HEALING OF EXPERIMENTAL INTESTINAL ANASTOMOSES

Effects of analgetics and suture line reinforcement

Rozemarijn Junelle van der Vijver
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Healing of experimental intestinal anastomoses

Effects of analgetics and suture line reinforcement

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General introduction and outline of the thesis
**Anastomotic leakage**

Anastomotic dehiscence is the most feared and potentially devastating complication after gastrointestinal surgery. Dehiscence, with a reported incidence varying between 1 and 30% is attended by high morbidity while its mortality rate is believed to fall between 10 and 15%. ¹⁻⁴ Thus, there is much to gain by the identification of its causes and the development of preventive measures. Impairment of anastomotic healing is probably multifactorial. Many clinical studies have aimed to identify risk factors for impaired anastomotic healing ¹⁻⁷ but the evidence remains equivocal. The finding that anastomotic leakage takes place in the absence of any currently known risk factor, illustrates that much remains unknown. ⁸ Apparently, some anastomotic leaks are inevitable, despite proper caution and excellent surgical technique. ⁹ Leakage of intestinal anastomoses therefore remains the single most important hazard which can result in generalized peritonitis due to fecal spill into the abdominal cavity. Consequently, reoperation with or without dismantling of the failed anastomosis and the construction of a temporary ileo- or colostomy may be necessary. The patient has to deal with this challenge while still recovering from the initial operation. Protecting the anastomotic integrity and finding ways to minimize, postpone or prevent the consequences of leakage remain important goals of ongoing research.

**Wound healing**

Wound healing is an evolutionary conserved, complex, multi-cellular process that consist of a series of carefully regulated steps, which are initiated after disruption of the normal structure and function of tissue. The classic sequential, yet overlapping, phases of wound healing are inflammation, proliferation and remodeling. ¹⁰
The process involves the coordinated efforts of several cell types. The migration, infiltration, proliferation and differentiation of these cells will culminate in an inflammatory response and the formation of new tissue. This complex process is regulated by a signaling network involving numerous growth factors and cytokines. In the inflammation or latent phase platelets create hemostasis by formation of an insoluble fibrin clot. Inflammatory cells, mostly neutrophils are recruited to the wound site. Debris and bacteria are phagocytosed. Influx of neutrophils is followed by monocytes which differentiate into macrophages. After 2-3 days these regulatory cells predominate the infiltrate and release a multitude of tissue growth factors and proteolytic enzymes.

Proteolytic degradation of the extracellular matrix, to remove damaged tissue and provisional matrix and to allow angiogenesis and cell migration, is an intrinsic feature of repair. Matrix metalloproteinases (MMPs), a family of proteolytic enzymes that degrade specific components of the extracellular matrix, play multiple roles in wound healing. Inflammation gradually resolves and the proliferation phase takes over from around day 4. In this phase new matrix is produced by fibroblasts which become the predominant cell type. Newly-formed collagen restores tissue strength. Once the collagen fibrils are formed they mature by formation of crosslinks and organize, resulting in increased wound strength. This process requires adequate oxygenation and nutrient supply provided by angiogenesis.

During the last, remodeling or repair, phase cells eventually disappear and cell-rich granulation tissue becomes cell-poor scar tissue. Further reorganization of collagen fibers occurs.

Wound healing in the gastrointestinal tract
Most of the intestinal tract consist of four layers: mucosa, submucosa, muscularis (muscle layer) and serosa.

The strength of the intestinal wall is mainly derived from collagen fibrils located in the submucosal layer. When the intestinal tract is surgically disrupted and continuity is restored by construction of an anastomosis the repair process starts. It follows the general pattern of wound healing, which is essentially similar in all tissues, although there are some subtle differences. Immediately after operation, the anastomosis is far weaker than the intact tissues. During the first days after surgery, in the inflammation phase, the anastomosis remains very weak. Upregulation of matrix metalloproteinase (MMP) activity may even result in further loss of strength. During this phase anastomotic strength depends solely on the suture holding capacity of the submucosal and muscular layers. After three days, new collagen is synthesized by fibroblasts but also by smooth muscle cells and new matrix starts to form. During the proliferation phase anastomotic strength increases consistently to pre-operative values. After seven days anastomotic strength may surpass that of the adjacent intact bowel wall. From the short description above it follows that the risk of wound failure in the intestinal tract is greatest during the earliest phase of the repair cascade. It seems logical to suppose that adverse conditions have most chance to disrupt the sutured intestinal wall before collagen accumulation and matrix deposition have restored initial strength.

Analgesia
Optimal management of postoperative pain remains a topic of ongoing research because it directly relates to postoperative patient well-being and clinical outcome. Peri-operative analgesia is ever increasing and expanding in patients after gastrointestinal surgery. Surprisingly, in the search for risk factors which contribute to anastomotic leakage little attention has been paid to the use of analgetic drugs. In addition, relatively little is known from preclinical studies about their potential effects on wound repair in general and anastomotic repair in particular. The cornerstones of perioperative pain relief are paracetamol, non steroidal anti-inflammatory drugs (NSAIDs) and opioids.

Paracetamol
Paracetamol, acetaminophen, is considered an effective analgetic and antipyretic drug, which is very often and quite routinely administered for perioperative pain relief, also after gastrointestinal surgery. It is not quite clear which analgesic pathway is affected by the administration of this non-opiod drug, but multisite activity in the central nervous system has been suggested. Potential mechanisms include interaction with both the serotonergic and cannabinoid pathways. However, an anti-inflammatory effect has also been reported through inhibition
of cyclooxygenase (COX). With the exception of a few studies on the muscoskeletal system, little has been reported on the potential effects of paracetamol on wound healing and no data are available at all pertaining to the healing of intestinal anastomoses.

Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are often added to paracetamol therapy immediately after surgery or after cessation of other analgetics such as epidural analgesia or patient controlled analgesia, usually around day 3 after surgery. NSAIDs inhibit cyclooxygenase enzymes, which catalyze the first two steps in prostanoid biosynthesis. Prostanoids influence inflammatory and immune responses, as well as sensitizing and augmenting the pain pathway. COX enzymes occur as the isoforms COX-1 and COX-2. COX-1 is expressed constitutively in housekeeping functions, whereas COX-2 is expressed only in response to external stimuli and is postulated to be mainly involved in essentially pathological conditions such as inflammation, pain and fever. The inflammatory reaction constitutes the first phase of wound healing and is an essential link within the wound repair cascade. Inhibitors of the COX enzymes might therefore interfere with inflammation and wound healing. Indeed, negative effects of NSAIDs (COX inhibitors) on anastomotic healing have already been described but have not lead to changes in their clinical use. In fact in the Netherlands diclofenac and naproxen are frequently used NSAIDs used for pain relief immediately after intestinal (fast track) surgery.

Opioids

Paracetamol and NSAIDs decreases morphine consumption and therefore morphine-related adverse effects like inhibition of the intestinal motility. The influence of morphine on anastomotic healing has not been described. Chronic use of morphine treatment has been reported to inhibited early recruitment of both neutrophils and monocytes towards an inflammatory signal and suppresses angiogenesis in general wound healing.

Sealants

Reinforcement of the suture or staple line is one of the possible strategies to diminish the risk of perioperative complications. Applying some kind of seal around the intestinal anastomosis to prevent fecal content from leaking into the abdominal cavity and causing peritonitis seems a logical way to contain the effects of dehiscence of the intestinal wound. Fibrin based sealants are increasingly used in different fields of surgery to provide a sealing barrier, hemostasis and bond tissue together. Two frequently used seals are fibrin glue and fibrin-thrombin coated patch, which both consists of homologous plasma-derived fibrin products from pooled donors combined with bovine thrombin and mimic the final stages of blood coagulation and fibrin clot formation. Their benefits in the field of gastrointestinal surgery need to be established.

Studying the intestinal anastomosis in the rat

Animal models are frequently used in experimental and preclinical studies. In order to study the fundamental processes of healing of the intestinal anastomosis rats are used almost routinely, far more frequently than mice, rabbits or bigger animals. Anastomotic healing in the rat is believed to essentially represent the human situation. Obviously, it must always be kept in mind that one should exert caution in translating results and conclusions drawn from such studies to the clinical situation. In our research laboratory we have gained broad experience using rats as a model for anastomotic healing. The animals tolerate surgery and the construction of an anastomosis very well and measurement of the various parameters for repair are reproducible. Next to macroscopic signs of anastomotic leakage like dehiscence of the anastomosis, the presence of faecal peritonitis, a puncture in the anastomotic line with or without an abscess near it, measurement of anastomotic strength constitutes the key parameter to assess healing. The latter can be measured as either resistance to intraluminal (bursting pressure) or longitudinal pressure (breaking strength). Both bursting pressure and breaking strength may be measured sequentially on the same segment and are thus essentially independent parameters for wound strength. Next to these main outcome parameters biochemical parameters are also measured. Early wound strength depends on the capacity of the existing extracellular matrix to retain sutures and matrix strength is determined by the presence of collagen. As hydroxyproline is an unique component of collagen, hydroxyproline levels reflect the presence of collagen. Loosening or degradation of the matrix is believed to be mediated by MMPs, which enzymatic activities can also be measured. The gelatinases MMP-2 and MMP-9 have been shown to be upregulated in the healing anastomosis. Finally, (semi) quantitative histological analysis of cells and structures relevant to healing can be used to study the repair sequence in the rat intestine.

A model for anastomotic leakage

Next to unraveling the mechanism and risk factors of anastomotic leakage, the incidence of anastomotic dehiscence and the severity of its consequences ask for
ways to protect anastomotic integrity, particularly if intestinal continuity needs to be restored in conditions which have been associated with high leakage rates. The development of such procedures requires preclinical evaluation and thus an animal model for anastomotic dehiscence.

So far, the only known way to mimic a leaking anastomosis consists of the creation of an iatrogenic defect during its construction, mostly by using an insufficient number of sutures. However, ideally, a complete and patent anastomosis should be constructed which develops dehiscence with time. Such a model would be less artificial and clinically more relevant and would offer an unique possibility for translational research. In particular, it would allow to investigate the efficacy of means to prevent leakage or contain or postpone its effects, such as peroperative application of biological sealants. To our knowledge, there is no such experimental model available.

Outline of the thesis
With respect to the problems and questions outlined above we have addressed the following aspects relevant to anastomotic repair in the intestine. All studies have been performed in the well-established rat model.

- **Chapter 2**: the effects of paracetamol on anastomotic healing in ileum, colon and abdominal fascia.
- **Chapter 3**: the effects of the NSAIDs naproxen and diclofenac on ileal and colonic anastomoses.
- **Chapter 4**: the effect of the NSAID carprofen on anastomotic healing in ileum and colon.
- **Chapter 5**: further characterization of the negative effects of carprofen on ileal healing.
- **Chapter 6**: the effects of application of fibrin glue to the anastomosis on the course of wound repair in ileum and colon.
- **Chapter 7**: the effects of fibrin glue and a fibrin coated patch around a high-risk anastomosis in the ileum.

Reference List


(13) Marjanovic J, Hopt UT. [*Physiology of anastomotic healing*]. Chirurg 2011; 82(1):41-47.


Thesis

Paracetamol does not compromise early wound repair in the intestine or abdominal wall in the rat

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Abstract

**Background** Paracetamol is a cornerstone for peri-operative pain relief. Its mechanism of action may include a local anti-inflammatory effect with inhibition of cyclooxygenase isoenzymes. The scarce literature available on its effects on wound healing consists of preclinical studies into the effect of paracetamol on healing of the musculoskeletal system. Although the drug is used abundantly for pain relief after surgery of the gastro-intestinal tract, there are no published data on the influence of paracetamol on anastomotic and abdominal healing. This also holds for the, crucial, early inflammatory phase of repair. The recovery of wound strength could therefore conceivably be affected by paracetamol.

**Methods** In 78 male Wistar rats an anastomosis was constructed in colon and ileum. The rats received either a low or a high dose (50 or 200 mg/kg/day, divided over two doses) paracetamol or vehicle (controls) until they were killed on day 3 or 7 after surgery (n=13 each). In anastomoses, the main outcome parameters were two independent measures for wound strength, bursting pressure and breaking strength, the latter being the primary outcome parameter. In addition, collagen levels were measured and histology was performed. In fascia, breaking strength was analysed.

**Results** No significant differences were found between control and paracetamol-treated groups at any time point for any of the parameters. Wound strength increased significantly from day 3 to day 7 in all groups. In the colon anastomosis the breaking strength increased from 130 ± 9 g (mean ± SEM) at day 3 to 232 ± 17 g at day 7 in the control group, from 144 ± 10 to 224 ± 9 g in the low dose group and from 130 ± 12 to 263 ± 28 g in the high dose group. The lower limit for the 95% CI was -11 for the difference between control and low dose groups at day 3 and -25 for the difference between control and high dose groups. No differences in collagen levels were found between the high dose and control groups. Histology did not indicate the presence of gross differences between groups.

**Conclusion** Peri-operative use of paracetamol in a rat model of intestinal surgery does not significantly impede wound repair in the early postoperative period.

Introduction

Optimal management of postoperative pain remains a topic of ongoing research because it directly relates to postoperative patient well-being and clinical outcome. Paracetamol, acetaminophen, is considered an effective analgetic and antipyretic drug that is a cornerstone in peri-operative pain relief, also in patients who have to go through intestinal surgery. Paracetamol decreases morphine consumption and therefore morphine-related adverse effects. With the exception of a few studies on the musculoskeletal system, surprisingly little has been reported on the potential effects of paracetamol on wound healing.

It is not quite clear which analgesic pathway is affected by the administration of this non-opoid drug, but multisite activity in the central nervous system has been suggested. Potential mechanisms include interaction with both the serotonergic and cannabinoid pathways. However, an anti-inflammatory effect has also been reported through inhibition of cyclooxygenase (COX) isoenzymes. The inflammatory reaction constitutes the first phase of wound healing and is an essential link within the wound repair cascade. Inhibitors of the COX enzymes might interfere with inflammation and wound healing. Indeed, negative effects of NSAIDs (COX inhibitors) on anastomotic healing have already been described.

Although the drug is used abundantly for pain relief after surgery of the gastro-intestinal tract, there are no published data on the influence of paracetamol on anastomotic and abdominal healing. This also holds for the, crucial, early inflammatory phase of repair. If paracetamol affects inflammation it conceivably could interfere with the recovery of wound strength. Therefore, we examined the effects of paracetamol on early healing in ileum and colon anastomoses and the abdominal wall of the rat.

Methods

**Study Design**

Seventy eight male Wistar rats (Harlan BV, Horst, The Netherlands) were housed 2 per cage and accustomed to laboratory conditions for five days before the start of the experiment. They were randomly divided over three groups of 26 animals each, one control group and two experimental groups. At day 0 all rats underwent intestinal resection and anastomoses were constructed in both colon and ileum. The two experimental groups received different doses (low and high) of paracetamol daily from operation onwards. Rats were observed closely and weighed daily and had free access to water and standard rodent chow (Hope Farms, Woerden, The Netherlands) throughout the entire experimental period. Half of the rats within
each group were killed at day 3 and day 7 after operation (Table 1). The Animal Ethics Review Committee of the Radboud University Nijmegen approved the study. The study was conducted in a manner that does not inflict unnecessary pain or discomfort upon the animal, as outlined by the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals (1996), prepared by the National Academy of Sciences Institute for Laboratory Animal Research.

Surgery and analgesics

Procedures were performed under semi sterile conditions using a Zeiss operation microscope (Carl Zeiss AG, Oberkochen, Germany). Animals were anesthetized by use of a mixture of isoflurane, oxygen and nitrogen, while breathing spontaneously through a mask. A midline laparotomy was performed and in each rat a 1-cm segment was resected from the descending colon 3 cm proximal to the peritoneal reflection. Colonic continuity was restored by constructing an end-to-end anastomosis with 8 single-layer, inverting, interrupted sutures (Ethilon 8-0; Ethicon, Norderstedt, Germany). A similar procedure was performed in the distal ileum, 15 cm proximal to the cecum. The abdominal wall was closed with a running suture (Vicryl 3-0; Ethicon, Norderstedt, Germany). The skin was closed with staples. During operations, body temperature was kept at 38°C using a heating pad and a lamp. Intestines were covered with gauze pads soaked with 0.9% NaCl to minimize desiccation. To prevent dehydration, 10 ml of 0.9% NaCl was administered subcutaneously after the operation.

All rats were administered buprenorphine (Temgesic, Schering Plough, Houten, the Netherlands), 0.02 mg/kg subcutaneously, every 12 hours (5 times in total) for 48 hours, the first dose just prior to surgery. The first experimental group received acetaminophen (Paracetamol, Sigma-Aldrich Chemistry, Steinheim, Germany) in a dose of 50 mg per kg each day (low dose) by oral gavage. The daily dose was divided in two parts which were given at least eight hours apart during the experimental period and starting just prior to surgery. The second experimental group (high dose) was given 200 mg/kg paracetamol per day. Paracetamol was dissolved in 0.5 ml 0.9% NaCl and 0.1% polysorbate 20 (Tween 20, Fluka Chemika, Buchs, Switzerland), the control group received 0.5 ml of this solution (vehicle) per gavage twice a day.

