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Olanders, Knut; Börjesson, Anna; Zhao, Xia; Andersson, Roland

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Effects of anticoagulant treatment on intestinal ischaemia and reperfusion injury in rats

K. OLANDERS[1], A. BÖRJESSON[2], X. ZHAO[2] and R. ANDERSSON[2]
Departments of [1]Anesthesiology and [2]Surgery, Lund University Hospital, Lund, Sweden

Background: In recent years it has become increasingly clear that a cross-talk between the inflammatory response and blood coagulation exists, although many of the underlying mechanisms remain unclear. In the present study we investigated the potential anti-inflammatory properties of two different anticoagulant compounds, i.e. active-site inactivated FVIIa (FVIIai) and fondaparinux sodium, a selective FXa inhibitor, administered as pretreatment in a model of intestinal I/R in rats.
Methods: Endothelial barrier permeability was assessed using the vascular leakage of radiolabelled human serum albumin, tissue neutrophil sequestration was quantitated by myeloperoxidase (MPO) activity, and plasma levels of macrophage inflammatory protein (MIP)-2 were examined using an enzyme-linked-immuno-sorbent assay after 40 min of intestinal ischaemia and 6 h of reperfusion in the rat (n = 34). Pretreatment with FVIIai or fondaparinux sodium was administered 90 min before initiation of ischaemia.
Results: Endothelial-barrier permeability in all examined organs, myeloperoxidase activity in the lungs, and ileum and MIP-2 levels in plasma increased after intestinal I/R. Pretreatment with FVIIai decreased the endothelial barrier permeability and MPO activity in the ileum, and a tendency towards decreased permeability was also observed in the lungs. Fondaparinux did not affect the endothelial barrier permeability or MPO activity. Both FVIIai and fondaparinux decreased the MIP-2 levels in plasma after intestinal I/R.
Conclusions: Inhibition of the TF-FVIIa complex by FVIIai can attenuate inflammatory responses in connection with intestinal I/R-injury and could represent a potentially important therapeutic strategy for the prevention of organ dysfunction. Potential anti-inflammatory properties of fondaparinux and other inhibitors of FXa are not excluded and need further investigation.

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Key words: Active-site-inactivated FVIIa (FVIIai, ASIS); cytokine; endothelium; fondaparinux; inflammation; intestine; leukocyte recruitment; tissue factor.

THE systemic inflammatory response syndrome (SIRS) has been recognized as a leading cause of morbidity and mortality during critical illness, represented by a combination of dysregulated inflammatory response, redistribution of microcirculatory blood flow and ischaemia-reperfusion injury. Intestinal ischaemia followed by reperfusion (I/R) is considered to be an important event in these conditions, frequently leading to organ dysfunction and multiple organ failure (MOF). The pathophysiological mechanisms behind the dysregulated inflammatory response are complex and involve not only activation of several inflammatory cell lines, but also local production of cytokines, chemokines and oxygen free radicals, mediating injury in both local and remote organs.

In recent years it has become increasingly clear that blood coagulation augments inflammation and that anticoagulants, particularly natural anticoagulants, can reduce the coagulation-induced increases in the inflammatory response (1). Tissue factor (TF) is the primary physiologic initiator of coagulation and is expressed on a variety of extravascular cells under normal conditions, and can also be expressed on blood monocytes and endothelial cells in inflammatory states (2). Tissue factor expression has been demonstrated to be greatly enhanced during I/R (3). Inflammation-induced TF expression activates coagulation by forming a TF-Factor VIIa (FVIIa) complex, initiating a downstream reaction eventually resulting in fibrin deposition, which in turn may potentiate inflammation. Results from previous studies have suggested that factors other than TF, FVIIa and fibrin, such as activated Factor X (FXa) and thrombin, are capable of altering vascular

Animals were handled in accordance with Swedish Physiological Society guidelines.

permeability, inflammatory cell migration and cytokine response (4, 5). In contrast to some of these findings it has been demonstrated that treatment with active-site-inactivated FXa (FXai) only inhibited the coagulopathic response and failed to block the inflammatory and lethal response to LD_{100} *E. coli* infusion in baboons (6). On the other hand, treatment with active-site-inactivated FVIIa (FVIIai) attenuated both the inflammatory as well as coagulopathic response, and it was hypothesized that the anti-inflammatory effect of FVIIai is elicited by inhibiting cytosolic Ca^{2+} flux by blocking the TF–VIIa interaction, which also may explain why FXai lacks anti-inflammatory properties, as it does not interfere with TF-FVIIa complex formation (7, 8).

