Intestinal barrier loss in sepsis

Introduction
In the intestine, the epithelial lining has the dual function of absorption and digestion of nutrients, while also protecting the body from food, microbiota and microbiota derived toxins [1,2]. Disruption of the physical intestinal barrier may play a role in the development and perpetuation of a variety of intestinal disease states. It is involved in the onset of sepsis and multiple organ failure and can be caused by intestinal hypoperfusion. This review will focus on the current insights into gut barrier integrity and function loss, which is important in order to improve our knowledge on disease aetiology and may contribute to early detection or prevention of sepsis.

Composition and function of the intestinal barrier
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The intestinal barrier can be subdivided into a physical and an immunological section [13-15]. The physical intestinal barrier is composed of a lining of epithelial cells, which are connected by tight junctions (Figure 1). These tight junctions serve as a fence sealing the paracellular pathway [8]. Tight junctions are anchored in the cell via the filamentous actin (F-actin) cytoskeleton, a dynamic structure that maintains cell shape and tight junction stability [16]. Experimental animal studies have generated the hypothesis that the intestine is central in the origin of postoperative and post-traumatic sequeae [5-9]. Human studies have insufficiently tested this hypothesis [4]. Recognition and treatment of patients at risk of developing postoperative or post-traumatic SIRS, sepsis and MOF is important, since such patients have the highest non-cardiac mortality rate of patients in the intensive care unit (ICU) [5-12].

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translocation. Paneth cells secrete antimicrobial peptides constitutively in order to keep intestinal crypts sterile, including lysozyme, phospholipase A₂, and α-defensins [25,26]. Moreover, exposure of Paneth cells to bacterial products induces increased levels of these antimicrobial peptides [25,27,28]. In addition to the local defence role of Paneth cells, they also have a key role in shaping the composition of the microbiota of the whole intestine by secretion of α-defensins [29]. Thereby, α-defensins may contribute to host-microbial symbiosis. Also enterocytes secrete a variety of antimicrobial proteins both constitutively and in response to microbials including β-defensins and cathelicidins [24].

**Intestinal barrier loss**

Intestinal barrier loss can occur after energy depletion as well as in energy rich environments. Energy depletion, due to inadequate intestinal blood flow (hypoperfusion or ischemia), is believed to underlie most intestinal problems in surgical or septic patients [30,31]. Hypoperfusion or ischemia can lead to adenosine triphosphate (ATP) depletion which results in activation (dephosphorylation) of actin depolymerizing factor/cofilin (AC), the regulator of cellular actin dynamics by binding to F-actin [32-35]. Activated AC binds F-actin and can sever F-actin. As a result the actin cytoskeleton is disrupted and connections with tight junction proteins are lost, causing disassembly of tight junctions [16]. Indeed, in renal ischemia-reperfusion injury ATP depletion causes activation of AC, its relocalization to the apical membrane, and consequent alterations in the apical actin cytoskeleton in renal tubular epithelial cells. The role of activated AC in intestinal cytoskeleton severing and intestinal tight junction loss remains to be clarified.

Energy depletion is a frequent but not exclusive cause of intestinal barrier loss. Inflammation can also lead to disruption of the intestinal barrier. In this context, myosin light chain (MLC) is described as a mediator of actin dynamics in the enterocyte [36-38]. Phosphorylation of MLC (pMLC) by myosin light chain kinase (MLCK) causes contraction of the actomyosin cytoskeleton, leading to tight junction opening in the intestine [39]. This cytokine-dependent mechanism is considered responsible for tight junction integrity loss in inflammatory bowel disease and other immune-mediated intestinal diseases [36,40]. In summary, actin filament reorganisation is regulated by AC or MLC, with tight junction opening caused by either AC after energy depletion or by MLC in an energy rich environment.

Loss of the paracellular barrier by tight junction damage can result in translocation of macromolecules, microbial products and microbiota from the intestinal lumen to surrounding tissues and the circulation [41-43]. Such translocation of microbial products can elicit a local and systemic inflammatory response, causing additional tight junction loss [9,41,44-47]. Tight junctions together with adherence junctions connect adjacent epithelial cells. Therefore, massive disruption of these junctions will result in translocation. Paneth cells secrete antimicrobial peptides constitutively in order to keep intestinal crypts sterile, including lysozyme, phospholipase A₂, and α-defensins [25,26]. Moreover, exposure of Paneth cells to bacterial products induces increased levels of these antimicrobial peptides [25,27,28]. In addition to the local defence role of Paneth cells, they also have a key role in shaping the composition of the microbiota of the whole intestine by secretion of α-defensins [29]. Thereby, α-defensins may contribute to host-microbial symbiosis. Also enterocytes secrete a variety of antimicrobial proteins both constitutively and in response to microbials including β-defensins and cathelicidins [24].

**Figure 1. Composition of the intestinal barrier in the small intestine**

The intestinal barrier can be subdivided into a physical and an immunological part. The physical intestinal barrier is composed of a lining of epithelial cells connected by tight junctions. The physical barrier is reinforced by a layer of mucus, produced by goblet cells. The immunological barrier is formed by intestinal epithelial cells and paneth cells, which sense bacteria and secrete antimicrobial peptides.
in loss of epithelial cells. Enterocyte loss and disruption of enterocyte integrity can also occur as a result of insufficient tissue perfusion with failure to meet the cellular demand for oxygen and nutrients. Mature enterocytes, at the tip of the villi or crypt of the small or large intestine, respectively, are particularly vulnerable to hypoperfusion or ischemia [48].

