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Effects of Fibrinogen Concentrate After Shock/Resuscitation: A Comparison Between In Vivo Microvascular Clot Formation and Thromboelastometry*

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Objectives: Dilutional coagulopathy after resuscitation with crystalloids/colloids clinically often appears as diffuse microvascular bleeding. Administration of fibrinogen reduces bleeding and increases maximum clot firmness, measured by thromboelastometry. Study objective was to implement a model where microvascular bleeding can be directly assessed by visualizing clot formation in microvessels, and correlations can be made to thromboelastometry.

Design: Randomized animal study.

Setting: University research laboratory.

Subjects: Male Syrian Golden hamsters.

Interventions: Microvessels of Syrian Golden hamsters fitted with a dorsal window chamber were studied using videomicroscopy.

After 50% hemorrhage followed by 1 hour of hypovolemia resuscitation with 35% of blood volume using a high-molecular-weight hydroxyethyl starch solution (Hextend, Hospira, MW 670 kD) occurred. Animals were then treated with 250 mg/kg fibrinogen IV (Laboratoire français du Fractionnement et des Biotechnologies, Paris, France) or an equal volume of saline before venular vessel wall injuries was made by directed laser irradiation, and the ability of microthrombus formation was assessed.

Measurements and Main Results: Thromboelastometric measurements of maximum clot firmness were performed at the beginning and at the end of the experiment. Resuscitation with hydroxyethyl starch and sham treatment significantly decreased FIBTEM maximum clot firmness from 32±9 mm at baseline versus 13±5 mm after sham treatment ($p < 0.001$). Infusion of fibrinogen concentrate significantly increased maximum clot firmness, restoring baseline levels (baseline 32±9 mm; after fibrinogen administration 29±2 mm). In vivo microthrombus formation in laser-injured vessels significantly increased in fibrinogen-treated animals compared with sham (77% vs 18%).

Conclusions: Fibrinogen treatment leads to increased clot firmness in dilutional coagulopathy as measured with thromboelastometry. At the microvascular level, this increased clot strength corresponds to an increased prevalence of thrombus formation in vessels injured by focused laser irradiation. (*Crit Care Med* 2013; 41:e301–e308)

Key Words: colloid resuscitation; dilutional coagulopathy; fibrinogen polymerization; hemorrhagic shock; laser injury; thrombus formation

The clinical scenario of dilutional coagulopathy accounts for a pronounced increase in early mortality of polytraumatized, severely bleeding patients (1–4). Resuscitation fluids, especially colloidal plasma expanders, which are used to restore normovolemia and thus guarantee perfusion pressure in vital organs, dilute clotting factors, thereby leading to a diffuse, microvascular bleeding tendency (5–8). Animal (9–12) and clinical investigations (13, 14) showed that

the presence of colloids decreases clot firmness due to direct interaction with fibrin polymerization by inducing changes to the ratio between fibrin fiber mass and fibrin length (6). Under these circumstances, plasma fibrinogen concentrations reach critical levels at an early stage and before any other coagulation factor. Interestingly, nearly 70% of lethal exsanguinations in trauma patients occur after hospital admission (15, 16), which could partly be attributed to coagulation disturbances.

Numerous studies have shown the effect of fibrinogen concentrate in reversal of dilutional coagulopathy after shock/resuscitation (12–14, 17–19). Nevertheless, in clinical practice, it is not possible to monitor and measure microvascular bleeding in real time. In the perioperative and trauma-management setting, thromboelastometry has been implemented as a "point of care" diagnostic tool, which acquires information about clot quality in terms of clot firmness and clot stability over time and has been shown to rapidly identify patients requiring massive transfusion (20). However, thrombus formation is a dynamic process, including the interaction between fibrinogen and platelets and between erythrocytes, leukocytes, microparticles (21) and being influenced by local blood flow and blood endothelial interactions (22). Thromboelastometry assesses clot formation and functionality; however, it does not provide information on the interaction among these factors in vivo.

The current study was performed in the hamster window chamber, an intravital microscopy model used for direct investigation of microvascular blood flow, capillary perfusion, and in vivo visualization of thrombus formation. The assessment of these variables in concert allows for a more physiological approach to understanding the mechanisms of clot formation under normal and pathological conditions such as shock/resuscitation. This study analyzed for the first time the complex interplay between blood flow conditions and hemostasis during dilutional coagulopathy and directly assessed the effect of fibrinogen concentrate on microvascular thrombus formation in vivo.

