Clinical and experimental studies on treatment of acute mesenteric ischemia

Schoots, I.G.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Enhancement of endogenous fibrinolysis does not reduce local fibrin deposition but modulates inflammation upon intestinal ischemia and reperfusion

Ivo G. Schoots¹,², Marcel Levi², Arlène K van Vliet¹, Adrie M. Maas¹, Paul J. Declerck³, and Thomas M. van Gulik¹.

Departments of ¹Surgery and Surgical Laboratory, ²Vascular and Internal Medicine, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands, and ³Laboratory for Pharmaceutical Biology and Phytopharmacology, Faculty of Pharmaceutical Sciences, University of Leuven, Belgium.

Microcirculatory failure – a limited role for suppressed endogenous fibrinolysis

Abstract

Background: Intestinal ischemia/reperfusion (I/R) causes local inhibition of endogenous fibrinolysis in combination with activation of coagulation. This may lead to thrombotic obstructions that compromise microcirculation and promote intestinal injury. Inhibited fibrinolysis following intestinal I/R is related to increased plasminogen activator inhibitor (PAI)-1. This study examined the effect of enhanced fibrinolysis in a rat model of intestinal I/R that results in local activation of coagulation, suppression of endogenous fibrinolysis and mucosal fibrin deposition.

Methods & Results: Fibrinolysis was enhanced by intravenous administration of recombinant tissue plasminogen activator (rt-PA) or by inhibition of PAI-1 by administration of MA-33H1F7. Although anti-PAI-1 antibody or rt-PA administration enhanced circulatory fibrinolytic activity, as evidenced by increased portal plasma plasminogen activator activity, elevation of fibrin degradation products and decreased levels of PAI-1, mucosal fibrin deposition and microthrombosis were not reduced in postischemic intestinal tissue. Furthermore, enhanced fibrinolysis did not attenuate I/R-induced intestinal injury or dysfunction, as demonstrated by morphological and functional analysis. However, both interventions resulted in decreased levels of interleukin-6, which indicates fibrin-induced modulation of inflammation.

Conclusions: Enhancement of fibrinolytic activity neither increased removal of mucosal fibrin deposition nor attenuated intestinal I/R injury. These results suggest a limited role of suppressed endogenous fibrinolysis in microcirculatory failure and consequent deterioration of intestinal function and structure following intestinal I/R.
Introduction

Patients subjected to circulatory shock, hypoxia and sepsis may sustain ischemia-reperfusion (I/R)-induced intestinal injury, due to decreased blood flow and oxygen delivery in combination with increased metabolic demand in sepsis. This results in (often clinically unrecognized) mucosal dysfunction and in local inflammatory response. In this regard, the gut plays a pivotal role in the development and maintenance of the systemic inflammatory response syndrome and has been speculated to be "one of the motors of multiple organ injury".

An important factor in the development of organ failure is the abundance of intravascular microthrombi associated with local or systemic intravascular coagulation. This widespread occurrence of microthrombi has also been demonstrated in ischemic intestinal tissue. Recently, we demonstrated fibrin deposits and microthrombotic obstructions in the intestinal microcirculation following experimental intestinal I/R, as a result of ongoing activation of coagulation and suppression of endogenous fibrinolysis. The endogenous fibrinolytic activity, mediated by endothelial release of tissue-type plasminogen activator (t-PA), was inhibited by the concomitant endothelial or platelet release of plasminogen activator inhibitor (PAI)-1, the physiological, fast-acting inhibitor of t-PA. This hypofibrinolytic state can hypothetically contribute to thrombotic obstruction and may compromise adequate microcirculation, thereby promoting intestinal injury. This led to the hypothesis that "recanalization" of the thrombotic microvasculature by fibrinolysis may attenuate the sequelae of intestinal post-ischemic, reperfusion injury.