Necropsy and analysis of wound strength

Ten rats from each group were killed by CO2 asphyxiation on postoperative day 3. (Table 1) At day 7, another 10 rats were killed for wound strength analysis by cardiac puncture and cervical dislocation, in order to allow blood sampling (see below). Adhesions were dissected carefully without manipulation of the anastomosis. Segments of 2 cm length, containing the anastomoses in the middle, were resected with sutures left in place. The segments were placed over a plastic tube and secured with a vessel loop on one end and closed with a clamp on the other side. To measure bursting pressure, the segments were infused (2 ml/min) with 0.9% NaCl containing methylene blue. The maximum pressure (mmHg) recorded immediately before sudden loss of pressure was taken as the bursting pressure. The site of rupture (within or outside the anastomotic line) was noted. Subsequently, the same segments were placed in a tensiometer, and the breaking strength (g) was measured. A rectangle (approximately 1 x 2 cm) containing the laparotomy wound in the middle was carefully cut out from the abdominal wall. After removing the running suture the segment was cut in half to create two samples with the midline laparotomy wound in the middle. The length of the laparotomy wound in each sample was noted before it was placed in the tensiometer. Subsequently, the breaking strength was measured and expressed in g/mm tissue by using the mean value obtained from both measurements. The anastomotic segments and abdominal wall were carefully cleaned from any adhering tissue and 5 mm samples, containing the suture line in the middle, were frozen in liquid nitrogen and stored at -80 °C until further processing.

Biochemical analysis

After weighing, tissue samples were frozen, lyophilized and pulverized. The hydroxyproline content, as a measure of the collagen content, was measured in the control and high dose groups by high-performance liquid chromatography (HPLC) after hydrolysis with 6 N hydrochloric acid and coupling to dabsyl-chloride. The detection limit of the assay is 0.25 μg and intra assay and inter assay coefficients of variation (at 6 μg levels) are 2.3 % and 10.3 %, respectively. In animals killed at day 7 blood was collected 1 h after the last gift of paracetamol (or vehicle) and immediately prior to killing the animals. Paracetamol levels were measured in heparin plasma by the Cobas Integra® (Roche) system. Detection limit is 0.2 mg/l.

Histology

The remaining 14 animals (Table 1) were killed as described above and used for descriptive histology. Intestinal samples of approximately 1 cm containing the entire anastomosis in the middle were carefully collected en bloc, opened at the mesenteric side, washed gently in 0.9% and spread out in a cassette for paraffin-embedding. From paraffin-embedded tissues, 4 μm sections were prepared and stained with hematoxylin and eosin (H&E). Sections were analyzed by using a binocular light microscope.
Statistics

Historical data from our own group show the breaking strength of colonic anastomoses at day 3 typically to be 117 ± 27 (SD) g. Sample size was determined as sufficient to detect a loss of wound strength of 30 g (25%). Using an α of 0.05, a power of 0.8 and an one-tail test the group size is calculated (G*Power 3.1.2) to be 10 (actual power 0.84).

All data passed the normality test (Kolmogorov-Smirnov, p>0.10). Comparison between control and both experimental groups was performed using a One-way Analysis of Variance (ANOVA). Comparisons between two groups (hydroxyproline content) or, within one group, between values at two different days were performed with a one-tailed unpaired t-test. The lower limit of the (one-sided) 95% confidence interval (CI) for the difference between each of the 2 paracetamol groups and the control group was determined using a t-test, with Fisher LSD procedure. (see Table 1 of Hayter, 1986) Results were considered statistically significant at p<0.05.

Results

General observations and paracetamol plasma concentrations

Four animals died prematurely. One animal from the low dose group did not survive the surgery. Two animals (one from the control group and one from the high dose group) were taken out of the experiment because of extreme weight loss and poor clinical condition. Both showed signs of ileus at obduction, but the anastomoses were all intact and conductant. One animal from the low dose group died from self mutilation on day 2 (Table 1).

At operation the rats weighed between 250 and 295 g (average 286 g) without differences between groups. All animals experienced transient postoperative weight loss which was maximal at day 3-4 and averaged approximately 9 % in all groups. At day 7 the mean (± SEM) relative body weight (compared to the preoperative weight) was 98 ± 1.2 %, 95 ± 2.1 % and 97 ± 0.9 % in controls, low dose and high dose groups, respectively. There were no signs of wound dehiscence of the laparotomy wound. No macroscopic signs of anastomotic leakage were found. Paracetamol levels were determined in plasma collected on day 7 one hour after drug administration. Average values (n=4, ± SEM) were 0 ± 0 mg/l, 0.4 ± 0.4 mg/l and 22.7± 6.5 mg/l in the controls and low and high dose groups, respectively.

Wound strength

In the control and both paracetamol groups the anastomotic bursting pressure remained low after 3 days and increased sharply (p<0.0001, both in ileum and in colon) thereafter (Figure 1). At day 7, in all groups, the bursting site had shifted

<table>
<thead>
<tr>
<th>Table 1 Experimental groups</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Control</td>
</tr>
<tr>
<td>Operated at day 0</td>
</tr>
<tr>
<td>Premature death (day)</td>
</tr>
<tr>
<td>Terminated at day 3 for biochemical analysis</td>
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<tr>
<td>Terminated at day 3 for histological analysis</td>
</tr>
<tr>
<td>Terminated at day 7 for biochemical analysis</td>
</tr>
<tr>
<td>Terminated at day 7 for histological analysis</td>
</tr>
</tbody>
</table>

Number of animals operated on day 0 and premature deaths. At day 3 and 7 10 animals in each group were analyzed for wound strength and the remaining animals for histology.

Figure 1 Anastomotic bursting pressure. Individual values and means (horizontal bars) are given for ileal (A) and colonic (B) anastomoses in both control (C) low dose (L) and high dose (H) groups. The bursting site was either within (●) or outside (○) the suture line.
On day 3, the strength of the abdominal fascia was only just measurable and varied widely between groups, e.g. 3.2 ± 0.7 g/mm in controls and 1.9 ± 0.5 and 4.8 ± 2.4 g/mm in low dose and high dose groups, respectively. In all groups, it increased manyfold (p<0.0001) until day 7 where control values averaged 87 ± 10 g/mm (Figure 2C). Paracetamol treatment did not affect wound breaking strength in any consistent or dose-dependent way: no significant differences (ANOVA) were found between control, low and high dose groups on either day. Table 2 shows average values together with the lower limit of the 95% CI for the difference between controls and paracetamol-treated groups, demonstrating that the probability for true loss of strength is quite limited.

Collagen and histology

Hydroxyproline levels were measured in anastomotic samples from the control and high dose groups. In both groups, the hydroxyproline content, expressed as μg/5 mm, increased significantly (p<0.05) from day 3 to day 7 (Figure 3). There was no significant difference between groups on day 3 or day 7, neither in the ileum nor in the colon. The same was true for the wound hydroxyproline concentrations, expressed as μg/mg dry weight (data not shown).

Although the number of animals available for histological analysis was limited, no gross differences between paracetamol treated rats and control rats were observed in the architecture of either the anastomosis in ileum and colon or the abdominal wound (not shown). Figure 4 illustrates normal healing and closing of the mucosal gap in the ileum between day 3 and 7 after surgery in the control and high dose animals.

Table 2 Wound breaking strength

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Low dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Mean</td>
<td>95% CI *</td>
</tr>
<tr>
<td>Colon</td>
<td>day 3</td>
<td>130</td>
<td>-11</td>
</tr>
<tr>
<td></td>
<td>day 7</td>
<td>232</td>
<td>-54</td>
</tr>
<tr>
<td>Ileum</td>
<td>day 3</td>
<td>59</td>
<td>-31</td>
</tr>
<tr>
<td></td>
<td>day 7</td>
<td>92</td>
<td>-8</td>
</tr>
<tr>
<td>Abdomen</td>
<td>day 3</td>
<td>3.2</td>
<td>-4.9</td>
</tr>
<tr>
<td></td>
<td>day 7</td>
<td>87</td>
<td>-28</td>
</tr>
</tbody>
</table>

Data represent mean values in the anastomoses (g) and abdominal wall (g/mm). * represents the lower limit of the (one-sided) 95% confidence interval for the difference between each of the paracetamol groups and the control group (t-test, Fisher LSD procedure).

Collagen and histology

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Although the number of animals available for histological analysis was limited, no gross differences between paracetamol treated rats and control rats were observed in the architecture of either the anastomosis in ileum and colon or the abdominal wound (not shown). Figure 4 illustrates normal healing and closing of the mucosal gap in the ileum between day 3 and 7 after surgery in the control and high dose animals.
Discussion

Paracetamol does not have a detrimental effect on the strength of the healing intestinal anastomosis in the ileum or colon of the rat within the first seven days after operation. Also the strength of the abdominal fascia is not compromised by paracetamol. The main outcome parameters to assess anastomotic healing in the intestine relate to mechanical strength. While the bursting pressure is a measure of the capacity to withstand intraluminal pressures the breaking strength represents longitudinal strength. Both can be measured sequentially on the same segment and are thus essentially independent parameters for wound strength. Since the breaking strength always represented the true strength of the suture line (while at day 7 the bursting site was often outside the wound area) it is considered to be the primary outcome parameter.

The first days after operation wound strength remains low and chances for dehiscence are believed to be highest. From 3 days onwards collagen deposition increases and therefore wound strength increases. Thus, the first postoperative week is most crucial to undisturbed healing and therefore measuring points were set at day 3, when anastomotic strength is lowest, and day 7, where strength should be considerably elevated.

Wound breaking strength, as the primary outcome parameter, did not change in any consistent or dose-dependent way by the administration of paracetamol. No significant differences existed between groups and analysis of the 95% CI for differences between control and paracetamol treated groups (Table 2) demonstrated the probability for considerable, and possibly clinically relevant, loss of strength to be very low. Although at day 3 the average bursting pressure in the ileum was below that of the controls in both paracetamol treated groups, this effect remained non-significant. Moreover, the bursting pressure increased sharply thereafter. Altogether, analysis of the parameters for wound strength and collagen levels in anastomoses and abdominal wounds did not reveal any differences, even when paracetamol was given at a high dose.

The scarce literature available consists of studies describing the effect of paracetamol on healing of tissues such as alveolar bone, femur, ligament or the patellar tendon. Here, paracetamol was given once a day or through the chow, while we divided the daily dose in two parts which were given at least 8 h apart. More importantly, the studies mentioned above examine repair at least 14 days after operation when the inflammatory phase of healing should long be over. For that reason, we studied parameters for repair at day 3, characterized by low wound strength during the inflammatory phase, and at day 7, characterized by increasing strength during the early proliferative phase. Presumably, wounds are most sensitive to disruption as their mechanical strength is lowest.

Figure 3 Hydroxyproline content of anastomotic segment. Bars represent mean and SEM in ileal and colonic segments from the control groups (grey bars) and the groups which received a high dose of paracetamol (white bars) on day 3 and 7. Asterix denote significant (p<0.05) difference between values obtained at day 3 and day 7 within the same group.

Figure 4 Anastomotic histology in the ileum. Each panel shows a tissue segment with the anastomosis in the middle and the mucosal layer at the bottom at a magnification of approximately x 40, representing typical examples obtained in the control group at day 3 (A) and day 7 (B) and in the high dose group at day 3 (C) and day 7 (D). In each figure an arrow identifies a typical finding. In A the mucosal villi of the intestine is pointed out. In B a suture is marked. In C the inflammation, shaped as a triangle, is marked. Note that is fills in the anastomosis. In D the submucosal muscular layer of the intestine is marked.
Although the minimum plasma paracetamol level required for analgesia is believed to be 10-20 mg/l \(^{13}\), the concentrations measured in humans 80 min after 1 g oral paracetamol were substantially lower and demonstrated to range between 0 and 13 (median 5) mg/l. \(^{13}\) The low dosed rats were administered 50 mg/kg of paracetamol a day, which compares on a per-weight basis with the maximum recommended human dose of 4 g. This dosage is based on studies examining the effect of paracetamol on wound healing and dose-related effect on hyperalgesia in the rat. \(^{15,13}\) Some studies on other processes in rodents \(^{31,32}\), although fairly dated, report a weak anti-inflammatory effect with higher doses of paracetamol.

The group receiving 200 mg/kg of paracetamol a day was therefore included to ensure any possible effect on wound healing would be noticed, even if it occurred after administration of supranormal dosages. Plasma concentrations in this group were comparable to those found after 2 g orally given to patients. \(^{17}\) The mechanism behind the suggested anti-inflammatory effect of paracetamol remains speculative. Paracetamol has periodically been proposed to inhibit one or more of the cyclooxygenase enzymes COX-1 and COX-2. \(^{4,21,25}\) COX enzymes catalyse conversion of arachidonic acid to prostanooids involved in inflammation. However, paracetamol generally lacks the other local effects of NSAIDs on platelet aggregation and gastric mucous production. This could be explained if paracetamol acts only centrally, and not peripherally, as a COX inhibitor. \(^{30}\) Perhaps this is why paracetamol does not influence the peripherally located processes of anastomotic and abdominal wall healing.

Anastomotic leakage remains a feared and potentially devastating complication after gastrointestinal surgery. A leaking anastomosis is associated with increased morbidity and considerable mortality. \(^{27,28}\) Many clinical studies have aimed to identify risk factors for impaired anastomotic healing, but so far very little attention has been paid to the use of analgetic drugs. The finding that anastomotic leakage takes place in the absence of any currently known risk factor, illustrates that much remains unknown. \(^{19}\) Since paracetamol is widely prescribed to patients who need construction of an intestinal anastomosis, there should be no doubt as to its safety regarding the development of anastomotic strength. The present report supplies the first preclinical data which demonstrate that the peri-operative use of paracetamol does not affect the progression of wound repair in the intestine or the abdominal wall in its most crucial early postoperative period.

Reference List


Diclofenac causes more leakage than naproxen in anastomoses in the small intestine of the rat

International Journal of Colorectal Disease
2013 Feb 10. Epub ahead of print
Abstract

Background Non steroid anti-inflammatory drugs (NSAIDs) such as the cyclooxygenase isoenzyme inhibitors diclofenac and naproxen are increasingly used for perioperative pain relief, while their potential effects on wound healing are scarcely investigated.

Methods In 104 male Wistar rats an anastomosis was constructed in both colon and ileum. The rats were divided into groups who received diclofenac (4 mg/kg/day) or naproxen (10 mg/kg/day) daily from the day of surgery or from day 3 after surgery. Animals were killed on day 3 or 7 and analysed for signs of anastomotic dehiscence and wound strength of anastomoses and abdominal fascia.

Results Anastomotic leakage in the ileum (p<0.0001) and mortality rates (p=0.001) were significantly increased in the diclofenac group. On day 7 the anastomotic bursting pressure in the ileum remained below that of the controls in the diclofenac and naproxen treated rats. When administration of diclofenac was postponed to day 3 after surgery, anastomotic dehiscence was almost absent. The colonic anastomosis and abdominal wall always remained unaffected.

Conclusion This study implies that immediate postoperative administration of diclofenac and, to a far lesser extent, naproxen can affect healing in the ileal anastomosis in the rat. This negative effect can be prevented by a short postoperative delay in administration. Non steroid anti-inflammatory drugs such as the cyclooxygenase isoenzyme inhibitors diclofenac and naproxen are increasingly used for perioperative pain relief, while their potential effects on wound healing are scarcely investigated.

Introduction

Non steroidal anti-inflammatory drugs (NSAIDs) are cornerstones in peri-operative pain relief because of their analgetic and anti-inflammatory features. NSAIDs decrease morphine consumption and therefore morphine-related adverse effects. NSAIDs inhibit cyclo-oxygenase (COX) enzymes, which catalyze the first two steps in prostanoid biosynthesis. These enzymes occur as isoforms COX-1 and COX-2. COX-1 is expressed constitutively in different cells and tissues, whereas COX-2 is expressed mainly in response to external stimuli and is postulated to be involved in essentially pathological conditions such as inflammation, pain and fever. Selective inhibitors of COX-2 supposedly allow specific targeting of inflammatory disease processes, without disruption of normal homeostatic mechanisms that account for side-effects of non-selective NSAID therapy. While the potency and selectivity of the various NSAIDs available vary widely, even a selective COX-2 inhibitor may also inhibit COX-1 to some extent, depending on its dosage.

Anastomotic dehiscence is the most feared complication after gastro-intestinal surgery. It is attended by high morbidity with a reported incidence varying between 1 and 30% 3-7, while its mortality rate is believed to fall between 10 and 15%. Any factors interfering with early anastomotic repair might increase the chances for dehiscence. Many of the patients who undergo gastro-intestinal surgery, receive NSAIDs for pain relief in the early postoperative period. If COX inhibitors interfere with inflammation they might also affect wound healing, since the inflammatory reaction constitutes the first, essential phase of the wound repair sequence. Indeed, negative effects of different NSAIDs on anastomotic healing have already been described. In the Netherlands diclofenac and naproxen are the NSAIDs most frequently used for pain relief immediately after intestinal (fast track) surgery. Sometimes they are administered after cessation of other analgetics such as epidural analgesia or patient controlled analgesia, usually around day 3 after surgery. Incidental and conflicting preclinical data have been published on the effects of diclofenac on intestinal repair 11-13, while data for naproxen are completely lacking. This study aims to comprehensively describe the effects of diclofenac and naproxen on early healing of anastomoses in the ileum and colon and abdominal wounds in the rat.

Methods

Study Design

Hundred and four male Wistar rats weighing 250-295 g (Charles River, Sulzfeld, Germany) were housed 2 per cage and accustomed to laboratory conditions for 5
days before the start of the experiment. They were randomly divided over 5 groups, a control group (C, n=24) and 4 experimental groups: a diclofenac group (D, n=28), a naproxen group (N, n=28) and 2 groups in which the administration of the COX-inhibitor was delayed until the 3rd day after surgery, the diclofenac delay (Dd, n=12) and naproxen delay (Nd, n=12) groups, respectively. The rats in the control, diclofenac and naproxen groups were scheduled to be killed on day 3 (10 in the C group and 12 in the D and N groups each) or day 7 (14 in the C group and 16 in the D and N groups each) after surgery, while all rats in de diclofenac delay and naproxen delay groups were scheduled for termination at day 7. The calculation of group size is given below.