Fondaparinux sodium is a synthetic pentasaccharide with antithrombotic activity through antithrombin III (AT III)-mediated selective inhibition of FXa. By selectively binding to AT III, fondaparinux sodium potentiates (about 300 times) the innate neutralization of FXa by AT III. This inhibition of FXa via antithrombin results in effective inhibition of thrombin generation (9), potentially leading to attenuation of the inflammatory response.

The objectives in the present study were to investigate the potential anti-inflammatory properties of active-site-inactivated FVIIa (FVIIai) and the selective FXa-inhibitor fondaparinux sodium in a murine intestinal ischaemia and reperfusion model, by evaluating endothelial permeability and tissue neutrophil recruitment, as well as plasma levels of macrophage inflammatory protein (MIP)-2, a potent neutrophil attractant belonging to the CXC-subfamily of chemokines, considered to be a functional murine homologue of human IL-8 (10, 11).

Methods

Animals

Adult male Sprague-Dawley rats weighing 250–300 g were fed standard rat chow (R_3, Astra-Ewos, Sweden) and water ad libitum. The rats were allowed to acclimatize to our laboratory conditions for 6 days and were subjected to a regime of 12-h day/night cycle living in mesh stainless-steel cages (three rats/cage) at a constant temperature (22°C). The protocol was approved by the Institutional Review Board for Animal Research at Lund University. All animals were handled in accordance with the guidelines set forth by the Swedish Physiological Society.

Induction of small intestinal ischaemia and reperfusion

The rats were operated under aseptic conditions using anaesthesia with a mixture of ketamine hydrochloride (Ketalar®, Pfizer AB, Täby, Sweden, 80 mg kg^{-1}) and xylazin hydrochloride (Rompun®, Bayer Corp, Monheim, Germany, 10 mg kg^{-1}) administered s.c. Intestinal ischaemia and reperfusion (I/R) was induced by separating and clamping the superior mesenteric artery with an atraumatic clamp for 40 min, followed by 6 h of reperfusion. The restoration of blood flow to the intestines after declamping of the artery was ensured by visual control of the intestinal colour change. Sham operation was performed by separating the superior mesenteric artery without clamping for 40 min, followed by 6 h of sham reperfusion. Animals in the control group were not subjected to laparotomy or any other challenge. The animals were divided into five groups of six to eight animals in each; group A: control (no laparotomy), group B: sham operation, group C: I/R without treatment, group, D: I/R and treatment with active site-inactivated FVIIa (Novo Nordisk, Copenhagen, Denmark) 10 mg kg^{-1} i.p., group E: I/R and treatment with fondaparinux sodium (Sanofi-Synthelabo, Paris, France) 200 μg kg^{-1} s.c. Treatment to groups D and E was administered 90 min before the start of ischaemia. The dose and route of administration of FVIIai were selected on the basis of dose–response data provided by Novo Nordisk from previous studies on rats. The dose of fondaparinux sodium was selected partly based on human safety studies and partly on previous studies on rats from our group. During the period of ischaemia (40 min), the animals were anaesthetised and were then allowed to wake up during reperfusion. Ninety minutes before the end of the reperfusion period the animals were anaesthetised again and a catheter (Medical-Grade Tubing; 0.51 mm, OD 0.94 mm, Dow Corning Co., Midland, MI) was put into the femoral vein. Sixty minutes before the end of reperfusion, blood samples for measurements of haemostasis (prothrombin complex, activated partial thromboplastin time, fibrinogen, platelets) were taken and 1 ml of ^{125}I-labelled human serum albumin (HSA, Isopharma AS., Kjeller, Norway) was injected. After 1 h of equibrilation, 1 ml of blood was drawn from the femoral vein, followed by injection of ^{51}Cr-labelled red blood cells (RBCs). The abdomen was then reopened and blood was withdrawn from the aorta and centrifuged for plasma analyses. A tracheostomy was performed and the animals were ventilated with room air using a ventilator (Rodent Ventilator UB 7025, Hugo Sachs Electronic, Harvard Apparatus, March –

