Postoperative ileus: Pathophysiology & treatment strategies
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Patients undergoing an abdominal surgical procedure develop a transient episode of impaired gastrointestinal motility or non-obstructive ileus. Postoperative ileus, characterized by impairment of coordinated propulsive intestinal motility, remains an almost inevitable consequence of surgery. Importantly, postoperative ileus is a major determinant of recovery after intestinal surgery and leads to increased morbidity and prolonged hospitalization, which is a great economic burden to health-care systems. Although a variety of strategies reduce postoperative ileus, none of these methods have been completely successful in shortening the duration of postoperative ileus. The aetiology of postoperative ileus is multifactorial, but activation of a local inflammatory response within the intestinal muscularis externa has become an accepted pathophysiological mechanism. The importance of this inflammatory response in postoperative ileus is underscored by the beneficial effect of pharmacological interventions that block the influx of leukocytes. In this thesis, we describe our latest insights into the mechanisms behind postoperative ileus and highlight new strategies to intervene in the postoperative inflammatory cascade.

Postoperative ileus is characterized by a transient inhibition of transit of gastrointestinal content after abdominal surgery. Currently, intestinal manipulation of the intestine in rodents is widely used as a preclinical model of postoperative ileus. In Chapter 2 we developed a new technique, using a purpose-designed device, to manipulate the intestine in a more controlled manner (Figure 1). Gastrointestinal transit, measured by evaluating the intestinal distribution of orally gavaged fluorescent labeled dextran, was used as a read-out to determine the degree of postoperative ileus. Our standardized manipulation technique resulted in a pressure-dependent decrease in intestinal transit with small variation and was associated with inflammation of the intestinal muscularis. This novel method provided a methodologically convenient and useful model to study the potential of new anti-inflammatory strategies (Chapter 5) in a reliable and adequately controlled manner.

The exact underlying molecular and cellular mechanisms of postoperative ileus are still under investigation. Animal models suggest that both neuronal and local inflammatory responses within the intestinal muscularis mechanistically contribute. The neuronal mechanism appears to involve the enhanced release of nitric oxide from inhibitory motor neurons. Likewise, nitric oxide and prostaglandins are released from inflammatory cells (macrophages and monocytes) via the induction of nitric oxide synthase (iNOS) and cyclooxygenase-2. The influx of leukocytes is not limited to the handled region, i.e. the small intestine, but is also present in the colon. Interestingly, we found that the severity of ileus results from an inflammatory response that is independent of the number of leukocytes infiltrating the small intestinal muscularis (Chapter 2), indicating that other mechanism are responsible for the more severe ileus.
These data bring forward the hypothesis that tissue trauma induced immune response via damage-associated molecular patterns may be involved in the pathophysiology of more severe ileus. In this line, enhanced local inflammation may result in a systemic inflammatory response and consequently trigger enhanced brain stem activation. To elucidate the contribution of tissue damage in the severe ileus, we analysed the local intestinal and systemic inflammatory response along with brain stem nuclei activation after different intensities of intestinal handling (Chapter 3). We showed that the manipulation-induced tissue damage led to not only an enhanced intestinal inflammatory response but also the release of pro-inflammatory molecules in the bloodstream. This tissue damage induced enhanced inflammatory response was also associated with enhanced brain activation in mice and correlated with the severity of postoperative ileus in both humans and mice. Together, our data provide evidence of an additional mechanism by which tissue damage mediators and pro-inflammatory cytokines released into the systemic circulation contribute to the impaired motility of non-manipulated intestine. Secondly, we demonstrated that severe ileus and tissue damage results in activation of brain stem areas such as the area postrema, most likely explaining the sickness behavior associated with postoperative ileus. To what extent this further contributes to the general hypomotility in postoperative ileus deserves further attention in future studies. Insights into how tissue damage triggers the release of systemic cytokines should aid in the development of therapeutics to prevent this response.

