Clinical and experimental studies on treatment of acute mesenteric ischemia
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Summary and conclusions
This thesis focuses on several issues regarding treatment of acute mesenteric ischemia.

**Chapter 1** provides an introduction into the lethal condition of acute mesenteric ischemia and gives an overview of the surgical revascularization procedures in acute mesenteric ischemia. The rapid onset of acute mesenteric ischemia and the potential rapidity with which bowel infarction may occur explain the lethality of this disease. Despite considerable advances in medical diagnosis and treatment over the past four decades, mesenteric vascular occlusion still has a poor prognosis with an in-hospital mortality rate of 59–93 per cent. The relative infrequency of acute mesenteric ischemia, the variable pathogenesis and the broad spectrum of ischemic injury of the small and large intestines make it almost impossible to study this disease and its diagnostic and therapeutic strategies in clinical randomized or case-controlled trials. Surgery is by far the most favorable treatment, as it includes the assessment of intestinal viability, determination or confirmation of the underlying cause, revascularization, and resection of the nonviable intestine. However, in the last decades of the previous century, a number of new treatment modalities have been introduced, which may benefit a selection of patients with acute mesenteric artery occlusion and may improve outcome of this lethal disease.

**Chapter 2** discusses the differentiation of acute mesenteric ischemia on the basis of etiology (arterial embolism, arterial thrombosis, venous thrombosis and non-occlusive mesenteric ischemia). This differentiation is of great importance because of variation in disease progression, response to treatment and outcome. However most of the previous retrospective studies assessed calculated a mortality rate based on data compiled from all etiological subsets taken together, and therefore obscure differences in clinical presentation and characteristics, diagnostic investigation, disease progression, mortality and response to therapeutic modalities that are specific to disease etiology. We therefore performed a systematic review of the available literature, which concludes: 1) the prognosis after acute mesenteric venous thrombosis is better than that following acute arterial mesenteric ischemia; 2) the prognosis after mesenteric arterial embolism is better than that after arterial thrombosis or non-occlusive ischemia; 3) the mortality rate following surgical treatment of arterial embolism and venous thrombosis (54.1 and 32.1 per cent respectively) is less than that after surgery for arterial thrombosis and non-occlusive ischemia (77.4 and 72.7 per cent respectively); and 4) the overall survival after acute mesenteric ischemia has improved over the past four decades. Taken together, there are large differences in prognosis after acute mesenteric ischemia depending on etiology. Surgical treatment of arterial embolism has improved outcome whereas the mortality rate following surgery for arterial thrombosis and non-occlusive ischaemia remains poor.

**Chapter 3** systematically reviews the current (observational) data of thrombolytic therapy in patients with acute thromboembolic mesenteric occlusion, in order to evaluate this treatment modality as an alternative or adjunctive therapy to surgery. Thrombolytic therapy of acute superior mesenteric artery thromboembolism is still to be considered as a relatively new treatment modality. Insufficient evidence is available from reviewed literature to determine the relative effectiveness and safety of thrombolytic treatment for acute superior mesenteric artery thromboembolism, however, initial results appear to be promising. The relative infrequency of acute mesenteric ischemia and the variation in
clinical presentation constitute an almost insurmountable obstacle to undertaking randomized or case-control trials. Nevertheless, this compilation of data gives insight into current status of thrombolytic therapy of acute superior mesenteric artery thromboembolism, may provide questions and answers for clinical investigators to address, and may give rise to directions for future evaluation and clinical guidelines (at least based on consensus).

Chapter 4 questions the idea that Riolan would have conceived an arterial collateral pathway in the mesocolon. Riolan's anastomosis or arc is eponymously used to indicate the arterial anastomosis between the superior and inferior mesenteric arteries. Vascular as well as gastro-intestinal surgeons are well-acquainted with this collateral mesenteric pathway for retrograde perfusion of the superior mesenteric artery when the origin of the latter is occluded. The eponym suggests that Jean Riolan (1580-1657), a famous 17th century French anatomist, was the first to describe this arterial anastomosis. Riolan was a strong defender of traditional Galenic doctrine in medicine and therefore, proved a vigorous opponent of the new concept of the circulation of the blood as exposed by William Harvey (1578-1657). This makes it unlikely that Riolan would have conceived an arterial collateral pathway in the mesocolon, a notion confirmed by examining his anatomy book published in 1649. He probably had observed vascular arcades running along the inner border of the colon which later associated him with the collateral circulation of the mesentery. It was not until 1743, that Albrecht von Haller (1708-1777) gave a detailed description of the anatomy of the mesenteric arteries, referring to the arterial collateral connection between the superior and inferior mesenteric arteries, as the "Arcus Riolani", in honour of an old master of anatomy.

Chapter 5 outlines current knowledge on the crosstalk between coagulation and inflammation and the potentially beneficial effects of restoration of the dysfunctional physiological anticoagulant pathways in the microvasculature, following hypoxia or ischemia and reperfusion. The endothelium plays a central role in all major pathways involved in the pathogenesis of hemostatic derangement during ischemia and reperfusion. Endothelial cells seem to be directly involved in the initiation and regulation of thrombin generation and the inhibition of fibrin removal. Proinflammatory cytokines are crucial in mediating these effects on endothelial cells, which themselves may also express cytokines, thereby amplifying the coagulative response. Rather than being a unidirectional relationship, the interaction between inflammation and coagulation involves significant cross-talk between the systems. This could result in inflammation-modifying effects of hemostatic interventions in patients with ischemia and reperfusion-syndromes.

