

Fibrinogen Concentrate Does Not Suppress Endogenous Fibrinogen Synthesis in a 24-hour Porcine Trauma Model

Christian Zentai, M.D., Till Braunschweig, M.D., Jonas Schnabel, M.D., Michael Rose, Ph.D., Rolf Rossaint, M.D., Oliver Grottke, M.D., Ph.D.

ABSTRACT

Background: Fibrinogen concentrate may reduce blood loss after trauma. However, its effect on endogenous fibrinogen synthesis is unknown. The authors investigated the effect of exogenous human fibrinogen on endogenous fibrinogen metabolism in a 24-h porcine trauma model.

Methods: Coagulopathy was induced in 20 German Landrace pigs by hemodilution and blunt liver injury. Animals were randomized to receive fibrinogen concentrate (100 mg/kg; infusion beginning 20 min postinjury and lasting approximately 10 min) or saline. Fibrinogen concentration, thromboelastometry, and quantitative reverse transcriptase polymerase chain reaction of fibrinogen genes in liver tissue samples were recorded. Internal organs were examined histologically for emboli.

Results: Coagulation parameters were impaired and plasma fibrinogen concentrations were reduced before starting infusion of fibrinogen concentrate/saline. Twenty minutes after starting infusion, exogenous fibrinogen supplementation had increased plasma fibrinogen concentration *versus* controls (171 ± 19 vs. 63 ± 10 mg/dl [mean \pm SD for Multifibren U]; 185 ± 30 vs. 41 ± 4 mg/dl [Thrombin reagent]; $P < 0.05$ for both comparisons). The between-group difference in plasma fibrinogen concentration diminished thereafter, with maximum concentrations in both groups observed at approximately 24 h, that is, during the acute-phase reaction after trauma. Fibrinogen supplementation did not down-regulate endogenous fibrinogen synthesis (no between-group differences in fibrinogen messenger RNA). Total postinjury blood loss was significantly lower in the fibrinogen group ($1,062 \pm 216$ vs. $1,643 \pm 244$ ml; $P < 0.001$). No signs of thromboembolism were observed.

Conclusions: Administration of human fibrinogen concentrate did not down-regulate endogenous porcine fibrinogen synthesis. The effect on plasma fibrinogen concentration was most pronounced at 20 min but nonsignificant at approximately 24 h. (ANESTHESIOLOGY 2014; 121:753-64)

IN recent years, interest in therapeutic use of fibrinogen for the control of major bleeding in a variety of settings, including trauma and surgery, has increased.¹⁻⁴ Fibrinogen concentrate can be effective for the treatment of dilutional coagulopathy^{5,6}; it may reduce transfusion of allogeneic blood products and improve clinical outcomes.⁷ High fibrin levels seem to have an antithrombin effect,^{8,9} and clinical evidence suggests a low risk of thromboembolic complications with fibrinogen concentrate.¹⁰ Conversely, increased plasma fibrinogen is a marker of risk for thrombotic conditions such as cardiovascular disease^{11,12} and there have been calls for robust safety data to support therapeutic use of fibrinogen concentrate.¹³⁻¹⁵

A key safety-related question is the extent to which exogenous fibrinogen concentrate affects the normal acute-phase reaction. Randomized controlled trials have shown no difference in plasma fibrinogen concentration at 24 h between patients treated with fibrinogen concentrate and/or those treated with allogeneic blood products, in the context of cardiovascular surgery^{16,17} and cystectomy.¹

What We Already Know about This Topic

- Fibrinogen supplementation with concentrates may reduce blood loss in different bleeding models and in human studies
- Fibrinogen repletion is an important therapeutic target in bleeding patients

What This Article Tells Us That Is New

- Administration of human fibrinogen concentrates did not down-regulate endogenous porcine fibrinogen biosynthesis in this animal model

The plasma fibrinogen half-life of human fibrinogen concentrate is 2.7 to 3.6 days in patients with congenital hypofibrinogenemia.¹⁸ This suggests that plasma concentration may be increased for at least a week after fibrinogen concentrate therapy. We hypothesized that the difference between this expectation and the observed duration of increase in plasma fibrinogen concentration (<24 h) may be explained by one or more of the following: down-regulation of endogenous fibrinogen synthesis; increased consumption or metabolism of fibrinogen in circulation; or a shift to the

Submitted for publication November 13, 2013. Accepted for publication April 25, 2014. From the Department of Anesthesiology (C.Z., J.S., R.R., O.G.), Institute for Laboratory Animal Science (C.Z.), and Department of Pathology (T.B., M.R.), RWTH Aachen University Hospital, Aachen, Germany.

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extravascular space. Currently, evidence to show which of these processes takes place is lacking.

We performed a novel study to evaluate the impact of fibrinogen supplementation on endogenous fibrinogen synthesis in an established 24-h porcine model of blunt liver injury.

Materials and Methods

Study Approval

This study was conducted according to the Principles of Laboratory Animal Care, as defined by German legislation. Official permission was granted by the Landesamt für Natur, Umwelt und Verbraucherschutz, Recklinghausen, Germany (study number 84-02.04.2011.A254).

Anesthesia

Twenty male German Landrace pigs from a disease-free breeding facility (bodyweight 34 to 41 kg) were housed in ventilated rooms for at least 5 days before the study to acclimatize. They were fasted overnight before surgery, with free access to water. Initially, 4 mg/kg azaperone (Stresnil, Janssen, Neuss, Germany) and 0.1 mg/kg atropine (Atropinsulfate; B. Braun, Melsungen, Germany) were administered by intramuscular injection. This was followed by anesthesia with 3 mg/kg propofol (Disoprivan; AstraZeneca, Wedel, Germany) administered by intravenous injection *via* an 18-gauge cannula located in the right ear vein, with oral intubation. Animals were ventilated in volume-controlled mode at 16 to 18 breaths/min, with a tidal volume of 8 ml/kg to maintain $p\text{CO}_2$ 34 to 40 mmHg with an oxygen fraction of 1.0 in a closed circuit (PhysioFlex; Draeger, Lübeck, Germany). Anesthesia was maintained with isoflurane (Forane; Abbott, Wiesbaden, Germany) at an end-tidal concentration of 1.2 to 1.4% and constant infusion of 2 $\mu\text{g kg}^{-1} \text{h}^{-1}$ fentanyl (Fentanyl-Rotexmedica; Rotexmedica, Trittau, Germany).