Both in humans and animals abdominal surgery has shown to affect the integrity of the intestinal epithelial barrier and may potentially influence postoperative recovery. Studies have enlightened the importance of mast cells in the regulation of the epithelial barrier in diverse intestinal inflammatory settings. Whether the integrity of the epithelial barrier is relevant or not in the pathogenesis of postoperative ileus is not fully proven, but irrespective thereof, the clinical impact of bacterial translocation during surgery is significant. In fact, in the course of abdominal surgery, barrier dysfunction has been associated with increased postoperative septic morbidity in surgical patients undergoing laparotomy. In Chapter 4 we describe that activated mast cells evoke a disturbance of intestinal barrier function. To this end we performed our experiments in two mast cell deficient mouse strains. We observed that intestinal manipulation during abdominal surgery in mice resulted in a mast cell dependent inflammation and barrier dysfunction. These data underscore the importance of mast cells in the pathogenesis of postoperative ileus and the potential of mast cell stabilizers to shorten the duration of postoperative ileus. In addition to activation of mast cells, the pathogenesis of postoperative ileus involves the activation of macrophages and dendritic cells that reside in the muscularis externa (Figure 2). The influx of bacteria and their antigens
across the epithelial barrier following intestinal manipulation may activate these cells. Alternatively, IL-1 that is quickly released as a response to tissue damage may be of importance in intestinal manipulation induced inflammation. Whether recognition of translocated bacteria is crucial in the initiation of surgically induced ileus or only plays a role in the perpetuation of ileus is currently under investigation.

![Figure 1](image1.png)

**Figure 1** | Standardized manipulation in an experimental postoperative ileus mouse model

![Figure 2](image2.png)

**Figure 2** | CD68 staining for macrophages and infiltrating monocytes in the muscle layer of small intestine. Whole mounts of small intestinal muscularis stained with anti-CD68 antibody at 200X magnification. Control laparotomy mouse (a); Laparotomy plus eventration small intestine and caecum without standardized pressure manipulation (b); Mouse with postoperative ileus after 9 grams standardized pressure manipulation resulting in post (c).

In the gut, cholinergic fibers are located in close proximity to immune cells and is therefore the ideal site for neuro-immune modulation. In the last decade, vagus nerve stimulation has been shown to dampen immune responses in a number of disease models; this is referred to as the ‘cholinergic anti-inflammatory pathway’. Recently, our group demonstrated that electrical stimulation of the vagus nerve in mice improves intestinal transit and dampens intestinal muscular inflammation through alpha 7 nicotinic acetylcholine receptor (α7nAChRs) expressed on resident macrophages. The cholinergic anti-inflammatory pathway is proposed to be part of the so-called vago-vagal ‘inflammatory reflex’. Inflammation is sensed by afferent nerve fibers and is subsequently relayed to the brain. After integration of afferent information, the motor neurons of the vagus nerve are activated and an integrated anti-inflammatory signal is sent back to the inflamed area. We provided the neuro-anatomical evidence that the
Figure 3 | The vagal anti-inflammatory pathway and postoperative ileus. The cholinergic anti-inflammatory reflex relies on activation of the vagal sensory afferents that subsequently creates an integrated anti-inflammatory response through vagal efferent fibres. In postoperative ileus, activation of the vagal sensory afferent is triggered by pro-inflammatory cytokines released within the muscle layers. Vagal afferents will trigger neuronal activity in the NTS (brainstem nuclei that receive visceral information) which results in the activation of motor neurons of the vagus nerve (located in the DMV). Once activated, the vagal efferent fibres release acetylcholine at the level of the myenteric plexus stimulating cholinergic enteric neurons. These neurons amplify vagal signalling by releasing acetylcholine in the muscle layer where the macrophages reside. Binding of acetylcholine to α7 nicotinic receptors on macrophages suppresses the release of pro-inflammatory cytokines. Stimulation of the sensory afferent limb of the neuronal circuit by enteral high fat diet through cholecystokinin release or electrical stimulation of the motor efferent limb of the vagus nerve prevent intestinal inflammation and ileus. Intra-cerebroventricular injection of semapimod (CNI 1493), acetylcholinesterase inhibitor (galantamine), ghrelin and muscarinic receptor 1 agonists (McN-A 343) also activate vagal efferents thereby suppressing inflammation. Abbreviations: Ach: acetylcholine; DMV: dorsal motor nucleus of the vagus; NTS: nucleus of the solitary tract VNS: vagal nerve stimulation.