All rats underwent intestinal resection and anastomoses were constructed in both colon and ileum. Rats were observed and weighed daily and had free access to water and standard rodent chow (Hope Farms, Woerden, The Netherlands) throughout the entire experimental period. The Animal Ethics Review Committee of the Radboud University Nijmegen approved the study.

Surgery and analgesics

Procedures were performed under semi sterile conditions using a Zeiss operation microscope (Carl Zeiss AG, Oberkochen, Germany). Animals were anesthetized by use of a mixture of isoflurane, oxygen and nitrogen, while breathing spontaneously through a mask.

A midline laparotomy was performed and in each rat a 1-cm segment was resected from the descending colon 3 cm proximal to the peritoneal reflection. Colonic continuity was restored by constructing an end-to-end anastomosis with 8 single-layer, inverting, interrupted sutures (Ethilon 8-0; Ethicon, Norderstedt, Germany). A similar procedure was performed in the distal ileum, 15 cm proximal to the cecum. The abdominal wall was closed with a running suture (Vicryl 3-0; Ethicon, Norderstedt, Germany). The skin was closed with staples. During operations, body temperature was kept at 38°C using a heating pad and a lamp. Intestines were covered with gauze pads soaked with 0.9% NaCl to minimize desiccation. To prevent dehydration, 10 ml of 0.9% NaCl was administered subcutaneously after the operation.

All rats were administered buprenorphine (Temgesic, Schering Plough, Houten, the Netherlands), 0.02 mg/kg subcutaneously, every 12 hours (5 times in total) for 48 hours, the first dose just prior to surgery. Diclofenac (Diclofenac (sodium salt) Cayman Chemical Company An Arbor, MI, USA) was given in a dose of 4 mg/kg a day and naproxen (Naproxen sodium, Sigma Aldrich Chemie, Steinheim, Germany) in a dose of 10 mg/kg a day, both by oral gavage. The daily dose was divided into two parts which were administered (in a volume of 0.5 ml and dissolved in 0.1% polysorbate in saline) at least eight hours apart during the experimental period and starting immediately prior to surgery. The control rats received 0.5 ml vehicle per gavage twice a day. Rats that were administered diclofenac or naproxen delay received 0.5 ml of vehicle from operation up to day 3 when they switched to the drug regimen.

Necropsy and analysis of wound strength

The rats were killed by CO/CO₂ asphyxiation on postoperative day 3 or day 7 respectively. The abdomen was inspected and in particular attention was paid to signs of anastomotic leakage such as macroscopic dehiscence of the anastomosis, the presence of faecal peritonitis, a puncture in the anastomotic line with or without an abscess near it. Adhesions were dissected carefully without manipulation of the anastomosis. Segments containing the anastomoses were resected. To measure bursting pressure, the segments were infused (2 ml/min) with 0.9% NaCl containing methylene blue. The maximum pressure (mmHg) recorded immediately before sudden loss of pressure was taken as the bursting pressure. The site of rupture (within or outside the anastomotic line) was noted. Subsequently, the same segments were placed in a tensiometer, and the breaking strength (g) was measured. 17 A rectangle containing the laparotomy wound in the middle was carefully cut out from the abdominal wall. After removing the running suture the segment was cut in half to create two samples with the midline laparotomy wound in the middle. The length of the laparotomy wound in each sample was noted before it was placed in the tensiometer. Subsequently, the breaking strength was measured and expressed in g/mm tissue by using the mean value obtained from both measurements.

The anastomotic segments and abdominal wall were carefully cleaned from any adhering tissue and 5 mm samples, containing the suture line in the middle, were frozen in liquid nitrogen and stored at -80 °C until further processing.

Biochemical analysis

After weighing, tissue samples were frozen, lyophilized and pulverized. The hydroxyproline content, as a measure of the collagen content, was measured by high-performance liquid chromatography (HPLC) after hydrolysis with 6 N hydrochloric acid and coupling to dabsyl-chloride. Preparation of tissue extracts for gelatin zymography, using a buffer containing 1% (v/v) Triton X-100, has been described elsewhere. 18 The protein concentration of the extracts was measured using the bicinchoninic acid reagent. All tissue samples were stored at -80°C until zymography. The technique of preparation and electrophoresis of the gels and quantification of both the active and latent forms of gelatinases ((pro)-MMP-2 and (pro)MMP-9), which were expressed as arbitrary units.
on the basis of the lysed area, using a Sharp Jx-330 scanner and Imagemaster 1D software (Amersham Pharmacia, Uppsala, Sweden) has been described previously. 18

Histology
The remaining 4 animals in the N, D and C groups on day 7 were killed as described above and used for histological observation. Intestinal samples of approximately 1 cm containing the entire anastomosis in the middle were carefully collected en bloc, opened at the mesenteric side, washed gently in 0.9% and spread out in a cassette for paraffin-embedding. From paraffin-embedded tissues, 4 μm sections were prepared and stained with hematoxylin and eosin (H&E). Sections were analyzed by using a binocular light microscope.

The presence of COX-1 and COX-2 protein in healing anastomoses was visualized by immunohistochemistry. From the paraffin-embedded tissues 4 μm sections were prepared and stained with COX-1 and COX2 antibodies as described previously. 14 After dewaxing, endogenous peroxidase activity was blocked with 3% H2O2 in phosphate-buffered saline. Antigen retrieval was performed by heating the sections in a citrate buffer solution. After preincubation with 20% normal goat serum, the sections were incubated overnight with a 1:1000 dilution of COX-1 or COX-2 antibody (rabbit polyclonal antiserum against human COX-1 and COX-2. Cayman Chemical, An Arbor, MI, USA). Subsequently, sections were incubated with a 1:200 dilution of goat anti-rabbit biotinylated secondary antibody. Following incubation with avidin-biotin complex, protein was visualized with diaminobenzidine. Sections were counterstained with haematoxylin. Another set of longitudinal sections from the control, diclofenac and naproxen group were stained with picrosirius red to identify collagen fibers in the anastomotic area collagen was quantified by digital image analysis. 19

Statistics and group size
Sample size was determined in order to detect a loss of wound strength of 25%. Historical data from our own group show the breaking strength of colonic anastomoses at day 3 typically to be 117 ± 27 (SD) g and 232 ± 57 (SD) g at day 7 in the control groups. Using an α of 0.05, a power of 0.8 and a one-tail test the group size is calculated (G’Power 3.1.2) to be 10 (actual power 0.81). To compensate for an expected mortality rate of 15% in rats that are administered a COX-2 inhibitor we added 2 animals to the four experimental groups. Furthermore we added 4 animals per group in the C, D and N groups to examine histology at day 7. Comparison between control and several experimental groups was performed using a One-way Analysis of Variance (ANOVA) followed by a Tukey-Kramer post test. Comparisons between 2 groups or, within one group, between values at 2 different days were performed with a one-tailed unpaired t-test. Results were considered statistically significant at p<0.05.

Results
Clinical observations and mortality
Seven animals in the diclofenac group died prematurely of dehiscence of the ileal anastomosis, significantly more so than in the control group (Table 1). Also, signs of anastomotic leakage were far more frequent (p<0.0001) in the diclofenac group. In the naproxen group only 1 animal died and four animals showed signs of anastomotic leakage. In the diclofenac delay group one rat died of ileal dehiscence on day 6, while no animals died in the naproxen delay group. In the latter groups no further signs of anastomotic leakage were observed.

<table>
<thead>
<tr>
<th>Table 1 Mortality and anastomotic leakage</th>
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<tbody>
<tr>
<td>Number of rats</td>
</tr>
<tr>
<td>Control (C)</td>
</tr>
<tr>
<td>Diclofenac (D)</td>
</tr>
<tr>
<td>Naproxen (N)</td>
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<tr>
<td>Diclofenac delay (Dd)</td>
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<td>Naproxen delay (Nd)</td>
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Differences between the experimental groups and the control group were analysed for significance using Fisher’s exact test: * p < 0.0001 and # p = 0.0114.

All animals experienced transient weight loss after operation. In controls, weight loss was maximal at day 3, while the animals in the diclofenac and naproxen groups lost some more weight afterwards. As a consequence, there was a significant (p<0.05, ANOVA) difference between groups from day 4 onwards (Figure 1). Post-tests revealed no difference between controls and groups where drug administration was delayed.
Wound strength

On day 3 the mean bursting pressure in the ileum anastomoses was lower in the diclofenac group, 49 ± 10 (SEM) mm Hg, than in the control and naproxen groups, which were 61 ± 6 and 61 ± 9 mm Hg, respectively (ANOVA: ns). On day 7 bursting pressures had risen considerably and significantly in these three groups (diclofenac group 215 ± 73 mm Hg, control group 311 ± 11 mm Hg and naproxen group 233 ±24 mm Hg), but only four animals remained in the diclofenac group. If values for the control, diclofenac and naproxen groups were analysed by ANOVA differences were nearly significant (p=0.067), due to the fact that the mean value in the diclofenac group was lower than in the others. If all groups, including those where drugs were withheld until day 3, were analysed by ANOVA the p-value was 0.0009. Post-testing demonstrated significantly higher values in the diclofenac (337 ± 11 mmHg) and naproxen delay (334 ± 16 mm Hg) groups than in the groups where drugs were started at the day of operation (Figure 2a).

The average bursting pressure of the colonic anastomosis at day 3 was 66 ± 3, 78 ± 9 and 63 ± 8 mm Hg in the control, diclofenac and naproxen groups, respectively (not significantly different). At day 7 the bursting pressure had risen to 199 ± 12, 249 ± 8 and 154 ± 25 mm Hg in the control, diclofenac and naproxen groups, respectively, and was 242 ± 13 and 193 ± 28 mm Hg in the diclofenac and naproxen delay groups (figure 2b). Comparison between the five groups together demonstrated significance (ANOVA: p=0.049), without any significant differences between any of the groups after pair-wise post-testing.

Analysis of the anastomotic breaking strength (figure 3) revealed a significant gain in strength between day 3 and day 7 and an absence of differences between groups, both at day 3 and day 7 in either ileum or colon. Finally, the strength of the abdominal fascia was very low 3 days after operation and increased manyfold afterwards, in a similar fashion in control, diclofenac and naproxen groups. Delay of drug administration did not lead to enhanced strength (Figure 4).

Biochemical analysis

Collagen quantification and gelatin zymography were only performed in ileal anastomoses because the colonic anastomoses did not display any significant difference in wound strength between groups (see above). Hydroxyproline levels were measured in ileal anastomotic samples from the control, diclofenac and naproxen treated rats. The hydroxyproline content, expressed as μg/5 mm, increased

Figure 1  Postoperative course of body weight. Points represent mean values for body weight as percentage of pre-operative weight. *, p<0.05 by ANOVA.

Figure 2  Anastomotic bursting pressure. Individual values and means (horizontal bars) are given for ileal (a) and colonic (b) anastomoses in control (C), diclofenac (D), naproxen (N) and the diclofenac and naproxen delay (Dd and Nd) groups. The bursting site was either within (●) or outside (○) the suture line. * or #, p<0.05 vs D and N group, respectively.
Gelatin zymography of extracts from 3 days old ileal anastomoses demonstrated the presence of various forms of matrix metalloproteinase MMP-2 and MMP-9. Highest total activities, expressed in arbitrary units/5 mm tissue, were found for proMMP-9. While mean values were higher in diclofenac and naproxen groups, 563 ± 62 (in arbitrary units + SEM) and 565 ± 77 respectively, than in controls (403 ± 67), the overall difference remained non-significant (p=0.205, ANOVA). For the other activities, no indications were found for the existence of differences between control and experimental groups (data not shown).

Histology
No gross differences were observed in the architecture of the 7 days old anastomoses (H&E staining) in either ileum or colon between control rats and animals treated with diclofenac or naproxen. In Figure 6 examples of ileal anastomoses from the control, diclofenac and naproxen groups are shown. The architecture of the wound area in the abdominal wall was also similar in all groups (not shown). COX-1 was abundantly present in mucosa and granulation tissue in controls and diclofenac-treated animals, but less so in naproxen-treated animals (Figure 6, middle row). COX-2 immunoreactivity was clearly seen in controls but seemed hardly present in both drug-treated animals (Figure 6, lower row).

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postoperative pain in adults. In our study we have used doses for both diclofenac and naproxen which exert relevant antinociceptive effects in the rat. The present data add to incidental reports on interference of NSAIDs, and especially those with an enhanced specificity for COX-2, in anastomotic healing. Celecoxib appears to be similar in its effects on ileal anastomoses to diclofenac, such as reported here, while rofecoxib affects colonic anastomoses but valdecoxib does not. Effects on anastomoses in the small bowel have not been investigated for the latter compounds. Clinically, enhanced anastomotic leakage is reported when celecoxib is administered after colonic surgery. To our knowledge, there are no data yet on naproxen and anastomotic healing, either clinically or experimentally. Negative effects for diclofenac on ileal anastomoses have been described in rabbits. Contrary to our experiment, the drug was administered intramuscularly. The same route was used in two investigations on its effect on colonic healing, although with contradictory results. While Inan et al. suggest reduced bursting pressures at 3 and 7 days after operation, Klein et al. report an unchanged breaking strength after 3 days. The latter is in agreement with the present data although we investigated colonic healing in animals which had an anastomosis in the ileum as well. In our opinion, with this experimental design, which halves the number of experimental animals needed, it is allowed to draw conclusions about both anastomoses separately. Previous work in our laboratory with celecoxib and the veterinary COX-2 inhibitor carprofen has shown that effects in animals with two anastomoses could be repeated in animals with one anastomosis only. Next to these experimental data there is some clinical, although retrospective, evidence that diclofenac can affect anastomotic healing and increase the anastomotic leakage rate. Evidence appears to be accumulating that may be taken as a warning to be aware of risks if this class of compounds is used to treat acute pain in the immediate postoperative period. The finding that the pronounced negative effect of diclofenac can be prevented by postponing its first use with a few days may be taken in consideration in choosing an appropriate drug regimen. The mechanism that causes diclofenac to interfere with the healing anastomosis remains to be elucidated. Generally speaking, COX-1 activity does not change during the inflammatory process, while there is a dramatic increase in COX-2 levels leading to increased production of proinflammatory prostaglandins. COX-2 was thought to be absent in undamaged tissue. This initial concept is now challenged and in fact the COX-2 enzyme appears to play a regulatory role in maintaining gastrointestinal barrier function and motility. It has been reported that constitutive COX-2 expression varies along the gastrointestinal tract of the rat and is highest in the ileum, which may explain why its inhibition is most severely felt around the ileal anastomosis.

Discussion

Administration of diclofenac immediately after intestinal surgery strongly affects ileal anastomotic healing in the rat as demonstrated by a very high leakage rate and substantial mortality. This effect is also observed for naproxen, although to a much lesser extent. When drug administration is withheld for three days these complications are prevented. Healing of the colonic anastomoses and the abdominal wall remains unaffected. It is very relevant to gain knowledge about the potential drawbacks of the use of these two drugs. Both have been proven to provide effective analgesia for acute

Figure 6  Anastomotic histology in the ileum at day 7 after surgery. Each panel shows a tissue segment with the ileal anastomosis in the middle and the mucosal layer on top at a magnification of approximately x100 in control (C), diclofenac (D) and naproxen (N) groups. The photomicrographs represent typical examples of hematoxylin and eosin-stained sections (first row) and specific staining for COX-1 (second row) and COX-2 (third row). The arrows indicate substantial deposition of immunoreactive material.
Peri-operative analgesia is ever increasing and expanding and drugs that inhibit COX isoenzymes like NSAIDs are invariably part of regimens used for this purpose after major surgery. Selective COX-2 inhibitors have been developed to prevent the side effects induced by classical NSAIDs through inhibition of COX-1 resulting far more selective for COX-2 than naproxen. It could very well be that in platelet aggregation and gastric mucous production. The COX-1/2 inhibitory mechanisms responsible remain to be unraveled, our data add to the increasing body of evidence which suggests that one should be aware that NSAIDs may negatively affect the outcome of the wound healing sequence. Within this context, the new finding that a short postoperative delay in their administration prevents this effect may be clinically relevant.

Reference List


PERI-OPERATIVE PAIN RELIEF BY A COX-2 INHIBITOR AFFECTS ILEAL REPAIR AND PROVIDES A MODEL FOR ANASTOMOTIC LEAKAGE IN THE INTESTINE

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- 20th congress European Tissue Repair Society, Gent, Belgium, Sep 2010.
- The 46th congress European Society for Surgical Research, Aachen, Germany, May 2011.

R. J. van der Vijver
C.J.H.M. van Laarhoven
B.M. de Man
R.M.L.M. Lomme
T. Hendriks
Abstract

Background We examined the potential of the cyclooxygenase (COX) 2 inhibitor carprofen to reproducibly induce anastomotic leakage.

Methods In experiment 1 an anastomosis was constructed in both ileum and colon of 20 rats and they were given carprofen (5 mg/kg subcutaneously every 24 hours) or buprenorphine (0.02 mg/kg subcutaneously every 12 hours). In another 20 rats an anastomosis was constructed in either ileum or colon and all received carprofen (experiment 2). Animals were killed after 3 days.