MATERIALS AND METHODS

Animal Preparation

Investigations were performed in 50–65 g male golden Syrian hamsters (Charles River Laboratories, Boston, MA) fitted with a dorsal window. Animal handling and care were provided following the procedures outlined in the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996), and the study was approved by the local Animal Subjects Committee. The hamster window chamber model is widely used for microvascular studies in the unanesthetized state and allows for noninvasive measurements over extended periods of time of an intact vasculature without exposure to ambient air or need for perfusates (23, 24).

The complete surgical technique for the preparation has been previously described in detail (25, 26). Briefly, hamsters were prepared for chamber implantation with a 50 mg/kg intraperitoneal injection of pentobarbital sodium anesthesia. After hair removal, sutures were used to lift the dorsal skin away from the animal, and one frame of the chamber was positioned on the animal's

back. A chamber consisted of two identical titanium frames with a 15 mm circular window. With the aid of backlighting and a stereomicroscope, one side of the skinfold was removed following the outline of the window until only a thin layer of retractor muscle and the intact subcutaneous skin of the opposing side remained. Saline and then a cover glass were placed on the exposed skin held in place by the other part of the chamber. The intact skin of the other side was exposed to the ambient environment. The animal was allowed at least 2 days for recovery; its chamber was then assessed under the microscope for any signs of edema, bleeding, or unusual neovascularization. Barring these complications, the animal was anesthetized again and arterial and venous catheters (polyethylene-50) were implanted in the carotid artery and jugular vein, respectively. Catheters were prefilled with saline, tunneled under the skin, exteriorized at the dorsal side of the neck, and attached to the chamber frame with tape.

Inclusion Criteria. Animals were evaluated for inclusion into the study 4 days after the initial surgery. Animals were suitable for the experiments if 1) systemic variables were within normal range, namely, heart rate > 320 beats/min, mean arterial blood pressure (MAP) > 80 mm Hg, systemic hematocrit (HCT) > 45%, arterial Po_2 > 50 mm Hg; and 2) microscopic examination of the tissue observed under ×40 magnification did not show signs of edema or bleeding.

Intravital Microscopy Setup

The unanesthetized animal was placed into a restraining tube for the period of the experiment. The tube containing the conscious animal was fixed to the stage of a transillumination intravital microscope (BX51 W1, Olympus, New Hyde Park, NY). Animals had 30 minutes to adjust to the tube environment prior to measuring baseline variables. The tissue image was projected onto a CCD camera (4815-2000, COHU, San Diego, CA) connected to a timer and viewed on a closed circuit monitor. Microvascular diameter and RBC velocity were measured online in arterioles and venules using a ×40 water immersion objective (LUMPFL-WOR, numerical aperture 0.7, Olympus, Central Valley, PA). Blood flow rates were calculated from evaluated diameters and velocities as previously reported (27). The same vessel sites were followed throughout the entire experiment so that direct comparison could be made to baseline levels.

Experimental Protocol

Figure 1 shows the experimental timeline and measurement time points. Baseline measurements (systemic variables, blood gases, and microhemodynamic variables) were followed by an acute hemorrhage (withdrawal of 50% of estimated total blood volume (BV), calculated as 7% of the body weight) via the carotid artery catheter within 5 minutes. Hypovolemia was maintained for 1 hour before a single volume infusion (35% BV) of hydroxyethyl starch (HES) (Hextend, Hospira Inc., Lake Forest, IL, mean MW 670 kDa; degree of substitution 0.75) occurred within 10 minutes via the jugular vein catheter. Animals did not receive additional fluids during the experiment. Previous investigations performed in the same model have shown that fluid resuscitation with 25% of total
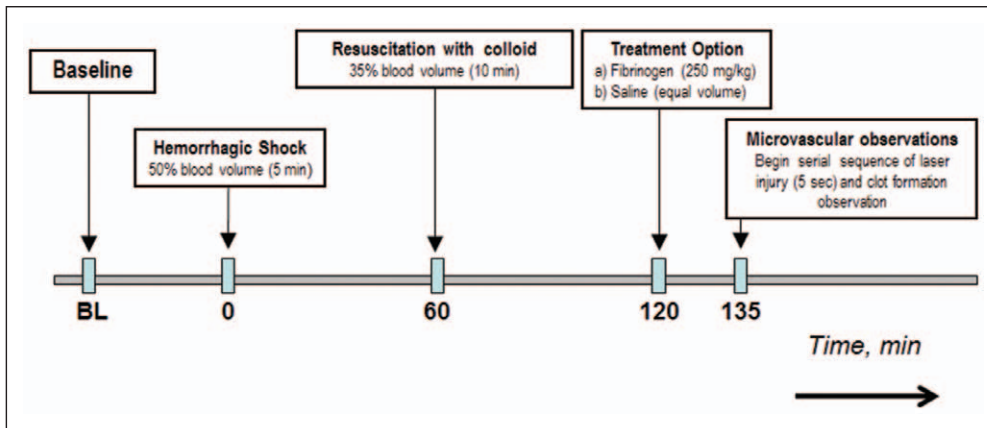
Figure 1. Experimental protocol. Fifty percent hemorrhagic shock was followed by colloid resuscitation (35% of blood volume). One hundred twenty min after shock animals were randomized to receive fibrinogen concentrate or saline. Fifteen min after treatment microvascular clot formation was observed in laser injured vessels.

