Effect of fibrinogen on reversal of dilutional coagulopathy: a porcine model

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Background. This study was conducted to determine whether replacement of fibrinogen is useful in reversing dilutional coagulopathy following severe haemorrhage and administration of colloids.

Methods. In 14 anaesthetized pigs, approximately 65% of the estimated blood volume was withdrawn and replaced with the same amount of gelatin solution to achieve dilutional coagulopathy. Animals were randomized to receive either 250 mg kg$^{-1}$ fibrinogen ($n=7$) or normal saline ($n=7$). A standardized liver injury was then inflicted to induce uncontrolled haemorrhage. Modified thrombelastography and standard coagulation tests were performed at baseline, after blood withdrawal, after dilution, after injection of the study drugs, and on conclusion of the protocol. Further, electron microscopy imaging of the blood clots was performed and blood loss after liver injury was determined.

Results. Severely impaired haemostasis was observed after haemodilution with gelatin substitution. With administration of fibrinogen, clot firmness and dynamics of clot formation reached baseline values. Median blood loss following liver injury was significantly less ($P=0.018$) in the fibrinogen-treated animals (1100 ml; 800–1400 ml) than in the placebo group (2010 ml; 1800–2200 ml).

Conclusions. Replacing 65% of the estimated blood volume with gelatin in swine resulted in dilutional coagulopathy; subsequent fibrinogen administration improved clot formation and reduced blood loss significantly.


Keywords: blood, fibrinogen; colloids; complications, coagulopathy; measurement techniques, thrombelastograph; pig

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Uncontrolled haemorrhage is responsible for 40% of early deaths in trauma patients. Interestingly, about two-thirds of fatal exsanguinations occur after hospital admission. At least in penetrating trauma, delayed fluid resuscitation revealed beneficial effects. While crystalloid fluid resuscitation predominates in the United States, the consensus in Europe is that a combination of both crystalloid and colloid infusion solutions is advantageous. Regardless of this discussion, fluid resuscitation induces coagulopathy because of haemodilution. Remarkably, an abnormal partial thromboplastin time (aPTT) on hospital admission may increase mortality by 300%. The underlying mechanism of this dilutional coagulopathy may be reduced clot weight, as well as impaired fibrinogen polymerization.

Thrombocytopenia was the first coagulation defect observed in the area of whole blood transfusion. Since the introduction of i.v. fluids and red cell concentrate for treating blood loss, deficiency of clotting factors and especially fibrinogen now occurs first. This coagulopathy results from dilution and consumption of clotting factors as neither red cell nor platelet concentrates contain sufficient amounts of plasma to maintain haemostatic competence.

An alternative approach to manage patients with life-threatening haemorrhagic shock in the early trauma management phase may be the administration of fibrinogen to reverse dilutional coagulopathy early before the occurrence of massive haemorrhage that may be extremely difficult to manage, especially when blood loss is uncontrolled.
The purpose of this study was therefore to assess the effects of fibrinogen vs saline on coagulation, blood loss, and survival rates in a porcine model of uncontrolled hemorrhage with haemodilution-induced coagulopathy.

Methods

This project was approved by the Austrian Federal Animal Investigational Committee, and the animals were managed in accordance with the American Physiological Society institutional guidelines, and the Position of the American Heart Association on Research Animal Use, as adopted on November 11, 1984. Animal care and use were performed by qualified individuals supervised by veterinarians, and all the facilities and transportation comply with current legal requirements and guidelines. Anaesthesia was used in all surgical interventions, all unnecessary suffering was avoided, and research was terminated if unnecessary pain or distress resulted. Our animal facilities meet the standards of the American Association for Accreditation of Laboratory Animal Care.

Surgical preparations and measurements

This study was performed in 14 healthy, 12–16-week-old swine weighing 30–40 kg. The animals were fasted overnight, but had free access to water. The pigs were premedicated with azaperone (4 mg kg\(^{-1}\) i.m., neuroleptic agent, Stresnil\(^{TM}\), Janssen, Vienna, Austria) and atropine (0.1 mg kg\(^{-1}\) i.m.) 1 h before surgery, and anaesthesia was induced with propofol (1–2 mg kg\(^{-1}\) i.v.). After intubation during spontaneous respiration, the pigs were ventilated with a volume-controlled ventilator (Draeger, EV-A, Lübeck, Germany) with oxygen 35% at 20 b.p.m. and with a tidal volume adjusted to maintain normocapnia. Anaesthesia was maintained with propofol (6–8 mg kg\(^{-1}\) h\(^{-1}\)), and a first injection of piritramid (30 mg, ~4–8 h half-life, Dipidolor\(^{TM}\), Janssen, Vienna, Austria) with subsequent doses of propofol and piritramid as clinically indicated. Muscle paralysis was achieved with 0.2 mg kg\(^{-1}\) pancuronium after intubation in order to facilitate laparotomy; 250 ml Ringer’s lactate was administered in the preparation phase. Body temperature was maintained between 38.0 and 39.0°C. An 18-gauge catheter was advanced into the femoral artery for collection of blood samples and continuous arterial pressure measurement; two 5 Fr catheters were advanced into both femoral veins for blood withdrawal and gelatin administration.