Promotion of microvascular fibrinolysis can be achieved by the administration of plasminogen activating drugs, such as streptokinase, recombinant t-PA, or recombinant single-chain urokinase, which all result in plasmin production and, subsequent, enhanced fibrinolytic activation. Such thrombolytic treatments have been demonstrated to reduce mortality in patients with acute myocardial infarction and represent a promising treatment strategy in acute mesenteric thromboembolic occlusion. Furthermore, administration of t-PA has been shown to reduce endotoxin-induced fibrin deposition and concomitant mortality in rabbits.

It should be noted, however, that the success of thrombolytic strategies has been restrained by the frequent occurrence of thrombotic reocclusion of initially reperfused vessels, presumably due to PAI-1-induced inhibition of endogenous fibrinolysis. PAI-1, as a serine protease inhibitor, is present in α-granules in platelets and in endothelial cells and is expressed by monocytes. In previous studies, we have demonstrated that inhibition of PAI-1 activity promotes endogenous fibrinolysis, inhibits thrombus extension and prevents fibrin deposition in experimental models of thrombosis and disseminated intravascular coagulation.

The aim of the present study was to investigate the contribution of endogenous suppression of fibrinolysis and increased fibrin deposition to intestinal dysfunction and injury in a rat model of intestinal I/R. Enhancement of fibrinolytic activity in the mesenteric circulation was induced by administration of recombinant t-PA. The role of PAI-1 in preventing t-PA-mediated fibrinolysis prompted us to investigate also the effect of inhibition of PAI-1 activity, using an anti-rat PAI-1 monoclonal antibody (MA-33H1F7).
Material and Methods

Biological and chemical agents
Recombinant tissue plasminogen activator (rt-PA) concentrate (Alteplase) was purchased from Genentech Inc. (San Francisco, CA) and anti-PAI-1 monoclonal antibody (MA)-33H1F7 was produced as described \(^2^7\). Goat anti-rat fibrin polyclonal antibody \(^2^8\) was kindly provided by Dr. J.J. Emeis (TNO Prevention and Health, Leiden, The Netherlands). All other reagents were of analytical grade.

Animal model of intestinal I/R
Adult male wistar rats (Charles Rivers, Broekman Instituut BV, Someren, The Netherlands), weighing 300-325 g, were fed standard rat chow (Hope Farms, Woerden, The Netherlands) and water ad libitum. The rats were allowed to acclimatize to our laboratory conditions for at least 4 days and were subjected to a regimen of 12:12 h/day-night cycle in mesh stainless-steel cages at constant temperature (22°C). The protocol was approved by the Animal Ethics Committee of the University of Amsterdam (The Netherlands). All animals were handled in accordance with the guidelines prescribed by the Dutch legislation and the International Guidelines on protection, care and handling of laboratory animals. The last 12 hours prior to the experiments, the animals had no access to solid food, but had free access to water.

Under anaesthesia with 1-2% isoflurane, rats were intubated and ventilated with air, \(O_2\) and 1-2% isoflurane. \(CO_2\) levels were kept between 37 and 43 mmHg. Continuous Ringer’s lactate solution was infused via the tail vein (10mL/hr.kg body weight) to correct for possible fluid loss during the experiment. A canula was inserted into the left carotid artery to measure the mean arterial pressure, which was kept between 90 and 110 mmHg during the experiment. Body temperature was maintained at 37 °C by use of a heating pad and lamp.

A 5 cm long midline laparotomy was performed and an intestinal loop of approximately 15 cm (10 cm proximal from the cecum) was isolated and canulated with soft silicon tubes. This loop was gently rinsed with saline prior to connection to a perfusion pump, a heat exchanger and a reservoir to obtain a closed circuit. The reservoir contained freshly made Ringer’s solution consisting of (in mmol/L): 117.5 NaCl; 5.7 KCl; 25.0 NaHCO\(_3\); 1.2 mM MgSO\(_4\); 1.2 NaH\(_2\)PO\(_4\); 2.5 CaCl\(_2\) (Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands). Glucose (25 mM) was added to this solution, in order to evaluate the absorptive function of the intestinal epithelium.

