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RECOMBINANT FACTOR XIII DIMINISHES MULTIPLE ORGAN DYSFUNCTION IN RATS CAUSED BY GUT ISCHEMIA-REPERFUSION INJURY

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Abstract

Plasma factor XIII (FXIII) is responsible for stabilization of fibrin clot at the final stage of blood coagulation. Because FXIII has also been shown to modulate inflammation and endothelial permeability, we hypothesized that FXIII diminishes multiple organ dysfunction caused by gut I/R injury. A model of superior mesenteric artery occlusion (SMAO) was used to induce gut I/R injury. Rats were subjected to 45-min SMAO or sham SMAO and treated with recombinant human FXIII A_2 subunit (rFXIII) or placebo at the beginning of the reperfusion period. Lung permeability, lung and gut myeloperoxidase activity, gut histology, neutrophil respiratory burst, and microvascular blood flow in the liver and muscles were measured after a 3-h reperfusion period. The effect of activated rFXIII on transendothelial resistance of human umbilical vein endothelial cells was tested *in vitro*. Superior mesenteric artery occlusion–induced lung permeability as well as lung and gut myeloperoxidase activity was significantly lower in rFXIII-treated versus untreated animals. Similarly, rFXIII-treated rats had lower neutrophil respiratory burst activity and ileal mucosal injury. Rats treated with rFXIII also had higher liver microvascular blood flow compared with the placebo group. Superior mesenteric artery occlusion did not cause FXIII consumption during the study period. *In vitro*, activated rFXIII caused a dose-dependent increase in human umbilical vein endothelial cell monolayer resistance to thrombin-induced injury. Thus, administration of rFXIII diminishes SMAO-induced multiple organ dysfunction in rats, presumably by preservation of endothelial barrier function and the limitation of polymorphonuclear leukocyte activation.

Keywords

Fibrin-stabilizing factor; superior mesenteric artery occlusion; oxidative stress; lung injury; microcirculatory disorders

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INTRODUCTION

Gut ischemia and intestinal permeability disorders caused by acute pathological conditions, such as trauma-hemorrhagic shock, burn, and sepsis, play an important role in the development of multiple organ dysfunction (MODS) (1–3). I/R injury of the gut can be a dangerous complication of other pathological states or interventions, for example, intestinal transplantation, abdominal aortic aneurysm surgery, and cardiopulmonary bypass (4). Gut-derived factors contained in intestinal lymph coming from the ischemic gut have been shown to be key factors in the pathogenesis of acute lung injury, bone marrow dysfunction, red blood cell alterations, endothelial cell injury, upregulation of endothelial cell adhesion molecule expression, and neutrophil activation (5–9). In addition, in severe inflammatory states, various blood and tissue cells, including polymorphonuclear granulocytes, release lysosomal proteinases that, together with other oxidizing agents produced during phagocytosis, enhance the inflammatory response by degrading connective tissue structures, membranes, and soluble proteins. Thus, agents that could reduce the severity of gut damage and inflammatory response after gut I/R injury are useful for the prevention and treatment of subsequent MODS.

Factor XIII (FXIII) or fibrin-stabilizing factor is a trans-glutaminase involved in the final stage of blood coagulation. It consists of two catalytic A subunits and two noncatalytic B subunits. Factor XIII circulates in the plasma as an inactive precursor and is activated by thrombin. Activated FXIII stabilizes fibrin clots by cross-linking fibrin monomers with covalent bonds, which increase the mechanical strength of the clot, retard fibrinolysis, and enhance platelet adhesion to the injured tissue (10).

The role of FXIII is not limited to the area of hemostasis. Activated FXIII has been shown to stabilize endothelial barrier function by reducing endothelial permeability (11,12). *In vitro* studies revealed that FXIII promotes intestinal healing (13). *In vivo* animal experiments confirmed that FXIII is effective in the treatment of trinitrobenzenesulfonic acid-induced colitis (14). In addition, limited clinical experience with FXIII has suggested its efficacy in the treatment of ulcerative colitis and chronic inflammatory bowel diseases (15,16). Finally, there is evidence that FXIII modulates the inflammatory response by retardation of macrophage migration (17).

Because FXIII has been suggested to improve endothelial function and modulate the inflammatory response, we hypothesized that treatment with FXIII could protect from the development of MODS after gut I/R.