Initial fluid therapy comprised 4 ml $\text{kg}^{-1} \text{h}^{-1}$ Ringer's lactate (RL) solution (Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany). Arterial, central venous, and pulmonary pressures, body temperature, tail pulse oximetry, and electrocardiography were constantly monitored using a standard anesthesia monitor (AS/3; Datex Ohmeda, Helsinki, Finland).

Surgical Preparation and Hemodilution

Two 8.5-French catheters were inserted percutaneously in the right and left femoral veins for volume substitution, and a pulmonary artery catheter was placed in wedge position through a third 8.5-French catheter that was implanted surgically in the right jugular vein. Hemodynamic variables were recorded through an 18-gauge catheter in the right arteria femoralis. After line placement, a midline laparotomy with splenectomy and cystostomy was performed. After this procedure, a bolus of RL equal to three times the weight of the removed spleen was

administered and the RL administration rate was increased to 8 ml $\text{kg}^{-1} \text{h}^{-1}$.

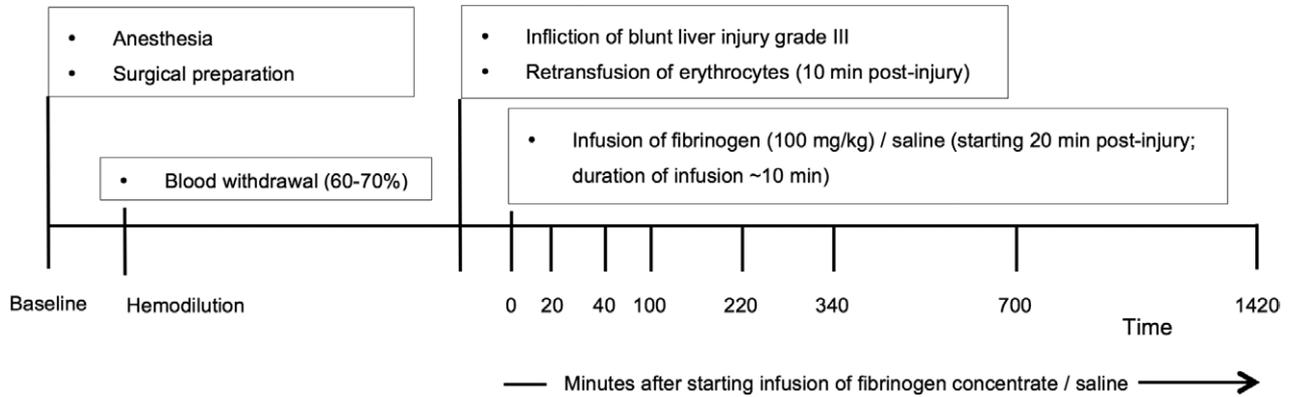
For hemodilution, approximately 60 to 70% of the animal's estimated blood volume was withdrawn (100 ml/min) and substituted with RL. Volume was substituted based on blood pressure measurements: infusion was initiated at a mean arterial pressure of less than 50% of the baseline value and continued until 80% of baseline mean arterial pressure was reached. Collected blood was processed in an autologous cell saver system (Cell Saver 5; Haemonetics, Braintree, MA) for later retransfusion. The use of room temperature solutions decreased the animals' body temperature to 34.6° to 35.8°C (baseline 38.5° to 39.0°C). A further decrease in temperature was avoided by using a warming blanket.

Liver Injury and Fibrinogen Administration

A standardized grade-III blunt liver injury was inflicted as described before using a custom-made instrument.¹⁹ In brief, the liver was gently retracted to allow adequate exposure. The base of the plate was positioned beneath the right middle lobe, and a single clamping of the instrument through the parenchyma with a force of 291 ± 57 N induced the injury. Five minutes after injury, all animals received a bolus of 35 ml/kg RL, and at 10 min postinjury, the continuous rate was set to 40 ml $\text{kg}^{-1} \text{h}^{-1}$ to hemodynamically compensate for hemorrhage. In addition, 12 ml/kg washed erythrocytes were retransfused to avoid early death from severe anemia. Animals were randomized in a 1:1 ratio using sealed envelopes to receive normal saline solution (control) or 100 mg/kg fibrinogen (Haemocomplettan® P; CSL Behring, Marburg, Germany) 20 min after injury at a rate of 20 ml/min. Thus, in a 40-kg animal, the fibrinogen dose was 100 mg \times 40 kg = 4,000 mg or 4 g; the administration volume was 4 g \times 50 ml/g = 200 ml; and the duration of administration was 200 ml/20 ml/min = 10 min. A summary of the study is shown in figure 1. Two hours after injury, the rate of RL infusion was decreased to 8 ml $\text{kg}^{-1} \text{h}^{-1}$ until the end of the experiment.

Blood Sampling and Analysis

Blood was collected and arterial blood gas analysis was performed before injury (baseline), at the end of hemodilution, immediately before starting infusion of fibrinogen concentrate/saline ($t = 0$), and at 20, 40, 100, 220, 340, 700, and 1,420 min after the start of infusion. For animals dying before the last time point, an assessment was performed immediately after death. Partial pressure of oxygen, pH, and carbon dioxide were measured with a blood gas analyzer (ABL725; Radiometer GmbH, Willich, Germany). A standard hematology analyzer (MEK-6108; Nihon Kohden, Tokyo, Japan) was used to measure platelet count and hemoglobin concentration. Fibrinogen concentration was determined *via* a steel-ball coagulometer (MC 4 plus; Merlin Medical, Lemgo, Germany). Measurements of plasma fibrinogen concentration were made using the Clauss assay with Multifibren U and Thrombin reagent from Dade Behring (Dade Behring,



- qRT-PCR: Measured using liver tissue samples obtained at baseline, 100, 340, 700 and 1420 minutes after starting infusion of fibrinogen concentrate / saline
- Blood samples obtained at baseline, hemodilution and at 0, 20, 40, 100, 220, 340, 700 and 1420 minutes after starting infusion of fibrinogen concentrate / saline

Fig. 1. Study overview. Flowchart summarizing the main steps of the study. qRT-PCR = quantitative reverse transcriptase polymerase chain reaction.

Marburg, Germany). Coagulation measurements included daily quality checks using control plasma. In addition, enzyme-linked immunosorbent assays (ELISAs), specific to porcine fibrinogen (Pig Fibrinogen; ICL Inc., Portland, OR) and to human fibrinogen (Human Fibrinogen; Abnova, Heidelberg, Germany), were performed.