seconds induced full thrombus development. Because the awake animal might move during such long exposure times, thereby shifting the laser beam out of focus, only venules were used in this study. Thrombus formation was readily identified on the site of injury using transillumination without the use of platelet staining. Results were quantified by calculating the ratio of number of vessels with microthrombi divided by the total number of vessels exposed to irradiation.

BV is adequate bacause the mechanism of autotransfusion restores about half of the shed volume during shock (28). In this study, however, 35% of total BV resuscitation was chosen to increase the volume effect and thereby produce severe dilutional coagulopathy.

In order to maximize the negative effect of the colloidal solution on the coagulation system and to allow the full clinical picture of dilutional coagulopathy to develop, animals remained in this resuscitated state for 1 hour. Then animals were randomized into two groups: 1) Treatment, 250 mg/kg fibrinogen IV (FGTW, Laboratoire français du Fractionnement et des Biotechnologies, Paris, France), or 2) Sham, treatment with IV infusion of an equal volume of normal saline. The dose of 250 mg/kg of fibrinogen concentrate was chosen due to the results of preliminary experiments (unpublished) indicating a normalization of ROTEM variables after shock/resuscitation in this model. The fibrinogen concentrate and the control volume (normal saline) were infused at a rate of 0.2 mL/min.

Fifteen minutes after fibrinogen or saline administration, laser injuries were induced by targeting the beam of a dye laser (425 nm, 25 mW, Power Technology, Alexander, AR) on venular vessel walls. The laser beam was introduced via an optical port to an upright microscope (BX51 W1, Olympus, New Hyde Park, NY). A micropoint laser system (Photonics Instruments, St. Charles, IL) and a ×60 objective (LUMPFL-WIR, numerical aperture 0.8, Olympus, Central Valley, PA) was used to collimate the beam. The beam was focused on the targeted vessel wall at 1/25 of the power for 5 seconds. Before, during, and after laser exposure, the vessel image was visualized using a video microscope system. After laser exposure, the vessel was observed for 1 minute, and the presence or absence of a thrombus was recorded. The event of thrombus formation was noted as "successful" when a flow disturbance was observed. Investigators were not blinded to the randomization status of the animal, which is a weakness of the study.

Cabrales et al (28) showed that high blood flow rates prevented injury to the arteriolar vessel wall when laser exposure times were below 20–30 seconds; however, in venules where the flow rates are much lower, exposure of only 2–5

Experimental Groups

Microvascular Analysis and Thromboelastometry. In each animal, microvessels were chosen for study based on their type (arteriole or venule), vessel diameter, blood flow, and visual clarity. Following these inclusion criteria, 8–11 venules and 4–6 arterioles were selected from each animal (n) (Treatment: number of animals, $n = 5$, and Sham: $n = 5$). Microvascular variables and thrombus formation were studied in total 98 venules (Treatment: number of vessels, $n = 48$ and Sham: $n = 50$). Additionally, microcirculation measurements were also performed in 45 arterioles (Treatment: $n = 22$ and Sham: $n = 23$). Blood was sampled from all animals in both groups for thromboelastometric evaluation.

Plasma Fibrinogen Concentration and Platelet Count. Changes of the plasma fibrinogen concentration and platelet count due to the experimental protocol were studied in another group of animals because of BV sampling limitations in this size of animal. For this additional analysis, animals ($n = 14$) underwent exactly the same surgical procedures were randomized for 1) Treatment: $n = 7$ or 2) Sham: $n = 7$. Blood for platelet and fibrinogen concentration measurements was withdrawn at the end of the experimental protocol.