MATERIALS AND METHODS

Study design

Male Sprague-Dawley rats weighing between 250 and 300 g received standard rat chow and water *ad libitum* and were allowed an acclimatization period of at least 1 week before the experiment. Animals were subjected to a cycle of 12-h light/12-h dark, controlled humidity, and room temperature between 18°C and 22°C. Animal study protocols were approved by Novo Nordisk Ethical Review Committee and the University of Medicine and Dentistry–New Jersey Medical School Animal Care and Use Committee. Experiments were performed in adherence to the guidelines of the Danish Animal Experiments Council, Danish Ministry of Justice, and in concordance with the National Institutes of Health Guidelines on the Use of Laboratory Animals.

Rats subjected to superior mesenteric artery occlusion (SMAO) or sham SMAO were treated in blinded fashion with placebo or recombinant human FXIII A_2 subunit (rFXIII; Novo

Nordisk A/S, Maaloev, Denmark). Animals were randomly divided into four groups (eight animals each): group 1: SMAO + plus vehicle treatment; group 2: SMAO + rFXIII treatment; group 3: sham SMAO + vehicle treatment, and group 4: sham SMAO + rFXIII treatment. The vehicle represented a buffer consisting of 40 mM histidine, 8.5% sucrose, and 0.02% Tween 20 at pH 8.0. Lyophilized rFXIII was resuspended in the same buffer to achieve a final concentration of 1 mg/mL. The vehicle (1.0 mL/kg) or rFXIII (1.0 mg/kg) was given intravenously after 45 min of ischemia (in SMAO groups) immediately after mesenteric blood circulation was restored or after 45 min of sham SMAO (in sham groups). The chosen dose of rFXIII was in alignment with available literature data (14).

The majority of the end-point parameters (lung permeability, lung and gut myeloperoxidase [MPO] activity, neutrophil respiratory burst, gut histology, and microvascular blood flow in the muscle and liver) were assessed after 3 h of reperfusion. Factor XIII activity in rat plasma was measured before SMAO/sham SMAO and at the end of reperfusion period.

SMAO protocol

Rats were anesthetized with pentobarbital (50 mg/kg, i.p.). Using aseptic technique, the femoral artery and internal jugular vein were dissected out and cannulated with PE-50 tubing containing trisodium citrate (0.13 M). The jugular vein and femoral artery lines were used for drug administration and blood withdrawal.

Through a 5-cm midline laparotomy, the superior mesenteric artery was isolated and temporarily occluded by placing a 2-0 suture around the artery at its origin from the aorta. The abdomen was then covered with a sterile moist gauze pad. After 45 min of intestinal ischemia, the ligature was removed from around the artery, and after return of the blood supply to the gut was verified, the laparotomy incision was closed.

Rats subjected to sham SMAO were anesthetized, had a laparotomy, and had their superior mesenteric artery looped with 2-0 suture, but the vessel was not occluded. After 45 min of sham SMAO, the suture was removed, and the laparotomy incision was closed.

Rats were killed 3 h after SMAO or sham SMAO using i.v. pentobarbital injection.

Lung permeability assay

Lung permeability was measured using the Evans blue dye (EBD) technique. Rats were injected with 10 mg of EBD through the internal jugular catheter at 2 h 40 min after SMAO/sham SMAO. After 5 min, to allow for complete circulation of the dye, a blood sample (1.0 mL) was withdrawn from the femoral artery catheter and centrifuged at 1,500 revolutions per minute (rpm) at 4°C for 20 min. The plasma was used to determine the plasma EBD concentration. Twenty minutes after injection of the dye, the rats were killed, and the tracheobronchial tree and lungs were harvested as a unit. Bronchoalveolar lavage was performed by lavaging the lungs three times with 5-mL aliquots of normal saline. The recovered bronchoalveolar lavage fluid (BALF) was then centrifuged at 1,500 rpm at 4°C for 20 min to remove any cells. The supernatant fluid was then assayed spectrophotometrically at 620 nm to measure the concentration of the EBD in the BALF. The concentration of EBD in the BALF was then expressed as the percentage of that present in the plasma.

Tissue perfusion and oxygenation measurements

Tissue perfusion and oxygenation were investigated using combination of OxyFlo and OxyLite monitors (Oxford Optotronix Ltd, Oxford, UK). OxyFlo is a multichannel system for measuring tissue blood flow (perfusion), which combines laser Doppler technology and

digital signal processing. OxyLite operates according to the principle of oxygen quenching of fluorescence and uses a small optical sensor featuring zero oxygen consumption for monitoring rapid temporal oxygen changes in a given tissue microregion. Precalibrated probes (sensor) are able to provide both spatial and continuous real-time measurement of dissolved oxygen (tissue Po_2) and temperature.

