reduction in the number and size of thrombi; *D*). All samples stained with hematoxylin/eosin. Magnification: 25× (*A*), 50× (*B*), 100× (*C*), and 200× (*D*).

concentrate 100 mg/kg plus PCC 50 IU/kg. As with PCC 50 IU/kg plus antithrombin 50 IU/kg *versus* PCC monotherapy, increases in peak height and endogenous thrombin potential were much reduced with fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg *versus* fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg. Lag-time results were similar at all timepoints in both the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg and fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg groups, with little difference compared to control (Supplemental Digital Content, http://links.lww.com/ALN/B960).

In the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg group, D-dimer levels increased during the study to a similar extent as with PCC monotherapy (table 1). D-Dimer levels also increased over time in the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg group, but to a lesser extent such that they were statistically significantly lower than in the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg group at 150 and 210 min after completion of study drug administration. The thrombin–antithrombin complex levels were similar in animals receiving fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg and fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg throughout the study and lower than those seen with PCC alone (table 1). The plasma level of antithrombin increased after intervention in the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg group and then decreased gradually over time, with comparable values to those observed with PCC 50 IU/kg plus antithrombin 50 IU/kg (table 1).

Clotting time and clot formation time values in the EXTEM and NATEM assays were similar in the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg and fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg groups (Supplemental Digital Content, http://links.lww.com/ALN/B960). Maximum clot firmness (both assays) was also similar in the two groups upon completion of study drug administration but subsequently, maximum clot firmness values in the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg group declined. Thrombi exceeding 1 mm in diameter were observed in 57% (8 of 14) of lung samples from the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg group (fig. 3), but no samples (0 of 14)

from the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg group contained such thrombi.

Coadministration of Tranexamic Acid with Fibrinogen Concentrate, PCC, and Antithrombin

The addition of tranexamic acid to fibrinogen plus PCC or to fibrinogen plus PCC plus antithrombin was associated with a trend toward reduced overall bleeding. All animals in the tranexamic acid 15 mg/kg plus fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg group survived for the whole observation period, but in the tranexamic acid 15 mg/kg plus fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg group, four of seven animals (57%) died because of pulmonary emboli (Supplemental Digital Content, http://links.lww.com/ALN/B960). This death rate was lower than in the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg group.

The addition of tranexamic acid to either PCC plus antithrombin 50 IU/kg or fibrinogen concentrate plus PCC plus antithrombin 50 IU/kg appeared to have no effect on PT or activated partial thromboplastin time (Supplemental Digital Content, http://links.lww.com/ALN/B960). Plasma fibrinogen concentrations were also unaffected by the administration of tranexamic acid.

Tranexamic acid had little impact on the thromboelastometry parameters (Supplemental Digital Content, http://links.lww.com/ALN/B960). Tranexamic acid also had no discernible effect on any of the thrombin generation parameters (Supplemental Digital Content, http://links.lww.com/ALN/B960). Antithrombin levels after study drug administration were slightly lower in the tranexamic acid 15 mg/kg plus fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg group than with fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg but slightly higher in the tranexamic acid 15 mg/kg plus fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg group than with fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg (Supplemental Digital Content, http://links.lww.com/ALN/B960). However, tranexamic acid did not affect levels of D-dimers or thrombin–antithrombin complex. Among lung samples from animals receiving tranexamic acid 15 mg/kg plus fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg and tranexamic acid 15 mg/kg plus fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg plus antithrombin 50 IU/kg, 29% (4 of 14) and 0% (0 of 14) were found to contain thrombi with diameters of at least 1 mm, respectively.