Experimental protocol

After assessing baseline haemodynamic and coagulation values, a midline laparotomy was performed. Propofol infusion was then adjusted to 2 mg kg\(^{-1}\) h\(^{-1}\). The animals subsequently underwent an isovolaemic and normothermic exchange of approximately 65% of their total blood volume of about 2500 ml with gelatin solution (Gelifusin\(^{®}\), Braun, Melsungen, Germany) over 30 min.\(^7\) Animals were randomly assigned to either 250 mg kg\(^{-1}\) fibrinogen concentrate (Haemocompletan\(^{®}\), Aventis Behring, Marburg, Germany) or to an equal amount of normal saline (investigators were blinded to the drugs). An incision was made in the right liver lobe (length, 12 cm; depth, 3 cm) to induce uncontrolled bleeding. At the end of the study protocol, blood was suctioned out of the abdomen and the total blood loss was determined. After liver incision, observation was conducted for 60 min. If an animal died within these 60 min, the last blood sampling was performed immediately before death, which was defined as pulseless electrical activity, a mean arterial pressure below 10 mm Hg, and an end-tidal carbon dioxide below 10 mm Hg. Animals surviving for more than 1 h were killed with an overdose of fentanyl, propofol, and potassium chloride.

Blood sampling and analytical methods

Arterial blood sampling was performed at baseline, after withdrawal of blood, after haemodilution, after administration of study drugs, and 60 min after liver injury. Fibrinogen concentration, prothrombin (PT) and partial thromboplastin time (PTT) (Amelung Coagulometer, Baxter, UK), haemoglobin values, and platelet count at corresponding time points were determined by standard laboratory methods. Further, antithrombin (AT) (Antithrom Stago, Boehringer Mannheim, Germany), D-Dimer (D-Dimer, Latex Immunoassay, Instrumental Laboratories) with good cross-reactivity to porcine fibrinogen, thrombin–antithrombin (TAT; Elisa Test, Dade Behring) and thrombelastographic measurements (ROTEM\(^{®}\), Pentapharm, Munich, Germany) were performed.\(^8\) The parameters of ROTEM\(^{®}\) analysis are ‘coagulation time’ (CT) corresponding to the reaction time in a conventional thrombelastogram, ‘clot formation time’ (CFT) meaning the coagulation time and ‘maximum clot firmness’ (MCF), which is equivalent to the maximum amplitude (Fig. 1).
Blood clots were examined by electron microscopy imaging using fresh whole undiluted blood, as well as diluted blood and diluted blood after fibrinogen administration. Blood clots were initially fixed with glutaraldehyde 2.5%, buffered with sodium cacodylate containing CaCl₂, washed in the same buffer and then fixed in osmium tetroxide for 1 h. Next, the clots were washed, dissected into small pieces in ethanol 70%, dehydrated, critical point-dried, and sputter-coated with gold palladium. All scanning electron microscopy specimens were examined with a Zeiss DSM 982 Gemini electron microscope.

Statistical analysis
A non-parametric Friedman ANOVA was applied to analyse a possible time effect within each group. Differences to baseline were compared between groups using the Wilcoxon test for unpaired observations. Thrombelastographic parameters are presented in box plots (minimum, first quartile, median, third quartile, maximum). A P value less than 5% was considered statistically significant.

Results
After withdrawal of 65% of the estimated total blood volume, haemoglobin values decreased only marginally, but reached critical levels between 3 and 4 g litre⁻¹ after compensating blood loss with gelatin solution (Table 1). Fibrinogen concentrations decreased slightly in both groups after blood withdrawal, but reached critical levels of approximately 100 mg dl⁻¹ after compensating blood loss with gelatin. After administering fibrinogen, fibrinogen values increased significantly as compared with the saline-treated animals and reached values similar to baseline (Table 1). Also, the platelet count decreased significantly after the administration of gelatin, but never reached critical values in either group (Table 1). PT decreased from about 130 to 66% following haemodilution. After the administration of fibrinogen, PT increased by 10%, while aPTT was prolonged during the whole study period without showing any differences between groups. After compensating blood loss with gelatin, AT decreased significantly in both groups. In the animals treated with fibrinogen, D-Dimer showed a statistically significant increase only at the end of the observation period, while TAT did not differ between groups (Table 1). ROTEM® parameters did not change immediately after blood removal, but were significantly impaired after compensating blood loss with gelatin. Clotting time increased and maximum clot firmness decreased significantly following gelatin infusion. The administration of fibrinogen did not shorten the prolonged clotting time (CT), while CFT decreased and MCF as well as the alpha angle increased significantly, achieving values similar to baseline (Fig. 2). Scanning electron microscopy of blood