Measured Variables

Thromboelastometry. Coagulation measurements were performed using a thromboelastometer (ROTEM Gamma, Tem Innovations GmbH; Munich, Germany) to assess functional coagulation variables. The device measures the elasticity of the developing and resolving clot over time, which is used to assess all phases of clot formation, stabilization, and degradation (29). Maximum clot firmness is the most important variable for this study because it is directly dependent on fibrinogen levels. ROTEM assays were run at 37°C immediately after drawing arterial blood in citrated 1 mL syringes (0.04 mL of citrate/1 mL of whole blood). Due to the small total BV of the hamsters (between 3.5 and 4.5 mL) and the relatively large sample size needed for ROTEM analysis (300 μL/test), only two ROTEM measurements could be performed in each animal. Baseline ROTEM analysis was performed using the first milliliter of

shed blood from hemorrhagic shock. The next ROTEM analysis was performed only at the end of the experiment by withdrawing 1 mL of blood from the arterial line before the animal was euthanized. Due to the limited BV available, only EXTEM and FIBTEM assays were performed. Because thromboelastometric values for hamsters have not been published yet, our data represent the first reference list.

Plasma Fibrinogen Concentrations and Platelet Count. Fibrinogen concentrations and platelet count were determined from the first milliliter of shed volume from hemorrhagic shock; after treatment with either fibrinogen concentrate or sham, blood was withdrawn again for the same measurements. Plasma fibrinogen concentrations were determined using the Clauss method. Platelet counts were measured using the Abbott Cell-Dyn 3500 Hematology Analyzer (GMI Inc., Ramsey, MN).

Microhemodynamics. Arteriolar and venular RBC centerline velocities (V) were measured online using the photodiode cross-correlation method (30) (Photo Diode/Velocity Tracker Model 102B, Vista Electronics, San Diego, CA). Measured V was corrected according to vessel size to obtain mean RBC velocity (31). Video image–shearing was used to measure vessel diameter (D; Image Shearing Monitor, Vista Electronics) (32). Blood flow (Q) was calculated from the measured values as $Q = V \times \pi (D/2)^2$.

Functional capillary density. Capillaries were considered functional if RBC transit was observed through the capillary segments during a 30 seconds period. Functional capillary density (FCD) was tabulated from capillary lengths with RBC transit in an area comprised of 20 successive microscopic fields under ×40 magnification. FCD (cm^{-1}) is the total length of RBC-perfused capillaries divided by the surface area. The relative change in FCD from baseline levels, at each time point, is indicative of the extent of capillary perfusion (33). FCD is a key variable for evaluation of microvascular perfusion and severity of shock.

Systemic Variables. MAP and heart rate were monitored continuously (MP 150, Biopac System, Santa Barbara, CA). Arterial blood samples taken in heparinized microcapillary tubes (50 μL) were centrifuged to determine HCT. Hemoglobin content (Hb) was determined spectrophotometrically from a single drop of blood (B-Hemoglobin, Hemocue, Stockholm, Sweden).

Blood Chemistry. Arterial blood was collected in heparinized glass capillaries (50 μL) from the carotid catheter and immediately analyzed for Pao_2, $Paco_2$, base excess (BE), and pH (Blood Chemistry Analyzer 248, Bayer, Norwood, MA). The animals have comparatively low Pao_2 and high $Paco_2$ as a consequence of their adaptation to a fossorial environment (34).

Measurement Points

Blood chemistry, systemic and microvascular variables were measured at baseline (BL), 1 hour after shock (S), and 1 hour after resuscitation (R). ROTEM variables were measured at BL and R.

Statistical Analysis

All variables besides the microhemodynamic variables were normally distributed and are presented as mean ± SD. One-way analysis of variance was performed among time points of interest within a treatment group. When appropriate, post hoc analyses were performed with the Dunn multiple-comparison test. Microhemodynamic variables were not normally distributed, and thus, results are presented using box-whisker plots (median, lower, and upper quartiles). All microhemodynamic measurements were compared with baseline levels. A ratio of 1.0 signifies no change from baseline, whereas lower and higher ratios are indicative of changes proportionally lower and higher than baseline. Statistical analysis with the chi-square test showed there was no difference in microhemodynamic variables among groups at baseline. Similarly, there was no difference in microhemodynamic variables among groups after hemorrhagic shock. Therefore, data from both groups at these individual time points were pooled allowing for more robust statistical analysis. Statistical comparison of thrombus formation was made with nonparametric methods using the Pearson chi-square with Fisher exact test. Statistical analyses were performed using computer software (Prism 4, Graphpad, San Diego, CA). Results were considered significant when p values are of less than 0.05.