Thromboelastometry

The FIBTEM assay specifically measures fibrin-based clotting by inhibiting platelets with cytochalasin-D, providing a sensitive measure of changes in plasma fibrinogen concentration.²⁰ However, it cannot be reliably used with porcine blood.²¹ As an alternative means of measuring fibrin-based clot strength without a contribution from platelets, we performed the EXTEM assay with platelet-poor plasma (PPP). The EXTEM assay was also performed using whole blood to assess overall strength of the clot formed *via* the extrinsic coagulation pathway. A thromboelastometry device (ROTEM; Tem International GmbH, Munich, Germany) was used to perform these tests. PPP was prepared by two centrifugation steps, both performed at room temperature: first 2,000g for 10 min and second 10,000g for 10 min.

Quantitative Reverse Transcriptase Polymerase Chain Reaction

For quantitative reverse transcriptase polymerase chain reaction analysis, liver tissue was obtained with a biopsy punch (Kai Standard Biopsy Punch, 4 mm; Kai Europe GmbH, Solingen, Germany) at baseline (before injury) and at 100, 340, 700, and 1,420 min after the beginning of fibrinogen concentrate/saline infusion. Samples were snap-frozen in liquid nitrogen for later analysis. The resulting liver laceration was closed using a 5x5 mm thrombin/fibrinogen-coated patch (Tacho-Sil; Nycomed, Roskilde, Denmark). Total RNA was extracted from 30 mg liver biopsy tissue using a commercially available RNA/protein extraction kit (NucleoSpin RNA/Protein; Macherey-Nagel, Dueren, Germany). Using a high-capacity reverse transcription kit (Applied Biosystems, Carlsbad, CA), 1 µg total RNA was reverse transcribed to complementary DNA. A polymerase chain reaction reaction was performed using 1 µl complementary DNA and Sybr Green reagent (IQ Sybr Green Supermix; BioRad, Munich, Germany) on a StepOne-Plus Cycler (Applied Biosystems). Specific primers were used for three different genes of fibrinogen synthesis, that is, α, β, and γ chains plus glyceraldehyde-3-phosphate dehydrogenase

Table 1. Primers for Reverse Transcriptase Polymerase Chain Reaction Analysis of Fibrinogen Messenger RNA Synthesis

Primer	Forward	Reverse	NCBI Gene Accession Number
FGA	5'-TCTCCAATGCTAACAAACATGA-3'	5'-GTCCACCTCCAGCCGTTT-3'	XM_003129130.3
FGB	5'-TCTGGCAAAGAATGTGAGGA-3'	5'-CTGTCCATCCTCCTCTTTCTG-3'	NM_001244113.1
FGG	5'-TGTGTTTCAGAAGAGGGTGA-3'	5'-GCAGTACTGGTTTTGCCACTC-3'	NM_001244524.1
GAPDH	5'-CACGATGGTGAAGGTCCGAG-3'	5'-TCAGATCCACAACCGACACG-3'	NM_001206359.1

Specific forward and reverse primers for FGA, FGB, and FGG were designed according to the NCBI gene accession number using the NCBI primer design tool. FGA = fibrinogen α; FGB = fibrinogen β; FGG = fibrinogen γ; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; NCBI = National Center for Biotechnology Information.

as housekeeping gene (table 1). All primers used spanned at least one intron (table 1). The gene expression levels of the fibrinogen chains were calculated as a relative quantity value according to the $\Delta\Delta C_t$ method, which reflects the differences in threshold for each target gene relative to glyceraldehyde-3-phosphate dehydrogenase and baseline measurements.²²

Pathological Examination

After death, internal organs (heart, lungs, liver, and kidneys) were removed immediately and fixed in 10% buffered formalin. Injured parts of the liver were cut into 3-mm-thick slices and examined macroscopically and microscopically to assess the degree of injury by a pathologist blinded to therapy. In addition, representative tissue sections of all four organs were processed to determine the occurrence of thromboembolic events. All samples were embedded in paraffin and stained, both by hematoxylin–eosin and by a standard elastic van Gieson protocol, for histologic examination under light microscopy (microscope: Nikon Eclipse 50i; Nikon Instruments Europe BV, Amsterdam, The Netherlands). Both staining methods were applied to sections from all of the tissues. Sections of lung and liver tissue from regions with a high likelihood of thrombus formation were immunostained to test for fibrinogen (antibodies and detection kit from Dako, Glostrup, Denmark) as described elsewhere.²³

Statistical Analysis

GraphPad Prism version 6.0b was used for statistical and graphing purposes (GraphPad Software, La Jolla, CA). The

D'Agostino and Pearson omnibus normality test was applied. For parameters measured at all 10 study time points, differences between groups were analyzed by a two-way ANOVA model, with group and time as factors, followed by Sidak multiple comparisons test. As an exception, this analysis was not done for human ELISA because of zero values at all times in the control group. Parameters with fewer time points (*e.g.*, blood loss and messenger RNA [mRNA] levels) were analyzed by separate comparison at each time point using the unpaired *t* test. Normally distributed data are expressed as mean with SD. Statistical tests were performed two tailed, and *P* values less than 0.05 were considered as statistically significant.

Additional *post hoc* analyses were performed to examine correlations between total fibrinogen concentration measured by ELISA (sum of human- plus porcine-specific ELISA values) and Clauss fibrinogen concentration. In addition, correlations between EXTEM PPP maximum clot firmness (MCF) and Clauss fibrinogen concentration were assessed. Spearman rank correlation was used for all of these analyses. Each time point was examined separately, increasing the number of analyses such that only *P* values less than 0.01 were considered to represent statistical significance.

Results

Baseline Measurements and Impact of Liver Trauma

Baseline parameters were comparable between groups (table 2). Baseline fibrinogen concentrations determined

Table 2. Laboratory Parameters at Time Points from Baseline to 1,420 min (Approximately 24 h) after Trauma