Intestinal barrier integrity loss, with disruption of paracelllular (tight junction) or cellular (enterocyte) barriers, can lead to translocation of luminal substances that cause systemic inflammation. This inflammatory response can lead to organ damage and therefore has a detrimental effect on the health of an individual [46,49-53].

**Prevention of intestinal barrier loss and treatment of its harmful consequences**

Early detection or prevention of systemic hypotension and intestinal hypoperfusion during surgery is of major importance, since the gut can only cope with relatively short periods of inadequate blood flow [54-56]. In recent studies in patients admitted to the intensive care unit (ICU) because of postoperative sepsis, we showed the development of intestinal hypoperfusion, objectivated by gastric mucosal PiCO2 [12]. Furthermore, a relationship was shown between this intestinal hypoperfusion and intestinal epithelial cell damage, assessed by circulating levels of I-FABP [12]. Intestinal Fatty Acid Binding Protein (I-FABP) is a low molecular weight (14-15 kDa) cytosolic protein found in high concentrations in tissues involved in the uptake and consumption of fatty acids. I-FABP is particularly highly expressed in cells present on the tops of the villi. Since the tops of the villi are the initial site of destruction in numerous intestinal diseases, circulating I-FABP is a potentially useful plasma marker in early stages of intestinal diseases. The kidneys remove approximately 30% of I-FABP in a single pass, leading to a calculated I-FABP half-life of 11 minutes [57]. This underscores that I-FABP reflects actual cell damage and that assessment of the urinary concentration is potentially useful in reflecting enterocyte damage.

Splanchnic hypoperfusion in the early phase of abdominal sepsis correlated strongly with intestinal mucosal damage. Moreover, elevated plasma I-FABP values on admission to the ICU were associated with a poor outcome in patients with abdominal sepsis [12]. Next, in children with meningococcal sepsis, it was shown that almost half of the patients presented with intestinal epithelial cell damage, as reflected by increased plasma I-FABP values, on admission to the paediatric ICU. Children who died were characterized by continued presence of gut damage, while in all survivors this injury came to an end within 12 hours after starting intensive treatment [58]. Recently, we showed that a significant proportion (93%) of adult trauma patients rapidly developed intestinal mucosal cell damage, measured by elevation of plasma I-FABP values [59]. The extent of intestinal damage was readily detectable in blood taken on admittance to the emergency department. Interestingly, the highest 10% of I-FABP values on admission belonged to patients with severe abdominal trauma that required acute surgical intervention, such as ruptures of the diaphragm, liver, and spleen. Circulatory concentrations of enterocyte damage marker I-FABP were related to the presence of shock and the extent of general injury as well as abdominal trauma, indicating that the level of intestinal cell damage was determined by both systemic and local factors. Moreover, early I-FABP values positively correlated with the inflammatory response that developed in the days following trauma [59]. In conclusion, evaluation of intestinal tissue damage in the early phase of sepsis is an adequate predictor for survival.

Several studies have focused on hemodynamic optimisation during surgery and during hypoperfusion in patients admitted to the ICU with the aim of preventing intestinal hypoperfusion or ischemia. Unfortunately, results of clinical trials investigating the effect of fluid therapy on patient outcome remain contradictory. Several authors report on improved postoperative recovery and reduced hospital stay by stroke volume-directed fluid administration, [28,60-63] while others emphasize the importance of conservative fluid administration with beneficial effects on surgical outcome [64-66]. In patients undergoing major surgery, we showed that intestinal barrier loss can be prevented by preserving the mean arterial pressure above 60 mmHg, which is accomplished by goal-directed fluid therapy (Thuijls et al., submitted). Besides preventing inflammatory complications of intestinal damage, fluid therapy is also the cornerstone of the treatment of sepsis in general [67].

An experimental, appealing approach to attenuate the inflammatory response is administration of high-lipid enteral nutrition. It has been shown that short-term administration of high-lipid feeding before or after hemorrhagic shock decreases cytokine release and reduces damage to several organs including the intestine and the liver [15,41]. A direct vasovagal reflex mediated these protective effects, through activation of peripheral cholecystokinin (CCK) receptors on the afferent vagal nerve [68]. Next, the so-called cholinergic anti-inflammatory pathway is stimulated; at the efferent vagus endings, the neurotransmitter acetylcholine is released, which binds to α7nAch-receptors on peripheral macrophages, thereby suppressing their cytokine release [68,69]. Apart from attenuating the inflammatory response, the vasovagal reflex is also involved in the reduction of intestinal epithelial damage [68,70]. However, it remains to be clarified whether high-lipid enteral nutrition decreases intestinal damage only by reducing systemic inflammation or whether it also has a direct protective effect on the intestine.

In summary, the human gut can cope well with a short period of ischemia followed by reperfusion. Intestinal barrier disruption, which occurs after longer periods of inadequate blood flow is associated with sepsis and MOF. Therefore, intestinal barrier loss should be prevented if possible. When barrier loss has already occurred, the harmful sequelae should be diminished.
Literature