RESULTS

Microvascular variables were analyzed in a total of 98 venules and 45 arterioles. Vessels were recruited from 10 animals, which after resuscitation with Hextend received either fibrinogen concentrate (Treatment: $n = 5$ animals) or an equal volume of saline (Sham: $n = 5$ animals). An extra group of 14 animals underwent exactly the same surgical procedures were randomized for treatment or sham but were then used for blood withdrawal for platelet and fibrinogen concentration measurements. A total of 24 animals were entered into this study, and all tolerated the protocol without visible signs of discomfort.

Coagulation Measurements

Thromboelastometric Variables. Hemorrhage/shock and following resuscitation significantly reduced FIBTEM and EXTEM MCF compared with BL. Treatment with fibrinogen restored BL MCF levels (**Fig. 2**). Hemorrhage/shock and resuscitation significantly decreased FIBTEM and EXTEM alpha angle compared with BL; fibrinogen treatment restored baseline values. Also clot formation time was significantly prolonged if fibrinogen was not substituted after resuscitation with Hextend (**Table 1**).

Plasma Fibrinogen Concentration and Platelet Count. Measurements of plasma fibrinogen concentration and platelets were performed in a separate group of animals ($n = 14$) as described above. Hemorrhage/shock significantly reduced plasma fibrinogen concentration. Resuscitation and treatment with fibrinogen restored BL plasma fibrinogen concentrations. Animals not receiving fibrinogen exhibited significantly lower fibrinogen values after resuscitation compared with BL and to animals who had received fibrinogen (**Table 1**).

Platelet count at the end of the experiment was not significantly changed from baseline in both experimental groups (**Table 1**).
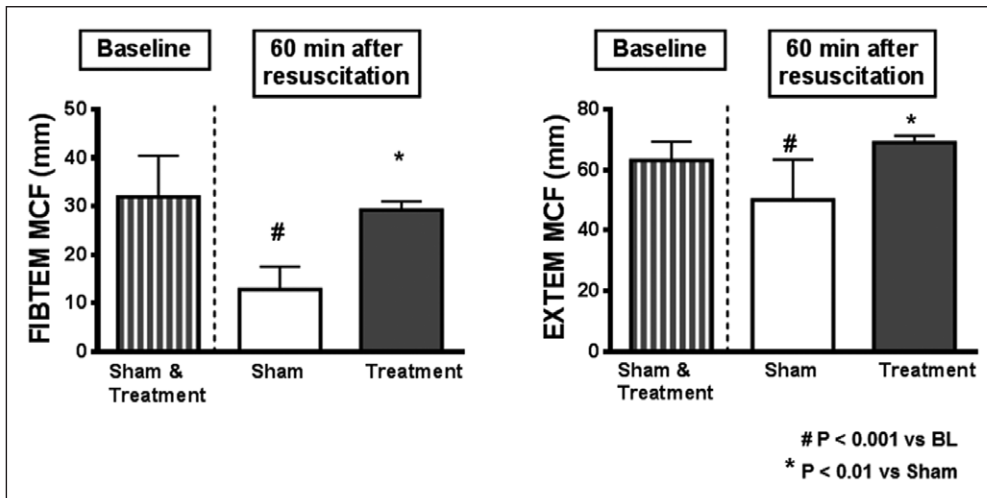
Figure 2. Changes in maximum clot firmness measured by thromboelastometry. Maximum clot firmness significantly decreased in FIBTEM and EXTEM after resuscitation with Hextend. Administration of fibrinogen concentrate restored BL levels. Baseline data were pooled for both groups to allow for more robust statistical analysis. #*p* < 0.001 versus BL. *p < 0.01 versus Sham. *Striped bars* = all animals, *white bars* = Sham, *gray bars* = Treatment.

Microcirculation

Venules. A total of 98 venules (Treatment: *n* = 48; Sham: *n* = 50; diameter range: 25–78 μm) were studied. After 1 hour of shock, venules exhibited a significant vasoconstriction compared with BL. Resuscitation restored venular diameters of the sham group back to baseline levels; venules of the fibrinogen group showed a significant vasodilation compared with shock (**Fig. 3***A*). Venular blood flow significantly decreased after hemorrhage/shock; resuscitation significantly increased venular blood flow in both groups (**Fig. 3***C*).