Discussion

This study reports combined treatment with PCC and antithrombin in an animal model of trauma-associated coagulopathy. Previous findings with PCC monotherapy were confirmed: this treatment reduced total blood loss and increased survival *versus* control. However, treatment with PCC alone (50 IU/kg) led to a DIC-like syndrome with low fibrinogen concentrations and reduced clot strength (as shown by EXTEM maximum clot firmness). The hypercoagulable state after PCC therapy was mitigated by antithrombin without impairing treatment efficacy. In the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg group, total blood loss was statistically significantly lower than in the control group and numerically lower than with PCC monotherapy, but early death occurred in four of seven animals (57%) because of pulmonary emboli, and the prevalence of pulmonary thrombi was higher than in the PCC-only group. Thrombin generation potential was increased by a similar amount with fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg as with PCC alone, but PT and activated partial thromboplastin time did not suggest the presence of DIC in the fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg group; this might be explained by the early deaths and the lack of imputation for missing values (*i.e.*, the true impact on these variables could have been masked).

Coadministration of antithrombin prevented DIC with PCC and prevented early deaths with fibrinogen concentrate 100 mg/kg plus PCC 50 IU/kg caused by reduced thrombin generation potential. The endogenous thrombin potential increase seen with PCC monotherapy was reduced by approximately one or two thirds by the 12.5 or 50 IU/kg doses of antithrombin, respectively. This effect was confirmed by reduced levels of coagulation activation markers. Antithrombin also reduced bleeding, with doses of 25 and 50 IU/kg having a greater impact than 12.5 IU/kg. In all study groups where antithrombin was administered, there were no deaths before the end of the 210-min observation period.

The observation of a DIC-like syndrome in this study should be considered against diagnostic criteria. The International Society on Thrombosis and Haemostasis (Carrboro, North Carolina) and the Japanese Association for Acute Medicine (Tokyo, Japan) have highlighted a low platelet count, elevated fibrin/fibrinogen degradation products, prolonged PT, and either a low plasma fibrinogen level (International Society on Thrombosis and Haemostasis) or fulfillment of systemic inflammatory response syndrome criteria (Japanese Association for Acute Medicine) as key determinants.[36,39] The International Society on Thrombosis and Haemostasis also identified excessive thrombin generation as being integral to DIC.[36] In animals, diagnostic criteria for DIC are not standardized, but the International Society on Thrombosis and Haemostasis/Japanese Association for Acute Medicine criteria are typically used for guidance.[40–42] We observed all of the International Society on Thrombosis and Haemostasis/Japanese Association for Acute Medicine features except systemic inflammatory response syndrome (not assessed) in the PCC-only group, together with thrombi in 30% of post-mortem lung samples. In the control group of our study, some posttrauma observations were

consistent with DIC (lower platelet counts, raised levels of D-dimers, and reduced fibrinogen levels were seen *vs.* baseline). However, in the PCC-only group, these changes were far more pronounced, and they were accompanied by a large increase in endogenous thrombin potential.

In trauma patients with uncontrolled coagulopathic bleeding, the first priority is to stop blood loss by restoring hemostasis. In Europe, trauma guidelines recommend administration of tranexamic acid "as early as possible to the trauma patient who is bleeding or at risk of significant hemorrhage," whereas the American Society of Anesthesiologists suggests that antifibrinolytic treatment should be considered if a patient is bleeding excessively and if fibrinolysis is documented or suspected.[38,43] In the present study, mortality rates suggested that the addition of tranexamic acid reduced but did not eliminate the negative effects of fibrinogen concentrate plus PCC, which were manifested as pulmonary emboli. By inhibiting fibrinolysis, it is likely that tranexamic acid reduced the overall extent to which coagulation was activated while also reducing the consumption of fibrinogen.

PCC is a valuable treatment option for patients with evidence of a need for increased thrombin generation, enabling quicker and greater supplementation of factors II, VII, IX, and X than fresh frozen plasma.[2,9,11] However, there are few data on the safety profile of PCCs outside the context of anticoagulation reversal, and preclinical data from this and other studies[16,22,23,44] suggest that their use may carry a risk of thromboembolic complications, consumptive coagulopathy, or DIC. The current study shows that the addition of antithrombin to PCC may be effective in both reducing hypercoagulopathic tendency and increasing the likelihood of achieving hemostasis, without evidence of any safety concerns. However, antithrombin is not available in every trauma center, and its licensing status may not support its use in this setting. In such circumstances, PCC therapy should be undertaken only after obtaining the best available diagnostic information to indicate whether the patient has a thrombin generation insufficiency, and a cautious approach to dosing is required.