	Preinjury Baseline	Hemodilution	Before Infusion of Fibrinogen Concentrate/Saline	Time after Starting Infusion of Fibrinogen Concentrate/Saline							
				20 min	40 min	100 min	220 min	340 min	700 min	1,420 min	
Hemoglobin (g/l)											
Control	8.6±0.6	4.5±0.4	6.1±0.3	5.4±0.4	5.2±0.5	5.3±0.3	5.2±0.4	5.1±0.5	5.1±0.5	4.6±0.5	
Fibrinogen	8.7±0.7	4.8±0.4	6.3±0.3	5.8±0.7	5.9±0.6	5.6±0.5	5.6±0.5	5.5±0.5	5.5±0.5	5.2±0.5	
Platelet count (10 ⁹ /μl)											
Control	235±29	140±23	95±14	90±14	90±12	85±11	84±14	82±17	75±16	60±10	
Fibrinogen	259±33	160±26	107±20	120±22*	113±20	109±11	107±12	102±11	95±14	78±12	
Fibrinogen concentration: Multifibrin U (mg/dl)											
Control	300±59	110±20	66±8	63±10	64±13	72±7	80±10	98±12	171±15	460±84	
Fibrinogen	280±37	115±15	70±11	171±19*	163±15*	147±18*	139±15*	145±17	212±34	459±77	
Fibrinogen concentration: Thrombin reagent (mg/dl)											
Control	147±32	64±11	43±5	41±4	42±7	42±7	51±7	62±9	107±15	189±64	
Fibrinogen	143±25	68±7	42±4	185±30*	176±23*	155±18*	140±16*	139±9*	165±30*	244±38*	
Fibrinogen concentration: porcine ELISA (mg/dl)											
Control	170±47	61±12	36±6	35±5	39±7	39±7	44±7	54±12	95±40	205±41	
Fibrinogen	159±39	49±12	40±10	53±14	54±7	54±7	51±9	57±9	90±35	197±71	
Fibrinogen concentration: human ELISA (mg/dl)											
Control	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	
Fibrinogen	0±0	0±0	0±0	154±10	131±10	116±15	105±16	88±16	77±18	57±11	

Data are presented as mean (SD).

**P* < 0.05 vs. control.

ELISA = enzyme-linked immunosorbent assay.

using Multifibren U appeared higher than those obtained by porcine ELISA or with Thrombin reagent (table 2). However, all three methods indicated that fibrinogen concentrations decreased upon hemodilution.

Combined hemodilution and liver injury was associated with impaired clot strength in both thromboelastometry assays immediately before infusion of fibrinogen concentrate/saline as compared with before injury. Platelet count decreased from baseline in response to hemodilution, with a further decrease after liver trauma. Hemoglobin was also decreased after hemodilution; despite subsequent retransfusion of washed erythrocytes, values for these parameters remained below baseline immediately before infusion of fibrinogen concentrate/saline (table 2).

Study treatment (fibrinogen concentrate or saline) was administered over approximately 10 min (range: 8 min, 30 s to 10 min, 15 s).

Protein Expression after Fibrinogen Supplementation

To elucidate fibrinogen synthesis, mRNA expression levels of the α , β , and γ fibrinogen chains were determined in liver tissue. In both groups, mRNA expression of the three different genes for fibrinogen synthesis (α , β , and γ chains) was up-regulated *versus* preinjury baseline, from 100 min to the end of the observation period (fig. 2). mRNA expression for all three chains peaked 700 min after starting infusion in the fibrinogen concentrate group; in controls, a peak at this time was apparent for the γ chain, but peak expression was poorly defined for the α and β chains. Despite these variations, no significant differences were found between the two groups for any of the fibrinogen chains at any time point.

Fibrinogen Concentration after Fibrinogen Supplementation

In control animals, plasma fibrinogen concentration remained unchanged from the start of saline infusion until an increase observed between 100 and 1,420 min later (the latter time point corresponding to almost 24 h). Among animals receiving fibrinogen concentrate, the initial effect was an increase in the total plasma fibrinogen concentration. As a result, plasma fibrinogen concentrations were significantly higher in treated *versus* untreated controls, as determined by both Clauss methods at 20, 40, 100, and 220 min after starting infusion ($P < 0.05$; table 2 and fig. 3). Between 20 and 700 min (almost 12 h) after starting infusion of fibrinogen concentrate/saline, the difference between treated and untreated animals was diminished (table 2 and fig. 3). Plasma fibrinogen concentration measured using Multifibren U showed no significant between-group differences between 340 and 1,420 min after the start of fibrinogen concentrate/saline infusion. There was also no significant between-group difference at 1,420 min with Thrombin reagent. In both groups of animals and with both Clauss reagents, total plasma

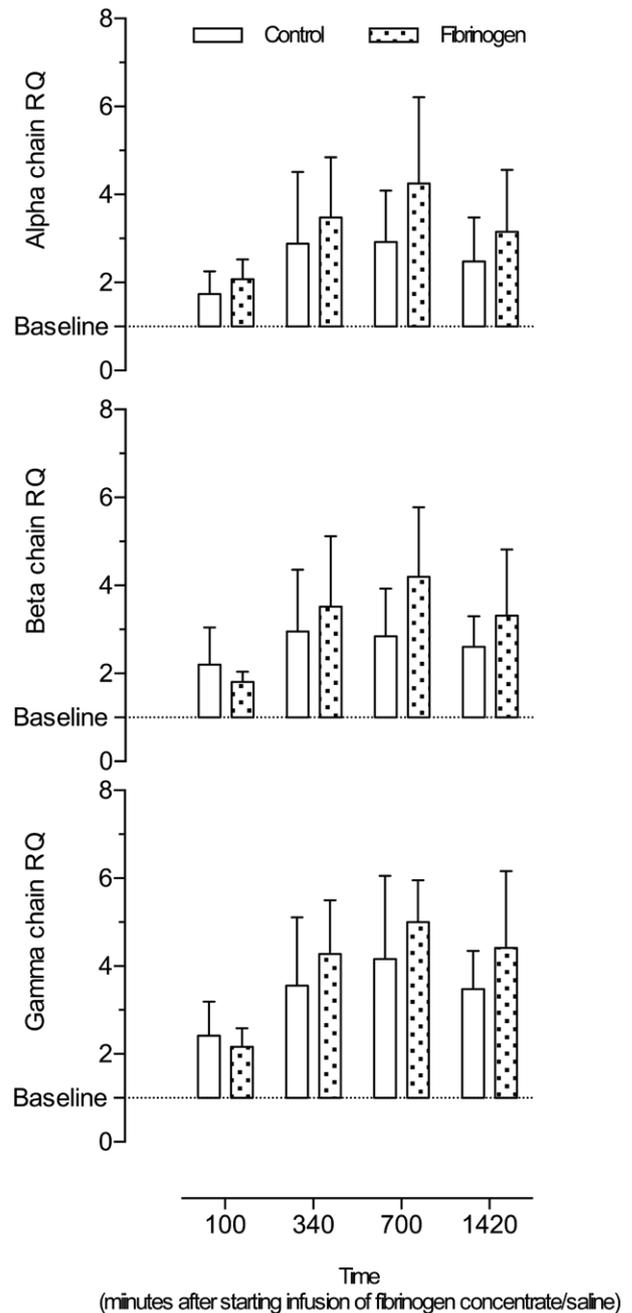


Fig. 2. Fibrinogen synthesis after trauma and infusion of fibrinogen concentrate or saline (control). Fibrinogen synthesis was evaluated by quantitative reverse transcriptase polymerase chain reaction. Messenger RNA levels for the α , β , and γ chains in liver tissue were quantified at several time points. The bars show the relative gene expression (relative quantity [RQ]) in relation to baseline measurements (RQ = 1). Data are presented as mean with error bars representing SD.

fibrinogen concentration reached a maximum 1,420 min after starting infusion, significantly higher than preinjury baseline concentrations ($P < 0.001$).