In Vivo *Thrombus Formation After Laser Injury.* After 5 seconds of exposure to laser light, animals treated with fibrinogen showed a significantly higher prevalence of thrombus formation compared with nontreated animals.

The success rate of microthrombus formation in irradiated venules was 37 out of 48 (77%) in the fibrinogen group and 9 out of 50 (18%) in the sham group (*p* < 0.0001). Thrombus formation was not detected in vessels beyond the laser irradiation area.

Other Microvascular Variables: Arterioles. A total of 45 arterioles were studied (Treatment: *n* = 22; Sham: *n* = 23; diameter range, 23–77 μm). Hemorrhage resulted in a significant arteriolar vasoconstriction compared with BL (**Fig.**

TABLE 1. Thromboelastometric Variables, Fibrinogen Concentration, and Platelet Count at Baseline and After Shock/Resuscitation

| Variables	Baseline	After Shock/Resuscitation	
		Sham	Treatment
FIBTEM			
CT (s)	53±20	59±17	57±35
CFT (s)	74±34	—[a]	76±33
Alpha (degree)	80±5	66±3[b]	77±6
MCF (mm)	32±6	13±5[c]	29±2[d]
EXTEM			
CT (s)	42±8	54±13	48±18
CFT (s)	55±18	106±51[b]	61±5
Alpha (degree)	82±2	72±9[b]	79±2
MCF (mm)	63±6	50±13[e]	69±2[d]
Fibrinogen (mg/dL)	440±112	261±62[b]	439±73[d]
Platelet count	350±120	296±71	327±53

CT = clotting time, CFT = clot formation time, MCF = maximum clot firmness.

[a]Sham animals never reached a CFT value in the FIBTEM test due to their low fibrinogen levels.

[b]*p* < 0.01 vs baseline.

[c]*p* < 0.001 vs baseline.

[d]*p* < 0.01 vs Sham.

[e]*p* < 0.05 vs baseline.
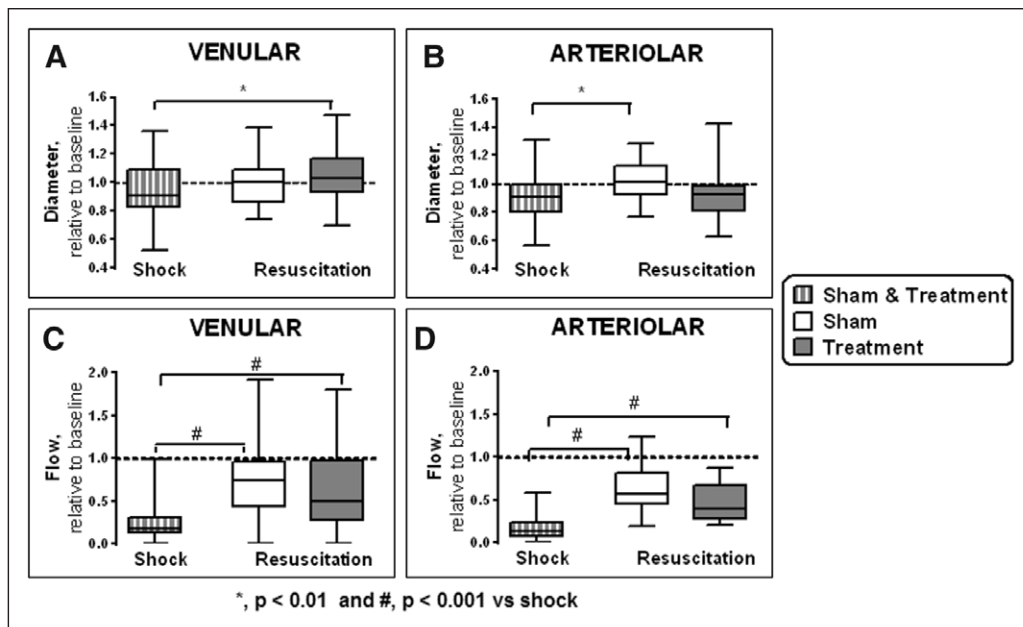
Figure 3. Microhemodynamic changes during shock/resuscitation. **A**, Venular diameters. **B**, Arteriolar diameters. **C**, Venular flow. **D**, Arteriolar flow. Shock resulted in a significant venular and arteriolar vasoconstrictions, leading to decreased microvascular blood flow. Resuscitation with hydroxyethyl starch significantly recovered venular and arteriolar blood flow compared with shock conditions; however, baseline levels were not restored. Data for baseline and shock between the two groups (Sham and Treatment) were not statistically different and thus were pooled to allow for more robust statistical analysis. *$p < 0.01$ versus shock. #$p < 0.001$ versus shock. *Striped bars* = all animals, *white bars* = Sham, *gray bars* = Treatment