The question of dosing is complicated by the fact that PCCs are dosed according to the quantity of factor IX, whereas the ratio of factor II to antithrombin may be of primary clinical importance. For the PCC used in this study, the unit ratio of factor II:factor IX was approximately 1:1, but this may vary across different PCC products. The effects of treatment with PCC on the factor II:antithrombin ratio were investigated in a theoretical modeling study.[19] PCC was shown to be highly effective in raising the plasma level of factor II, but the factor II:antithrombin ratio was increased.

Diagnosing thrombin generation insufficiency is also complicated in clinical practice. Thrombin generation potential is based not only on levels of procoagulant factors but also on coagulation inhibitors to which neither standard laboratory tests (*e.g.*, PT) nor thromboelastometry variables (*e.g.*, clotting time) are sensitive.[16,45–47] These tests can suggest a need for increased thrombin generation when levels of inhibitors are low, meaning that there could be a hidden risk of PCC therapy triggering hypercoagulopathy. If we are to use PCCs to treat trauma-related bleeding without putting patient safety at risk, a rapid (point-of-care), accurate diagnostic test to assess thrombin generation potential is required. The importance of using a low dose of PCC is increased if antithrombin coadministration is not possible and reliable evidence of thrombin generation insufficiency cannot be obtained.

The study has several limitations. Injury was induced in healthy, anesthetized animals with breathing controlled by a ventilator. Injuries to humans are associated with factors such as pain and inflammation; these factors were absent from our animal model, and they may affect hemostasis. In addition, initial hemostatic therapy would be administered within 10 min of injury in only a small minority of patients. Differences between species mean that we cannot be certain the results are applicable to humans, and in this regard, it would be interesting to repeat our study using porcine instead of human coagulation factors. The benefits of coadministering antithrombin with PCC could vary according to the timings and doses of the treatments; further studies would be needed to confirm optimal timing and dosing of antithrombin therapy. In addition, in the present study, PCC was only tested at the upper dose level recommended for vitamin K antagonist reversal.

Physiologic responses to trauma typically include a degree of hypercoagulation. This study suggests that high-dose PCC monotherapy for trauma-related bleeding can increase hypercoagulation and escalate the risk of DIC. Coadministration of fibrinogen concentrate appears to aggravate the risk, potentially causing early death. Additional treatment with antithrombin was effective in mitigating PCC-induced hypercoagulation, supporting future investigation of this treatment approach in human patients. Clinical use of PCCs in trauma or perioperative coagulopathy would, ideally, be supported by assessment of thrombin generation potential and/or coadministration of antithrombin concentrate as required. Without these measures, we would suggest giving only low doses of PCCs in settings other than warfarin reversal to limit the potential dangers of DIC and other thromboembolic complications.

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Competing Interests

Dr. Grottke has received research funding from Bayer Healthcare (Wuppertal, Germany), Boehringer Ingelheim (Ingelheim, Germany), Novo Nordisk (Bagsværd, Denmark), Biotest (Dreieich, Germany), CSL Behring (Marburg, Germany), and Nycomed (Konstanz, Germany); he has also received honoraria for lectures and consultancy support from Bayer Healthcare, Boehringer Ingelheim, CSL Behring, Octapharma (Lachen, Switzerland), Sanofi (Berlin, Germany), Tem International (Munich, Germany), Pfizer (Berlin, Germany), and Portola (San Francisco, California). Dr. Schöchl has received honoraria for participation in advisory board meetings for Bayer Healthcare and Boehringer Ingelheim, as well as study grants and speaker fees from CSL Behring and Tem International. Dr. Rossaint has received honoraria for lectures and consultancy from CSL Behring, Boehringer Ingelheim, and Novo Nordisk. Dr. Honickel has received travel support from Boehringer Ingelheim. 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. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. Anesthesiology's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