Table 1
<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After blood WD</th>
<th>Dilution</th>
<th>After DA</th>
<th>60 min DA</th>
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</thead>
<tbody>
<tr>
<td>Haemoglobin (g litre⁻¹)</td>
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<tr>
<td>Saline</td>
<td>9.5 (8.8–9.9)</td>
<td>8.5 (7.2–9.4)</td>
<td>3.0 (2.3–4.2)</td>
<td>3.0 (2.3–4.2)</td>
<td>4.0 (3.5–4.1)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>9.3 (8.4–9.6)</td>
<td>8.7 (8.0–9.2)</td>
<td>2.9 (2.4–3.1)</td>
<td>2.9 (2.6–4.0)</td>
<td>3.6 (3.5–3.9)</td>
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<tr>
<td>Platelet count (g litre⁻¹)</td>
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<tr>
<td>Saline</td>
<td>223 (160–314)</td>
<td>231 (153–279)</td>
<td>96 (91–120)</td>
<td>96 (91–120)</td>
<td>119 (99–139)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>172 (157–384)</td>
<td>143 (137–369)</td>
<td>115 (92–128)</td>
<td>114 (110–163)</td>
<td>119 (112–210)</td>
</tr>
<tr>
<td>Fibrinogen (g dl⁻¹)</td>
<td></td>
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<tr>
<td>Fibrinogen</td>
<td>306 (248–405)</td>
<td>252 (228–298)</td>
<td>103 (86–130)</td>
<td>274 (243–294)*</td>
<td>241 (231–266)*</td>
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<tr>
<td>PT (%)</td>
<td></td>
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<tr>
<td>Saline</td>
<td>130 (119–130)</td>
<td>121 (110–124)</td>
<td>67 (52–72)</td>
<td>67 (52–72)</td>
<td>63 (50–71)*</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>130 (130–130)</td>
<td>119 (113–126)</td>
<td>66 (43–90)</td>
<td>78 (68–96)*</td>
<td>79 (67–96)*</td>
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<tr>
<td>aPTT (s)</td>
<td></td>
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<tr>
<td>Saline</td>
<td>83 (75–104)</td>
<td>84 (72–88)</td>
<td>74 (65–96)</td>
<td>74 (65–96)</td>
<td>69 (58–74)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>88 (75–92)</td>
<td>78 (72–101)</td>
<td>67 (52–81)</td>
<td>80 (58–113)</td>
<td>91 (65–96)</td>
</tr>
<tr>
<td>AT (%)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>103 (99–113)</td>
<td>98 (82–100)</td>
<td>43 (37–52)</td>
<td>43 (37–52)</td>
<td>42 (37–56)*</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>103 (99–120)</td>
<td>94 (87–106)</td>
<td>39 (33–45)</td>
<td>41 (38–55)</td>
<td>43 (40–46)</td>
</tr>
<tr>
<td>D-Dimer (ng ml⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TAT (µg litre⁻¹)</td>
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<td></td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>34 (27–39)</td>
<td>19 (18–25)</td>
<td>35 (11–38)</td>
<td>19 (12–20)</td>
<td>23 (17–34)</td>
</tr>
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</table>
clots showed a reduced reticular network, and fibrin strands appeared to be thinner after administration of gelatin as compared with clots before dilution and after fibrinogen administration (Figs 3–5). Median blood loss after liver injury was significantly less ($P=0.018$) in the fibrinogen-treated animals (1100 ml; 800–1400 ml) as compared with the saline-treated animals (2010 ml; 1800–2200 ml). A median survival time of 25 min (23–39 min) following liver injury was observed in the fibrinogen-treated animals as compared with 55 min (41–60 min) in the saline-treated animals (N.S.). Three of seven fibrinogen-treated animals (43%), and one of seven saline-treated animals (14%) survived the 1-h observation period following liver injury (N.S.).