Hemorrhage/shock introduced significant changes in acid-base balance: pH significantly decreased from 7.37 ± 0.04 to 7.22 ± 0.06; BE significantly decreased from 5.6 ± 3.6 to -7.9 ± 7.0. pH was restored to baseline levels after resuscitation (7.33 ± 0.06 for Sham and 7.34 ± 0.03 for Treatment group), whereas BE remained significantly lower than BL (1.1 ± 5.0 for Sham and -1.0 ± 4.0 for Treatment group). There were no significant differences in pH and BE between the two groups.

DISCUSSION

This study shows that administration of fibrinogen significantly increased the incidence of microthrombus formation after shock/resus-

3*B*). After resuscitation, arterioles of the sham group showed a significant vasodilation and returned to baseline levels; arteriolar diameters of the fibrinogen group showed a tendency toward vasodilation; however, the changes were not significant (Fig. 3*B*). Arteriolar flow was significantly reduced after hemorrhage/shock compared with BL (Fig. 3*D*); resuscitation significantly increased arteriolar flow in both groups; however, baseline conditions were not restored (Fig. 3*D*).

Functional Capillary Density. Functional capillary density was significantly reduced during shock compared with BL. After resuscitation, FCD was partially restored but remained significantly different from BL. There was no difference in FCD at any time point between groups.

Systemic Variables and Blood Chemistry
Hemorrhage induced a significant drop in HCT compared with BL ($31.7\% \pm 4.0\%$ vs $49.6\% \pm 1.8\%$ at BL). Resuscitation and treatment with fibrinogen/saline significantly decreased HCT further to $21.8\% \pm 2.4\%$ (Sham) and $22.2\% \pm 0.8\%$ (Treatment). As expected, hemoglobin showed the same trend as HCT: hemorrhage significantly decreased Hb from 14.6 ± 0.6 g/dL at BL to 9.3 ± 1.0 g/dL. Resuscitation significantly decreased Hb further to 6.6 ± 0.5 g/dL (Sham and Treatment). The changes in HCT and Hb values were not significantly different between groups.

Following hemorrhage, MAP significantly decreased from 116 ± 7 mm Hg to 57 ± 7 mm Hg. Resuscitation partially restored MAP (85 ± 9 mm Hg for Sham and 83 ± 11 mm Hg for Treatment); however, MAP was still significantly lower than at baseline. No statistically significant differences in MAP were found among the two study groups.

citation with high–molecular weight HES (Hextend) compared with untreated animals. These findings are corroborated by thromboelastometric measurements showing that fibrinogen was able to normalize reduced clot firmness following dilutional coagulopathy. Additionally, thrombus formation was only observed in vessels exposed to laser irradiation. In both the fibrinogen and the untreated groups, there was no prevalence of spontaneous clot formation. Thus, the presence of excess fibrinogen did not lead to random thrombogenesis. It is, therefore, suggested that at the microvascular level, the increased clot strength, measured by thromboelastometry, corresponds to an increased ability to form a thrombus in laser-injured vessels.

Current guidelines for the treatment of hemorrhagic shock emphasize damage control resuscitation, where hypotensive resuscitation, a strategy restricting fluid administration until hemorrhage is controlled, is combined with hemostatic treatment and damage control surgery (35). Hemostatic treatment addresses the issue that rapid volume resuscitation needed to restore perfusion pressure, induces dilutional coagulopathy that might cause additional blood loss, and therefore deteriorates the condition of a severely injured person (36–39). In this scenario, fibrinogen reaches critically low levels before any other clotting factor, including platelets (40). Substitution of fibrinogen has been shown to diminish blood loss and improve clot firmness in large animal and patient studies (11, 13, 14). Administration of fibrinogen concentrate in patients undergoing radical cystectomy reduced the need for postoperative RBC transfusions (13) and could rapidly correct hypofibrinogenemia, thereby controlling bleeding (41) during massive obstetric hemorrhage. Despite these findings, in clinical practice, the effect of fibrinogen

substitution on clot formation in vivo is unknown and treatment is mainly based on in vitro laboratory variables. Although thromboelastometry is considered to provide a more functional coagulation analysis, it still lacks the input of physiological circumstances. The data of this study suggest that thromboelastometric measurements are valid indicators for the clotting situation in vivo. One has to be cautious, however, in extrapolating these results to a clinical situation because this model does not reflect a clinical situation and is not a trauma model.