References

1. Franchini M, Lippi G: Prothrombin complex concentrates: An update. Blood Transfus 2010; 8:149–54
2. Grottke O, Levy JH: Prothrombin complex concentrates in trauma and perioperative bleeding. Anesthesiology 2015; 122:923–31
3. DeAngelo J, Jarrell D, Cosgrove R, Camamo J, Edwards C, Patanwala AE: Comparison of 3-factor *versus* 4-factor prothrombin complex concentrate with regard to warfarin reversal, blood product use, and costs. Am J Ther 2018; 25:e326–32
4. Sørensen B, Spahn DR, Innerhofer P, Spannagl M, Rossaint R: Prothrombin complex concentrates: Evaluation of safety and thrombogenicity. Crit Care 2011; 15:201
5. Goldstein JN, Refaai MA, Milling TJ Jr, Lewis B, Goldberg-Alberts R, Hug BA, Sarode R: Four-factor prothrombin complex concentrate *versus* plasma for rapid vitamin K antagonist reversal in patients needing urgent surgical or invasive interventions: A phase 3b, open-label, non-inferiority, randomised trial. Lancet 2015; 385:2077–87
6. Hickey M, Gatien M, Taljaard M, Aujnarain A, Giulivi A, Perry JJ: Outcomes of urgent warfarin reversal with frozen plasma *versus* prothrombin complex concentrate in the emergency department. Circulation 2013; 128:360–4
7. Rodgers GM: Prothrombin complex concentrates in emergency bleeding disorders. Am J Hematol 2012; 87:898–902
8. European Medicines Agency. Core SPC for human prothrombin complex products. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003518.pdf. Accessed June 8, 2018
9. Bruce D, Nokes TJ: Prothrombin complex concentrate (Beriplex P/N) in severe bleeding: Experience in a large tertiary hospital. Crit Care 2008; 12:R105
10. Jehan F, Aziz H, O'Keeffe T, Khan M, Zakaria ER, Hamidi M, Zeeshan M, Kulvatunyou N, Joseph B: The role of four-factor prothrombin complex concentrate in coagulopathy of trauma: A propensity matched analysis. J Trauma Acute Care Surg 2018; 85:18–24
11. Schick KS, Fertmann JM, Jauch KW, Hoffmann JN: Prothrombin complex concentrate in surgical patients: Retrospective evaluation of vitamin K antagonist reversal and treatment of severe bleeding. Crit Care 2009; 13:R191
12. Matsushima K, Benjamin E, Demetriades D: Prothrombin complex concentrate in trauma patients. Am J Surg 2015; 209:413–7
13. Peralta RM, Chowdary P: The use of new procoagulants in blunt and penetrating trauma. Curr Opin Anaesthesiol 2019; (in press)
14. US National Library of Medicine. Early administration of prothrombin concentrate complex in patients with acute hemorrhage following severe trauma (PROCOAG). Available from: https://clinicaltrials.gov/ct2/show/NCT03218722. Accessed February 12, 2019
15. Hanke AA, Joch C, Görlinger K: Long-term safety and efficacy of a pasteurized nanofiltrated prothrombin complex concentrate (Beriplex P/N): A pharmacovigilance study. Br J Anaesth 2013; 110:764–72
16. Grottke O, Braunschweig T, Spronk HM, Esch S, Rieg AD, van Oerle R, ten Cate H, Fitzner C, Tolba R, Rossaint R: Increasing concentrations of prothrombin complex concentrate induce disseminated intravascular coagulation in a pig model of coagulopathy with blunt liver injury. Blood 2011; 118:1943–51
17. Honickel M, Rieg A, Rossaint R, Braunschweig T, Spronk HM, ten Cate H, van Oerle R, Tolba R, Grottke O: Prothrombin complex concentrate reduces