Blood flow and Po_2 in muscles of the medial surface of the thigh were measured using NP/O/E/4 needle-encased sensor inserted percutaneously via an 18-gauge guiding cannula. Liver blood flow was measured by reusable MSP300XP (Oxford Optotronix Ltd., Oxford, UK) miniature surface sensor. Blood flows are expressed in conditional units. The Win Daq software package (Dataq Instruments Inc, Akron, Ohio) was used to record and display tissue blood flow, Po_2, and temperature in real time on a computer screen as well to save these data for further analysis.

MPO assay

Myeloperoxidase activity was measured in the ileum and lung. Harvested tissue samples were homogenized for 30 s in 4 mL of 20 mmol/L potassium phosphate buffer (pH 7.4) and centrifuged (40,000*g*) at 4°C. The pellet was resuspended in 4 mL of 50 mmol/L potassium phosphate buffer (pH 6) containing 0.5 g/dL hexadecyltrimethylammonium bromide. Samples were sonicated for 90 s, incubated in a 60°C water bath for 2 h, and centrifuged. The supernatant in the amount of 0.1 mL was added to 2.9 mL of 50 mmol/mL potassium phosphate buffer (pH 6) containing 0.167 mg/mL o-dianisidine and 0.0005% hydrogen peroxide. Absorbance at 460 nm of visible light (A_{460}) was measured for 3 min. Myeloperoxidase activity was calculated using the following formula: MPO activity [in units per gram of tissue] = ($\delta A_{460} \times 13.5$)/weight [in grams], where δA_{460} equals the rate of change in absorbance at 460 nm between 1 and 3 min. The coefficient 13.5 was empirically determined such that one-unit MPO activity is the amount of enzyme that will reduce 1 μmol peroxide per minute (18).

Neutrophil respiratory burst assay

Flow cytometry was used to assess neutrophil respiratory burst. Heparinized whole-blood samples (100 μL) were placed into 5-mL polystyrene round-bottom tubes containing an equal volume of Dulbecco modified Eagle medium, and the red blood cells were lysed by means of 1% Pharm Lyse solution (BD Biosciences, Franklin Lakes, NJ). The tubes were spun at 1,135 rpm for 5 min at 25°C. The supernatants were discarded, and the cells were washed twice with Hanks balanced salt solution. After the white blood cell pellets were resuspended in 400 μL of Hanks balanced salt solution, 15 ng/mL of dihydrorhodamine was added to the tubes. Five minutes after dihydrorhodamine was added, polymorphonuclear neutrophils were stimulated with phorbol myristyl acetate. After 15-min incubation at 37°C, the polymorphonuclear neutrophil respiratory burst was measured by flow cytometry.

Histological examination

After the rats were killed, a segment of the terminal ileum was excised and fixed in 10% buffered formalin. After processing, semithin (2–4 μm) sections were cut and stained with 1% hematoxylineosin. Five random fields with 100 to 250 villi were analyzed in a blinded fashion by means of light microscopy at 100× magnification. The overall percentage of ileal villous damage was determined by dividing the number of injured villi by the total number of villi examined. Ileal mucosal damage score was calculated as described by Chiu et al. (19).

FXIII activity assay

The photometric FXIII activity assay (Berichrom FXIII; Dade Behring, Newark, Del) was performed on rat plasma samples according to the manufacturer's instructions but adapted to a microtiter plate format read on a SpectraMax (Molecular Devices, Sunnyvale, Calif) at 340 nm in the kinetic mode. Plasma samples were diluted 1:3 in 20 mM HEPES and 150 mM NaCl, pH 7.4, and rFXIII standards were diluted in FXIII-deficient plasma (George King Biomedical, Overland Park, Kans).

Measurement of endothelial cell electrical resistance

Endothelial cell electrical resistance was measured on human umbilical vein endothelial cells (HUVECs). They were grown in endothelial growth medium (EGM-2) (Lonza Inc, Allendale, NJ) containing epidermal growth factor (EGF), hydrocortisone, GA-1000, 2% fetal bovine serum (FBS), vascular endothelial growth factor (VEGF), human fibroblast growth factor, recombinant insulin-like growth factor (R-IGF-1), heparin, and ascorbic acid.