The technique of visualizing thrombus formation in vivo has been primarily used to better understand the interactions between protein and cellular components involved in the clotting cascade (21, 22, 42). The classical doctrine of an intricate cascade of protease complexes resulting in thrombin generation and finally leading to the conversion from fibrinogen to fibrin derives mainly from in vitro observations, lacking many components that under physiological circumstances play an important role in the process of clot formation. Not only are active cellular components such as endothelial cells, platelets, erythrocytes, and leukocytes involved in the hemostatic process but also microparticles deriving from platelets and leukocytes. Additionally, physical forces, for example, shear forces, generated by the flowing blood have a major impact on clot formation, especially on primary platelet adhesion to the vessel wall (43). From a clinical view point, this factor plays an important role because resuscitation fluids increase perfusion pressure and therefore augment shear forces on the vessel wall, which might interfere with firm platelet adhesion.

Until now the effect of shear rates on thrombus formation has only been studied in vitro. Studies on excised vessels found that at low shear rates, platelet aggregation to the vessel wall is highly dependent on fibrinogen (43), whereas at high shear rates ($\geq 1.500/s$), platelet adhesion to the vessel wall only occurred in the presence of von Willebrand factor. Nevertheless, the establishment of a stable thrombus after primary adhesion requires the presence of both fibrinogen and von Willebrand factor in order to resist the high shear forces produced by dynamic flow conditions (43). These observations underline that fibrinogen plays a dual role, promoting primary endothelial adhesion at first but eventually contributing to the formation of a stable clot. Considering that shear rates in vessels of the hamster window chamber are low (100–400/s), our in vivo findings could be in concordance with these previous results. However, this conclusion is limited because this model does not allow for investigation of high shear rate vessels.

It is important to note, that the dose of fibrinogen concentrate used in this study is very high compared with doses administered to humans. Comparative physiology studies across species show that slight variations in the fibrinogen molecule affect its function (44, 45). Therefore, it is not possible to simply transferring results of this study to humans, especially in regards to the fibrinogen dose. This study was designed to establish an animal model that allows to study coagulation function in the microcirculation and to compare it to a clinical measurement (thromboelastometry). Our results show that this animal model has the potential to becoming useful tool for further in-depth in

vivo coagulation studies that would include factors such as clot size, stability, and formation velocity as a function of time, which cannot be ascertained from in vitro measurements.

The small BV of the animals used in this study limits our study design because all variables cannot be measured in the same animal. The baseline levels for fibrinogen and platelet counts were established using a different group of animals from those used in the microcirculation and ROTEM measurements. This is a confounding factor that can lead to a bias in the interpretation of the results, especially in a setting of small experimental groups as in our study. The bias is reduced because the platelet count and fibrinogen levels in the separate group are within the range established for these animals (34).

The classical understanding of thrombus formation postulates that platelet adhesion occurs first, leading to a platelet thrombus, followed by stabilization of the primary thrombus through fibrin strands. Interestingly, even though platelet count was equal at the time point of laser injury between treatment and sham groups, the prevalence of thrombus formation was highly different between groups. This suggests that the presence of fibrinogen is crucial for primary adhesion of platelets to the vessel wall, and that platelet thrombus formation and stabilization through fibrin strands are not strictly sequential steps but are more likely simultaneously occurring events.

In this study, dilutional coagulopathy was induced by using Hextend, a plasma expander currently used in the United States. In European clinical practice, smaller molecular weight starches (e.g., HES 130/0.4) or gelatins are mainly used as colloidal plasma expanders. It might be speculated that the use of HES 130/0.4 or gelatin would not have induced the same degree of coagulopathy as seen in this study.

CONCLUSIONS

This study shows that the hamster window chamber model can be effectively used for analyzing the microvascular events portrayed by thromboelastometric measurements of clotting ability. The visualization of microscopic blood flow and its consequences on local shear stress provides additional data with which to support conclusions derived from thromboelastometry and should aid in devising hemostatic therapy in patients. Ultimately, studies based on this type of animal model should contribute to the understanding of trauma-associated coagulopathy.

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