In chapter 6 we evaluate the role of intravascular coagulation in microvascular reperfusion injury after acute mesenteric occlusion. We demonstrated that intestinal ischemia and reperfusion result in local generation of thrombin and subsequent conversion of fibrinogen to fibrin. Simultaneously, intestinal fibrinolysis is impaired, ultimately leading to intravascular fibrin deposition. These findings suggest that microvascular thrombotic obstruction plays a role in the pathogenesis of structural and functional intestinal injury induced by ischemia and reperfusion.
In chapter 7 we briefly review the involvement of the protein C system in a selected number of models of ischemia-reperfusion injury. Experimental studies indicate that an impaired function of the protein C pathway plays a major role in the pathogenesis of sepsis and associated organ dysfunction. Also clinical trials in patients with sepsis have shown a beneficial effect of recombinant human activated protein C. It is tempting to speculate that other clinical situations that are characterized by endothelial dysfunction and microvascular failure may benefit from the administration of recombinant activated protein C. Thereby, a prominent role of activated protein C may be envisaged in ischemia-reperfusion syndromes. Ischemia-reperfusion injury is characterized by a local inflammatory response and local activation of coagulation, reminiscent of the systemic situation in sepsis. Virtually all organs may suffer from ischemia-reperfusion injury, which can play an important role in major clinical entities, such as myocardial infarction, acute renal failure, stroke, acute lung injury and intestinal ischemia. There is interesting evidence to support a role of the protein C system in ischemia-reperfusion injury. Consequently, administration of activated protein C may be a promising therapeutic option in these situations. The efficacy of this approach deserves further study in experimental and clinical studies.

In chapter 8 we show that activated protein C or antithrombin inhibits local and systemic derangement of coagulation and inflammation following intestinal ischemia and reperfusion in rats, diminishes mucosal fibrin deposition and attenuates ischemia/reperfusion-induced intestinal injury. Intestinal ischemia and reperfusion resulted in considerable local and systemic derangement of the coagulation and inflammatory system, compromising mucosal and submucosal microcirculation by widespread microthrombosis and deposition of fibrin. Activated protein C or antithrombin treated animals showed less thrombin generation, fibrin degradation products and fibrin deposition compared to control animals, as confirmed by histological examination, whereas heparin administration showed only a limited reduction of portal fibrin degradation products levels. Furthermore, activated protein C or antithrombin administration markedly inhibited the inflammatory response, as reflected by reduced interleukin-6 plasma levels to baseline values whereas heparin had no effect. Furthermore, activated protein C or antithrombin treated animals demonstrated less ischemia/reperfusion-induced intestinal dysfunction and histological changes, compared to control animals. These observations suggest that activated protein C or antithrombin reduces ischemia and reperfusion-induced intestinal injury, both through their anticoagulant and anti-inflammatory effects.

In chapter 9 we demonstrate that enhancement of fibrinolytic activity by intravenous administration of recombinant tissue plasminogen activator (rt-PA) or by inhibition of PAI-1 by administration of MA-33H1F7 neither increased removal of mucosal fibrin deposition nor attenuated intestinal ischemia and reperfusion injury in a rat model of acute mesenteric occlusion. Intestinal ischemia/reperfusion 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. However, enhanced fibrinolysis did not result in improvement of any of the measured parameters. 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 ischemia and reperfusion-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. These results suggest a limited role of suppressed endogenous fibrinolysis in microcirculatory failure and consequent deterioration of intestinal function and structure following intestinal ischemia and reperfusion.

In contrast to the previous in vivo studies, in chapter 10 we developed an in vitro Ussing chamber model to study the effect of hypoxia and reoxygenation on the functional characteristics of the intestinal epithelium, focusing on the dysfunctional properties of barrier function and absorptive and secretory capacity of the intestinal epithelial layer. Intestinal ischemia and reperfusion may lead to profuse secretion of water and electrolytes. The underlying mechanisms have been related to increased hydrostatic pressure, to denudation of intestinal villi and recently, to adenosine-mediated enhancement of chloride secretion. By studying these mechanisms in an in vitro system, we avoid the complex interplay between vascular, subepithelial and epithelial factors in in vivo models of ischemia and reperfusion. We conclude that hypoxia and reoxygenation differentially impair nutrient absorption, corroborating recent absorption data in in vivo models of ischemia, and that it differentially affects secretory capacity in crypts, dependent on the intracellular messenger pathway. The relative persistence of Ca\(^{2+}\)/PKC-mediated secretion to hypoxia and reoxygenation indicates that secretagogues that activate this pathway play a significant role in the intraluminal fluid sequestration and diarrhea observed after intestinal ischemia and reperfusion.

In addition to the studies focusing on mesenteric ischemia, we fundamentally analyze the role of reactive oxygen species in intracellular mechanisms of the endothelial cell in response to vascular endothelial growth factor (VEGF) in chapter 11. Reactive oxygen species (ROS) have traditionally been viewed as cytotoxic molecules, predominantly generated in pathological conditions such as ischemia and reperfusion syndromes, but they are now recognized to play a critical role in signal transduction and transcriptional regulation within the vascular tree. It has recently been shown that VEGF-induced proliferation, migration, and downstream expression of some but not all genes in endothelial cells are dependent upon a Rac1-regulated NADPH oxidase-derived ROS, suggesting that VEGF signaling in the endothelium is tightly coupled to NADPH oxidase activity. We demonstrate that NADPH oxidase-derived ROS serve to modulate selective VEGF-dependent signaling pathways, including PI3-kinase/Akt, MAPK, and PKC, transcriptional profiles and biological functions in endothelial cells.