The superior mesenteric artery (SMA) was identified after deflecting the intestinal loops to the right side of the abdomen. The SMA was temporarily occluded by an atraumatic clamp at the origin of the aorta, avoiding the accompanying lymphatic trunk. Immediate blanching of the small intestine and cecum confirmed that the blood supply to the intestinal segments had been shut off. The abdomen was then covered with a sterile moist gauze pad. After the period of ischemia, the clamp was removed from the SMA and restoration of blood flow to the gut was confirmed by returning to its original colour. The rats in the sham-operation group underwent the same procedure except that clamping of the SMA was omitted. During reperfusion, luminal perfusion (1.0 mL/min) of the isolated intestinal loop was performed with the Ringer-glucose solution. Samples from the perfusate (0.25 mL each) were obtained after 1, 2 and 3 hours of reperfusion and stored at -20 °C until further analysis.

Portal blood samples (1.25 mL each) were collected after 5 minutes, and after 1 and 3 hours of reperfusion. Three times of portal blood sampling during the experiment was chosen to limit blood extraction (<15% of total blood volume). An equivalent volume of a warmed volume expander of 6% polyhydroxyethyl-starch and 0.9% NaCl (eloHaes, Fresenius Kabi, The Netherlands) was injected through the tail vein to maintain blood volume. Blood samples were collected in sodium-citrate buffer (final citrate concentration 0.32%) and in EDTA tubes and were centrifuged at 2,000g at 4 °C for 20 minutes and the plasma samples were stored at -80 °C until further analysis.
Rats were sacrificed by bleeding after final blood sampling and subsequent administration of heparin (2000 IU/kg) to prevent intravascular clotting. Biopsies of the small intestine were collected 5 cm proximal from the isolated rat intestinal loop and were fixed in 10% formaldehyde for histological examination.

To exclude different influences of anaesthesia upon the outcome parameters, all rats were anaesthetized for approximately 4.5 hours, which includes the duration of the surgical procedure and the period of ischemia and reperfusion.

**Experimental design of intestinal I/R**

In total, 54 rats were randomly allocated to one control group (saline) and two experimental groups (rt-PA and anti-PAI-1 monoclonal antibody, respectively), consisting of 18 rats each. rt-PA (1.0 mg/kg of body weight), anti-PAI-1 MA-33H1F7 (1.5 mg/kg) or 0.9% saline was administered intravenously into the penal vein during ischemia, 15 minutes before reperfusion. In each group, intestinal ischemia and reperfusion was induced by isolating and clamping the SMA with an atrumatic clamp for 0 minutes (sham operation, n = 6), 20 minutes (n = 6) or 40 minutes (n = 6), followed by 3 hours of reperfusion.

**Assessment of coagulation and fibrinolysis**

Plasma samples in sodium-citrate buffer, stored at -80 °C were utilized. Thrombin generation was assessed by measuring the thrombin-antithrombin (TAT) complexes with an enzyme-linked immunosorbent assay (ELISA) kit (Behring, Marburg, Germany). ATIII was measured by an automated amidolytic technique according to methods previously described. Fibrin degradation products (D-dimers) were assessed by an ELISA (Asserachrom D-Di, Diagnostica Stago, Asnieres-sur-Seine, France). Plasminogen activator activity (PAA) was measured by an automated amidolytic assay. Briefly, 25 µL of plasma was mixed with 0.1 M TrisHCl, pH 7.5, 0.1% (v/v) Tween-80, 0.3 mM S-2251 (Chromogenix, Mölndal, Sweden), 0.13 M plasminogen and 0.12 mg/mL cyanogen bromide-digested fibrinogen fragments of fibrinogen to a final volume of 250µL. The amount of plasmin formed under these conditions is proportional to the concentration of PAA present, and can be spectrophotometrically detected by conversion of the chromogenic substrate. Plasminogen activator inhibitor (PAI)-1 activity was measured with the amidolytic method. Briefly, plasma was incubated with a fixed excess of t-PA (40 IU/mL) for 10 min at room temperature. The residual t-PA activity was determined by incubation with 0.13 µM plasminogen (Chromogenix, Sweden), 0.12 mg/mL cyanogen bromide-digested fibrinogen fragments and 0.1 mM S-2251 (Chromogenix, Sweden). Under these circumstances, the plasmin generated is inversely proportional to the amount of PAI-1 present.