Porcine-specific ELISA showed no significant difference in endogenous porcine fibrinogen levels between treated and untreated animals at any time point. The same

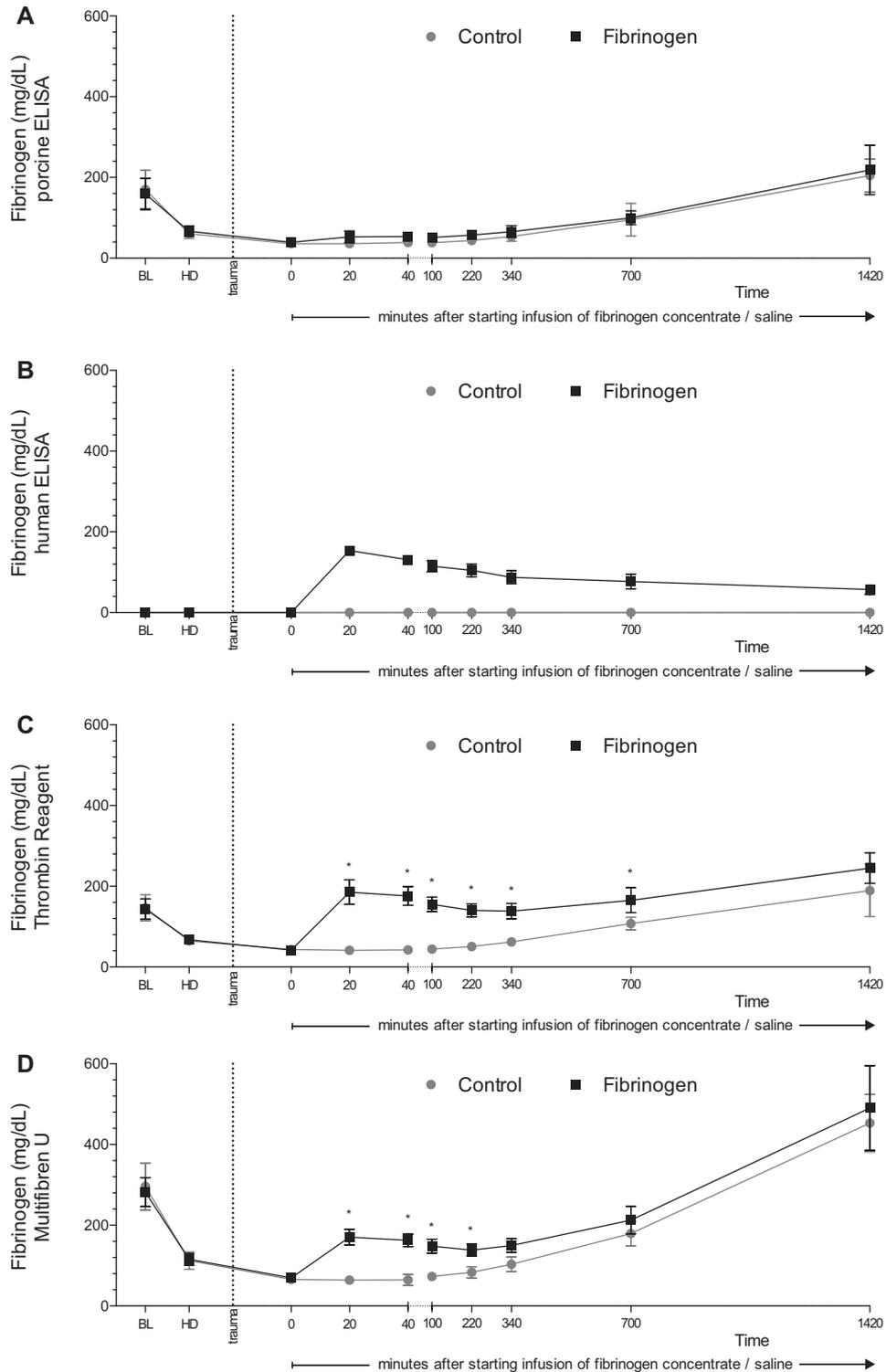


Fig. 3. Plasma fibrinogen concentrations before and after trauma and infusion of fibrinogen concentrate or saline (control). Fibrinogen concentrations in plasma were determined by porcine enzyme-linked immunosorbent assay (ELISA) (A) and human ELISA (B), as well as by the Clauss method using Thrombin reagent (C) and Multifibrin U (D). Data are presented as mean with error bars representing SD; * $P < 0.05$ versus control.

continual increase in endogenous fibrinogen was observed in both groups of animals from 100 to 1,420 min after starting infusion of fibrinogen concentrate/saline, so that plasma concentrations above preinjury baseline were reached

($P < 0.001$, 1,420 min *vs.* baseline, both groups; porcine ELISA). Human ELISA measurements were zero in the control group as expected. In animals treated with fibrinogen concentrate, the data showed an increase to a maximum of