vagal feedback loop is activated in course of local intestinal inflammation (Chapter 6), offering new approaches to modulate undesired inflammatory processes (Figure 3). Currently, we are studying whether intra-operative electrical stimulation of the intra-abdominal vagus nerve reduces the inflammatory response to abdominal surgery and can shorten the duration of postoperative ileus in patients undergoing open rectal resections. In addition, a multicenter trial is currently investigating if stimulating the vagus nerve in the neck of patients with rheumatoid arthritis can decrease joint inflammation.
In the second part of this thesis, we evaluated potential therapeutic strategies for postoperative ileus and its clinical aspects. With novel treatments for postoperative ileus in development, there is a definite need for reliable outcome measures to evaluate clinical success in new drug trials. Up to date validated clinical hallmarks of gastrointestinal recovery to evaluate new treatments and readiness for discharge from the hospital were lacking. In **Chapter 7**, we established what clinical hallmarks best identify recovery of gastrointestinal transit after intestinal surgery. We objectively determined colonic transit using scintigraphy and established that clinical recovery following abdominal surgery is indeed associated with recovery of colonic transit. Using the latter as objective criterion for clinical improvement, we showed that the combined hallmark of tolerance of solid food and having had defecation best predicted clinical recovery and indicates readiness for discharge. In contrast, time to first flatus was not associated with recovery of colonic transit or time to discharge. Our next step was to validate the outcome parameter tolerance of solid food and having had defecation in the large cohort of patients of the multicenter LAFA trial. These analyses confirmed that the presence of both clinical parameters is the best clinical marker of gut recovery and should be preferred as primary outcome measure in future clinical trials on postoperative ileus.

Postoperative ileus is a major determinant of recovery after colorectal surgery. Recent studies suggest substantial improvements in perioperative care (namely multimodal enhanced recovery programs and laparoscopic surgery), resulting in faster recovery of the gut and shorter hospital stay during the last decade. However, objective measures supporting faster gastrointestinal recovery are lacking. In **Chapter 8** we demonstrated faster recovery of gastrointestinal transit after laparoscopic surgery and the fast-track program, providing objective data that laparoscopy and fast-track care lead to faster recovery of motility and concomitant enhanced clinical recovery. The faster clinical recovery observed after laparoscopic surgery compared with open surgery could be explained by decreased tissue trauma with concomitant decreased immune cell activation leading to attenuated intestinal inflammation and thus a quicker gastrointestinal recovery. The mechanisms behind the beneficial effect of the fast-track program remain unclear, but in a rat model enteral feeding was shown to improve postoperative gastrointestinal transit through activation of the vagus nerve-mediated anti-inflammatory pathway. Correspondingly, within the fast-track program patients are not only mobilized faster but also earlier resume oral feeding. This last observation leads us to speculate that feeding may activate the vagal anti-inflammatory pathway contributing to faster recovery in these patients. To what extent vagal activation in response to early feeding may explain improved transit in the fast-track groups in this randomized double-blind study remains to be studied. Despite the apparent
effectiveness of the fast-track approaches, this approach has not been fully implemented in the majority of surgical wards. Strategies to increase the implementation of these fast-track approaches should be encouraged. If this implementation can be achieved, it will markedly affect the study design of clinical trials as new therapeutic strategies being tested will have to show clinical benefit in the setting of a fast-track programme.