**Immunohistochemical assessment of fibrin deposition**

Fibrin deposition was detected on formaldehyde-fixed tissue sections using immunohistochemistry according to standard procedures. Briefly, paraffin sections of jejunal tissue (4 µm) were deparaffinized and rehydrated through a graded series of xylene-ethanol. Endogenous peroxidase activity was blocked by treatment with 1.5% (v/v) H₂O₂ in phosphate buffered saline (PBS) for 30 minutes. Non-specific protein binding was blocked using Blocking Agent. All sections were incubated overnight at 4°C using polyclonal goat anti-rat fibrin antibody (TNO Prevention and Health, Leiden, The Netherlands) diluted in PBS (1:1600). Immunoreaction was detected using the VECTASTAIN ABC Elite Kit (Vector Laboratories, Burlingame, England) and staining was developed using 0.5 mg/mL 3,3'-diaminobenzidine (DAB) (Sigma, Zwijndrecht, The Netherlands), 0.02% (v/v) H₂O₂ in 30 mM imidazole and 1mM EDTA (pH 7.0). The time of development was equal for all the tissues. Finally, sections were counterstained with hematoxylin, dehydrated and mounted. As negative controls, parallel sections consisted of the omission of the primary antibody and yielded no immunohistochemical reaction. Evaluation of fibrin deposition was performed by microscopical examination.
Role of fibrinolysis in intestinal ischemia/reperfusion injury

Measurement of cytokines
In EDTA plasma samples, stored at -80 °C, levels of tumor necrosis factor-α, cytokine induced neutrophil chemoattractant, interleukin-1β and interleukin-6 were determined with the use of rat-ELISA kits (RnD Systems, Minneapolis, MN).

Histological assessment of intestinal injury
The in formaldehyde fixed jejunal tissues were embedded in paraffin, sectioned, and stained with haematoxylin and eosin for histological grading. The histological grading classification of Park-Chiu was used by two independent, non-informed examiners to assess intestinal injury. Briefly, the scores used were 0: normal mucosa, 1: subepithelial space at villus tips, 2: extension of subepithelial space with moderate lifting, 3: massive lifting down sides the villi, some denuded villi, 4: denuded villi, dilated capillaries, 5: disintegration of the lamina propria, 6: crypt layer injury, 7: transmucosal infarction and 8: transmural infarction.

Assessment of intestinal transport
Intestinal water transport (Clwater) was assumed to be reflected by the clearance of water from the total volume of the perfusion solution in the closed circuit (including the reservoir, connecting tubes and isolated intestinal loop) and was used to estimate net intestinal absorption and secretion. Glucose transport (Clglucose) was determined to measure the active-transport capacity of the epithelium. The glucose concentration (CGlucose) was determined by a glucose-detection kit (Sigma Diagnostics, St. Louis, MO, USA). The transport rate of water and glucose was determined as the clearance from the luminal perfusate per minute per gram intestine and calculated from the formula: Clearance (in µL/g.min) = (Ci*Vi-Cf*Vf) / (0.5*(Ci+Cf)*T*W) in which C is the detectable glucose concentration of the initial solution (i) and final solution (f), V the volume of the same solutions, T the time in minutes (min), and W the weight of the intestinal loop in grams (g).