Hustetten, Germany) with a tidal volume of 1 ml and 60 breaths min^{-1}. The animals were then rapidly exsanguinated by transection of the distal part of the abdominal aorta and caval vein. The thorax and left atrium were opened and the organs were perfused with phosphate buffer saline (PBS) + Heparin through a catheter in the right atrium. The lungs (lower right lobe), ileum (5 cm of the distal part), colon (3 cm of the proximal part) and liver (lateral lobe) were harvested and cleared of external blood by blotting dry, and then placed in tubes for gamma-counter measurements. The left lung and the remaining distal parts of the ileum were harvested, frozen immediately in liquid nitrogen and stored at −70°C for later measurement of myeloperoxidase (MPO).

Evaluation of endothelial permeability

Red blood cells were labelled with ^{51}Cr (Amersham Health, Buckinghamshire, UK) during 20 min of incubation at room temperature and then washed three times with physiological saline. The radioactivity was about 1.5×10^6 cpm ml^{-1}. Endothelial permeability was assessed by the passage of ^{125}I-labelled human serum albumin (Isopharma, Kjeller, Norway) from blood to the tissues. Five hours after the sham operation or the end of intestinal ischaemia, 1 ml of ^{125}I-labelled human serum albumin (^{125}I-HSA), with about 2.5×10^6 cpm, was injected through the femoral vein catheter. After 1 h of equibrilation, 1 ml of blood was drawn from the femoral vein, followed by injection of ^{51}Cr-labelled RBCs. The animals were then rapidly exsanguinated by transection of the distal part of the abdominal aorta and caval vein 2 min after the RBC injection. Organs were harvested and cleared of external blood by blotting dry. The intestinal samples were also cleared from its intraluminal contents. The radioactivity of ^{51}Cr and ^{125}I in blood and tissue samples were measured in a gamma-counter (1272 Clinigamma, LKB, Wallac OY, Finland).

Endothelial permeability was assessed by leakage of radiolabelled albumin from blood into the interstitial space and expressed as isotopic flux, defined as the proportion of ^{125}I-radioactivity per gram tissue compared with per gram blood as described previously (12). To assay possible redistribution of tissue blood, tissue blood content (TBC) was calculated by the proportion of ^{51}Cr counts per gram tissue and ^{51}Cr counts per gram blood. In order to correct for potential differences in the vascular surface area available for exchange of albumin, the albumin leakage index was calculated by dividing the isotopic flux in each tissue by assuming that all ^{51}Cr-labelled RBCs remained intravascularly, using the formula:

albumin leakage index (ALI) = ^{125}I counts per gram tissue/^{125}I counts per gram blood)/TBC

Measurement of leukocyte recruitment

After that the animals were exsanguinated and perfused, the left lung and the distal part of the ileum were harvested, frozen immediately in liquid nitrogen and stored at −70°C until measurements. For MPO measurement we used a modification of the method described by Komatzu et al. (13). All chemicals were purchased from Sigma Chemical Co, St. Louis, MO. The samples were weighed (130–170 mg), put in 1 ml of ice-cold potassium phosphate buffer (20 mM, pH 7.4), homogenized (Homogenizer Ommi 1000, Lambda Polynom, Sollentuna, Sweden) for 15 s on ice and then centrifuged at 20,000 r.p.m. for 15 min at 4°C (Sorvall RC-5B, Refrigerated superspeed centrifuge, Lambda Polynom). The supernatant was discharged to avoid the haemoglobin influence. The precipitates were rehomogenized for 15 s in 0.5 ml 50 mM PBS, pH 6.0, with 0.5% HTAB (hexadecyltrimetylammoniumbromide) and 10 mM EDTA, followed by sonication in an ultrasonic bath (ULTRA sonik 28X, Neytech, Göteborgs Termometerfabrik AB, Gothenburg, Sweden) for 20 s and freezing (−70°C) and thawing in order to permeate cellular membranes. Sonication, freezing and thawing were repeated once. The samples were then centrifuged at 20,000 r.p.m. for 15 min at 4°C. Of the final supernatant, 0.1 ml was put to 1.0 ml 0.5% HTAB and 0.3 mM H_2O_2. After incubation for 3 min at 37°C the reaction was terminated by adding 4 ml of 0.2 mM sodium acetate, pH 3.0, with the samples kept on ice. The tissue leukocyte recruitment was reflected by the units of myeloperoxidase activity in the samples, defined as the change in absorbence at 660 nm in 1 min per gram tissue (A660 min^{-1} g^{-1}) (Hitachi U 2000, KEBO Laboratory, Lund, Sweden).