The barrier function of confluent endothelial cell layers was measured using the Electrical Cell-substrate Impedance Sensing (ECIS) system, 1600R (Applied Biophysics, Troy, NY). The ECIS arrays consisted of eight-well chamber slides containing gold film surface microelectrodes through which current was flowing using culture medium as the electrolyte. As it has been shown by Keese et al. (20), the attachment of cells to the electrodes causes changes in resistance that are measured by the ECIS system. Human umbilical vein endothelial cells were grown to confluent monolayers on gelatin-coated ECIS chamber slides, as confirmed by morphology and impedance measurement, and then treated with various combinations of thrombin (0.05–0.2 nM), activated rFXIII (rFXIIIa) (0.5–100 nM), or albumin, which served as a control. The response to the treatment was measured for 20 h. Human α-thrombin was obtained from Haematological Technologies Inc (Essex Junction, Vt). Recombinant human FXIII A_2 subunit was activated by incubating with thrombin-agarose beads (Sigma, St. Louis, Mo) for 4 h at room temperature with shaking, which provided more than 80% activation of the purified FXIII as determined by a fluorometric FXIIIa assay (N-zyme Biotech GmbH, Darmstadt, Germany), as described by Oertel et al. (21) Human albumin (Sigma) received similar thrombin-agarose treatment before adding to control wells.

Resistance values for each microelectrode were normalized as the ratio of measured resistance to baseline resistance and plotted as a function of time. Concentration-response relationships were established from at least three experiments/concentration of FXIIIa and thrombin.

Statistics

Data were analyzed using SPSS 9.0 for Windows (SPSS, Chicago, Ill) and presented as the mean ± SD. Mean values were compared using one-way ANOVA followed by Tukey test. The level of statistical significance was set at $P < 0.05$.

RESULTS

In vivo experiments

Lung permeability in rats subjected to SMAO and treated with rFXIII was significantly lower than in placebo-treated animals and did not differ from sham values (Fig. 1).

Treatment with rFXIII improved regional microvascular blood flow and oxygenation after SMAO. Muscle Po_2 as well as muscle and liver blood flows after SMAO were significantly

higher in rFXIII-treated rats; however, these values were lower than in sham animals (Table 1).

Lung and gut MPO activity after SMAO was also significantly lower in rFXIII-treated rats; however, these values were higher than in sham animals (Fig. 2).

Neutrophil respiratory burst in rats subjected to SMAO and treated with rFXIII was significantly lower than in placebo-treated animals and did not differ from sham values (Fig. 3).

The number of injured ileal villi after SMAO did not differ in placebo- or rFXIII-treated rats and was significantly higher than in sham animals (Table 2). However, ileal mucosal injury score after SMAO was significantly less with rFXIII treatment. Rats subjected to SMAO and placebo treatment demonstrated severe disruption of villi tips (Fig. 4A). Ileal mucosal injury was less profound after SMAO and rFXIII treatment. However, an extension of the subepithelial space as well as villi edema was recorded (Fig. 4B). Ileal damage was minimal in both sham groups (Fig. 4, C and D).

Superior mesenteric artery occlusion did not cause a drop in FXIII activity. Factor XIII activity in rats subjected to SMAO and treated with placebo did not differ from sham placebo-treated animals (Fig. 5). Similarly, FXIII activity increased equally in SMAO and sham animals if they were treated with rFXIII.

In vitro experiments

Thrombin-induced barrier disruption of HUVECs was attenuated by rFXIIIa (Fig. 6). Treatment with unactivated rFXIII did not affect endothelial permeability (not shown). In the absence of thrombin, neither rFXIII alone nor albumin caused detectable alterations in endothelial cell electrical resistance.

Immunostaining of HUVEC monolayers demonstrated that thrombin disrupted adherens junctions as evidenced by the decreased vascular endothelial (VE)–cadherin signal starting at 5 min after treatment (not shown) and continues to be lower at 60 min (Fig. 7). Addition of rFXIIIa caused less disruption of VE-cadherin along cell-cell contact points than thrombin alone, with almost complete recovery by 60 min.