Statistical analysis
The data analysis was performed using Graphpad Prism version 3.0 (Graphpad Software, Inc) for Windows 95. All quantitative data were presented as mean values ± standard error of the mean (SEM). Statistical analysis for repeated measurements was performed by analysis of variance and subsequent Bonferroni’s post-hoc test. Differences between experimental groups were analyzed by the unpaired student-t and where appropriate by the Mann-Whitney U test. P values <.05 were considered to be statistically significant.

Results
Effects of rt-PA and MA-33H1F7 on intestinal I/R-induced activation of coagulation and fibrinolysis
Intestinal I/R resulted in local intravascular coagulation activation and suppression of fibrinolysis. Thrombin generation and conversion of fibrinogen to fibrin was demonstrated by increase of the portal plasma levels of TAT-complexes and FDP (Figure 1). During 3 hours of reperfusion this thrombin generation resulted in local consumption of ATIII to 92±1% (P<.001) and 86±1% (P<.001) of baseline values after 20 and 40 minutes of ischemia, respectively, which was associated with a 3-fold and 5-fold rise of TAT-complexes, respectively. The initial rise of plasminogen activating activity after 1 hour of reperfusion, demonstrating local activation of fibrinolysis, was blunted by fibrinolytic inhibition to 91±2% (P<.002) and to 81±2% (P<.001) of baseline levels, 3 hours after 20 and 40 minutes of ischemia, respectively. This decrease of plasminogen activating activity was accompanied by an increase in portal plasma levels of PAI-1, starting at 1 hour of reperfusion and reaching levels of 17±1 IU/mL (P<.001) and 25±2
Figure 2. Effects of rt-PA and MA-33H1F7 on intestinal I/R-induced fibrin deposition and microvascular thrombosis

Representative results of fibrin deposition after 3 hours of reperfusion are shown of animals in the sham-operated group (A,B), and of animals subjected to 40 minutes of ischemia, treated with saline (C,D), rt-PA (E and F), or MA-33H1F7 (G,H). For A, C, E, and G, original magnification x200 (bar in A represents 50 μm); for B, D, F and H, original magnification x500 (bar in B represents 20 μm).
Role of fibrinolysis in intestinal ischemia/reperfusion injury

A

TAT (ng/mL)

0 60 120 180

Time of reperfusion (min)

B

TAT (ng/mL)

Sham  Saline + I/R 20  Saline + I/R 40  tPA + I/R 20  tPA + I/R 40  MA-33H17 + I/R 20  MA-33H17 + I/R 40

C

ATIII (%)

0 60 120 180

Time of reperfusion (min)

D

ATIII (%)

Sham  Saline + I/R 20  Saline + I/R 40  tPA + I/R 20  tPA + I/R 40  MA-33H17 + I/R 20  MA-33H17 + I/R 40

E

FDP (ng/mL)

0 60 120 180

Time of reperfusion (min)

F

FDP (ng/mL)

Sham  Saline + I/R 20  Saline + I/R 40  tPA + I/R 20  tPA + I/R 40  MA-33H17 + I/R 20  MA-33H17 + I/R 40
Figure 1. Effects of rt-PA and MA-33H1F7 on intestinal I/R-induced activation of coagulation and fibrinolysis

Activation of coagulation and suppression of fibrinolysis were determined by the measurement of portal plasma levels of thrombin-antithrombin (TAT)-complexes (A,B), antithrombin III (ATIII) (C,D), fibrin degradation products (FDP) (E,F), plasminogen activator activity (PAA) (G,H) and plasminogen activator inhibitor (PAI)-1 (I,J). Animals were intravenously administered saline, rt-PA (1.0 mg/kg of body weight) or anti-PAI-1 MA-33H1F7 (1.5 mg/kg), 15 minutes before reperfusion. Data (n = 6, in each group) are expressed as mean values ± SEM. Portal plasma levels of repeated measurements during 3 hours of reperfusion in saline treated animals subjected to sham operation ( ), or to 20 minutes ( ) or 40 minutes ( ) of ischemia are depicted in A, C, E, G and I. Portal plasma levels of animals treated with saline (closed bars), rt-PA (crossed bars), or MA-33H1F7 (blocked bars), subjected to 20 minutes or 40 minutes of ischemia after 3 hours of reperfusion are depicted in B, D, F, H and J. *P<.05 compared with the sham-operated group; †P<.05 compared with 20 minutes I/R plus saline group; ‡P<.05 compared with 40 minutes I/R plus saline group.