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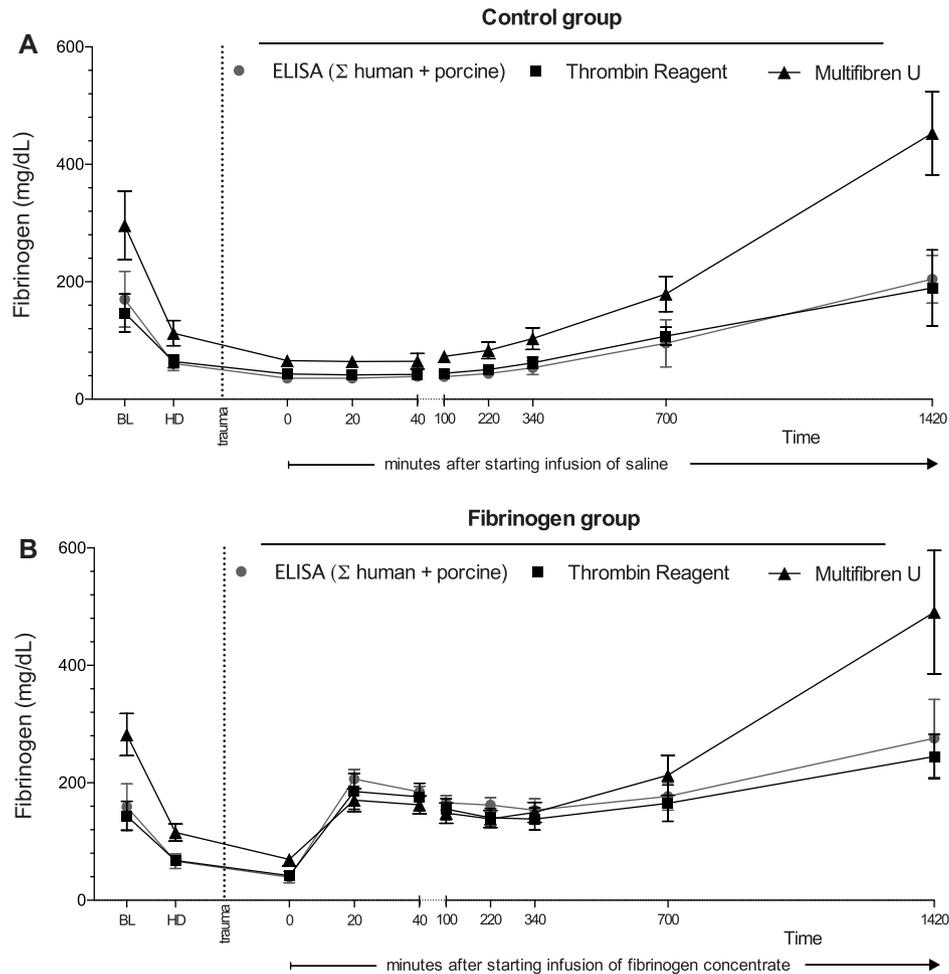


Fig. 4. (A: Control group; B: Fibrinogen group) Plasma fibrinogen concentrations measured by enzyme-linked immunosorbent assay (ELISA) or Clauss methods, before and after trauma and infusion of fibrinogen concentrate or saline (control). ELISA results are the sum of measurements obtained with human and porcine ELISA tests. Data are presented as mean with *error bars* representing SD.

154 mg/dl 20 min after the start of infusion, with a continual decrease during the remainder of the follow-up period.

The sum of human ELISA measurement and porcine ELISA measurement (total ELISA) showed similar plasma fibrinogen concentrations to Thrombin reagent (fig. 4), and strong positive correlations were observed between these parameters at all study time points ($P < 0.005$; table 3). Total ELISA and Thrombin reagent appeared to yield lower plasma fibrinogen concentrations than Multifibren U at preinjury baseline, immediately before starting infusion, and at 340, 700, and 1,420 min after the start of infusion. The relation between total ELISA and Multifibren U showed strong positive correlations at the eight time points between hemodilution and 700 min after the start of infusion, but not at preinjury baseline or 1,420 min (table 3).

Statistically significant correlation between Clauss fibrinogen concentration, measured by either Thrombin reagent or Multifibren U, and fibrin-based clot strength (measured by EXTEM MCF with PPP) was observed at hemodilution and all time points between 20 and 700 min after starting infusion of fibrinogen concentrate/saline ($P < 0.001$;

table 4). With Thrombin reagent, significant correlation was also observed at 1,420 min ($P = 0.003$), whereas the P value for correlation at preinjury baseline was on the borderline for significance ($P = 0.01$).

Thromboelastometry

EXTEM clot strength (MCF) with both whole blood and PPP increased significantly after therapy with fibrinogen (fig. 5). The difference between the study groups in these parameters reduced over time. At 1,420 min after beginning infusion of fibrinogen concentrate/saline, no significant difference was detected between the treatment and the control group in whole-blood clot strength. At the same time point, a significantly higher EXTEM MCF was observed with PPP among animals receiving fibrinogen concentrate although the difference between these animals and controls appeared to have decreased over time.

Blood Loss, Survival, and Histopathological Analysis

With regard to blood loss 10 min after injury, there was no difference between fibrinogen-treated animals (624 ± 72 ml) and

Table 3. Associations between Fibrinogen Measurements by ELISA and the Clauss Assay

Time Point	Thrombin Reagent		Multifibren U	
	Coefficient of Correlation with ELISA (95% CI)	P Value	Coefficient of Correlation with ELISA (95% CI)	P Value
Baseline	0.67 (0.33–0.86)	0.001	0.17 (–0.30 to 0.57)	0.48
Hemodilution	0.70 (0.38–0.87)	<0.001	0.67 (0.32–0.86)	0.001
Time after starting infusion of fibrinogen concentrate/saline, min				
0	0.72 (0.41–0.88)	<0.001	0.70 (0.36–0.87)	<0.001
20	0.82 (0.60–0.93)	<0.001	0.84 (0.64–0.94)	<0.001
40	0.91 (0.78–0.96)	<0.001	0.92 (0.80–0.97)	<0.001
100	0.86 (0.67–0.94)	<0.001	0.89 (0.74–0.96)	<0.001
220	0.92 (0.81–0.97)	<0.001	0.88 (0.71–0.95)	<0.001
340	0.97 (0.93–0.99)	<0.001	0.90 (0.76–0.96)	<0.001
700	0.75 (0.46–0.90)	<0.001	0.71 (0.39–0.88)	<0.001
1,420	0.61 (0.23–0.83)	0.004	0.36 (–0.10 to 0.69)	0.12

ELISA values are the sum of human- plus porcine-specific measurements; associations assessed for the Clauss assay performed using either Thrombin reagent or Multifibren U.
 ELISA = enzyme-linked immunosorbent assay.