DISCUSSION

Intestinal ischemia followed by reperfusion injury causes severe alterations in gut permeability and the loss of its barrier function as well as the initiation of an inflammatory cascade that can lead to MODS. A significant increase in intestinal permeability has been well documented using the experimental model of SMAO (22–25). Gut I/R activates circulating neutrophils, which promote elastase-mediated injury in distant organs, especially in the lungs (26). Similarly, SMAO has been shown to amplify neutrophil accumulation in the lungs and to increase pulmonary permeability (27,28). Different treatment strategies have been proposed to ameliorate gut I/R-induced lung permeability alterations and to modulate the inflammatory response following this injurious event. These therapies include the use of nutrition agents (29), elastase inhibitors (26), antioxidants (25), complement blockers (27), inhibitors of nuclear factor κB activation and proinflammatory cytokine production (23), and NO synthase inhibitors (28). In our study, we tested the hypothesis that rFXIII would decrease SMAO-induced endothelial permeability alterations in the lung and modulate the inflammatory response. The main rationale for this hypothesis is based on the ability of activated FXIII to stabilize endothelial barrier function by reducing endothelial permeability, as shown in monolayers of cultured porcine aortic endothelial cells (11,12). In

these studies, the flux of albumin across the endothelial monolayer served as a measure of paracellular permeability. Treatment with activated FXIII led to a 30% reduction in permeability. The authors demonstrated that FXIII not only lowered basal permeability of the endothelial monolayer but also prevented the increase in permeability provoked by an inhibition of endothelial energy production. In addition, the promotion of intestinal epithelial wound healing by enhancement of epithelial cell restitution has been achieved by the *in vitro* use of FXIII (13). The mechanisms by which FXIII might stimulate tissue repair and remodeling include stimulation of endothelial cell proliferation and migration, inhibition of apoptosis (30), and mediation of platelet adhesion to endothelial cells through $\alpha_v\beta_3$ and glycoprotein IIb/IIIa integrins (31).

Data from our current study indicate that rFXIII partially protects distant organs after gut I/R injury. Specifically, lung permeability at 3 h after SMAO was significantly lower in the rFXIII-treated animals compared with placebo-treated ones. Similarly, rFXIII treatment favorably influenced microvascular blood flow and tissue oxygenation in both liver and muscle tissue after gut I/R injury. Less severe gut and lung injury can be explained by lower levels of inflammatory response and oxidative stress achieved by rFXIII treatment because placebo-treated animals had significantly higher levels of gut and lung MPO activity and neutrophil respiratory burst.

The concentration of FXIII A subunit in human plasma is reported to be 10 to 15 μg/mL (32). Our data show that endogenous levels of FXIII A subunit in rat plasma are slightly lower and range from 5 to 12 μg/mL (mean, 7 μg/mL). In our series of *in vivo* experiments, we used the dose of recombinant human FXIII A subunit that is equal to 28 μg/mL. It means that supraphysiological levels of FXIII are needed to achieve a protective effect after gut I/R injury. It should be mentioned that the half-life of rFXIII when used in presumably pharmacological doses is 270 to 320 h (33).

We suggest that one of the key elements of rFXIII-induced protection of distant organs after gut I/R injury is a reduction of SMAO-induced intestinal damage. The severity of ileal mucosal injury was lower in rFXIII-treated rats, compared with placebo-treated ones.

In vitro experiments with HUVEC were performed to reveal the direct influence of rFXIIIa on injured endothelium. Thrombin was applied to cause barrier disruption of HUVECs, which was confirmed by the lowering of their electrical resistance. Activated rFXIII attenuated the thrombin-induced endothelial barrier dysfunction. These data are in alignment with findings of Dardik et al. (30,31), who revealed the effect of rFXIII on endothelial cell proliferation and inhibition of apoptosis. However, the authors used higher concentrations of rFXIII (50–70 μg/mL).

Factor XIII deficiency has been recorded after trauma, burn, and sepsis (34–36). However, we did not reveal any drop in FXIII activity 3 h after gut I/R injury: rats subjected to SMAO or sham SMAO demonstrated an equal increase in FXIII activity if rFXIII was given. The increased FXIII levels were sufficient to show a protective effect in rats that were subjected to SMAO.

We conclude that rFXIII ameliorates MODS caused by gut I/R injury in this rat model. The mechanism of the protective effect of rFXIII might be explained by preservation of the gut endothelial barrier function, amelioration of permeability alterations in distant organs, and by modulation of the inflammatory response. Future studies are necessary to clarify the mechanisms of rFXIII action.