IU/mL (P<.001) after 20 and 40 minutes ischemia and 3 hours of reperfusion, respectively.

Administration of rt-PA enhanced circulatory plasminogen activator activity (PAA exceeded beyond detection limit) (Figure 1H). This increased fibrinolytic activity resulted in a 2.5-fold and 4-fold increase of baseline FDP portal plasma levels (272±22 ng/mL)
after 20 and 40 minutes of ischemia and 3 hours of reperfusion, respectively (Figure 1F). Administration of rt-PA eliminated approximately all free active PAI-1 (Figure 1J).

Administration of anti-PAI-1 monoclonal antibody abolished the I/R-induced suppression of fibrinolysis by decreasing PAI-1 activity resulting in approximately a 2-fold increase of plasminogen activating activity after 20 and 40 minutes of ischemia (Figure 1H). This increased fibrinolytic activity led to a 3-fold and 4.5-fold rise of baseline FDP portal plasma levels (266±23 ng/mL) after 20 and 40 minutes of ischemia and 3 hours of reperfusion, respectively (Figure 1F).

Effects of rt-PA and MA-33H1F7 on intestinal I/R-induced fibrin deposition and microvascular thrombosis

Microscopical assessment of intestinal stainings of saline treated rats revealed mucosal and submucosal fibrin deposits, microvascular thrombotic obstructions and villus microvessel hemorrhage after 20 (data not shown) and 40 minutes of intestinal ischemia (Figure 2C and 2D) and 3 hours of reperfusion, whereas histological examination of intestinal stainings after sham operation did not reveal any mucosal fibrin deposits, microvascular thrombotic obstructions or villus microvessel hemorrhage (Figure 2A and 2B).

Administration of rt-PA and anti-PAI-1 antibody had no effect at all on mucosal and submucosal deposition of fibrin, microvascular thrombotic obstructions or villus microvessel hemorrhage after 20 and 40 minutes of intestinal ischemia and 3 hours of reperfusion (Figure 2E-H).

Figure 3. Effects of rt-PA and MA-33H1F7 on intestinal I/R-induced inflammation

The local pro-inflammatory response to intestinal I/R was determined by the detection of interleukin-6 in portal plasma. Data (n = 6, in each group) are expressed as mean values ± SEM. Interleukin-6 portal plasma levels of saline treated, sham operated animals ( ), or subjected to 20 minutes ( ) or 40 minutes ( ) of ischemia during 3 hours of reperfusion are depicted in A. Interleukin-6 portal plasma levels of animals treated with saline (closed bars), rt-PA (crossed bars), or MA-33H1F7 (blocked bars), subjected to 20 minutes or 40 minutes of ischemia after 3 hours of reperfusion are depicted in B. *P<.05 compared with the sham-operated group; †P<.05 compared with 20 minutes I/R plus saline group; ‡P<.05 compared with 40 minutes I/R plus saline group.

184
Effects of rt-PA and MA-33H1F7 on intestinal I/R-induced inflammation

Portal plasma levels of tumor necrosis factor-α, cytokine induced neutrophil chemoattractant and interleukin-1β were below the 15 pg/mL detection limit. Portal plasma concentrations of interleukin-6 were significantly higher in rats subjected to 20 (P=.046) and 40 (P=.002) minutes of intestinal ischemia as compared to sham-operated rats after 3 hours of reperfusion (Figure 3). The increases of portal plasma interleukin-6 after 20 and 40 minutes of ischemia followed by reperfusion were significantly reduced to sham-operated values by rt-PA administration (P=.028 and P=.049, respectively) and anti-PAI-1 antibody administration (P=.022 and P=.010, respectively).

