General Introduction
Introduction

Postoperative ileus is an iatrogenic condition that occurs following abdominal surgery, characterized by a transient cessation of coordinated propulsive motility. The clinical manifestations include abdominal distention, nausea, vomiting, and inability to pass stools or tolerate a solid diet. Besides the discomfort experienced by patients, postoperative ileus is also an important risk factor for complications such as wound dehiscence and for pulmonary and thromboembolic complications. Current management strategies consist of perioperative anaesthetic and analgesic management, avoidance of nasogastric tube feeding and the use of supportive therapies. Although a variety of strategies have been proposed to reduce postoperative ileus, including feeding soon after surgery, early ambulation, epidural analgesia, fluid restriction, and minimally invasive surgery, none of these have been completely successful in preventing postoperative ileus. The treatments currently available are reviewed in Chapter 1.

Since the beginning of the 20th century, postoperative ileus has been recognized as a highly prevalent consequence of abdominal surgery. Initially, inhibition of gastrointestinal motility immediately after surgery was shown to primarily result from anaesthetics and opioid analgesics. In addition, evidence was provided that handling of the intestine during surgery activates inhibitory neuronal reflexes involving both adrenergic and non-adrenergic pathways and leads to intestinal oedema by excessive intravascular fluid loading. These events, however, fail to explain the prolonged inhibition of gastrointestinal motility seen during several days after abdominal surgery. At the end of the 20th century, the inflammatory-mediated ileus hypothesis was introduced, derived from data illustrating that inflammation of the intestinal muscularis externa is the main mechanism underlying postoperative ileus. Handling of the intestine during abdominal surgery activates resident innate immune cells located within the muscularis externa, triggering the release of inflammatory cytokines and chemokines, as well as increased expression of adhesion molecules on endothelial cells, which causes circulating leukocytes (mainly neutrophils and monocytes) to invade the muscularis externa. Invading monocytes and activated resident macrophages produce nitric oxide and prostaglandins, compromising the contractile activity. This inflammatory response has also been confirmed in human intestinal surgical samples and is now considered to mediate impaired contraction of handled and inflamed tissue. Postoperative ileus, however, is not restricted to the small intestine but involves the entire gastrointestinal tract. The mechanisms underlying the generalized inhibition of gastrointestinal motility in response to this local inflammation comprise a complex neuronal and immunological response.
involving activation of inhibitory neural pathways that affect the entire gut\textsuperscript{16} and production of inflammatory cytokines and nitric oxide by resident muscularis macrophages.\textsuperscript{14} In addition, it has recently been shown that also the adaptive immune system is triggered in response to bowel manipulation. This is mediated through the activation of dendritic cells with subsequent migration of T helper (T\textsubscript{H}) cells into the systemic circulation leading to inflammation to distant non-manipulated areas of the intestine.\textsuperscript{11}

Currently, intestinal manipulation of the intestine is generally used as a preclinical model of postoperative ileus. The technique used to manipulate the intestine is however highly variable and difficult to standardize, leading to large variations and inconsistent findings between different investigators. To overcome these problems, we decided to develop a new method for studying postoperative ileus in mice (Chapter 2). An important point of consideration was that the new technique of intestinal manipulation should be performed in a controlled manner, providing a reproducible model with small variation. To this end, we developed a device allowing application of a fixed pressure during intestinal manipulation. Using this device, we first examined the effect of graded manipulation on postoperative gastrointestinal transit by evaluating the intestinal distribution of orally gavaged fluorescein isothiocyanate (FITC)-labeled dextran postoperatively. Next, we compared this new technique to the widely used conventional manipulation technique, focusing on gastrointestinal transit, infiltration of myeloperoxidase positive cells and cytokine production in the muscularis externa of the intestine.

Despite the recent insights underlying the inhibition of gastrointestinal motility, the mechanisms involved in more severe postoperative ileus remain unclear.\textsuperscript{7, 17} Animal studies suggest that the severity of the ileus is linked to the extent of intestinal handling and the provoked tissue trauma.\textsuperscript{17-19} 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. Indeed, several studies have reported an increased postoperative inflammatory response related to increased operative trauma with systemic release of cytokines and systemic spread of the inflammatory response.\textsuperscript{18, 20, 21} Moreover, we found that the severity of ileus seems to be explained by an inflammatory response that is independent of the number of leukocytes infiltrating the small intestinal muscularis, indicating that other mechanism are responsible for the more severe cases of ileus. One possibility is that the severity of tissue trauma determines the intensity of the immune response and thus the severity ileus. Enhanced local inflammation will result in a more systemic inflammatory response with increased serum levels of pro-inflammatory cytokines. The latter will consequently affect distant regions of the gut and contribute to the generalized
aspect of postoperative ileus (Figure 1). Therefore our aim in Chapter 3 was to investigate the mechanism behind severe postoperative ileus by studying the local and systemic inflammatory response, including brain stem activation after different intensities of intestinal handling.

Figure 1 | Postoperative ileus caused by local surgical manipulation induces the influx of leukocytes into the intestinal muscularis. This inflammatory response activates visceral sensory afferents and brainstem nuclei such as the nucleus of the solitary tract (NTS). We hypothesized that intense manipulation triggers tissue damage and release of systemic inflammatory mediators that activate the area postrema (AP) in the brainstem.