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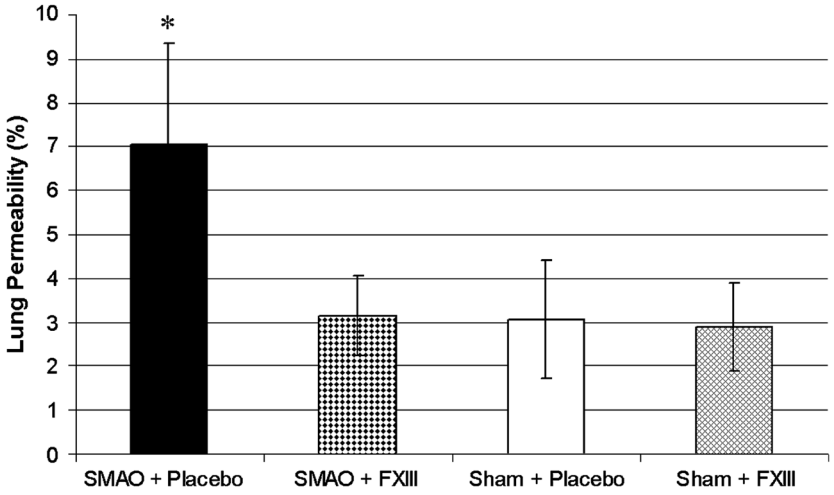

Fig. 1. rFXIII diminishes lung permeability alterations after gut I/R injury

Lung permeability is evaluated by measuring concentration of EBD in BALF and expressed as the percentage of that present in the plasma. Data are expressed as means ± SD (n = 8 in each group). *$P < 0.05$ vs. all other groups.

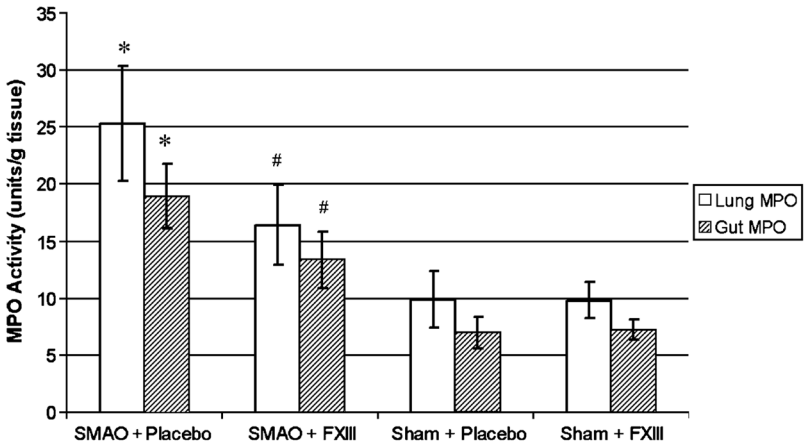

Fig. 2. rFXIII reduces neutrophil sequestration in lung and gut after gut I/R injury

The level of neutrophil sequestration is evaluated by measuring MPO activity. Data are expressed as means ± SD (n = 8 in each group). *$P < 0.05$ vs. all other groups; $^{\#}P < 0.05$ vs. shams.

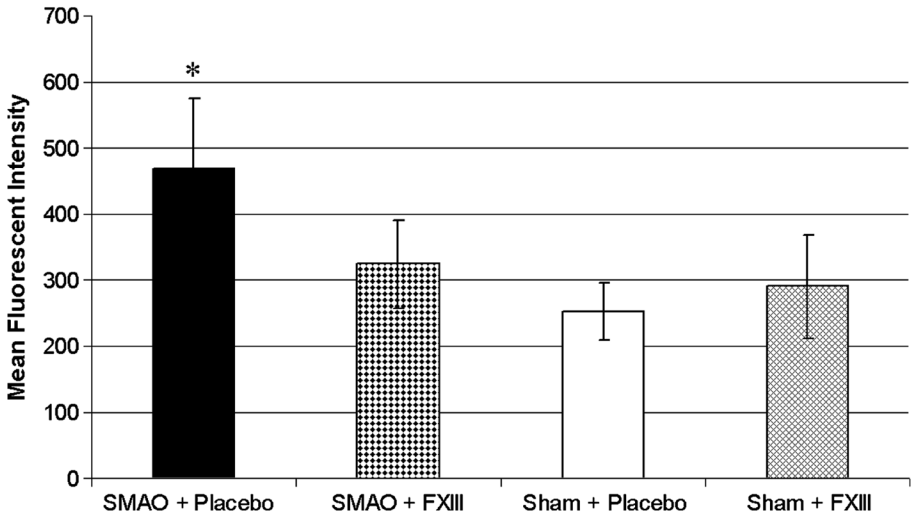

Fig. 3. rFXIII decreases the level of oxidative stress after gut I/R injury

The level of oxidative stress is assessed by neutrophil respiratory burst activity. Data are expressed as means ± SD (n = 8 in each group). *$P < 0.05$ vs. all other groups.