Measurement of MIP-2 in plasma

Plasma for the MIP-2 assay was obtained by centrifuging blood for 15 min at 3500 r.p.m. at 4°C, then keeping it at −70°C until the assay was performed. Plasma levels of MIP-2 were determined using a sandwich enzyme-linked-immunosorbent assay (ELISA) specific for rat MIP-2 (DRG Instruments GmbH, Marburg, Germany). All samples were tested in duplicate. The plate was read in a microplate reader (Multiskan Plus, Thermo Labsystems OY, Helsinki, Finland) at

450 nm, and the MIP-2 concentration ($pg\,ml^{-1}$) in experimental samples was calculated from a standard curve.

Statistics

The data were analyzed using one-way analysis of variance by ranks (Kruskal–Wallis test), followed by the Wilcoxon rank sum test (Mann–Whitney *U*-test). Values are presented as means ±SD. Statistical significance was set at $P < 0.05$.

Results

Endothelial permeability

Albumin leakage index (ALI) increased significantly in the lungs and the ileum ($P < 0.05$) following 40 min of intestinal ischaemia and 6 h of reperfusion as compared to both sham-operated animals and controls (Figs 1 and 2). In the colon, ALI also increased significantly after intestinal I/R as compared to controls ($P < 0.01$), but did not reach statistical significance as compared to sham-operated animals ($P = 0.055$; Fig. 1). Increased endothelial albumin leakage was also noted in the liver after intestinal I/R as compared to controls ($P < 0.01$), but not as compared to sham-operated animals (Fig. 2).

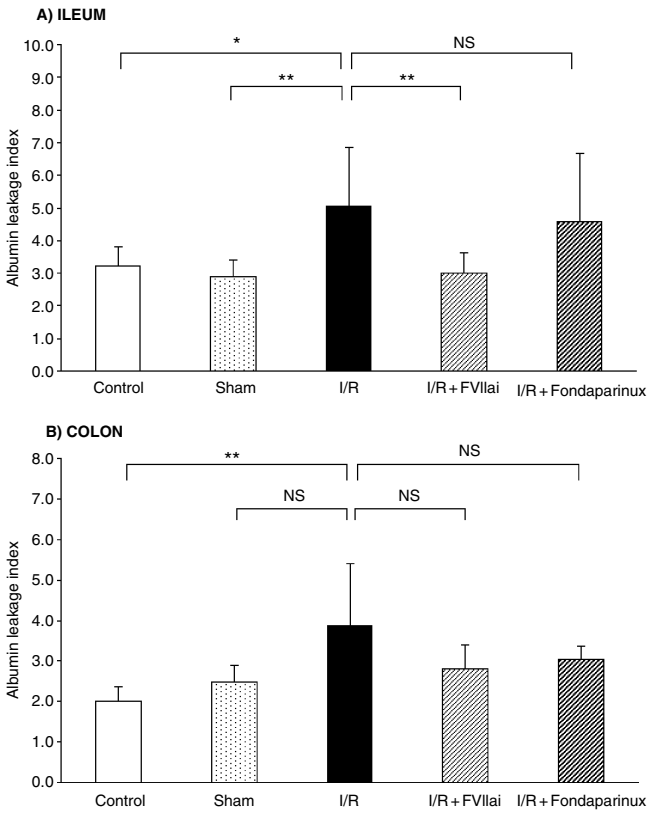

*Fig. 1. Albumin leakage index (ALI) in the ileum (A) and colon (B) in controls, sham-operated animals and in animals subjected to small intestinal ischaemia for 40 min and reperfusion for 6 h. Pretreatment with FVIIai or Fondaparinux was administered to two groups 90 min before start of ischaemia. *P<0.05 and **P<0.01 vs. I/R.*