Table 4. Associations between Platelet-poor Plasma EXTEM MCF and Fibrinogen Concentration Measured by the Clauss Assay

Time Point	Thrombin Reagent		Multifibren U	
	Coefficient of Correlation with EXTEM MCF (95% CI)	P Value	Coefficient of Correlation with EXTEM MCF (95% CI)	P Value
Baseline	0.56 (0.15–0.80)	0.01	0.19 (–0.28 to 0.58)	0.38
Hemodilution	0.68 (0.35–0.87)	<0.001	0.61 (0.22–0.83)	0.005
Time after starting infusion of fibrinogen concentrate/saline, min				
0	0.30 (–0.16 to 0.66)	0.19	0.38 (–0.08 to 0.70)	0.10
20	0.85 (0.65–0.94)	<0.001	0.83 (0.62–0.93)	<0.001
100	0.83 (0.61–0.93)	<0.001	0.79 (0.53–0.91)	<0.001
240	0.84 (0.64–0.94)	<0.001	0.87 (0.70–0.95)	<0.001
340	0.86 (0.68–0.95)	<0.001	0.86 (0.68–0.95)	<0.001
700	0.92 (0.80–0.97)	<0.001	0.75 (0.47–0.90)	<0.001
1,420	0.63 (0.26–0.84)	0.003	0.45 (0.01–0.75)	0.04

EXTEM performed in platelet-poor plasma to obtain an assessment of fibrin-based clotting, equivalent to the FIBTEM assay; associations assessed for the Clauss assay performed using either Thrombin reagent or Multifibren U.
 MCF = maximum clot firmness.

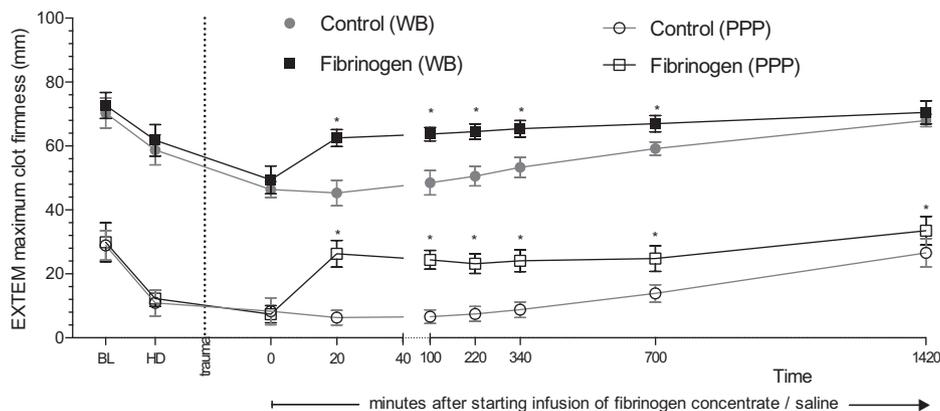


Fig. 5. Clot strength measured by thromboelastometry before and after trauma and infusion of fibrinogen concentrate or saline (control). A ROTEM EXTEM assay was performed to assess maximum clot firmness in whole blood (WB) and platelet-poor plasma (PPP). Data are presented as mean with error bars representing SD; *P < 0.05 versus control.

the control group (565 ± 177 ml; $P = 0.741$). In contrast, total blood loss after injury was significantly lower in the fibrinogen group ($1,062 \pm 216$ ml) as compared with control animals ($1,643 \pm 244$ ml; $P < 0.001$). Except for one animal in the control group (which died 1,184 min after the start of infusion), all animals from both groups survived the whole observation period.

Macroscopic and histological evaluation by immunostaining of injured liver sections revealed equal tissue damage and comparable laceration of venous vessels of a maximum of 3 to 4 mm. No evidence of thrombus formation or microthrombi was found in the hematoxylin–eosin and fibrinogen stain of kidney, heart, liver, or lung tissues.

Discussion

This experimental animal study is the first to examine the effects of fibrinogen concentrate on a long-term (24 h) experimental model of injury. Early administration of exogenous fibrinogen increased plasma concentrations of total fibrinogen and had no impact on concentrations of endogenous porcine fibrinogen. Differences between animals receiving fibrinogen concentrate and controls, in both plasma fibrinogen concentration and fibrin-based clotting, were highest 20 min after starting infusion of fibrinogen concentrate/saline (duration of infusion was approximately 10 min). The difference in plasma fibrinogen concentration between the two groups of animals diminished thereafter, becoming nonsignificant within 24 h. Increased fibrinogen mRNA expression was apparent postinjury, but there were no significant differences between animals that did or did not receive fibrinogen.

The short duration of increase in fibrinogen concentration after administration of fibrinogen concentrate is consistent with previous clinical studies.^{1,16,17} Our results indicate that endogenous synthesis of fibrinogen is increased after trauma; this is in accordance with previous data showing a 2- to 20-fold increase in the synthesis of fibrinogen after trauma or infection.^{24,25} However, the rate of synthesis was unaffected by administration of fibrinogen concentrate. The shorter-than-expected duration of increase in plasma fibrinogen concentration may be related to increased consumption or metabolism of fibrinogen in circulation, or transportation of fibrinogen from plasma to the extravascular space. Experimental investigations using labeled fibrinogen may provide some insight, and they could also increase our understanding of how fibrinogen contributes to the hemostatic process in stopping massive bleeding.

The effect of fibrinogen supplementation on plasma fibrinogen concentration decreased continually between 20 and 1,420 min after the start of infusion. This is consistent with previous clinical studies reporting that the effect of fibrinogen concentrate therapy on plasma fibrinogen concentration disappears within 24 h.^{1,16,17}

The goal of perioperative fibrinogen supplementation is immediate correction of coagulopathy and restoration of hemostasis. The current study indicates by ELISA analysis

that approximately two thirds of exogenous fibrinogen is metabolized/consumed within 24 h after administration. Nevertheless, because of endogenous fibrinogen synthesis, the plasma concentration of fibrinogen was higher at 1,420 min than either baseline or the concentration observed 20 min after the start of infusion. Similarly, Solomon *et al.*¹⁷ reported 24-h plasma fibrinogen concentrations at least as high as baseline in aortic surgery patients treated with fibrinogen concentrate. Fibrinogen is an acute-phase protein, and physiological plasma fibrinogen concentrations up to 700 mg/dl can be expected in humans after trauma or surgery.^{26–29} The fact that physiological acute-phase concentrations are above those observed after administration of fibrinogen concentrate may help to explain the low risk of complications with fibrinogen concentrate.^{10,30,31} A recent porcine dilutional coagulopathy study reported no signs of hypercoagulability or thromboembolism in response to high doses up to 600 mg/kg (equivalent to a dose of 48 g in an 80-kg patient; tissue samples from lungs, heart, kidneys, and liver were dissected and examined macroscopically and microscopically for the presence or absence of thrombi).³¹ Thus, high doses in pigs seem to be well tolerated. In the same study, a fibrinogen dose of 150 mg/kg was sufficient to restore baseline levels of fibrinogen in pigs, whereas higher doses achieve higher levels of fibrinogen and higher EXTEM MCF.³¹ Such data should be considered in the context of previous evidence that clot strength increases with increasing concentration of fibrinogen, with no “plateau” at levels up to 900 mg/dl.^{32,33} In humans, a dose of 150 mg/kg would be considered as high. In a study of aortic replacement surgery, where the dose of fibrinogen concentrate was determined by FIBTEM MCF (target value 22 mm), median dose of fibrinogen was in the region of 100 mg/kg, and this led to a significant reduction in the use of allogeneic blood products.³⁴ In trauma, coagulation management based on coagulation factor concentrates showed positive results from the administration of a slightly lower dose, approximately 75 mg/kg.³⁵ The optimal dosing of fibrinogen concentrate in clinical practice, to achieve the right balance of safety, efficacy, and cost, is not well defined. A large prospective study, appropriately powered to detect low-frequency adverse events, would be needed to confirm the safety of fibrinogen concentrate in humans, and this would help inform dosing decisions.