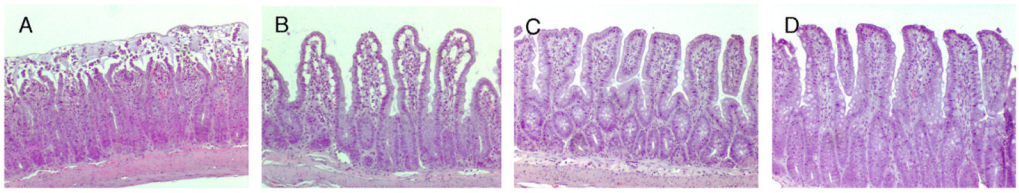

Fig. 4. rFXIII reduces the severity of ileal mucosal damage after gut I/R injury

Hematoxylineosin staining. Original magnification ×100. A, Rat is subjected to SMAO and placebo treatment. Villi tips are disrupted. Red blood cell congestion is seen. Gut injury level is classified as grade 3 mucosal damage score. B, Rat is subjected to SMAO and rFXIII treatment. Extension of the subepithelial space as well as villi edema is seen. Gut injury level is classified as grade 2 mucosal damage score. C, Rat is subjected to sham SMAO and placebo treatment. Almost normal villi are seen (grade 0–1 mucosal damage score). D, Rat is subjected to sham SMAO and rFXIII treatment. Almost normal villi are seen (grade 0–1 mucosal damage score).

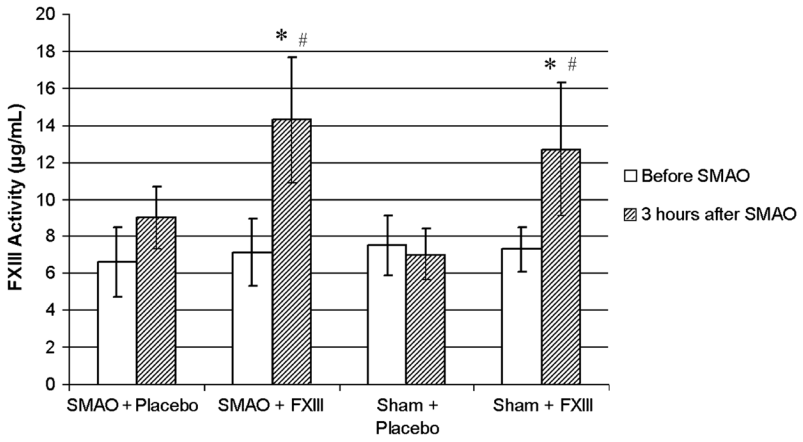

Fig. 5. FXIII activity does not change after gut I/R injury

Factor XIII activity in rat plasma is determined using Berichrom assay before and 3 h after SMAO or sham SMAO. Data are expressed as means ± SD (n = 8 in each group). **P* < 0.05 vs. before SMAO; $^{\#}P < 0.05$ vs. sham + placebo and SMAO + placebo.