Effects of rt-PA and MA-33H1F7 on I/R-induced intestinal injury and dysfunction

Histological analysis of rat intestinal tissues, subjected to sham operation, or to 20 or 40 minutes of intestinal ischemia, was performed after 3 hours of reperfusion according to the Park-Chiu classification. Microscopical assessment of intestinal injury revealed subepithelial spaces in the villus tips with moderate to massive lifting, together with villus denudation in saline treated animals after 20 minutes of ischemia with a mean score of 3.2±0.4 (range 2-4, P=.007) (Figure 4A). Intestinal injury was increased to disintegration of the lamina propria in most intestinal tissues of saline treated animals after 40 minutes of ischemia, with a mean score of 4.7±0.2 (range 4-5, P=.001); such changes were not observed in sham-operated animals. Neither rt-PA nor anti-PAI-1 monoclonal antibody administration reduced I/R-induced intestinal injury.

Intestinal clearances of glucose and water during 3 hours of reperfusion were significantly and dose-dependently decreased after 20 and 40 minutes of intestinal ischemia in saline treated animals (Figure 4B and 4C). Administration of rt-PA or anti-PAI-1 antibody did not attenuate I/R-induced intestinal dysfunction.

Discussion

Intestinal I/R-induced endothelial cell injury results in a procoagulant and fibrinolysis-suppressing environment giving rise to fibrin deposition, which may further compromise the microcirculation of the intestine and promote necrosis in distal intestinal tissue. Mechanisms that play a role in the procoagulant response are the upregulation of tissue factor in combination with dysfunctional anticoagulant pathways, along with suppression of fibrinolysis mainly due to increased levels of the inhibitor of fibrinolysis: PAI-1. In the present study we investigated the relative contribution of suppressed endogenous fibrinolysis to the development of fibrin deposition and microthrombosis following intestinal reperfusion injury, by restoring the suppressed fibrinolytic activity either by intravenous administration of rt-PA or by inhibition of PAI-1 by monoclonal antibody 33H1F7.

Intestinal I/R resulted in inadequate removal of mucosal and submucosal fibrin deposits and microthrombotic obstructions in postischemic intestinal tissue in saline treated animals. This was presumably the result of a dysfunctional fibrinolytic system, as reflected by the suppression of plasminogen activator activity following increased plasma levels of PAI-1. Restoration of the dysfunctional fibrinolytic system by rt-PA administration or PAI-1 inhibition enhanced intravascular fibrinolytic activity, as reflected by elevated plasminogen activating activity and total abolishment of PAI-1 in the
Role of fibrinolysis in intestinal ischemia/reperfusion injury

Figure 4. Effects of rt-PA and MA-33H1F7 on I/R-induced intestinal injury and dysfunction

Histological analysis of intestinal tissues was performed after 3 hours of reperfusion and assessed according to the Park-Chiu classification. Intestinal clearances of glucose and water during 3 hours of reperfusion were determined in an intestinal loop of approximately 15 cm. Data of animals treated with saline (closed bars), rt-PA (crossed bars), or MA-33H1F7 (blocked bars) are expressed as means ± SEM (n = 6, in each group). *P<.05 compared with the sham-operated group.
mesenteric circulation. Subsequent increased levels of FDP indicated augmented removal of intravascular fibrin in both groups, which is supported by a previous study, in which administration of anti-PAI-1 antibody reduced thrombosis and restored postsischemic reperfusion in mesenteric arterioles. Although in the current study adequate intravascular, thrombolytic activity increased intravascular fibrin removal and restored reperfusion in arterioles, mucosal and submucosal fibrin deposition and microthrombosis in combination with continuing activated coagulation in postsischemic intestinal tissue was not reduced either after rt-PA administration or after PAI-1 inhibition. Also, the enhanced fibrinolytic activity in our model did not attenuate I/R-induced intestinal injury, as demonstrated by morphological and functional analysis. Postischemic intestinal structure and water and glucose clearances were equally affected in saline, rt-PA or anti-PAI-1 treated animals. Although a hypofibrinolytic state may hypothetically contribute to thrombotic obstruction and may hamper adequate microcirculation thereby promoting distal injury, “recanalization” of the thrombotic microvasculature by fibrinolysis did not attenuate the sequela of intestinal post-ischemic, reperfusion injury.