blood loss and enhances thrombin generation in a pig model with blunt liver injury under severe hypothermia. Thromb Haemost 2011; 106:724–33
18. Mitrophanov AY, Szlam F, Sniecinski RM, Levy JH, Reifman J: A step toward balance: Thrombin generation improvement via procoagulant factor and antithrombin supplementation. Anesth Analg 2016; 123:535–46
19. Schöchl H, Grottke O, Sutor K, Dony K, Schreiber M, Ranucci M, Collins PW: Theoretical modeling of coagulation management with therapeutic plasma or prothrombin complex concentrate. Anesth Analg 2017; 125:1471–4
20. Sanfelippo MJ, Engel JM, Onitilo AA: Antithrombin levels are unaffected by warfarin use. Arch Pathol Lab Med 2014; 138:967–8
21. Schöchl H, Voelckel W, Maegele M, Kirchmair L, Schlimp CJ: Endogenous thrombin potential following hemostatic therapy with 4-factor prothrombin complex concentrate: A 7-day observational study of trauma patients. Crit Care 2014; 18:R147
22. Moe DM, Lallemand MS, McClellan JM, Smith JP, Marko ST, Eckert MJ, Martin MJ: Three- *versus* four-factor prothrombin complex concentrates for "factor-based" resuscitation in a porcine hemorrhagic shock model. J Trauma Acute Care Surg 2017; 83:1114–23
23. Harrison J, Abildgaard C, Lazerson J, Culbertson R, Anderson G: Assessment of thrombogenicity of prothrombin complex concentrates in a porcine model. Thromb Res 1985; 38:173–88
24. Dunbar NM, Chandler WL: Thrombin generation in trauma patients. Transfusion 2009; 49:2652–60
25. Gando S, Hayakawa M: Pathophysiology of trauma-induced coagulopathy and management of critical bleeding requiring massive transfusion. Semin Thromb Hemost 2016; 42:155–65
26. Hayakawa M, Gando S, Ono Y, Wada T, Yanagida Y, Sawamura A, Ieko M: Noble–Collip drum trauma induces disseminated intravascular coagulation but not acute coagulopathy of trauma-shock. Shock 2015; 43:261–7
27. Karkouti K, Callum J, Wijeysundera DN, Rao V, Crowther M, Grocott HP, Pinto R, Scales DC; TACS Investigators: Point-of-care hemostatic testing in cardiac surgery: A stepped-wedge clustered randomized controlled trial. Circulation 2016; 134:1152–62
28. Schöchl H, Voelckel W, Grassetto A, Schlimp CJ: Practical application of point-of-care coagulation testing to guide treatment decisions in trauma. J Trauma Acute Care Surg 2013; 74:1587–98
29. Theusinger OM, Madjdpour C, Spahn DR: Resuscitation and transfusion management in trauma patients: Emerging concepts. Curr Opin Crit Care 2012; 18:661–70
30. Theusinger OM, Spahn DR, Ganter MT: Transfusion in trauma: Why and how should we change our current practice? Curr Opin Anaesthesiol 2009; 22:305–12
31. Weber CF, Görlinger K, Meininger D, Herrmann E, Bingold T, Moritz A, Cohn LH, Zacharowski K: Point-of-care testing: A prospective, randomized clinical trial of efficacy in coagulopathic cardiac surgery patients. Anesthesiology 2012; 117:531–47
32. Petersen LC, Karpf DM, Agersø H, Hermit MB, Pelzer H, Persson E, Nichols TC, Ezban M: Intravascular inhibition of factor VIIa and the analogue NN1731 by antithrombin. Br J Haematol 2011; 152:99–107
33. Dusel CH, Grundmann C, Eich S, Seitz R, König H: Identification of prothrombin as a major thrombogenic agent in prothrombin complex concentrates. Blood Coagul Fibrinolysis 2004; 15:405–11
34. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council: Guide for the care and use of laboratory animals, 7th edition. Washington DC, National Academy Press, 1996
35. Grottke O, Braunschweig T, Philippen B, Gatzweiler KH, Gronloh N, Staat M, Rossaint R, Tolba R: A new model for blunt liver injuries in the swine. Eur Surg Res 2010; 44:65–73
36. Taylor FB Jr, Toh CH, Hoots WK, Wada H, Levi M; Scientific Subcommittee on Disseminated Intravascular Coagulation (DIC) of the International Society on Thrombosis and Haemostasis (ISTH): Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. Thromb Haemost 2001; 86:1327–30
37. Grottke O, Braunschweig T, Henzler D, Coburn M, Tolba R, Rossaint R: Effects of different fibrinogen concentrations on blood loss and coagulation parameters in a pig model of coagulopathy with blunt liver injury. Crit Care 2010; 14:R62
38. Rossaint R, Bouillon B, Cerny V, Coats TJ, Duranteau J, Fernandez-Mondejar E, Filipescu D, Hunt BJ, Komadina R, Nardi G, Neugebauer EA, Ozier Y, Riddez L, Schultz A, Vincent JL, Spahn DR: The European guideline on management of major bleeding and coagulopathy following trauma: Fourth edition. Crit Care 2016; 20:100
39. Sawamura A, Hayakawa M, Gando S, Kubota N, Sugano M, Wada T, Katabami K: Application of the Japanese Association for Acute Medicine disseminated intravascular coagulation diagnostic criteria for patients at an early phase of trauma. Thromb Res 2009; 124:706–10
40. Stokol T: Laboratory diagnosis of disseminated intravascular coagulation in dogs and cats: The past, the present, and the future. Vet Clin North Am Small Anim Pract 2012; 42:189–202