Results In experiment 1 the ileal dehiscence rate was 60% in the carprofen group and 0% in the buprenorphine group (p=0.0108). Colonic anastomoses in both groups remained patent. In experiment 2 the anastomotic leakage rate was 80% in ileum and 0% in colon.

Conclusion Thus, COX-2 inhibitors can severely interfere with intestinal healing, particularly in the ileum. Peri-operative administration of carprofen yields an unique model for anastomotic leakage which allows translational research on the effectiveness of perisuture line reinforcement.

Introduction

Despite advances in surgical techniques, anastomotic leakage remains a feared and dangerous complication after gastrointestinal surgery. Dehiscence, with a reported incidence varying between 1 and 30% is attended by high morbidity while its mortality rate is believed to fall between 10 and 15%.

The incidence of anastomotic dehiscence and the severity of its consequences ask for ways to protect anastomotic integrity, particularly if intestinal continuity needs to be restored in conditions which have been associated with high leakage rates. The development of such procedures requires preclinical evaluation and thus an animal model for anastomotic dehiscence. So far, the only known way to mimic a leaking anastomosis consists of the creation of an iatrogenic defect during its construction, mostly by using an insufficient number of sutures. However, ideally, a complete and patent anastomosis should be constructed which develops dehiscence with time. Such a model would be less artificial and clinically more relevant and would offer an unique possibility for translational research. In particular, it would allow to investigate the efficacy of means to prevent leakage or contain or postpone its effects, such as peroperative application of biological sealants. To our knowledge, there is no such experimental model available. Previous work in our laboratory has indicated that inhibitors of cyclooxygenase (COX) may interfere with anastomotic repair in the intestine. Peri-operative celecoxib, an inhibitor with a preference for COX-2, even induces leakage of ileal, but not colonic, anastomoses resulting in considerable mortality. This finding suggests that controlled application of a suitable drug from this class might result in reproducible leakage rates, without excessive mortality, in initially patent small bowel anastomoses.

Carprofen is an analgesic, widely used in veterinary medicine, which is believed to possess a certain degree of selectivity for COX2 and is suitable for pain relief during surgery in the rat. The present prospective study aims to demonstrate that the perioperative use of carprofen affects the integrity of ileal anastomoses and yields a much-needed reproducible model for anastomotic leakage in the rat intestine.

Methods

Study Design

Forty male Wistar Rats were used for the study: 20 (experiment 1) were obtained from Harlan BV, Horst, The Netherlands and another 20 (experiment 2) form Charles River, Sulzfeld, Germany. All were housed 2 per cage and accustomed to
laboratory conditions for five days before the start of the experiment. Body weights ranged between 260 and 300 g at operation. For experiment 1, the animals were divided randomly over two equal groups, which underwent intestinal resection and anastomotic construction in both colon and ileum. Starting before operation, group 1 received buprenorphine as an analgesic and group 2 carprofen. For experiment 2, the animals were again randomly divided over two equal groups, which underwent resection and anastomosis of either ileum (group 3) or colon (group 4). Both groups received analgesia by carprofen (Table 1). All rats were observed closely and weighed daily until termination at day 3 after operation. They had free access to water and standard rodent chow (Hope Farms, Woerden, The Netherlands). The Animal Ethics Review Committee of the Radboud University Nijmegen approved the study.

Procedure

Procedures were performed under semi sterile conditions using a Zeiss operation microscope (Carl Zeiss AG, Oberkochen, Germany). Animals were anesthetized by use of a mixture of isoflurane, oxygen and nitrogen, while breathing spontaneously through a mask. A midline laparotomy was performed and a 1-cm segment was resected from the descending colon 3 cm proximal to the peritoneal reflection. Colonic continuity was restored by constructing an end-to-end anastomosis with 8 single-layer, inverting, interrupted sutures (Ethilon 8-0; Ethicon, Norderstedt, Germany). A similar procedure was performed in the distal ileum 15 cm proximal to the cecum.

During operations, body temperature was kept at 38°C using a heating pad and a lamp. Intestines were covered with gauze pads soaked with 0.9% NaCl to minimize desiccation. To prevent dehydration, 10 ml of 0.9% NaCl was administered subcutaneously during the operation. Group 1 was administered buprenorphine (Temgesic, Schering Plough, Houten, the Netherlands), 0.02 mg/kg, every 12 hours (5 times in total) for 48 hours. The other three groups were administered carprofen (Rimady, Pfizer Animal Health, Cappele aan de IJssel, the Netherlands), 5 mg/kg every 24 hours (three times in total) for 48 hours. Both analgesics where administered subcutaneously and the first dose was given immediately prior to surgery.

Necropsy and analysis of wound strength

Rats were killed by CO₂/CO₃ asphyxiation on postoperative day 3 and the abdomen was inspected. The observers were blinded to the treatment groups. In particular, attention was paid to signs of anastomotic leakage such as macroscopic dehiscence of the anastomosis, the presence of faecal peritonitis, a puncture in the anastomotic line with or without an abscess near it. All fibrin-encased, purulent collections were considered to represent abscesses.

The presence of abscesses alone was not considered to be a sign of leakage. Adhesions were dissected carefully without manipulation of the anastomosis. Segments containing the anastomoses were resected and bursting pressure and breaking strength were measured consecutively. To measure bursting pressure, the segments were infused (2 ml/min) with 0.9% NaCl containing methylene blue. The maximum pressure (mm Hg) recorded immediately before sudden loss of pressure was taken as the bursting pressure. The site of rupture (within or outside the anastomotic line) was noted. Subsequently, the same segments were placed in a tensiometer, and the breaking strength (g) was measured.

Biochemical analysis

After weighing, tissue samples were frozen, lyophilized and pulverized. The hydroxyproline content, as a measure of the collagen content, was measured by high-performance liquid chromatography (HPLC) after hydrolysis with 6 N hydrochloric acid and coupling to dabsyl-chloride.

Preparation of tissue extracts for gelatin zymography, using a buffer containing 1% (v/v) Triton X-100, has been described elsewhere. The protein concentration of the extracts was measured using the bicinchoninic acid reagent. All tissue samples were stored at −80°C until zymography. The technique of preparation and electrophoresis of the gels and quantification of the various enzyme activities, which were expressed as arbitrary units on the basis of the lysed area, using a Sharp Jx-330 scanner and Imagemaster 1D software (Amersham Pharmacia, Uppsala, Sweden) has been described previously. In-between comparison of values obtained on different gels was performed using collagenase type 1 (from clostridium histolyticum; Sigma, St. Louis, MO, USA) as an internal standard. The presence of true matrix metalloproteinase (MMP) activity was confirmed by adding 10 mM EDTA or 1,10 phenanthroline to the buffers used after electrophoresis.

Statistics

Group size was estimated on the basis of the expectation (based on prior experiments in our laboratory) of a 0% ileal leakage rate in the control group and at least 60% leakage in the carprofen group. Using an α of 0.05 and a power of 0.8 an one-tailed Fisher’s exact test puts the group size at 10 (G*Power). Further comparisons between control and experimental groups was performed using a two-tailed unpaired t-test. Results were considered statistically significant at p<0.05.
Results

General observations
Postoperative weight loss at day 3 was around 10% and similar in all groups. Two rats died prematurely after 2 days, one in group 2 and one in group 3. In both, a complete dehiscence of the ileal anastomosis was seen with faecal peritonitis (Figure 1a), while in the animals in group 2 the colonic anastomosis was intact. At termination of experiment 1, another five rats in the carprofen group (group 2) showed signs of ileal anastomotic leakage (Figure 1b). The colonic anastomoses all remained intact. In the buprenorphine group (group 1) there were no signs of anastomotic leakage in either ileum or colon. Thus, the ileal leakage rate was significantly (p=0.0108) elevated by carprofen (Table 1). In the second, independent, experiment in animals with one anastomosis the use of carprofen led to ileal leakage in 80% of the animals while all colonic anastomoses remained intact (p=0.0007 vs ileum).

Wound Strength
During bursting pressure analysis all anastomoses ruptured within the suture line. The mean bursting pressure of the ileal anastomoses was significantly (p = 0.0005) lower in the carprofen treated animals in experiment 1 (group 2; 25 ± 8 mm Hg, mean ± SE) than in the control group (group 1; 67 ± 6 mm Hg), while the difference in breaking strength (39 ± 7 vs 50 ± 9 g) did not reach significance (Figure 2). This low wound strength was also found in the ileal anastomoses from group 3, where bursting pressure and breaking strength averaged 31 ± 10 mm Hg and 23 ± 7 g, respectively. In both groups 2 and 3 the bursting pressure in animals which showed macroscopic signs of anastomotic leakage at necropsy was considerably lower than that in the rats without these signs (Figure 3). The colonic anastomotic strength in experiment 1 was similar in control (group 1) and carprofen (group 2) animals.

Table 1  Experimental design and anastomotic leakage rate

<table>
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<th>Group</th>
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<td>Ileum</td>
<td>Colon</td>
</tr>
<tr>
<td>Leakage rate (%)</td>
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</table>

In experiment 1 an anastomosis was constructed in ileum and colon. In experiment 2 an anastomosis was constructed either in ileum or colon.

Figure 1  Macroscopic aspects of anastomotic leakage. a: complete dehiscence with faecal peritonitis; b: a defect in the suture line.

Figure 2  Anastomotic strength in experiment 1. Bars represent mean ± SEM for bursting pressure (A) and breaking strength (B) in control (n=10, grey bars) and carprofen (n=9, open bars) group. * p = 0.0005 versus control group.
Discussion

Healing of the ileal anastomosis in the early postoperative phase is impaired by the administration of carprofen to such extent that it results in reduced wound strength and a dehiscence rate of 60-80%. The fact that this COX inhibitor can elicit such profound effects in the ileum, though not in the colon, should be taken as a further warning that anti-inflammatory analgesics may interfere with tissue repair. However, at the same time it offers an opportunity to establish a long-wanted model for anastomotic leakage, without iatrogenic disruption of the anastomotic integrity.

Leakage of intestinal anastomoses remains a relatively frequent disaster which can result in generalized peritonitis and a need for reoperation. The patient has to deal with this challenge while still recovering from the initial operation. Any methods which would either prevent peritonitis to occur, or postpone its clinical effects, would constitute enormous progress. For this purpose, the potential benefits of peroperative suture or staple line reinforcement 15, for example by means of fibrin sealant 16,17, need investigation.

Finally, mean anastomotic strength in animals with only a colonic anastomosis which had received carprofen (group 4) was high: 64 ± 9 mm Hg and 155 ± 10 g for bursting pressure and breaking strength, respectively.

Collagen and proteolytic activity

Analysis of collagen and proteolytic activity was measured in experiment 1 where both an ileal and a colonic anastomosis were constructed within the same rat: group 1 (control) and group 2 (carprofen). The mean hydroxyproline content was similar in the ileal anastomosis of group 1 (161 ± 16 μg/5 mm) and group 2 (140 ± 28 μg/5 mm) group. The respective hydroxyproline concentrations were 10.5 ± 0.5 and 9.7 ± 0.8 μg/mg dry weight. Likewise, no differences between groups were found in colon anastomoses for these parameters.

Gelatin zymography of anastomotic extracts demonstrated the presence of multiple gelatin-degrading activities, representing various forms of matrix metalloproteinase MMP-2 and MMP-9. Administration of carprofen did not influence the activity of any of the gelatinases present in either the ileal or colonic anastomoses. Figure 4 shows both the specific and total activity in ileal anastomoses of proMMP-2 and active MMP-2 as an example.

![Figure 3](image1.png)
**Figure 3** Anastomotic bursting pressure in the ileum. Bars represent mean ± SEM for animals which showed macroscopic signs of dehiscence at obduction (open bars) and animals without these signs (grey bars). Results are given for both experiment 1 (group 2, n=4 for grey bar and n=5 for open bar) and experiment 2 (group 3, n=2 for grey bar and n=7 for open bar) * P < 0.05.

![Figure 4](image2.png)
**Figure 4** MMP-2 activity in ileal anastomoses from experiment 1. Bars represent mean ± SEM for specific (A) and total (B) activity of both pro- and active MMP-2 in control (n=10, grey bars) and carprofen (n=9, open bars) groups.
In order to assess such procedures a sound preclinical model is required. So far, basic research on intestinal healing is mostly performed in models with intact anastomoses. If constructed properly, intestinal anastomoses in rodents do not leak. Therefore, anastomotic strength is taken as a parameter which reflects its propensity to leak. However, a model which displays actual dehiscence of essentially patent anastomoses with time would be unique and clinically very relevant. The present data demonstrate that the administration of carprofen before and after anastomotic construction in the ileum results in the desired sequence of events in a high percentage of the animals. The fact that the expected leakage rate is somewhat less than 100% might be taken as a limitation of the model. Still, in our opinion the leakage rate is such that the effectiveness of procedures to lower the dehiscence rate can be established with reasonable numbers of animals. This will allow preclinical examination of, for instance, the efficacy of various sealing procedures for preventing anastomotic dehiscence. The mechanism underlying the effects described are as yet poorly understood.

Carprofen is an analgesic widely used in veterinary medicine. 11 It is used here in the dose recommended for peri- and post-operative pain relief. Carprofen inhibits COX and exhibits a preference for COX-2. 11 Preclinical 15 and clinical 23 data have been reported suggesting that COX-2 inhibitors can negatively affect anastomotic repair. Early wound strength depends on the capacity of the existing subcellular matrix to retain sutures. Loosening of the matrix by degradation of its structural components, mediated by MMP-activity, could precede anastomotic leakage. In our experiments, carprofen reduces the anastomotic breaking strength to a lesser extent than the bursting pressure. This observation may indicate matrix degradation to be very localized since a minute gap in the bowel wall will only lower the bursting pressure. 13 If so, this would also explain why hydroxyproline levels, as a measure for collagen in the anastomotic segments, remain unchanged. Also unchanged are both the gelatinase activities which are abundantly present in the wound area during the first postoperative days. 14 Since activities were only measured after 3 days, it cannot be ruled out that MMP-2 or -9, or possibly other members from the MMP family, are elevated at an earlier stage, although it is believed that COX-2 inhibitors generally suppress MMP activity. 22 The fact that anastomotic dehiscence was observed in the 2 rats that died prematurely suggests that the process responsible may originate in the very first postoperative period. The mechanism responsible for the negative effect of carprofen on the healing anastomosis remains unknown as yet. At first, COX-2 levels were thought to be virtually absent in undamaged tissue, while the enzyme is strongly expressed during the inflammatory process. Indeed, anastomotic construction leads to upregulation of COX-2 in the wound area in both small and large bowel. 4 However, it now seems likely that COX-2 plays a regulatory role in maintaining gastrointestinal barrier function and motility and is needed to maintain small bowel integrity. 21,24 The fact that constitutive COX-2 expression appears to vary along the gastrointestinal tract of the rat and is highest in the ileum 25 may explain why its inhibition is most severely felt around the newly-made ileal anastomosis.

Peri-operative analgesia is ever increasing and expanding and drugs that inhibit COX isoenzymes are invariably part of regimens used for this purpose after major surgery. 26,27 Although the mechanisms responsible remain to be fully understood, there appears to be an increasing body of evidence which suggests that one should be aware that analgesic drugs may affect the outcome of the wound healing sequence.

In conclusion it should be emphasized that we do not propose carprofen-induced ileal dehiscence to be a model to study the aetiology of leakage of intestinal anastomoses. Rather, it provides a means to perform much needed translational research on the effectiveness of peroperative suture line reinforcement.
Reference List


Carpofen for perioperative analgesia causes early anastomotic leakage in the rat ileum

BMC Veterinary Research
2012 Dec 27;8(1):247
Abstract

Background There is increasing evidence that perioperative use of non-steroidal anti-inflammatory drugs (NSAIDs) may compromise the integrity of intestinal anastomoses. This study aims to characterize the negative effects of carprofen on early anastomotic healing in the rat ileum.

Methods In 159 male Wistar rats an anastomosis was constructed in the ileum. In experiment 1 eighty-four rats were divided over control and experimental groups, which received daily buprenorphine or carprofen, respectively, as an analgesic and were killed on day 1, 2 or 3 after surgery. In experiment 2 three groups of 15 rats received carprofen either immediately after surgery or with a delay of 1 or 2 days. Animals were killed after 3 days of carprofen administration. In experiment 3 three groups of 10 rats received different doses (full, half or quarter) of carprofen from surgery.

Results In significant contrast to buprenorphine, which never did so, carprofen induced frequent signs of anastomotic leakage, which were already present at day 1. If first administration was delayed for 48 hours, the leakage rate was significantly reduced (from 80 to 20%; \( p=0.0028 \)). Throughout the study, the anastomotic bursting pressure was lowest in animals who displayed signs of anastomotic leakage. Loss of anastomotic integrity did not coincide with reduced levels of hydroxyproline or increased activity of matrix metalloproteinases.

Conclusion Carprofen interferes with wound healing in the rat ileum at a very early stage. Although the mechanisms responsible remain to be fully elucidated, one should be aware of the potential of NSAIDs to interfere with the early phase of wound repair.