Currently, there is no “definitive standard” method for measuring fibrinogen concentration in the perioperative setting. The Clauss assay is often used, but it typically has a long turnaround time of 30 to 60 min.^{36,37} The FIBTEM assay, performed in whole blood, is increasingly being used to gauge the need for fibrinogen supplementation because the results are available more quickly (*e.g.*, clot amplitude at 5 min can be used).^{37–40} We measured MCF of the fibrin-based clot by performing the EXTEM assay with PPP. The significant positive correlations we observed between EXTEM MCF with PPP and fibrinogen concentration measured by two Clauss methods are consistent with previous reports of statistically

significant correlation between EXTEM MCF with human PPP and concentration of fibrinogen in plasma.⁴¹

Using our model of blunt liver injury, we have shown that early supplementation with fibrinogen substantially reduced blood loss; this is in line with findings from previous animal studies.^{42,43} In addition, numerous clinical studies have shown that early supplementation of fibrinogen is effective in restoring hemostasis and decreasing blood loss in a perioperative setting.^{34,35,44} The beneficial effects of fibrinogen are attributed mainly to improved strength of fibrin-based clotting.⁴⁵

This study has shown important differences between methods of measuring fibrinogen concentration. The plasma fibrinogen concentrations measured *via* Multifibren U were considerably higher than those from Thrombin reagent or total (porcine + human) ELISA at preinjury baseline and at 700 and 1,420 min after starting infusion of fibrinogen concentrate/saline, time points with the highest porcine plasma fibrinogen concentrations. A previous study in humans has similarly shown higher values for fibrinogen concentration with Multifibren U than with other, “conventional” Clauss assays, and the magnitude of discrepancy correlated with fibrinogen concentration.⁴⁶ In addition, a high coefficient of variation (24%) has been reported with Multifibren U measurements.⁴⁷ Discrepancies between Multifibren U and other Clauss assays could be related to a limitation acknowledged in the package insert for Multifibren U, “some analyzers in the KC 4/10/40 series may yield false results.” (The MC4 device used in the current study is similar to a KC4 analyzer.). Different assay constituents could also contribute to the differences; for example, fibrin-aggretion retarding peptide is used with Multifibren U but not with Thrombin reagent. Overall, the accuracy of laboratory-assessed fibrinogen concentration measurement may be questioned if Multifibren U is used, and the United Kingdom National External Quality Assessment Scheme for blood coagulation has recommended that Multifibren U results are analyzed separately from conventional Clauss assay results.⁴⁶

Several limitations of our study need to be acknowledged. Species differences mean that our study would need to be replicated in humans to confirm clinical applicability of the findings. In humans, unlike in our porcine study, there would be little or no molecular difference between endogenous fibrinogen and exogenous fibrinogen concentrate, and this could conceivably affect how the protein is cleared. Hemodilution had to be induced before the infliction of injury to standardize the degree of coagulopathy. Clinically, hemodilution results from blood loss (*e.g.*, caused by major trauma) and the subsequent infusion of large volumes; hemostatic agents are administered as coagulopathy occurs. In addition, physiologic responses to factors such as pain and inflammation may have additional effects on hemostasis, and these are not reflected in our model. Our study included only one dose of fibrinogen concentrate, and it is conceivable that different outcomes would be obtained with

different doses. Another limitation with this study relates to the interpretation of ELISA results: there is a degree of cross-reactivity of the porcine ELISA assay (approximately 10%) with human fibrinogen, meaning that the sum of human plus porcine values is liable to be slightly higher than the true total fibrinogen concentration. Finally, the study could potentially have been improved by assessing fibrin-based clotting *via* the EXTEM assay on platelet-free plasma as opposed to PPP. However, in some samples, we compared FIBTEM with EXTEM results for PPP and found no difference, suggesting lack of significant platelet component in the PPP coagulation.

Conclusions

Our results from a 24-h porcine trauma model show that human fibrinogen concentrate did not lead to down-regulation of endogenous fibrinogen synthesis. A significant reduction in blood loss was observed, attributable to initial increases in plasma fibrinogen concentration and fibrin-based clot strength. These effects then diminished over time: at approximately 24 h, there was no difference between animals receiving fibrinogen concentrate or saline in all tests with the exception of a small residual effect on strength of the fibrin-based clot. The mechanisms of metabolism and the clinical meaning of our findings warrant further investigation.

Acknowledgments

The authors thank Renate Nadenau and Christian Bleilevens, M.Sc. (Department of Anesthesiology, RWTH Aachen University Hospital, Aachen, Germany), for their excellent support.

This research project was supported by funding from CSL Behring, Marburg, Germany (to Dr. Grottke), Meridian HealthComms (Plumley, Cheshire, United Kingdom) provided editorial assistance and statistical advice in the final stages of article development and this was funded by CSL Behring, Marburg, Germany.

Competing Interests

Dr. Grottke has received research funding from CSL Behring (Marburg, Germany), Novo Nordisk (Bagsvaerd, Denmark), Biotest (Dreieich, Germany), and Nycomed (Roskilde, Denmark) and consultancy fees from Boehringer Ingelheim (Ingelheim, Germany) and Bayer Healthcare (Leverkusen, Germany). Dr. Rossaint has received honoraria for lectures and consultancy from CSL Behring (Marburg, Germany) and Novo Nordisk (Mainz, Germany). The other authors declare no competing interests. The sponsor of this study had no influence on the interpretation of the data.

Correspondence

Address correspondence to Dr. Grottke: Department of Anesthesiology, RWTH Aachen University Hospital, Pauwelsstrasse 30, D-52074 Aachen, Germany. grottke@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.

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