41. Soerensen KE, Olsen HG, Skovgaard K, Wiinberg B, Nielsen OL, Leifsson PS, Jensen HE, Kristensen AT, Iburg TM: Disseminated intravascular coagulation in a novel porcine model of severe *Staphylococcus aureus* sepsis fulfills human clinical criteria. J Comp Pathol 2013; 149:463–74
42. Wiinberg B, Jensen AL, Johansson PI, Kjelgaard-Hansen M, Rozanski E, Tranholm M, Kristensen AT: Development of a model based scoring system for diagnosis of canine disseminated intravascular coagulation with independent assessment of sensitivity and specificity. Vet J 2010; 185:292–8
43. American Society of Anesthesiologists Task Force on Perioperative Blood Management: Practice guidelines for perioperative blood management: An updated report by the American Society of Anesthesiologists Task Force on Perioperative Blood Management. Anesthesiology 2015; 122:241–75
44. Mitterlechner T, Innerhofer P, Streif W, Lödl M, Danninger T, Klima G, Hansson K, Fries D: Prothrombin complex concentrate and recombinant prothrombin alone or in combination with recombinant factor X and FVIIa in dilutional coagulopathy: A porcine model. J Thromb Haemost 2011; 9:729–37
45. Tripodi A, Caldwell SH, Hoffman M, Trotter JF, Sanyal AJ: The prothrombin time test as a measure of bleeding risk and prognosis in liver disease. Aliment Pharmacol Ther 2007; 26:141–8
46. Hasan S, Abuelkasem E, Williams B, Henderson R, Mazzeffi MA, Tanaka KA: Factor IX from prothrombin complex concentrate augments low dose tissue factor-triggered thrombin generation *in vitro*. Br J Anaesth 2018; 121:936–43
47. Mannucci PM: Abnormal hemostasis tests and bleeding in chronic liver disease: Are they related?: No. J Thromb Haemost 2006; 4:721–3