Modulation of fibrinolysis was shown to cause similar results in experimental models of acute myocardial ischemia and reperfusion. Administration of rt-PA or streptokinase did not benefit ventricular function and structure in canine models of coronary I/R. The results of these studies suggest that fibrin deposition and associated injury is of limited importance for the total amount of necrosis. Consequently, based on these reports and our present observations, it may be suggested that the relative contribution of suppressed endogenous fibrinolysis to microcirculatory fibrin deposition and I/R-injury is of limited importance.

As the inflammatory response plays a pivotal role in I/R injury, inhibition of inflammatory cell recruitment/migration and cytokine release has demonstrated to reduce I/R-injury. It is therefore noteworthy that, in addition to their effects on local fibrinolytic activity, administration of rt-PA or anti-PAI-1 antibody significantly inhibited the inflammatory response following intestinal I/R, as demonstrated by the reduction of interleukin-6 portal plasma levels to baseline values. Interestingly, these results illustrate the tight cross-talk between coagulation and fibrinolysis on the one hand and activation of inflammation on the other hand.

Previous studies have shown that tumor necrosis factor-α and interleukin-1β are pivotal regulators of plasminogen activators and inhibitors. Inhibition of these inflammatory mediators by monoclonal antibodies, soluble receptors, or receptor antagonists resulted in an abolishment of endotoxin-induced effects on fibrinolysis. Experiments in mice with targeted disruptions of genes encoding components of the plasminogen-plasmin system confirm that fibrinolysis itself may also affect inflammation. Mice with a deficiency of plasminogen activators have more extensive inflammation in various tissues when challenged with endotoxin, whereas PAI-1 knockout mice, in contrast to wild-type controls, display a reduction of inflammatory activity. It is thought that fibrinolytic activators and inhibitors may modulate the inflammatory response by their effect on inflammatory cell recruitment and migration. In particular the plasminogen activator u-PA and its receptor (u-PAR) play a central role in this respect. Taken together, it seems that the fibrinolytic shutdown as occurs during intestinal I/R, may contribute to the inflammatory activity and results in enhanced release of interleukin-6, whereas promotion of endogenous fibrinolysis as achieved in our experiments by
administration of r-tPA or inhibition of PAI-1, may modulate this inflammatory activity and result in lower interleukin-6 expression. However, in spite of inflammatory response modulation, administration of r-tPA or inhibition of PAI-1 did not attenuate I/R-induced intestinal injury.

In conclusion, intestinal I/R resulted in considerable derangement of the coagulation and inflammatory system, and compromised the enteric microcirculatory system by widespread deposition of fibrin and microthrombosis. Despite enhancement of fibrinolytic activity, administration of rt-PA or anti-PAI-1 antibody neither increased removal of mucosal and submucosal fibrin deposition nor attenuated intestinal I/R injury. These results suggest a limited role of suppressed fibrinolysis in compromising enteric microcirculation with subsequent deterioration of intestinal function and structure following intestinal I/R.

Acknowledgements

The authors gratefully thank Mrs. A.M. Korteland-Van Male (Laboratory of Paediatrics and Sophia Children's Hospital, Erasmus MC, Rotterdam, the Netherlands) for providing technical assistance.
References

Role of fibrinolysis in intestinal ischemia/reperfusion injury