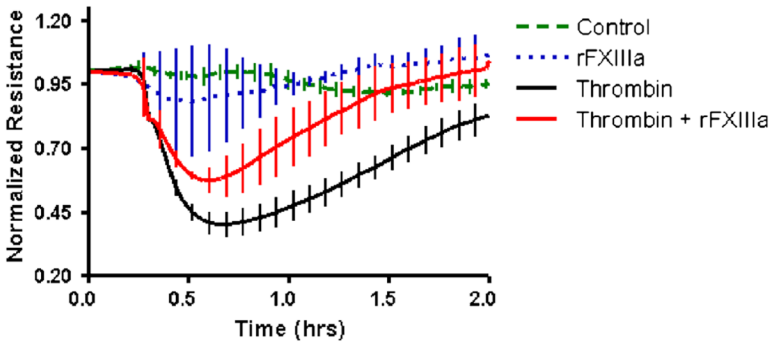

Fig. 6. rFXIIIa protects from thrombin-induced HUVEC resistance alterations

Transendothelial electrical resistance of HUVECs after the following types of treatment is shown: 0.1 nM thrombin, 50 nM rFXIIIa, combination of thrombin and rFXIIIa, or albumin (control). Data are expressed as means ± SE (n = 8 in each group). Human umbilical vein endothelial cell resistance after thrombin administration (starting from 0.5 h after treatment) is lower than in all other groups ($P < 0.05$). Human umbilical vein endothelial cell resistance after thrombin + rFXIIIa administration (starting from 0.5 h and up to 1.2 h after treatment) is higher than after sole thrombin administration alone but lower than after sole rFXIIIa treatment or albumin administration ($P < 0.05$).

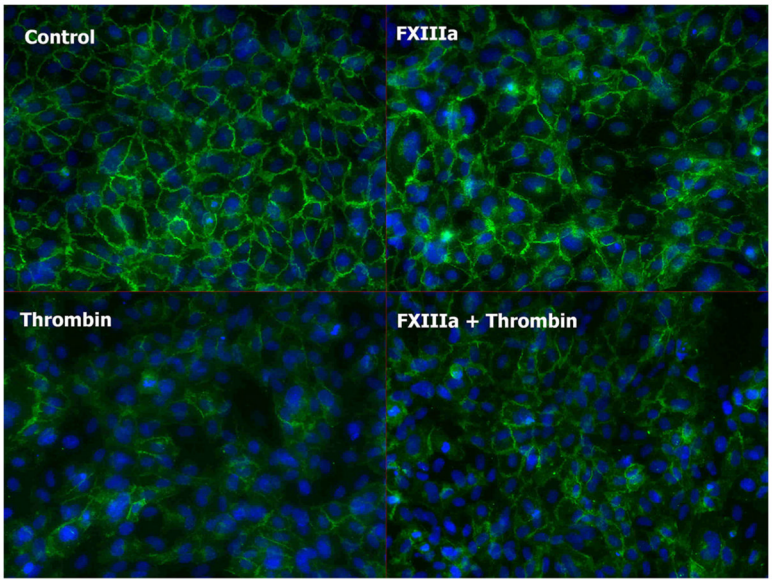

Fig. 7. Immunofluorescent staining of HUVECs treated with the following agents: thrombin, combination of thrombin and rFXIIIa, rFXIII, or albumin (control)

Cells are fixed in 4% paraformaldehyde and stained with anti–VE-cadherin antibody (Cell Signaling, Beverly, Mass), Alexa-Fluor488–conjugated secondary antibody (Invitrogen, Carlsbad, Calif), and DAPI (blue) at 60 min after treatment. Disruption of adherens junctions by thrombin is seen by the decrease in green signal intensity. Addition of rFXIIIa reduces HUVEC alterations.

Table 1

rFXIII improves tissue oxygenation and perfusion after gut I/R injury

Groups of animals	Muscle Po_2 (mmHg)	Muscle blood flow (conditional units)	Liver blood flow (conditional units)
SMAO + placebo	32.0 ± 7.2*	163.5 ± 82.1*	509.6 ± 138.8*
SMAO + FXIII	56.2 ± 4.1†	265.9 ± 54.2†	841.3 ± 137.4†
Sham + placebo	66.2 ± 7.3	483.9 ± 238.7	1,211.7 ± 272.4
Sham + FXIII	68.2 ± 13.2	464.8 ± 385.3	1,159.3 ± 250.7

*$P < 0.05$ vs. all other groups.

†$P < 0.05$ vs. shams.

Table 2

rFXIII reduces the severity of ileal mucosal damage after gut I/R injury

Groups of animals	Villi examined	Injured villi (%)	Injury score
SMAO + placebo	240 ± 13	56.7 ± 14.0*	2.88 ± 0.35†
SMAO + FXIII treatment	256 ± 19	58.3 ± 14.5*	2.25 ± 0.46†
Sham + placebo	257 ± 23	11.5 ± 4.7	1.0 ± 0
Sham + FXIII treatment	247 ± 18	9.8 ± 3.6	1.12 ± 0.35

*$P < 0.05$ vs. shams.

†$P < 0.05$ vs. all other groups.