Introduction

The use of analgetics in veterinary medicine is still relatively low, but adequate management of animal pain is becoming increasingly important in companion, farm and laboratory animals. Human pain control strategies using opioids or non-steroidal anti-inflammatory drugs (NSAIDs) can be applied to animals, because most animals possess neuronal mechanisms for pain perception which are similar to humans. NSAIDs inhibit cyclo-oxygenase (COX), enzymes which catalyze the first two steps in prostanoid biosynthesis. These enzymes occur as isoforms COX-1 and COX2. COX1 is expressed constitutively in housekeeping functions, whereas COX2 is expressed mainly in response to external stimuli. One widely used NSAID in veterinary medicine is carprofen, which is believed to possess a certain preference for COX2 and is suitable for pain relief. It is mostly used in dogs and cats but also in cattle and in laboratory animals. If carprofen is used as an analgetic during and after surgery of the intestine in rats the healing of ileal, but not of colonic, anastomoses is seriously compromised. A similar effect was found previously for celecoxib, another inhibitor with a certain degree of specificity for COX-2. Such a negative effect could have implications for the use of carprofen in veterinary practice and laboratory animals, particularly if used in the peri-operative period.

The present experiments were conducted to expand our earlier preliminary findings and further investigate the effects of carprofen on the healing of anastomoses in the small intestine of the rat.

Methods

Study design

Hundred and fifty-nine male Wistar rats weighing 250-290 g (Charles River, Sulzfeld, Germany) were housed 2 per cage and accustomed to laboratory conditions for five days before the start of the experiment. All rats underwent intestinal resection and an anastomosis was constructed in the ileum. Animals were observed closely and weighed daily and had free access to water and standard rodent chow (Hope Farms, Woerden, The Netherlands) throughout the entire experimental period. When observing the rats attention was paid to activity, colour and aspect of nose, presence of diarrhoea, aspect of fur and tenderness of the abdomen. If the rats scored as abnormal on all of these points they were taken out of the experiment, always after consultation of one of the experienced biotechnicians.

In experiment 1, 84 rats were randomly divided into two cohorts of 42 rats, each consisting of three groups of 14 animals to be killed on day 1, 2 or 3 after surgery.
The controls were administered buprenorphine and the experimental animals received carprofen as an analgesic (Table 1). Ten rats from each group were analysed for wound strength and the remaining animals were used for histology.

In experiment 2, 45 rats were randomly divided over three equal groups. All received carprofen for three days, starting immediately after surgery or 1 or 2 days afterwards. Animals in the three groups were killed on day 3, 4 or 5 after surgery, respectively (Table 2). Twelve rats from each group were analysed for wound strength and the remaining animals were used for histology.

In experiment 3, 30 rats were divided into three equal groups which each received a different daily (full, half or quarter, see below) dose of carprofen from the day of surgery. All animals were killed at day 3 (Table 3) and analysed for wound strength. The Animal Ethics Review Committee of the Radboud University Nijmegen approved the study (RU-DEC 2011-015 and 2011-127).

**Surgery and analgesics**

Procedures were performed under semi sterile conditions using a Zeiss operation microscope (Carl Zeiss AG, Oberkochen, Germany). Animals were anesthetized by use of a mixture of isoflurane, oxygen and nitrogen, while breathing spontaneously through a mask.

A midline laparotomy was performed and in each rat a 0.5-cm segment was resected from the distal ileum, 15 cm proximal to the cecum. Ileal continuity was restored by constructing an end-to-end anastomosis with 8 single-layer, inverting, interrupted sutures (Ethilon 8-0; Ethicon, Norderstedt, Germany). The abdominal wall was closed with a running suture (Vicryl 3-0; Ethicon, Norderstedt, Germany). The skin was closed with staples. During operations, body temperature was kept at 38°C using a heating pad and a lamp. Intestines were covered with gauze pads soaked with 0.9% NaCl to minimize desiccation. To prevent dehydration, 10 ml of 0.9% NaCl was administered subcutaneously after the operation.

All rats in experiment 2 and 3 and the rats in the control group of experiment 1 received carprofen as an analgetic (Table 1). Ten rats from each group were analysed for wound strength and the remaining animals were used for histology.

In experiment 1 by high-performance liquid chromatography (HPLC) after hydrolysis with 6 N hydrochloric acid and coupling to dabsyl-chloride. In experiment 1 gelatin zymography was performed. Tissue extracts were prepared using a buffer containing 1% (v/v) Triton X-100. The protein concentration of the extracts was measured using the bicinchoninic acid reagent. All tissue samples were stored at -80°C until further processing.

Biochemical analysis

After weighing, tissue samples were frozen, lyophilized and pulverized. The hydroxyproline content, as a measure of the collagen content, was measured in experiment 1. The hydroxyproline content was measured using the bicinchoninic acid reagent. All tissue samples were stored at -80°C until zymography. The techniques for preparation and electrophoresis of the gels and quantification of the various enzyme activities have been described previously.

Histology

Intestinal samples of approximately 1 cm containing the entire anastomosis in the middle were carefully collected en bloc, opened at the mesenteric side, washed gently in 0.9% NaCl and spread out in a cassette for paraffin-embedding. From the paraffin-embedded tissues, 4 μm sections were prepared and stained with hematoxylin and eosin (H&E). The presence of COX-1 and COX-2 protein in healing anastomoses was visualized by immunohistochemistry. From the paraffin-embedded tissues 4 μm sections were prepared and stained as described previously. COX-1 and COX-2 proteins were visualized using a rabbit polyclonal antiserum against human COX1 and COX2 (Cayman Chemical, An Arbor, MI, USA).
Statistics
Comparison between more than two groups was performed using a One-way Analysis of Variance (ANOVA). Comparisons between two groups or, within one group, between values at two different days were performed with a two-tailed unpaired t-test. Results were considered statistically significant at p<0.05.

Results
Presence of COX during normal healing
Both COX-1 and COX-2 are expressed during normal healing, if buprenorphine is used for peroperative pain relief. Figure 1 shows COX-1 to be present in undamaged intestine and also at day 1, 2 and 3 after surgery (Figure 1 panels a-d). COX-2 is absent in undamaged intestine but appears at day 1 and, more strongly, at days 2 and 3 (Figure 1 panels e to h).

Experiment 1: carprofen versus buprenorphine
All animals lost weight after operation. In the buprenorphine group the mean (+ SEM) relative weight (vs weight at operation) was 91 ± 1 % at day 3. The corresponding value in the carprofen group was 94 ± 2 % (p=0.0356). One rat from the buprenorphine group died immediately after surgery of no discernible reason. In the carprofen group another rat died prematurely from anastomotic leakage. No signs of anastomotic leakage were observed in the buprenorphine groups. These signs were found increasingly with time, and significantly (p<0.05) more frequently at all days, in the carprofen groups (Table 1). The anastomotic bursting pressure is depicted in Figure 2A. The biggest difference between groups was observed at day 3 where values in the buprenorphine group averaged 56 ± 6 (SEM) mm Hg and in the carprofen group 34 ± 10 mm Hg (p=0.0915). Clearly, in the carprofen groups the bursting pressure was lowest in animals who displayed signs of anastomotic leakage. At day 3, the average anastomotic breaking strength (Figure 2B) was also lower (p=0.0729) in the carprofen group than in the buprenorphine group, at 26 ± 7 vs 44 ± 6 g, respectively.

Gelatin zymography revealed the presence of both pro- and active metalloproteinase MMP-2 and -9 in anastomotic extracts. At days 1 and 2 no differences were found for any of these activities between buprenorphine and carprofen groups (data not shown). At day 3, total activities (in arbitrary units/sample) for both pro-MMP-2 and active MMP-2 were higher in the buprenorphine groups: 571 ± 62 vs 313 ± 35 (p=0.0038) and 223 ± 21 vs 133 ± 9 (p=0.0024) respectively. The anastomotic hydroxyproline content did not significantly differ between groups at any of the days (Figure 3).
Table 1 Characteristics of experiment 1

<table>
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<th>Group name</th>
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<td>0 0 1 (2)</td>
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<tr>
<td>Anastomotic leakage</td>
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</table>

After termination 10 animals from each group were analysed for wound strength and the remaining animals for histology. At obdution, special attention was paid to signs of anastomotic leakage. P-values denote significant differences between each carprofen group and the corresponding buprenorphine group (Fisher’s two-sided test).

Experiment 2: delayed administration of carprofen

Weight loss was similar to that observed in experiment 1, with the relative weight at day 3 averaging 93, 91 and 93 % in the groups receiving carprofen from day 0, 1 or 2, respectively. Two rats which received carprofen from day 1 after surgery died prematurely on day 3 due to anastomotic leakage. As before, signs of anastomotic leakage were seen frequently if carprofen was given from day 0 (Table 2). Delaying the first gift reduced leakage, significantly so if carprofen was only given from day 2 onwards, although in that group signs were still observed in 20 % of the animals after termination at day 5. Anastomotic strength did increased with time. Despite the fact that the animals also received a three day course of carprofen both anastomotic bursting pressure and breaking strength at day 5 were considerably and significantly (p<0.001) higher than at day 3 (Figure 4). Again, the bursting pressure was lowest in animals who displayed signs of anastomotic leakage.

Experiment 3: different dosage of carprofen

The weight loss was similar to that in the preceding experiments, with a mean relative weight (vs weight at operation) at day 3 of 94, 96 and 94 % in the groups receiving full, half or quarter doses of carprofen, respectively. One rat died prematurely of anastomotic dehiscence in the group which received the full dose of carprofen and another one, of unknown reasons, in the group that received a quarter dose.
The average bursting pressure rose slightly with decreasing dosage of carprofen but did not differ significantly between groups (Figure 3). Animals without signs of anastomotic leakage showed the highest anastomotic bursting pressure. Breaking strength was similar in all groups.

Signs of anastomotic leakage were abundant in all groups (Table 3). Although the incidence decreased with dose, this effect remained non-significant with the current cohort size.

<table>
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Table 2 Characteristics of experiment 2

After termination 12 animals in each group were analysed for wound strength and the remaining animals for histology. Signs of anastomotic leakage were noted at obduction. P-value denotes a significant difference with the group administered carprofen from day 0 (Fisher’s two-sided test).

The average bursting pressure rose slightly with decreasing dosage of carprofen but did not differ significantly between groups (Figure 3). Animals without signs of anastomotic leakage showed the highest anastomotic bursting pressure. Breaking strength was similar in all groups.
Carprofen interferes with early repair of ileal anastomoses in the rat. Signs of anastomotic leakage comprise the main outcome parameter in this study. If carprofen is used from the day of operation, these signs are already abundantly present 24 hours later. Delaying the first gift of carprofen for 48 hours significantly reduces signs of leakage, without completely preventing them. Reducing the dose fourfold lowers the incidence of this complication, but not significantly so.

NSAIDs are commonly used in small animals and equine practice for peri-operative pain relief because of their analgetic and anti-inflammatory features. NSAIDs inhibit the family of COX enzymes. COX-2 is expressed mainly in response to external stimuli and is postulated to be involved in essentially pathological conditions such as inflammation, pain and fever. Selective inhibitors of COX-2 supposedly allow specific targeting of inflammatory disease processes, without disruption of normal homeostatic mechanisms that accounts for many side-effects of non-selective NSAID therapy. Both COX-1 and -2 are believed to be involved in wound healing and Figure 1 demonstrates their presence in the healing anastomosis. There is a growing understanding that NSAIDs, and especially those with a certain preference for COX-2, may increase the risk of anastomotic leakage. Carprofen is such a drug that is used in domestic animals, but is also effective as an analgesic in laboratory animals undergoing laparotomy. Recent evidence shows that its long-term use can inhibit bone healing in dogs, but nothing is known about its potential effects on soft tissue healing. Recent incidental reports suggest that carprofen administration to dogs alters functions of platelets, which are relevant to the repair sequence. Also, carprofen has been shown to compromise the integrity and barrier function of the gastrointestinal mucosa in dogs. The present data, which expand on a previous short communication unequivocally demonstrate that carprofen can interfere significantly with anastomotic integrity in the rat small bowel.

While signs of leakage in the carprofen group greatly surpass those in the buprenorphine group (Table 1), the average anastomotic strength is not dramatically reduced (Figure 1). It seems likely that in the majority of animals the leakage can be contained, e.g. by the formation of fibrinous adhesions. This way, some degree of strength is restored to the anastomotic segment. On the whole though,

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**Table 3 Characteristics of experiment 3**

<table>
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All animals, except those who died prematurely, were terminated at day 3 and analysed for wound strength. Signs of anastomotic leakage at obduction were noted.

**Figure 5** Anastomotic strength in experiment 3. Animals received different doses of carprofen from the day of operation and were terminated at day 3 after operation.

Panel A gives the individual bursting pressures (and means as horizontal bars) for all animals: ● denotes an absence of signs of anastomotic leakage and ○ the presence of such signs. The bursting site was always in the anastomotic line. In panel B bars represent mean (+ SEM) for breaking strength.

**Discussion**

Carprofen interferes with early repair of ileal anastomoses in the rat. Signs of anastomotic leakage comprise the main outcome parameter in this study. If carprofen is used from the day of operation, these signs are already abundantly present 24 hours later. Delaying the first gift of carprofen for 48 hours significantly reduces signs of leakage, without completely preventing them. Reducing the dose fourfold lowers the incidence of this complication, but not significantly so. NSAIDs are commonly used in small animals and equine practice for peri-operative pain relief because of their analgetic and anti-inflammatory features. NSAIDs inhibit the family of COX enzymes. COX-2 is expressed mainly in response to external stimuli and is postulated to be involved in essentially pathological conditions such as inflammation, pain and fever. Selective inhibitors of COX-2 supposedly allow specific targeting of inflammatory disease processes, without disruption of normal homeostatic mechanisms that accounts for many side-effects of non-selective NSAID therapy. Both COX-1 and -2 are believed to be involved in wound healing and Figure 1 demonstrates their presence in the healing anastomosis. There is a growing understanding that NSAIDs, and especially those with a certain preference for COX-2, may increase the risk of anastomotic leakage. Carprofen is such a drug that is used in domestic animals, but is also effective as an analgesic in laboratory animals undergoing laparotomy. Recent evidence shows that its long-term use can inhibit bone healing in dogs, but nothing is known about its potential effects on soft tissue healing. Recent incidental reports suggest that carprofen administration to dogs alters functions of platelets, which are relevant to the repair sequence. Also, carprofen has been shown to compromise the integrity and barrier function of the gastrointestinal mucosa in dogs. The present data, which expand on a previous short communication unequivocally demonstrate that carprofen can interfere significantly with anastomotic integrity in the rat small bowel.

While signs of leakage in the carprofen group greatly surpass those in the buprenorphine group (Table 1), the average anastomotic strength is not dramatically reduced (Figure 1). It seems likely that in the majority of animals the leakage can be contained, e.g. by the formation of fibrinous adhesions. This way, some degree of strength is restored to the anastomotic segment. On the whole though,
from our own laboratory suggest that diclofenac, another inhibitor with specificity for COX-2, shows effects similar to carprofen, while naproxen, with a suspected lesser specificity for COX-2, leaves ileal anastomoses intact (unpublished results). Older data indicate increased complications in rats with anastomoses in both ileum and colon after preoperative administration of either ibuprofen or indomethacin. 23 It thus remains to be determined if preference for COX-2 is essential for a drug to exert the negative effects observed.

It is necessary to gain knowledge about the potential drawbacks of NSAIDs like carprofen, as they are rapidly becoming cornerstones in peri-operative pain relief and are able to minimize post-operative opioid requirement in veterinary practice and in experimental studies. 2;24 The findings reported here may also be relevant to the human situation where NSAIDs are frequently used after gastro-intestinal surgery and are even incorporated in protocols for fast track surgery, despite emerging evidence that they may affect repair. 12;13

Thus, we conclude that carprofen interferes with wound healing in the rat ileum at a very early stage. If it does not kill the animal, at least it renders it more vulnerable to second hits after initial surgery. Although the mechanisms responsible remain to be fully understood, there appears to be an increasing body of evidence which suggests that one should be aware that NSAIDs may affect the outcome of the wound healing sequence.

throughout the three experiments anastomoses which display signs of leakage at necropsy display lower bursting pressures than those that are free of such signs. These findings raise the question what the fate would have been of the animals which survive until day 3 or later, but which show signs of leakage. Most likely, those with zero bursting pressure, thus in fact with a gap in the suture line at that time, will eventually die from secondary peritonitis. However, the possibility cannot be excluded that in some cases a fibrinous adhesion barrier will prevent full faecal leakage into the abdominal cavity. The resulting subclinical leakage could eventually allow complete healing of the intestinal wall, just as the majority of animals with anastomotic strength compatible with normal repair at day 3 may be expected to survive and show increasing strength in the proliferative phase of healing (as observed in the animals in experiment 2).

From the data presented it is clear that, whatever mechanism is responsible for leakage to occur, the origin of the phenomenon must lie within the first days after operation. It is not confined to the first 24, or even 48, hours since in the group where carprofen was first given at day 2, 20% of the animals still showed proof of leakage 3 days later (Table 2).