We previously reported that mast cell activation may play an important role in triggering the inflammatory process underlying postoperative ileus. One of the earliest observations in rodent models is indeed the activation of mast cells and the subsequent release of mediators such as β-hexosaminidase and mMCP-1 in the peritoneal cavity. Moreover, W/Wv mutant mice that lack mast cells, fail to develop an intestinal infiltrate following intestinal manipulation. Reconstitution with wild-type mast cells on the other hand restores the capacity of mutant animals to recruit leukocytes to the intestine after surgery. Also in man, mast cell mediators are detected in peritoneal lavage fluid very early during surgery. Even very gentle inspection of the intestines at the beginning of the abdominal procedure increased the level of peritoneal tryptase. In contrast, patients undergoing a laparoscopic or a vaginal hysterectomy hardly showed an increase in tryptase. However, it remains unclear whether local release of mast cell mediators may directly activate the inflammatory cascade, or alternatively, that mast cells increase mucosal permeability shortly after intestinal manipulation. The latter may lead to bacterial translocation activating intestinal leukocytes with subsequent inflammation of the muscularis externa. Therefore, in Chapter 4, we further investigated the role of
mast cells in intestinal manipulation-induced barrier disruption using mast cell deficient mouse strains.

Previously de Jonge et al. have shown that the mast cell stabilizers ketotifen and doxantrazole reduced muscular inflammation and shortened postoperative ileus in our mouse model.\textsuperscript{22} This observation has led to a pilot study in which 60 patients undergoing abdominal surgery were treated with 4 or 12 mg ketotifen for 6 days.\textsuperscript{23} Although gastric emptying was statistically significant improved by ketotifen, no improvement of colonic transit was observed. These data suggest that more potent mast cell stabilizers might be more effective. One potential approach resulting in more potent mast cell stabilization might be blockade of the intracellular spleen tyrosine kinase (Syk). Syk is one of the critical tyrosine kinases involved in mast cell degranulation induced by IgE crosslinking. Crosslinking of the FceRI receptor causes phosphorylation of Syk subsequently activating intracellular pro-inflammatory pathways.\textsuperscript{24} Therefore, Syk inhibitors will suppress the signaling cascades that normally lead to degranulation of mast cells.\textsuperscript{25} Interestingly, inhibition of Syk signaling also diminishes macrophage activation.\textsuperscript{26-28} Hence, modulation of the Syk pathway may be a potential new therapeutic strategy for postoperative ileus. Therefore, in Chapter 5 we evaluated the effect of the Syk-inhibitor GSKcompound143 (GSK143) as potential future treatment to shorten postoperative ileus in patients. To this end we evaluated the effect of GSK143 on cultured peritoneal mast cells and bone marrow derived macrophages and subsequently tested its effect in our postoperative ileus mouse model.

An alternative approach to reduce the inflammatory response evoked by intestinal handling is electrical stimulation of the vagus nerve. During the past decade, the importance of the vagus nerve in modulating the immune system has been repeatedly demonstrated. In a rat model of sepsis,\textsuperscript{29} electrical stimulation of the vagus nerve was shown to have anti-inflammatory properties: it reduced tumour necrosis factor (TNF) levels and improved survival, an effect mediated by neuronal acetylcholine α7 receptors.\textsuperscript{30} 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 (\(\alpha7n\)AChRs) expressed on resident macrophages. The anti-inflammatory effect of the vagus nerve is part of a reflex by which the brain senses inflammatory information in the periphery through vagal afferents and subsequently creates an integrated anti-inflammatory response through vagal efferent fibres.\textsuperscript{31} This so-called cholinergic anti-inflammatory pathway is suggested to represent an additional system controlling the inflammatory response to a wide range of threats to the organism.\textsuperscript{31} Still, the presence of such a feedback loop (i.e. reflex) and its anatomical connections have not been demonstrated. Hence, in Chapter 6, we investigated whether the anti-inflammatory pathway is indeed a hard-wired neural circuit. As
such, we tested whether intestinal inflammation indeed triggers a vagus-nerve-mediated circuit leading to activation of vagal motor neurons in the brainstem connected to the inflamed intestine. To this end, a retrograde neuronal tracer was used to show neural connections from the intestine to the central nervous system.

Considerable progress has been made in understanding the mechanism behind postoperative ileus using experimental animals, and several translational studies show that the pathophysiological mechanisms described above also apply to humans. Hence, this new insight will ultimately lead to the development of new drugs to treat postoperative ileus. Obviously, these compounds will have to be tested in large clinical trials, obviating the need of validated outcome measures to assess their clinical efficacy. However, to date, parameters such as first defecation and flatus are often used as primary outcome parameters in clinical trials. Time to first flatus strongly depends on patient reporting whereas passage of stool might simply reflect rectal emptying and provides no reliable information on recovery of whole gut transit. Thus, with novel treatments for postoperative ileus in development, there is a definite need for more reliable outcome parameters in order to evaluate new treatments. In Chapter 7 we determined the relationship between clinical symptoms and gastrointestinal transit, assessed using scintigraphy, to identify clinical hallmarks associated with recovery of gastrointestinal transit in a large cohort of postoperative patients.

Postoperative ileus is a major determinant of recovery after colorectal surgery. Laparoscopic surgery and the implementation of an enhanced recovery after surgery program, also referred to as ‘fast-track’ perioperative care, are the two most important recent advances in modern surgical care. Both laparoscopic surgery and fast-track multimodal perioperative care have been reported to be safe and effective with earlier recovery of gastrointestinal function and less morbidity compared to open colorectal surgery and standard care. Clinical hallmarks of gastrointestinal function are less accurate and reliable to objectively evaluate the effect of different treatment strategies on postoperative ileus, as such clinical parameters not necessarily adequately reflect recovery of effective gastrointestinal motility. However, objective measures supporting faster gastrointestinal recovery are lacking. Up to date, scintigraphic recording of gastrointestinal transit is considered the gold standard. Therefore, in Chapter 8 we conducted a randomized double-blind study assessing gastrointestinal transit following open and laparoscopic colorectal surgery with or without fast-track care.
References