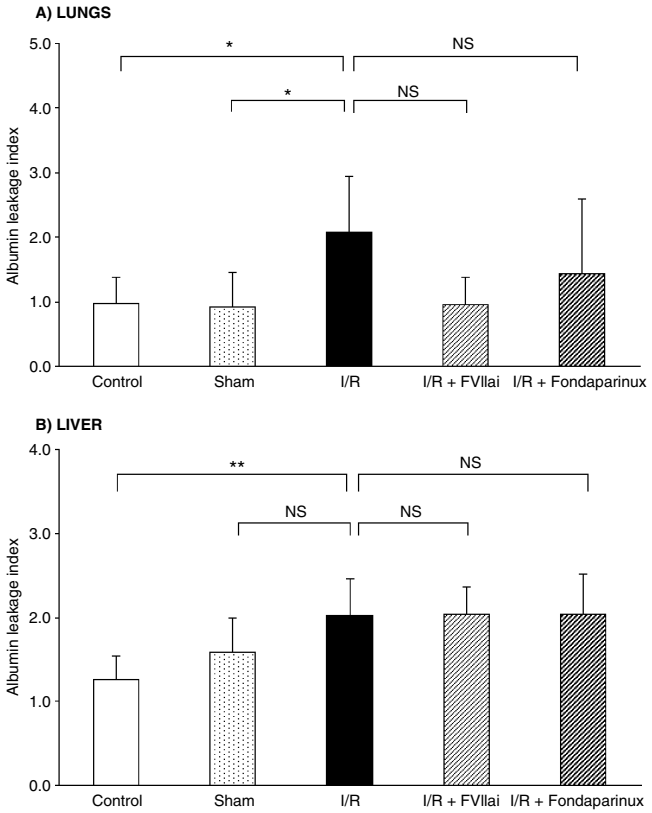

*Fig. 2. Albumin leakage index (ALI) in the lungs (A) and liver (B) in controls, sham-operated animals and in animals subjected to small intestinal ischaemia for 40 min and reperfusion for 6 h. Pretreatment with FVIIai or Fondaparinux was administered to two groups 90 min before start of ischaemia. *P<0.05 and **P<0.01 vs. I/R.*

Pretreatment with FVIIai decreased the albumin leakage index significantly in the ileum after intestinal I/R as compared to I/R-challenged animals without treatment ($P < 0.05$; Fig. 1). In the lungs, although not statistical significant ($P = 0.058$), pretreatment with FVIIai resulted in a tendency towards decreased leakage (Fig. 2). In the colon and liver, pretreatment with FVIIai did not affect the albumin leakage index after 40 min of intestinal ischaemia and 6 h of reperfusion (Figs 1,2). Pretreatment with fondaparinux did not affect the albumin leakage index significantly after intestinal I/R in any of the organs examined.

Tissue myeloperoxidase activity

Tissue leukocyte recruitment, measured as myeloperoxidase (MPO) activity (Fig. 3), significantly increased

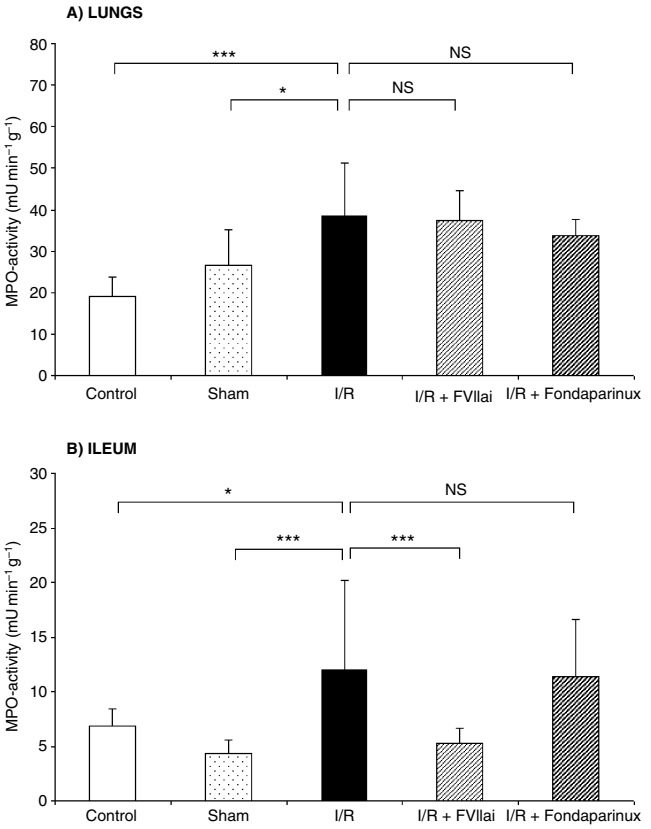

*Fig. 3. Tissue myeloperoxidase (MPO) content in the lungs (A) and the ileum (B), in controls, sham-operated animals and in animals subjected to small intestinal ischaemia for 40 min and reperfusion for 6 h. Pretreatment with FVIIai or Fondaparinux was administered to two groups 90 min before start of ischaemia. *P<0.05 and ***P<0.001 vs. I/R.*