Thus, carprofen somehow affects the inflammatory phase of healing, where anastomotic integrity is determined by the capacity of the existing submucosal matrix to retain sutures. Induction of massive matrix degradation seems unlikely since hydroxyproline (collagen) levels in the anastomotic segments remain unaffected. Also, total activities of MMP-2 and MMP-9 remain unchanged during the first 2 days. These results do not exclude the possibility of limited and local matrix degradation, e.g. around the sutures, by one of the other enzymes from the MMP family. Still, it is believed that COX2 inhibitors generally suppress MMP activity. 19

It has been suggested that COX2 plays a regulatory role in maintaining gastrointestinal barrier function and motility and is needed to maintain small bowel integrity. 20;21 Interestingly, the effect described here does not occur in colonic anastomoses 5 while the highest level of COX2 expression in normal uninjured rats is located on the ileal side of the ileocaecal junction. 22 The question then arises if the carprofen effect is mediated specifically through COX2 and if it is species specific. It has been suggested that determining the exact specificity of any inhibitor for either of the COX enzymes is fraught with practical difficulties and may depend on the test and the laboratory that uses it. 4 Still, carprofen seems to possess a certain degree of specificity for COX2, although it may vary between species. Thus, as yet the question remains if these findings are limited to laboratory animals or that they can also occur in dogs and cats. Using a commercially available formulation and the manufacturer’s recommended dose it seems likely that carprofen also produces significant inhibition of COX1. 4 Very recent data
Reference List


The effect of fibrin glue on the early healing phase of intestinal anastomoses in the rat

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Abstract

Background: Anastomotic dehiscence remains the most feared complication after gastrointestinal surgery. Protecting the anastomotic integrity remains an important goal for ongoing research. It seems logical to investigate if a seal, such as fibrin glue, around the anastomosis can prevent leakage. The paucity of any clinical studies investigating such a procedure is surprising and possibly caused by incidental preclinical data which suggest a negative effect on anastomotic strength. We performed a comprehensive study into the effect of fibrin glue on the early phase of anastomotic healing.

Methods: In 108 young adult male Wistar rats resection and anastomosis of ileum and colon was performed. Fibrin glue applied around anastomoses in the experimental group.

Wound strength, both bursting pressure and breaking strength were measured at day 1, 3 and 5 after operation. Hydroxyproline content and histology were also measured, the latter also after 7 days.

Results: Anastomotic breaking strength was always similar in both control and fibrin glue groups. Anastomotic bursting pressures remained low at day 1 and day 3, without any differences between groups. At day 5 the bursting pressure in the fibrin glue group remained below that in the controls, although only significantly (p=0.0138) so in the ileum. Still, in both groups the bursting pressure increased sharply between days 3 and 5, by a factor 3.17 (p<0.001) and 2.85 (p<0.001) in controls and the experimental group, respectively. At day 5, but not at day 7, the wounds in the fibrin glue group appeared to contain less collagen. Other aspects of microscopic wound architecture appeared to be the same.

Conclusion: During normal healing, fibrin glue does not affect early anastomotic strength in a clinically relevant way. Caution with extrapolation to clinical situation is warranted.

Introduction

Anastomotic dehiscence is the most feared and potentially devastating complication after gastrointestinal surgery. A leaking anastomosis is associated with increased morbidity and considerable mortality. Protecting the anastomotic integrity and finding ways to minimize, postpone or prevent the consequences of leakage therefore remain important goals of ongoing research. In theory it seems logical to apply some kind of seal around the anastomosis to prevent leakage. Very recently, it has been emphasized again that the potential benefits of such suture or staple line reinforcement need investigation. In this respect a possible role for tissue glues should not be overlooked.

Tissue adhesives or glues, and in particular fibrin glue, are already used in different fields of surgery to promote tissue union, sealing, hemostasis and wound healing. Fibrin glue consists of homologous plasma-derived fibrin products from pooled donors combined with bovine thrombin and mimics the final stages of blood coagulation and fibrin clot formation. While the concept that fibrin glue may be used to strengthen the intestinal anastomosis is certainly not new, it has not led to consistent and reliable data which demonstrate its effectiveness and supply indications for its use. So far, clinical data are mainly limited to a few observational reports involving anastomoses in gastric bypass patients which might indicate a potential benefit in terms of a reduced frequency of leakage. Very recently, a similar suggestion has been reported for stapled anastomoses during laparoscopic resection of rectal cancer. The question still stands if the routine application of fibrin glue to sutured or stapled intestinal anastomoses may contribute to successful healing. Incidental data from preclinical studies seem to suggest that fibrin glue applied to patent sutured colonic anastomoses can negatively affect their strength. If true, such an effect would limit the range of possible indications for its clinical use. In order to evaluate if fibrin glue can indeed have a relevant negative effect on anastomotic healing in the intestine, we conducted a comprehensive experimental study in the rat, examining anastomoses in both ileum and colon and measuring multiple parameters for wound repair on three different days during the early crucial phase of healing.

Methods

Experimental Design

One hundred and eight male Wistar rats weighing 250-295 g (Harlan BV, Horst, The Netherlands) were housed 2 per cage and accustomed to laboratory conditions for five days before the start of the experiment. They were randomly divided over
the control group (n=54) or the group where fibrin glue was applied around the anastomoses (n=54). All rats underwent intestinal resection and anastomoses were constructed in both colon and ileum. Within each group, 16 animals were sacrificed on day 1, 3 and 5 after surgery and 6 animals 7 days after operation. All animals were observed closely and weighed daily and had free access to water and standard rodent chow throughout the entire experimental period (Hope Farms, Woerden, The Netherlands). The Animal Ethics Review Committee of the Radboud University Nijmegen approved the study.

Operative Procedure

Procedures were performed under semi-sterile conditions using a Zeiss operation microscope (Carl Zeiss AG, Oberkochen, Germany). Animals were anesthetized by use of a mixture of isoflurane, oxygen, and nitrogen, while breathing spontaneously through a mask. A midline laparotomy was performed and in each rat a 1-cm segment was resected from the descending colon 3 cm proximal to the peritoneal reflection. Colonic continuity was restored by constructing an end-to-end anastomosis with 8 single-layer, inverting, interrupted sutures (Ethilon 8-0; Ethicon, Norderstedt, Germany). A similar procedure was performed in the distal ileum, 15 cm proximal to the cecum. During operations, body temperature was kept at 38°C using a heating pad and a lamp. Intestines were covered with gauze pads soaked with 0.9% NaCl to minimize desiccation. To prevent dehydration, 10 ml of 0.9% NaCl was administered subcutaneously during the operation. If the animal was randomized into the fibrin glue group, 0.2 ml of fibrin glue (Tissucol Duo 500 0.5 ml; Baxter AG, Vienna, Austria) was applied around the anastomosis, immediately after its completion. All rats were administered the analgesic buprenorphine (Temgesic, Schering Plough, Houten, the Netherlands), 0.02 mg/kg, before operation and then every 12 hours for two days after surgery.

Wound Strength

Sixty (30 control and 30 fibrin glue) rats were killed by CO2 asphyxiation on postoperative day 1, 3, 5 or 7 and the abdomen was inspected. Segments containing the anastomoses were resected and adhesions were dissected carefully without manipulation of the anastomosis, while fibrin glue was left in place. In order to measure bursting pressure, the segments were infused (2 ml/min) with 0.9% NaCl containing methylene blue. The maximum pressure (mm Hg) recorded immediately before sudden loss of pressure was taken as the bursting pressure. The site of rupture (within or outside the anastomotic line) was noted. Subsequently, the segments were placed in a tensiometer, and the breaking strength (g) was measured. The anastomotic segment was carefully cleaned from any adhering tissue and fibrin glue and a 5 mm sample, containing the suture line in the middle, was frozen in liquid nitrogen and stored at -80°C until further processing.

Biochemical analysis

After weighing, tissue samples were frozen, lyophilized and pulverized. The hydroxyproline content, as a measure of the collagen content, was measured by high-performance liquid chromatography after hydrolysis with 6 N hydrochloric acid and coupling to dabsyl-chloride. Preparation of tissue extracts for gelatin zymography, using a buffer containing 1% (v/v) Triton X-100, has been described elsewhere. The protein concentration of the extracts was measured using the bicinchoninic acid reagent. All tissue samples were stored at 80°C until zymography. The technique of preparation and electrophoresis of the gels and quantification of the various enzyme activities, which were expressed as arbitrary units on the basis of the lysed area, using a Sharp Jx-330 scanner and Imagemaster 1D software (Amersham Pharmacia, Uppsala, Sweden) has been described previously. In-between comparison of values obtained on different gels was performed using collagenase type I from clostridium histolyticum; Sigma, St. Louis, MO, USA as an internal standard. The presence of true matrix metalloproteinase (MMP) activity was confirmed by adding 10 mM EDTA or 1,10 phenanthroline to the buffers used after electrophoresis.

Histology

The remaining 44 animals (see results), divided evenly over the groups, were killed as described above at 1, 3, 5 or 7 days after surgery and used for histological evaluation. Adhesions were dissected carefully without manipulation of the anastomosis, while fibrin glue was left in situ. Intestinal samples of approximately 1 cm containing the entire anastomosis in the middle were carefully collected en bloc, opened at the mesenteric side, washed gently in 0.9% and spread out in a cassette for paraffin-embedding. From paraffin-embedded tissues, 4 μm sections were prepared and stained with hematoxylin and eosin (H&E). Sections were analyzed by using a binocular light microscope. Another set of longitudinal sections were stained with picrosirius red to identify collagen fibers in the anastomotic area collagen was quantified by digital image analysis. Images were recorded by a 3-chip CCD RGB camera (DXC-325P, Sony) mounted on a conventional light microscope (Axioskop, Carl Zeiss), using a 5x objective. Image acquisition and analysis were performed using a complimentary software program (Zeiss KS 400™ Axiovision 3.0). Microscopic images were digitized and the area positive for picrosirius red staining was recognized by segmentation in RGB using fixed threshold values. The anastomotic area between the two inverted apposite muscular layers was interactively defined on the computer screen. The ratio of picrosirius red positive and the total amount of pixels yielded the percentual area of anastomotic collagen.
Statistics
Comparison between control and experimental groups was performed using a two-tailed unpaired t-test or Fisher’s exact test. Comparisons within one group (different time points) were performed by an one-way ANOVA followed by a Tukey-Kramer multiple comparisons test. Results were considered statistically significant at p<0.05.

Results
General observations
Three animals in the fibrin glue group did not survive surgery, while one animal in the control group died during the first night after operation. No abnormalities or signs of anastomotic leakage were seen in these animals. Up to day 3 the animals lost approximately 10% of their pre-operative weight (Figure 1). At day 3 the rats in the control group started to gain weight again, while the rats in the fibrin glue group decreased in weight up till 4 days after surgery. As a consequence, body weight in the latter group was significantly (p<0.05) lower from day 3 onwards. At day 3, ten out of sixteen rats from the fibrin glue group showed signs of a wide colon filled with hardened faeces immediately proximal to the anastomosis (Figure 2; panel e), which nevertheless remained conductant. This phenomenon, which was absent in controls, was seen in only one rat at day 5 and in none of the rats on day 7. In addition, figure 2 represents the macroscopic findings at termination at day 1, 3 and 5 in the colonic anastomosis. On day 1 the fibrin glue was still present (lifted by the forceps on panel d) and on day 5 there were some adhesions to the fibrin glue (panel f), but adhesions were also present in the control group.

Figure 1 Postoperative course of body weight. Data represent mean (± SD) relative body weight, in relation to the weight prior to operation, for the control (●, n=16) and the fibrin glue group (●, n=15). *: p<0.05 vs fibrin glue group.

Figure 2 Macroscopic findings in the colonic anastomosis on day 1 (a and d), 3 (b and e) and 5 (c and f) in the control (left side) and fibrin glue (right side) groups.
Collagen and proteolytic activity

In the controls, but not in the fibrin glue treated animals, the hydroxyproline content in anastomotic segments increased significantly (p<0.001) from day 3 to day 5 (Figure 5). As a consequence, it was significantly lower in the fibrin glue group than in the control group at day 5. This effect also appeared to be present if collagen was quantitated histologically in the true wound area, although not significantly (p=0.054 in colon) because of large inter-animal variations (Figure 6). Still, at day 7 no such effect was seen any more as a result of the enormous increase observed in the fibrin glue group. Here, mean values for collagen as percentage of wound surface area increased between days 5 and 7 from 4 to 29% (p=0.0002) in ileum and from 2 to 27% (p<0.0001) in colon, respectively.

Gelatin zymography of anastomotic extracts, obtained after 3 or 5 days, demonstrated the presence of multiple gelatin-degrading activities, representing proMMP-9, proMMP-2 and active MMP-2. No differences between the two experimental groups

Wound Strength

The early anastomotic breaking strength remained low, but increased in both groups between days 3 and 5 (Figure 3). At all time points, average values in both groups were similar, both in ileum and colon. Anastomotic bursting pressures remained low at day 1 and day 3, without any differences between groups, and increased sharply thereafter (Figure 4). From day 3 to day 5 in the ileum, the average bursting pressure was increased by a factor 3.17 (p<0.001) and 2.85 (p<0.001) in the controls and fibrin glue treated animals, respectively. In the colon these factors were 2.44 (p<0.001) and 2.51 (p<0.001), respectively. However, at day 5 the bursting pressure in the fibrin glue group remained below that in the controls, although only significantly (p=0.0138) so in the ileum. Only at day 5 some bursting sites were outside the suture line (Figure 4).

**Figure 3** Anastomotic breaking strength. Data represent mean and SD for strength in ileal (A) and colonic (B) anastomoses from the control groups (white bars) and the groups which received fibrin glue (grey bars). #: p<0.05 vs day 3.

**Figure 4** Anastomotic bursting pressure. Individual values and medians (horizontal bars) are given for ileal (A) and colonic (B) anastomoses in both control (C) and fibrin glue (FG) groups. The bursting site was either within (○) or outside (●) the suture line. ★: p<0.05; #: p<0.05 vs day 3.

**Collagen and proteolytic activity**

In the controls, but not in the fibrin glue treated animals, the hydroxyproline content in anastomotic segments increased significantly (p<0.001) from day 3 to day 5 (Figure 5). As a consequence, it was significantly lower in the fibrin glue group than in the control group at day 5. This effect also appeared to be present if collagen was quantitated histologically in the true wound area, although not significantly so (p=0.054 in colon) because of large inter-animal variations (Figure 6). Still, at 7 days no such effect was seen any more as a result of the enormous increase observed in the fibrin glue group. Here, mean values for collagen as percentage of wound surface area increased between days 5 and 7 from 4 to 29% (p<0.0002) in ileum and from 2 to 27% (p<0.0001) in colon, respectively.
Anastomotic leaks are detected anywhere from 3 to 45 days postoperatively and the diagnosis is mostly made between days 6 and 9 or even later. However, it stands to reason that the processes which lead to failure start much earlier, probably in the immediate postoperative period when wound strength is believed to be low. The incidence and severity of the complication being as they are, it seems logical and even necessary to seek ways to protect anastomotic integrity. There exists a renewed interest in staple or suture line reinforcement by topically applied sealants, which is also reflected in recent preclinical studies.

Fibrin sealant is a well established tool with multiple FDA-approved indications which are ever expanding. Although its potential is often suggested, the application of fibrin glue to strengthen anastomoses in the gastrointestinal tract has not become common practice. Mostly, its reported clinical application in gastrointestinal

were found, with the exception of proMMP-9 in the ileum at day 3, where it was higher (p=0.003) in the fibrin glue group than in the controls (data not shown).

**Histology**

Typical examples of colonic anastomoses are presented in Figure 7. No obvious or consistent differences in microscopic wound architecture were seen between groups. Fibrin glue was clearly present in the experimental group at day 3. At day 7, the fibrin glue appeared to be mostly gone. The same was observed in the ileum (not shown).

**Discussion**

Anastomotic leaks are detected anywhere from 3 to 45 days postoperatively and the diagnosis is mostly made between days 6 and 9 or even later. However, it stands to reason that the processes which lead to failure start much earlier, probably in the immediate postoperative period when wound strength is believed to be low. The incidence and severity of the complication being as they are, it seems logical and even necessary to seek ways to protect anastomotic integrity. There exists a renewed interest in staple or suture line reinforcement by topically applied sealants, which is also reflected in recent preclinical studies.

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**Figure 5** Hydroxyproline content of anastomotic segment. Bars represent mean and SD in ileal (A) and colonic (B) segments from the control groups (white bars) and the groups which received fibrin glue (grey bars). *: p<0.05 vs controls; #: p<0.05 vs day 3.

**Figure 6** Wound collagen. Bars represent mean and SD in ileal (A) and colonic (B) segments from the control groups (white bars) and the groups which received fibrin glue (grey bars). #: p<0.05 vs day 5.
the existing submucosal layer while restoration of original tissue strength depends on the deposition of newly-made collagen fibrils. Interestingly, the application of fibrin glue appears to result in a reduced hydroxyproline content and a lessened presence of collagen fibrils in anastomotic segments at day 5. This phenomenon may explain the somewhat lower bursting pressures observed, although apparently sufficient collagen is still synthesized in both groups. It is presently unknown how fibrin glue around the anastomosis would cause the slight delay in collagen deposition, since fibrin is believed to be an excellent template for cellular migration. 28

The bursting pressure is a parameter for anastomotic strength only if the bursting site is within the suture line, which will mostly not be the case anymore at day 7 after operation. For that reason, and the fact that no differences in breaking strength are seen between groups up until day 5, we only assessed histology after 7 days.

The present data extend earlier, limited, findings where significant loss of colonic bursting pressure was described at days four 16 and five 15 after surgery. In our study the bursting pressure at day 5 is still quite high and much elevated compared to day 3. Still, a certain delay in recovery of intestinal strength cannot be denied, while wound strength during the first three days appears unaffected. Altogether, the available evidence indicates fibrin glue not to be beneficial, and at its best irrelevant, to anastomotic healing in the intestine.