**Discussion**

After major blood loss in our pigs, administration of 2500 ml (~60 ml kg$^{-1}$) gelatin solution resulted in dilutional coagulopathy with thrombelastographic parameters being severely impaired; however, normalization occurred rapidly after fibrinogen administration. These beneficial fibrinogen-mediated effects not only partially reversed dilutional coagulopathy, but also significantly decreased blood loss...
after liver injury as compared with the normal saline control group. Further, fibrinogen had a recovery effect on the reticular network as shown by electron microscopy images of the blood clots.

In the case of massive blood loss, aggressive fluid resuscitation is crucial, at least in blunt trauma. A systematic review of randomized controlled trauma resuscitation trials employing colloid vs crystalloid solutions in critically injured patients reported an absolute increase of 4% in mortality after colloid fluid resuscitation.9 Worsened bleeding may have increased mortality, which probably resulted not only from increased perfusion pressure following fluid therapy, but also from diluted clotting factors. Gelatin solutions are known to disturb the reticular fibrin mesh and usually cause a reduction in blood clot quality as measured by thrombelastography, clot weight, and electron microscopy.5 10 11 A change in clot organization and a decrease in clot elasticity are probably responsible for these effects. Substitution of fibrinogen may reverse this effect but has never been studied in this context previously. We here demonstrate that fibrinogen indeed resulted in increased clot firmness and therefore reduced blood loss in our porcine model during uncontrolled haemorrhage.

During the era of whole blood transfusion, thrombocytopenia developed early in the course of massive haemorrhage.12 Since the introduction of blood component therapy, clotting factors are diluted first, because red blood cell concentrates contain only insufficient plasma to sustain haemostatic levels of clotting factors.13 Nevertheless, guidelines for managing massive transfusion have not been modified to reflect these fundamental changes in transfusion strategy.

Furthermore, indirect fibrinogen assays should be interpreted with caution, as synthetic colloids interfere with fibrinogen assays. For example, samples diluted with colloids showed higher fibrinogen values than samples diluted with saline.14 Thus, in the case of major bleeding with dilution and consumption of coagulation factors and after administration of large amounts of colloids, the actual functional plasma fibrinogen value may be overestimated by standard laboratory tests. In this situation, platelet-independent modified thrombelastography may be useful for prompt and accurate analysis of the functional fibrinogen actually available for polymerization.15 16

Fibrinogen deficiency develops earlier than any other clotting factor deficiency, which has been shown in vivo, in mathematical models, in patients and in animals.6 17 18 A decrease in plasma fibrinogen below 150 mg dl⁻¹ is associated with bleeding complications not only during surgery, but also postoperatively. In 876 patients who underwent intracranial surgery, a decrease in fibrinogen and factor XIII concentrations increased the postoperative haematoma risk by about 12-fold.19 Our strategy of injecting fibrinogen concentrate rapidly raised fibrinogen plasma levels above this critical threshold, which reduced blood loss.

One concern associated with high plasma levels of fibrinogen is thrombosis and thromboembolic complications. D-Dimer as a laboratory parameter of this phenomenon was
elevated at the end of the observation period in the animals treated with fibrinogen, while TAT did not differ between the groups. We found no evidence for thromboembolic problems upon autopsy in our animals. Elevated D-Dimer values are also a marker for disseminated intravascular coagulopathy. Histological examination did not detect any microvascular thrombosis in the lungs, heart, gut, spleen, or liver. However, D-Dimer values between 200 and 300 ng ml\(^{-1}\) can be interpreted as an adequate response to liver injury. Furthermore, thrombelastographic measurements after fibrinogen administration did not show any signs of hypercoagulopathy.

In contrast to fresh frozen plasma (FFP), fibrinogen concentrates are immediately available, contain a defined concentration of clotting factors, are not associated with volume exposure, and are much safer in respect to transmission of infectious diseases or transfusion-associated lung injury. To our knowledge, fibrinogen concentrate is licensed for congenital and acquired fibrinogen deficiency in most European countries except the UK. However, it is available in nearly all countries and the actual price depends on the local supplier.

In conclusion, correction of fibrinogen deficiency was able to restore impaired clot firmness and clot formation time and to reduce blood loss after standardized liver injury in a diluted coagulopathic porcine model.

Limitations need to be noted. In order to enable the exact measurement of study end points, haemodilution and fibrinogen administration had to be induced before liver injury. However, in a multiple-traumatized patient, injury occurs before haemodilution. Further animal and clinical studies are needed to confirm our hypothesis that administration of fibrinogen may be a useful first step toward reversing dilutional coagulopathy, thereby reducing FFP supply, total blood loss and further volume resuscitation demand.

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References