in the lungs after intestinal I/R as compared to controls ($P < 0.001$) and sham-operated animals ($P < 0.05$). In the ileum, the MPO activity also increased after intestinal I/R as compared to controls ($P < 0.05$) and sham ($P < 0.001$).

Pretreatment with FVIIai significantly decreased the MPO activity in the ileum after intestinal I/R as compared to I/R-challenged animals without treatment ($P < 0.001$). In the lungs, pretreatment with FVIIai did not affect the MPO activity after intestinal I/R. Pretreatment with fondaparinux did not influence the MPO activity in any of the organs examined after 40 min of intestinal ischaemia followed by 6 h of reperfusion.

MIP-2 plasma levels

The plasma levels of MIP-2 (Fig. 4) increased significantly after intestinal I/R as compared to both controls ($P < 0.001$) and sham-operated animals ($P < 0.01$). Pretreatment with FVIIai, as well as with fondaparinux, reduced the I/R-induced increase in

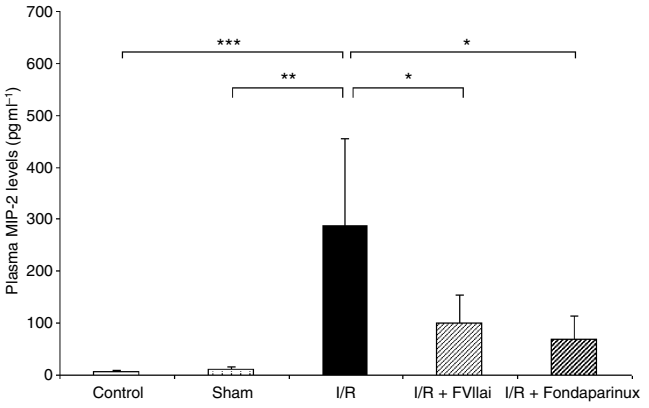

*Fig. 4. Plasma MIP-2 levels in controls, sham-operated animals and in animals subjected to small intestinal ischaemia for 40 min and reperfusion for 6 h. Pretreatment with FVIIai or Fondaparinux was administered to two groups 90 min before start of ischaemia. **P<0.01 and ***P<0.001 vs. I/R.*

MIP-2 plasma levels significantly ($P < 0.05$) as compared to animals challenged with intestinal I/R without pretreatment.

Haemostasis

The prothrombin complex, expressed as international normalized ratio (INR), increased significantly ($P < 0.001$), but the activated partial thromboplastin time (APT-t) was unaffected after treatment with FVIIai, whereas fondaparinux did not influence any of these parameters, as compared to I/R-challenged animals (Table 1). The plasma levels of fibrinogen and blood levels of platelets did not differ significantly in any of the groups.

Discussion

The cross-talk between blood coagulation and the inflammatory response has gained increasing interest in recent years and it is now recognized that initiation of coagulation is an integral and consistent element of the response to inflammatory stimuli. In the present study, we focused on the potential anti-inflammatory properties of two different anticoagulant compounds, i.e. active-site inactivated FVIIa (FVIIai) and fondaparinux sodium, a selective FXa inhibitor, administered as pretreatment in a model of intestinal ischaemia and reperfusion in rats.

Intestinal I/R is considered to be an important initiating event in several pathophysiological conditions, e.g. shock, trauma, sepsis and pancreatitis, frequently leading to concomitant organ dysfunction and ultimately multiple organ failure (MOF).

Table 1

Parameters of haemostasis.