The conclusion must therefore be that there is no justification for using fibrin glue on patent anastomoses constructed under low-risk conditions. It remains to be determined if it can play a beneficial role in other situations. In a very recent study on partial colonic anastomoses constructed with a limited number of sutures, a positive effect of fibrin glue was observed on the anastomotic bursting pressure at days 3 and 5 after operation. 29 Clinically, its application over stapled anastomoses during laparoscopic resection of rectal cancer has been reported, where a declining tendency of the anastomotic leakage was suggested. 14 Thus, it may be that it can play a protective role under conditions where chances for anastomotic leakage are enhanced. Under those conditions, its potential benefit may outweigh its unwanted effects.

surgery seems to be limited to bariatric surgery 13 and treatment of fistulae. 26,27 One question which needs an answer is if the application of fibrin glue, as a precaution against leakage, could be indicated for all intestinal anastomoses. The present experimental data show fibrin glue to be not entirely without harm if applied to intestinal anastomoses. Application of fibrin around patent anastomoses in both ileum and colon transiently affects repair. This effect presents clinically by a temporary and resolving ileus and a delayed weight gain and experimentally by a delay in collagen deposition in the true anastomotic area, which may cause the transient and relatively minor reduction in anastomotic bursting pressure. Loss of body weight, always observed after operation, is more quickly compensated in the control group. In the fibrin glue group signs of a wide colon, filled with hardened faeces, just proximal of the anastomosis are seen on day 3, but not anymore at day 5. Possibly, fibrin glue can interfere with peristaltic movement of the bowel which leads to transient constipation in the large bowel only, because the consistency of the faeces is different from the small bowel. Interestingly, ileus was also described after the application of collagen fleece which was kept in place by fibrin glue. 25 Early wound strength depends on the suture-holding capacity of

Figure 7 Anastomotic histology in the colon. Each panel shows a tissue segment with the anastomosis in the middle and the mucosal layer at the bottom at a magnification of approximately x 40, representing typical examples obtained at day 3 (a: control and b: fibrin glue) and day 7 (c: control and d: fibrin glue). The asterisk (*) denotes fibrin.
Reference List


THE EFFECT OF SEALING A HIGH-RISK ANASTOMOSIS IN THE RAT ILEUM WITH FIBRIN GLUE OR A FIBRIN COATED COLLAGEN PATCH

To be submitted
Abstract

Background Since leakage of intestinal anastomoses is associated with high morbidity and mortality, protecting anastomotic integrity and finding ways to minimize, postpone or prevent the consequences of fecal spill in the abdominal cavity remain important goals for ongoing research. The value of two frequently used fibrin-based sealants in protecting anastomotic integrity was investigated in a recently developed model where the analgesic carprofen induces leakage of patent anastomoses in the rat ileum.

Methods In 110 Wistar rats an anastomosis was constructed in the ileum. In experiment 1, where 20 animals were divided over a control (n=10) and a fibrin glue group (n=10), all rats were administered carprofen (5mg/kg/day) starting prior to surgery. In experiment 2 the rats were divided over two groups (n=45) which received either carprofen (1.25 mg/kg/day) starting prior to surgery (group 2A) or starting 24 h after surgery (group 2B) in a dosage of 5 mg/kg/day. Both group 2A and 2B were divided into a control (n=15), a fibrin glue (n=15) and a fibrin coated patch (n=15) group. The animals in experiment 1 and experiment 2A were sacrificed at day 3 and those in experiment 2B at day 4.

Results In experiment 1 anastomotic leakage rates, ileus and wound strength did not significantly differ between groups. In experiment 2A ileus was significantly more frequently present in the fibrin glue (p=0.02) and the fibrin coated patch group (p=0.001) compared to the control group, but anastomotic leakage rates and wound strength were similar. In experiment 2B the bursting pressure was significantly lower in the fibrin glue (p=0.001) group (p=0.01) than in the control group. Anastomotic leakage rates were elevated in the fibrin glue group, although not significantly so.

Conclusions Protecting anastomotic integrity must remain an import goal of ongoing research to lower morbidity and mortality rates. In this study, sealing the high-risk anastomosis with fibrin glue or a fibrin coated patch does not lower leakage rates and causes ileus and therefore these measures should be treated with caution.

Introduction

Anastomotic failure remains the single most important hazard within gastrointestinal surgery. A leaking anastomosis is associated with increased morbidity and considerable mortality. Protecting the anastomotic integrity and finding ways to minimize, postpone or prevent the consequences of leakage therefore remain important goals of ongoing research. Even a temporary protection would probably be valuable. Delaying leakage of intestinal contents into the peritoneal space until the patient has recovered to some extent from the operation would probably enhance the capacity to deal with a second event. In theory it seems logical to apply some kind of seal around the anastomosis to minimize, prevent or postpone leakage.

Fibrin based sealants are increasingly used in different fields of surgery to provide a sealing barrier, to induce hemostasis or to bond tissue together. Two frequently used seals are fibrin glue and fibrin-thrombin coated fleece, which both consists of homologous plasma-derived fibrin products from pooled donors combined with bovine thrombin, thus mimicking the final stages of blood coagulation and fibrin clot formation. The potential benefit of suture or staple line reinforcement with these type of products needs investigation. The clinical application of fibrin glue around a gastrointestinal anastomosis seems to be mainly limited to gastric bypass surgery where it is suggested, but not proven, to reduce the leakage rate. While data from some preclinical studies imply that fibrin glue can negatively affect anastomotic strength, we found no compelling reason why its efficacy in protecting high-risk anastomoses should not be investigated further. Most data on the use of a fibrin-thrombin coated fleece in gastrointestinal surgery are preclinical and concern esophageal and ileal anastomoses. It was suggested to be safe and to even strengthen the anastomosis. It appears especially worthwhile to investigate the effects of suture line reinforcement if anastomoses are to be constructed under conditions where chances for leakage are high. Very recently, we described a model in the rat where a complete and patent ileal anastomosis develops signs of dehiscence during the first three days after construction.

The current study aims to determine if leakage of such high-risk anastomoses can be prevented by peroperative suture line sealing with fibrin glue or a fibrin coated patch.
Methods

Experimental Design
Altogether 110 male Wistar rats weighing 250-295 g (experiment 1: n=20 from Harlan BV, Horst, The Netherlands; experiment 2: n=90 from Charles River, Sulzfeld, Germany) were housed 2 per cage and accustomed to laboratory conditions for five days before the start of the experiment. All rats underwent intestinal resection and an anastomosis was constructed in the ileum. Postoperative development of anastomotic leakage depended on the administration of carprofen. All animals were observed closely and weighed daily and had free access to water and standard rodent chow (Hope Farms, Woerden, The Netherlands) throughout the entire experimental period. The Animal Ethics Review Committee of the Radboud University Nijmegen approved the study.

In experiment 1, 20 rats were randomly divided over a control group (n=10) and a group (n=10) where fibrin glue was applied to the anastomosis. The animals received carprofen from the day of operation and were sacrificed at day 3 (Table 1).

In experiment 2, ninety rats were randomly divided over experiment A or B, each consisting of three groups: a control group (n=15) and groups (n=15 each) where the anastomosis was reinforced with fibrin glue or a fibrin-thrombin coated patch, respectively. The animals in experiment A received carprofen (low dose, see below) from the day of operation and were sacrificed at day 3. In experiment B the animals received carprofen from the first postoperative day and they were sacrificed at day 4 (Table 2).

Analgesia and operative procedure
All rats in experiment 1 were administered carprofen (Rimadyl, Pfizer Animal Health, Capelle aan de IJssel, the Netherlands) in a dosage of 5 mg/kg subcutaneously before operation and 24 h and 48 h after surgery.

All rats in experiment 2 were administered buprenorphine (Temgesic, Schering Plough, Houten, the Netherlands), 0.02 mg/kg subcutaneously, before operation and then 12 h for two days after surgery. Next to the buprenorphine, the animals in experiment 2A were also administered carprofen in a low dose, 1.25 mg/kg, prior to operation and 24 h and 48 h later. The animals in experiment 2B received carprofen in a dosage of 5 mg/kg every 24 h (three times in total), starting 24 h after surgery.

Procedures were performed under semi sterile conditions using a Zeiss operation microscope (Carl Zeiss AG, Oberkochen, Germany). Animals were anesthetized by use of a mixture of isoflurane, oxygen, and nitrogen, while breathing spontaneously through a mask. A midline laparotomy was performed and in each rat a 1-cm segment was resected in the distal ileum, 15 cm proximal to the cecum. Ileal continuity was restored by constructing an end-to-end anastomosis using 8 single-layer, inverting, interrupted sutures (Ethilon 8-0; Ethicon, Norderstedt, Germany). During operations, body temperature was kept at 38°C using a heating pad and a lamp. Intestines were covered with gauze pads soaked with 0.9% NaCl to minimize desiccation. To prevent dehydration, 10 ml of 0.9% NaCl was administered subcutaneously during the operation.

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* p<0.05 vs controls (Fisher’s two-sided test).
Necropsy and analysis of wound repair

All rats were killed by CO/CO₂ asphyxiation. The abdomen was inspected and all findings were recorded, especially signs of anastomotic leakage like dehiscence of the anastomosis, the presence of fecal spill or a puncture in the anastomotic line with or without an abscess near it. Also, the presence of severe adhesions and ileus were noted. Where possible, segments containing the anastomosis were resected and adhesions were dissected carefully without manipulation of the anastomosis, while any sealing material was left in place. In order to measure bursting pressure, the segments were infused (2 ml/min) with 0.9% NaCl containing methylene blue. The maximum pressure (mm Hg) recorded immediately before sudden loss of pressure was taken as the bursting pressure. The site of rupture (within or outside the anastomotic line) was noted. Subsequently, the segments were placed in a tensiometer, and the breaking strength (g) was measured.¹⁹

In a number of animals from experiment 2 (Table 2) tissue was used for histological analysis. Intestinal samples of approximately 1 cm containing the entire anastomosis in the middle were carefully collected en bloc, opened at the mesenteric side, washed gently in 0.9% and spread out in a cassette for paraffin-embedding. From paraffin-embedded tissues, 4 μm sections were prepared and stained with hematoxylin and eosin (H&E).

Statistics

Differences relating to dichotomous variables were analysed by a (two-tailed) Fisher’s exact test (two groups) or a Chi-square test (three groups). Comparison of continuous variables was performed using a two-tailed unpaired t-test or Mann-Whitney test (two groups) or a One-way Analysis of Variance (ANOVA) followed by a Tukey-Kramer post test (three groups). Results were considered statistically significant at p<0.05.

Results

Experiment 1

Postoperative weight loss was around 10% at day 3 and similar in the two groups. One rat died prematurely on day 3 in the control group due to ileal dehiscence and fecal spill. In the fibrin glue group four rats died prematurely on day 3, two of these exhibited signs of anastomotic leakage. In these two animals wound strength was measured because their death occurred immediately prior to the planned time of sacrifice. Altogether, anastomotic leakage was seen in eight rats in the control group and six rats in the fibrin glue group. Ileus was observed in three rats from the control group and four rats from the fibrin glue group (Table 1).
On day 3 the wound strength was similar in the ileal anastomoses from both groups. The median bursting pressure was 0 mm Hg in both groups. Median breaking strength (with range) was 21 (0-62) g in controls and 0 (0-24) g in the fibrin glue group. The breaking strength in the latter group could only be measured in seven rats, because two died prematurely and one anastomosis was completely disrupted by the bursting pressure measurement (Figure 2).

**Figure 2** Anastomotic bursting pressure and breaking strength in experiment 1. Individual values for the bursting pressure (panel A) and the breaking strength (panel B) in the control and the fibrin glue group. Horizontal bars indicate median, if no horizontal bar is present median value is zero. The bursting site was always within the suture line. Since two rats in the fibrin glue group died immediately prior to scheduled termination, wound strength could still be measured.

**Experiment 2A**

The mean relative weight at day 3 (compared to pre-operative values) was 93.2 ± 0.7 (SD) % in the control group. Weights were significantly (p<0.0001, ANOVA) lower in both the fibrin glue (90.0 ± 0.6 %, p<0.05 post test) and the fibrin coated patch (89.3 ± 0.5 %, p<0.05) groups.

Two rats died prematurely in each experimental group; only one of these (from the fibrin coated patch group) showed signs of anastomotic leakage. However, the fibrin coated patch that was applied contained the fecal spill. The other rats died of ileus (Figure 1f). Leakage rates in the groups with suture line reinforcement were not significantly different from that in controls. Ileus was more frequently observed in the fibrin glue (p=0.0169) and the fibrin coated patch (p<0.0001) groups than in the control group where it was absent (Table 2).

Neither anastomotic bursting pressure nor breaking strength were significantly different in the three groups (Figure 3A). Typical examples of ileal anastomoses are presented in Figure 4. No obvious or consistent differences in microscopic

**Figure 3** Anastomotic bursting pressure and breaking strength in experiment 2. Panels A and B depict individual values and means (red horizontal bars) for the bursting pressure in the control, fibrin glue and fibrin coated patch groups of experiment 2A (panel A) and 2B (panel C). The bursting site was always within the suture line. In panel B (experiment 2A) and D (experiment 2B) data represent mean and SEM for breaking strength in the three groups. * p<0.0001 vs controls (two-tailed Mann-Whitney test).
wound architecture were seen between groups. Fibrin glue and the fibrin coated patch were present and marked in the figures.

**Experiment 2B**

At day 4 the mean values for relative weight were 92.1 ± 3.8 % in the controls and 86.9 ± 2.2 (p<0.001) and 86.8 ±1.3 % (p<0.001) in the fibrin glue and fibrin coated patch groups, respectively.

In the control group, one rat died of ileus. Two rats died prematurely in the fibrin glue group, both of ileal dehiscence (Figure 1c). In the fibrin coated patch group seven rats died prematurely (p=0.0352 vs the control group). None of them displayed signs of anastomotic leakage, but all showed signs of ileus. Altogether nine rats (60 %) had an ileus in the fibrin coated patch group, which frequency was higher, but not significantly so, than in both other groups. The anastomotic leakage rate was highest (67%) in the fibrin glue group, but differences with the fibrin coated patch (40%) and control (27%) groups remained non-significant (Table 2).

Due to premature mortality and technical problems, anastomotic bursting pressure and breaking strength in the fibrin coated patch group could only be measured in two and one rat, respectively.

The mean bursting pressure on day 4 was significantly (p<0.0001) lower in the fibrin glue group  (24 ± 8 (SEM) mm Hg than in the control group (134 ± 15 mm Hg).

No such difference was observed for the anastomotic breaking strength (Figure 3C,D)

**Discussion**

In theory, reinforcement of the suture line in high-risk anastomoses may reduce the frequency of anastomotic leakage. In practice, in this particular animal model, neither fibrin glue nor a fibrin coated patch reduces the leakage rate of ileal anastomoses. They even increase postoperative complications and, in case of the patch, mortality.

Any method which prevents peritonitis to occur, or minimizes or even postpones the deleterious effects of anastomotic dehiscence, constitutes enormous progress. For this purpose, two frequently used, fibrin-based, sealants have been examined, using a new model for anastomotic leakage. So far, this type of experiments have been performed in models where anastomotic dehiscence is induced by creation of an iatrogenic defect, mostly by using an insufficient number of sutures. In the present model, peri-operative use of carprofen induces dehiscence with time of a patent anastomosis in the rat ileum. Although the mechanisms responsible remain to be elucidated, we believe the model to be clinically more relevant than those where a suture line defect is created on purpose during surgery.

It has been suggested that fibrin glue applied to patent sutured colonic anastomoses can negatively affect their strength. However, in a previous study we have found fibrin glue not to have any clinically relevant effect on the early healing of intestinal anastomoses.
The current study shows no advantage of the application of fibrin glue around the high-risk ileal anastomosis. It does not reduce anastomotic leakage rates. On the contrary, in experiment 2B, it even seems to increase them, although not significantly so (Table 2). Another important finding is that fibrin glue appears to correlate with ileus, particularly in experiment 2A. The peristaltic movement of the bowel can be decreased once a rigid glue clot is formed on the anastomosis and the area around it. Such a phenomenon may also explain the additional weight loss in the fibrin and fibrin coated patch groups. Since the rat is not capable of vomiting it just stops eating and loses weight. This effect has been previously found by Chmelnik 22 who made an insufficient ileo-ileal anastomosis in rats and found severe preanastomotic dilatation after wrapping it with a fibrin coated patch. Indeed, when a fibrin coated patch was applied in our study ileus rates were even higher. In experiment 2B, all premature deaths in the fibrin coated patch group were the result of ileus and not of anastomotic leakage. Although anastomotic leakage rates are not significantly reduced by applying the fibrin coated patch, there were two cases in which the patch prevented the fecal content from leaking into the abdominal cavity.

Pantelis 16 wrapped a fibrin coated patch around an incomplete colonic anastomosis in mice and reports less anastomotic leakage without mentioning ileus rates. Perhaps the colonic wall is more firm and therefore less prone to compression of the lumen than the ileal wall. However, in a previous study where fibrin glue was applied around a colonic anastomosis, we also found the rats to suffer from colonic ileus. 14 In addition, Schreinemacher finds a collagen fleece, which is kept in place around a colonic anastomosis with fibrin glue, to induce bowel obstruction and not to improve healing. 23 Nordentoft 17 tested a fibrin coated patch around a patent anastomosis in the small intestine of a pig and reports no difference in degree of stenosis, but also no differences in healing compared to the control anastomosis. Recently clinical studies using a fibrin coated patch have been published. Wrapping a fibrin coated patch around a colonic anastomosis is feasible and well tolerated. 24 A very small series describes less anastomotic leakage and a shorter hospital stay when a fibrin coated patch is used. 25 In addition, there is one clinical report on pancreatic surgery 26 showing promising results in reducing leakage. Tissue adhesives and glues have already proven to be very useful in different fields of surgery to promote tissue, union, sealing, hemostasis and wound healing. Despite their multi-faceted use they are not frequently used in gastro-intestinal surgery. This study finds fibrin glue and a fibrin coated patch not to be useful if applied around a leakage-prone anastomosis. Obviously, one should keep in mind that the results stem from an experimental model where the exact mechanism that induces leakage to occur remains unknown. Still, the adverse events observed constitute a warning against the random application of such seals around intestinal anastomoses. Since anastomotic leakage remains such a severe complication after gastro-intestinal surgery, the search for an effective seal around the anastomosis that prevents leakage without causing complications must remain an important goal for ongoing research.
Reference List


SUMMARY AND FUTURE DIRECTIONS
Summary

A leaking intestinal anastomosis is a relatively frequent disaster associated with increased morbidity and considerable mortality. Many clinical and preclinical studies have aimed to identify risk factors for impaired anastomotic healing, but the finding that anastomotic leakage may take place in the absence of any currently known risk factor illustrates that much remains to be learned. In this respect very little attention has been paid to the analgetics that are frequently used perioperatively. In addition, recently there exists renewed interest in the possibilities of suture line reinforcement as a means to prevent anastomotic leakage or to contain its effects.