Finally, in Chapter 5 we describe a novel anti-inflammatory strategy to improve postoperative ileus in mice. We evaluated whether Spleen tyrosine kinase (Syk), one of the critical intracellular tyrosine kinases involved in mast cell degranulation and macrophage activation, can be a potential target for intestinal inflammation. As both mast cells and macrophages are involved in the pathophysiology of postoperative ileus, we established whether blockade of Syk could represent an alternative approach to inhibit immune cell activation evoked by intestinal manipulation and thus represent a new tool to shorten postoperative ileus. We showed here that the potent and highly selective Syk inhibitor GSK143 prevented FcεRI and Substance P induced mast cell degranulation and endotoxin-induced macrophage activation in vitro. Secondly, in vivo GSK143 dampened the inflammatory cascade leading to postoperative ileus, without exerting a direct effect on gastrointestinal motility as it did not affect intestinal transit in mice that underwent laparotomy. Taken together, inhibition of Syk significantly reduced the inflammatory response to intestinal manipulation thereby preventing postoperative ileus. This suggests that Syk can be a potential target for intestinal inflammatory diseases and may be a new tool to shorten postoperative ileus.

Future Perspectives
In this thesis, new insights into the pathogenesis of postoperative ileus were revealed, which has led to the identification of new targets for treatment and novel therapeutic approaches. This includes Syk-inhibitors as novel anti-inflammatory strategy, and the implementation of multimodal postoperative rehabilitation (fast-track care) and minimally invasive surgery.

A clear implication of the data presented here is that it is of utmost importance to limit the amount of tissue damage. The identification of exact trigger that initiates the inflammatory response (mast cell activation, tissue damage, bacterial translocation) and more insight into how this inflammatory response spreads to the rest of the (unmanipulated) intestine should aid in the development and evaluation of therapeutics. Beyond this, we do not understand what controls the postoperative systemic inflammatory response and how this modulates the central nervous system and motility. Given the medical importance of the inflammatory response in postoperative ileus, these are key issues that must be addressed.
Recently, the group of Kalff discovered that besides the innate, also the adaptive immune system is triggered in response to bowel manipulation. It is suggested that this is mediated through the activation of dendritic cells that secrete IL-12 at the site of intestinal manipulation thereby stimulating memory Th1 cells in the inflamed muscularis, which subsequently egress into the systemic circulation to migrate to distant non-manipulated areas of the intestine. There, they release IFN-γ thereby inducing resident muscularis macrophages to produce NO and inflammatory cytokines. Depleting dendritic cells, the use of immunosuppressants such as anti-IL-12 antibodies or inhibiting Th1 cell migration by FTY-720 could reduce postoperative ileus, although the efficacy of these approaches still first have to be investigated in humans.

Taken together, a new avenue of potential targets for treatment has opened. Inhibiting intestinal macrophage or mast cell function by Syk modulation, or intervening in the adaptive immune response and systemic spread of inflammation might reduce the duration of postoperative ileus in patients following abdominal surgery. In addition interventions that activate the cholinergic anti-inflammatory pathway might also embody an attractive therapy.

From our work, it is becoming increasingly clear that new treatments of postoperative ileus should try to reduce the inflammatory response evoked by intestinal handling. Such anti-inflammatory strategies may however interfere with wound healing, the defense against micro-organism and ultimately with the clinical recovery of patients. Therefore it is important that before introduction of promising anti-inflammatory therapies in the clinic, their effect on wound healing in animal models is investigated. Another aspect that has to be taken into account when studying postoperative ileus is that the susceptibility to postoperative ileus following abdominal surgery increases with advancing age. There is both an age-dependent increase in the pro-inflammatory mediator expression and an age-dependent decrease in anti-inflammatory mediator expression following minor insult to the bowel in rodents. To what extent such imbalances between pro- and anti-inflammatory mechanisms may form the basis for increased susceptibility to ileus and for the increased severity and duration of ileus observed in the elderly is unknown. As more and more elderly will need abdominal surgery in the future, hopefully our insight on the role of altered inflammatory gene expression with advancing age in postoperative ileus will increase.

In summary, the data presented in this thesis have provided considerable new insight into the pathogenesis of postoperative ileus and have identified new therapeutic targets and strategies. Our work is also a direct plea for minimal invasive surgery as our data clearly indicate that intestinal handling and tissue injury during surgery should be avoided as much as possible.