	I/R	Control	Sham	FVIIai	Fondaparinux
Prothrombin complex (INR)	1.5 ± 0.08	1.4 ± 0.06	1.5 ± 0.20	5.4 ± 1.76***	1.6 ± 0.16
APT-t (s)	42 ± 20.6	21 ± 2.5**	22 ± 3.5**	32 ± 11.4	38 ± 14.2
Fibrinogen ($g\,l^{-1}$)	3.0 ± 0.35	3.2 ± 0.21	2.9 ± 0.17	2.9 ± 0.50	2.9 ± 0.58
Platelets ($\times 10^9\,l^{-1}$)	716 ± 145	698 ± 60	815 ± 173	659 ± 212	807 ± 292

**$P < 0.01$,
***$P < 0.001$ *vs.* I/R.

Intestinal I/R-injury induced by superior mesenteric artery (SMA) occlusion represent an isolated gut injury model which not only result in intestinal tissue injury but also in secondary distant organ dysfunction. We have in previous studies demonstrated that intestinal I/R result in tissue injury and multiple organ dysfunction with increased permeability over the vascular endothelium, tissue leukocyte recruitment, consumption of protease inhibitors and increased levels of proinflammatory cytokines such as IL-1β and IL-6 (14–16). In the present study, we demonstrated that 40 min of intestinal ischaemia followed by 6 h of reperfusion resulted in an increased endothelial albumin leakage in the lungs and ileum, whereas the endothelial permeability in the colon and liver was less affected. This is in line with our previous studies and implies that the lungs and small intestines are the organs most vulnerable to tissue injury after intestinal I/R. In parallel with the endothelial permeability dysfunction, an increased neutrophil recruitment was observed in the lungs and in the ileum. Moreover, the plasma levels of MIP-2, which is considered to be a functional murine homologue of human IL-8 (10), also increased after intestinal I/R. MIP-2 is a potent neutrophil attractant and activator belonging to the CXC-subfamily of chemokines and is produced by a variety of cell types, including intestinal epithelium, macrophages, astrocytes and fibroblasts (11). Increased MIP-2 expression has been found to be associated with neutrophil influx in various inflammatory conditions and experimental models, including I/R-induced injury in the liver (17–19), kidney (20) and heart (21). It has been suggested that MIP-2 activates neutrophils by inducing CD11b/CD18 on the cell surface (22) and that the expression of MIP-2 in turn is regulated by oxygen-free radicals (OFRs) during I/R (21).

Treatment with active-site-inactivated FVIIa (FVIIai) 90 min before initiation of the ischaemic insult resulted in a significant reduction of the albumin leakage over the endothelial barrier in the ileum. A similar response was observed in the lungs and in the colon, though not reaching statistical significance in this study, with a rather small number of animals in each group. The MPO activity in the perfused ileum, reflecting both extravasated and remaining intravascular neutrophils, decreased after treatment with FVIIai, suggesting that inhibition of the TF-FVIIa complex have pivotal effects on the adhesion and migration of neutrophils. In parallel, plasma levels of MIP-2 decreased, indicating that one possible mechanism for the decreased tissue neutrophil recruitment may be due to the reduced influence of this neutrophil chemoattractant. In opposition to some other reports (23, 24), we did not note a corresponding effect in the lungs, where neutrophil recruitment was unaffected by FVIIai. However, data exist indicating that neutrophil adhesion mechanisms may have organ-specific as well as stimuli-specific differences and thus could inhibition of TF result in various effects on neutrophils in different tissues and after different inflammatory stimuli. It has, for example, been suggested that the CD11/CD18 complex, mediating neutrophil adherence in the systemic circulation, may occur by either CD18-dependent or CD18-independent mechanisms specific to the inciting stimulus in the pulmonary circulation (25). Furthermore, neutrophil adhesion per se may not completely explain neutrophil retention in pulmonary capillaries and increased cytoplasmic stiffness, induced by chemoattractants, could represent an important factor for the adhesive interactions to take place (26, 27). Nevertheless, this is the first study to our knowledge that demonstrates a specific reduction of organ injury after inhibiting the initiation of coagulation by TF after intestinal I/R and thereby supporting the current hypothesis that TF blockade could represent a potentially important therapeutic strategy for the prevention of organ dysfunction in hyperinflammatory states. FVIIai competitively inhibits binding of FVIIa to TF and suppresses both TF-VIIa signalling and coagulant activities (24). In the present study an evident anticoagulant effect was observed after treatment with FVIIai by a significant increase in the prothrombin complex ratio, whereas the APT-t, which does not depend on FVII activity, was unaffected.

Previous studies by other groups have demonstrated protective effects of TF blockade in sepsis-induced end-organ dysfunction in conjunction with an attenuated inflammatory response (7, 23, 24). An important issue to be answered is whether a later inserted therapy with TF blockade, i.e. when organ dysfunction is established and therapy thereby more clinically relevant, result in the same protective effects. The mechanisms by which inhibition of the TF–FVIIa interactions lead to anti-inflammatory effects are not fully clear, but may include inhibition of cytosolic Ca^{2+} flux (7, 8) and possibly inhibition of other intracellular signalling pathways, including phosphorylation of mitogen-activated protein kinases (MAPK) (28).

Fondaparinux did not influence endothelial permeability or neutrophil recruitment in this model of intestinal I/R, although plasma MIP-2 levels decreased. The explanation for this is unclear, but suggests that inhibition of the coagulation downstream of the TF-FVIIa complex formation just attenuate the inflammatory response to a certain degree, which may not be enough to protect from neutrophil recruitment and tissue injury in this I/R model. Moreover, neutrophil adhesion and migration mechanisms are complex and may not fully depend on MIP-2 effects, e.g. as in the lungs, as previously discussed. Other factors that have been implicated as mediators in I/R-induced neutrophil recruitment and tissue injury, such as OFRs, platelet activating factor (PAF) and leukotriens (29), may not be influenced unless TF-FVII complex formation is inhibited. The synthetic pentasaccharide fondaparinux is the first of a new class of antithrombotic agents that selectively inhibit FXa via binding to antithrombin III (AT III), which in turn inhibits thrombin generation, without any direct effect on thrombin activity (30–33). Several clinical studies have demonstrated a reduced risk of venous thromboembolism with fondaparinux after major orthopaedic surgery, without an increased bleeding rate. It has been suggested that FXa may function as a mediator of acute inflammation (34), and it has furthermore been implied that the active site of FXa is involved in FXa-stimulated cytokine production (4). In line with the present study, FXa was found to stimulate C-X-C chemokine CINC production by macrophages after hepatic I/R-injury and that selective inhibition of FXa inhibits this production (35). Mechanism could involve an inhibition of FXa-induced TF expression or blocking of thrombin generation (36). Although enzymatic activity of FXa seems to be needed for cytokine production (4), it has been demonstrated that administration of FXa blocked in the active centre (FXai) only inhibit the coagulopathic pathway induced by infusion of *E. coli*, but fail to block the lethal effects of the bacterial infusion (6). Also active site-independent FXa-mediated inflammation have been demonstrated (34) and the exact biological mechanisms of factor Xa-induced proinflammatory responses remain unclear and need to be further investigated. The lack of a protective effect by fondaparinux in the present study on tissue injury and tissue neutrophil recruitment, although plasma levels of MIP-2 decreased, might be due to an insufficient dose administered to the animals. Although it is reported that fondaparinux does not affect coagulation parameters such as APT-t and prothrombin complex (INR) in humans using a dose of 2.5 mg once-daily for profylaxis of venous thromboembolism (33), sufficient inhibition of FXa should result in increases in both these parameters. Limited dose–response data exist on fondaparinux used in rats when focusing on potential anti-inflammatory effects and it is not known whether an anticoagulatory effect visible on APT-t and prothrombin complex (INR) is necessary to reach an effect on the inflammatory response. We do not exclude that an anti-inflammatory effect of fondaparinux could exist and further dose–response studies are needed to elucidate this.

In conclusion, the present study provides novel information by demonstrating that inhibition of the TF-FVIIa complex formation by FVIIai can attenuate inflammatory responses, such as endothelial barrier dysfunction, tissue neutrophil recruitment and chemokine production in connection with intestinal I/R-injury. Selective inhibition of FXa did not result in a corresponding effect, although decreased plasma levels of MIP-2 were observed. Anticoagulant treatment in hyperinflammatory states could represent a potentially important therapeutic strategy for the prevention of organ dysfunction, and further studies are needed to clarify the effects and mechanisms of the cross-talk between coagulation and inflammation.

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Address:
Roland Andersson
Department of Surgery
Lund University Hospital
S-221 85 LUND
Sweden
e-mail: roland.andersson@kir.lu.se