The background for the studies described hereafter is outlined in chapter 1. All studies have been performed in a well established rat model suitable for the assessment of anastomotic healing in the intestine. Primary outcome measurements for wound repair are wound strength, measured as bursting pressure and breaking strength, and anastomotic leakage. In addition, collagen levels are measured and wound histology is examined.

Paracetamol is widely used for pain relief after surgical interventions. Its potential effects on soft tissue repair have hardly been investigated. Chapter 2 describes the first study into the effect of paracetamol on early healing of intestinal anastomoses and abdominal fascia. In 78 rats an anastomosis was constructed in both ileum and colon. The rats received either a low or a high dose (50 or 200 mg/kg/day, respectively, divided over two doses) paracetamol or vehicle (controls) until they were killed on day 3 or 7 after surgery. No significant differences were found between control and paracetamol-treated groups at any time point for any of the parameters investigated. Wound strength increased significantly from day 3 to day 7 in all groups, both in anastomoses and in the fascia. No differences in collagen levels were found nor did histology indicate the presence of gross differences between groups. This study suggests that paracetamol can be given safely to patients for postoperative analgesia after gastrointestinal surgery.

Next to paracetamol, non-steroidal anti-inflammatory drugs (NSAIDs) are important constituents of protocols for postoperative pain relief. There is an emerging consciousness that perhaps their use may not be without consequences for the repair sequence, particularly the inflammatory phase. Chapter 3 reports on a study into the effects of the NSAIDs diclofenac and naproxen. An anastomosis was constructed in both colon and ileum of 104 rats. They were divided into groups who received either diclofenac (4 mg/kg/day) or naproxen (10 mg/kg/day) daily from the day of surgery or from day 3 after surgery. Animals were killed on day 3 or day 7.
Anastomotic leakage in the ileum \((p=0.0001)\) and mortality rates \((p=0.001)\) were significantly increased in the diclofenac group. On day 7 the anastomotic bursting pressure in the ileum remained below that of the controls in the diclofenac and naproxen treated rats. When administration of diclofenac was postponed to day 3 after surgery, anastomotic dehiscence was almost absent. The colonic anastomosis and abdominal wall always remained unaffected. This study implies that administration of diclofenac immediately after intestinal surgery strongly affects ileal anastomotic healing in the rat. This effect is also observed for naproxen, although to a much lesser extent. Interestingly when drug administration is withheld for three days these complications are prevented. NSAIDs differ in their selectivity for COX-1 and COX-2. Diclofenac is reported to be far more selective for COX-2 than naproxen.

Although the more selective NSAIDs may be preferred for their supposed lack of side effects, one should realize that this is more relevant for chronic users. In the short postoperative period it is more important to choose an analgesic drug that does not interfere with the healing process.

Earlier work from our laboratory and the data reported in chapter 3 indicate that NSAIDs with a preference for COX-2 inhibition may induce anastomotic failure in small bowel anastomoses. This suggests that controlled application of a suitable drug from this class might result in reproducible leakage rates, without excessive mortality, in initially patent small bowel anastomoses. Therefore, we examined the potential of the COX-2 inhibitor carprofen, widely used in veterinary medicine and laboratory animals, to reproducibly induce anastomotic leakage in the intestine (chapter 4). In twenty rats an anastomosis was constructed in ileum and colon carprofen or buprenorphine was administered (experiment 1). In another 20 rats an anastomosis in either ileum or colon was constructed and they were given carprofen (experiment 2). Animals were killed after 3 days. In experiment 1, the ileal dehiscence rate was 60% in the carprofen group and 0% in the buprenorphine group \((p=0.0108)\). Colonic anastomoses in both groups remained patent. In experiment 2, the anastomotic leakage rate was 80% in ileum and 0% in colon. Anastomotic bursting pressure was also lowered in the carprofen groups. The fact that the COX inhibitor carprofen elicits such profound effects in the ileum, though not in the colon, should be taken as a further warning that anti-inflammatory analgesics may interfere with tissue repair. However, at the same time it offers an opportunity to establish a long-wanted model for anastomotic leakage, without iatrogenic disruption of the anastomotic integrity. This model provides a means to perform much needed translational research on the effectiveness of, for instance, peroperative suture line reinforcement (see also chapter 7).

Chapter 5 describes experiments to further characterize the previously mentioned negative effects of carprofen on early anastomotic healing. In 159 rats an anastomosis was constructed in the ileum. In experiment 1 eighty-four rats were divided over control and experimental groups, which received daily buprenorphine or carprofen, respectively, as an analgesic and were killed on day 1, 2 or 3 after surgery. In experiment 2 three groups of 15 rats received carprofen daily either immediately after surgery or with a delay of 1 or 2 days. Animals were killed after 3 days of carprofen administration. In experiment 3 three groups of 10 rats received different doses \((\text{full, half or quarter})\) of carprofen daily from surgery. Animals were killed after 3 days.

In significant contrast to buprenorphine, which never did so, carprofen induced frequent signs of anastomotic leakage, which were already present at day 1. If first administration was delayed for 48 hours, the leakage rate was significantly reduced \((\text{from 80 to 20\%}; \ p=0.0028)\). Throughout the study, the anastomotic bursting pressure was lowest in animals who displayed signs of anastomotic leakage. Loss of anastomotic integrity did not coincide with reduced levels of hydroxyproline or increased activity of matrix metalloproteinases. Thus, we conclude that carprofen interferes with wound healing in the rat ileum at a very early stage. If it does not kill the animal, at least it renders it more vulnerable to second hits after initial surgery.

Although the mechanisms responsible remain to be fully understood, there appears to be an increasing body of evidence which suggests that one should be aware that NSAIDs may affect the outcome of the wound healing sequence.

In theory, it seems logical to apply some kind of seal around the anastomosis to prevent leakage. Very recently, it has been emphasized again that the potential benefits of such suture or staple line reinforcement need investigation. While the concept that fibrin glue may be used to strengthen the intestinal anastomosis is certainly not new, it has not yet led to consistent and reliable data which demonstrate its effectiveness and supply indications for its clinical use. Incidental data from preclinical studies seem to suggest that fibrin glue applied to patent sutured colonic anastomoses can negatively affect their strength. Therefore, the effects of applying fibrin glue around a normal intestinal anastomosis have been carefully examined and the results are reported in chapter 6. In 108 rats an anastomosis was constructed in the ileum and colon. In half, fibrin glue was applied around anastomoses. Animals were killed at day 3, 5 or 7. A transient colonic ileus was observed in the experimental group. Anastomotic breaking strength was always similar in both the control and fibrin glue groups. Anastomotic bursting pressures remained low at days 1 and 3, without any differences between the groups. In both groups, the bursting pressure increased sharply \((p<0.001)\).
between days 3 and 5. At day 5, the bursting pressure in the fibrin glue group remained below that in the controls, although only significantly (p=0.0138) so in the ileum. At day 5, but not at day 7, the wounds in the fibrin glue group contained less collagen. Other aspects of microscopic wound architecture appeared to be the same. Thus, a certain delay in the recovery of intestinal strength cannot be denied, while wound strength during the first 3 days appears unaffected. Altogether, the available evidence indicates fibrin glue to be not beneficial, and at its best irrelevant, to anastomotic healing in the intestine and there is no justification for using fibrin glue on patent anastomoses constructed under low-risk conditions. Its potential benefit under conditions where chances for anastomotic leakage are enhanced needs further investigation.

As described above, the use of carprofen leads to frequent signs of leakage of anastomoses in the rat ileum. Therefore, we conducted a study to investigate if the use of fibrin glue, or a fibrin coated patch, could protect anastomotic integrity in such a model for high-risk anastomoses (chapter 7). In 110 rats an anastomosis was constructed in the ileum. In experiment 1, where 20 animals were divided over a control and a fibrin glue group, all rats were administered carprofen (5mg/kg/day) starting prior to surgery. In experiment 2 the 90 rats were divided over two groups which received either carprofen (1.25 mg/kg/day) starting prior to surgery (group 2A) or starting 24 h after surgery (group 2B) in a dosage of 5 mg/kg/day. Both group 2A and 2B were divided into a control, a fibrin glue and a fibrin coated patch group, each consisting of 15 animals. The animals in experiment 1 and experiment 2A were killed at day 3 and those in experiment 2B at day 4 after surgery.

In experiment 1 anastomotic leakage rates, ileus and wound strength did not significantly differ between groups. In experiment 2A ileus was significantly more frequently present in fibrin glue and the fibrin coated patch group compared to the control group (p=0.02 and p=0.001, respectively), but anastomotic leakage rates and wound strength did not significantly differ. In experiment 2B the bursting pressure on day 4 was significantly lowered in the fibrin glue (p=0.001) and fibrin coated patch group (p=0.01) compared to the control group. Anastomotic leakage rates were highest in the fibrin glue group, although not significantly so. Protecting anastomotic integrity must remain an import goal of ongoing research to lower morbidity and mortality rates. In the present model, sealing the high-risk anastomosis with fibrin glue or a fibrin coated patch does not lower leakage rates and causes ileus. This suggests that these measures should be treated with caution.

Future directions
Mostly, in science the outcome of experiments will lead to new questions and new experiments. Fresh data always raise new questions. This also goes for the experiments and results described here. All data originate from preclinical experiments and it remains to be established to what extent they are valid in the clinical situation. Thus, in very general terms, clinical validation of the most salient facts must certainly be part of future research. In addition, certain results also ask for further investigation in preclinical setting. Our data warrant caution against an abundant and indiscriminate use of NSAIDs in the peri-operative period. Three different NSAIDs are shown to interfere, to various extent, with anastomotic healing in the intestinal tract. This seems especially disturbing in the case of diclofenac, which is used with increasing frequency after (fast-track) surgery. Over the last few years incidental clinical reports have surfaced on retrospective studies linking the use of NSAIDs to anastomotic leakage. The question, whether perioperative use of NSAIDs indeed can be a risk factor for anastomotic leakage can only be answered in the setting of a randomized controlled clinical trial. Perhaps, such a trial should be preceded by experimental studies which focus on our hypothesis that the COX-2 selectivity of the drugs decides the degree of interference with anastomotic healing. Next to diclofenac and naproxen, other frequently used NSAIDs exhibiting a wide range in selectivity for COX-1 and COX-2 should be examined. Also, the role of COX-2 in the early repair sequence and its expression during healing should be topics of further research. The fact that, in rats, only the ileum seems affected and not the distal colon also ask for further study into basal and induced expression of (COX-1 and) COX-2 throughout the gastrointestinal tract. In the end, it may well be that this line of research would offer enough incentive to change our current use of analgetics for postoperative pain relief.

It is obvious and often stated that anastomotic leakage remains a topic worthwhile for further study. Its frequency, certainly under ‘high-risk’ conditions, appears unacceptable and its consequences can be disastrous. Over the last decades it has often been argued that, in order to study anastomotic dehiscence in a preclinical setting, one should have an (animal)model at one's disposal where leakage is the primary outcome parameter. However, it can also be argued that wound strength is a good measure for the risk for anastomotic leakage to occur and, indeed, by far most studies use this as the primary outcome parameter. Still, if a model would be available where a patent anastomosis fails with time, it would add a much needed, and often wished for, tool. The results of our experiments into the effects of NSAIDs, and particularly carprofen, have demonstrated that administration of this drug may have the desired effect and cause a patent
anastomosis to fail. A goal for immediate follow up should be to determine the mechanism and to optimize the model in terms of leakage rates and other outcome parameters. Then, it could be used to further study methods that can minimize, prevent or postpone (the consequences of) leakage. In this respect, suture line reinforcement by (fibrin)glues or surgical tapes warrants further attention.
Nederlandse Samenvatting
Samenvatting

Een lekkende darmanastomose is een relatief veel voorkomende ernstige complicatie van gastrointestinale chirurgie die geassocieerd is met een verhoogde morbiditeit en mortaliteit. Veel klinische en preklinische studies hebben gepoogd factoren te identificeren welke een risico vormen voor ongestoorde genezing van de anastomose. Dat naadlekkage nog steeds optreedt als geen van de vermoede risicofactoren aanwezig is, illustreert dat er nog veel onbekend is over bijvoorbeeld de invloed van veel gebruikte perioperatieve pijnstillers op de genezing van darmanastomosen. Daarnaast is er een hernieuwde interesse naar de mogelijkheden van het verstevigen van de anastomose om naadlekkage en de gevolgen ervan te voorkomen.

De achtergrond voor de studies die zijn gedaan wordt uiteengezet in hoofdstuk 1. Alle experimenten zijn uitgevoerd in een diermodel (rat). Binnen ons laboratorium bestaat veel ervaring met dit model en het is geschikt gebleken voor het bestuderen van de (wond)genezing van darmanastomosen. Primaire uitkomstmaten voor wondgenezing zijn wondsterkte, gemeten als barstdruk en trekkracht, en lekkage van de anastomose. Daarnaast worden ook de hoeveelheid collageen in de wond en de histologische architectuur van de anastomose als parameters voor genezing gebruikt.

Paracetamol wordt zeer veel toegediend voor pijnstilling na een operatieve ingreep. Het effect van paracetamol op de wondgenezing is echter nauwelijks onderzocht. In hoofdstuk 2 wordt de eerste studie beschreven naar de effecten van paracetamol op de vroege genezing van darmanastomosen en de abdominale fascie. In 78 ratten werd een anastomose in zowel ileum als colon aangelegd. De ratten werden oplosmiddel of, respectievelijk, een lage of een hoge dosis paracetamol (50 of 200 mg/kg/dag, verdeeld over twee doseringen) toegediend, totdat ze werden gedood op dag 3 of 7 na operatie. Voor geen van de onderzochte parameters werd, op dag 3 of dag 7, een significant verschil gevonden tussen de controle en de paracetamol-groepen. De wondsterkte van de anastomose en de abdominale fascie nam significant toe in alle groepen van dag 3 naar dag 7. Er werden geen verschillen gevonden in collageenhoeveelheden en de histologische architectuur van de anastomosen. Paracetamol lijkt dan ook geen negatief effect te hebben op de wondgenezing van de hier bestudeerde weefsels. Deze studie suggereert dat paracetamol veilig kan worden toegediend als postoperatieve pijnstiller na gastro-intestinale chirurgie.

Naast paracetamol vormen non-steroid anti-inflammatory drugs (NSAIDs) een belangrijk onderdeel van de protocollen voor postoperatieve pijnstilling. Men begint echter steeds meer te beseffen dat het gebruik van NSAIDs wellicht consequenties kan hebben voor de wondgenezing, met name in de inflammatoire
fase. In hoofdstuk 3 wordt een studie beschreven naar het effect van de NSAIDs diclofenac en naproxen. In zowel colon als ileum van 104 ratten werd een anastomose aangelegd. De ratten werden verdeeld in groepen die dagelijks diclofenac (4 mg/kg/dag) of naproxen (10 mg/kg/dag) kregen toegediend vanaf de dag van operatie of vanaf 3 dagen na de operatie. De dieren werden gedood op dag 3 of dag 7. Naadlekkage in het ileum (p<0.0001) en mortaliteit (p=0.001) waren significant verhoogd in de diclofenac-groep. In dag 7 was de barstdruk in het ileum lager in de diclofenac en naproxen-groepen vergeleken met de controle-groep.

Wanneer diclofenac pas vanaf dag 3 na operatie werd toegediend werd er nauwelijks naadlekkage gezien. De colonanastomose en abdominale fascie waren nooit aangedaan. Deze studie impliceert dat diclofenac, indien het meteen na de operatie wordt toegediend, de genezing van de anastomose in het ileum van de rat sterk negatief beïnvloedt. Dit effect wordt ook in mindere mate bij naproxen gezien. Een interessante bevinding is dat het uitstellen van het toedienen van de medicatie naar dag 3 na operatie deze complicaties voorkomt. NSAIDs verschillen in hun selectiviteit voor COX-1 en COX-2. Diclofenac wordt selectiever bevonden voor COX-2 dan naproxen. Selectieve NSAIDs worden vaak geprefereerd omdat ze minder bijwerken geven. Men moet zich echter realiseren dat dit vooral bij chronisch gebruik van belang is. In de korte postoperatieve periode is het belangrijker een analgetisch medicijn te kiezen dat niet interferereert met de wondgenezing.

Uit eerder werk verricht op ons laboratorium en de data beschreven in hoofdstuk 3 blijkt dat NSAIDs en een voorkeur voor COX-2 inhibitie juist naadlekkage induceren in de dunne darm. Dit suggereert dat een gecontroleerde toediening van een geschikt medicijn van deze klasse kan resulteren in reproduceerbare naadlekkage zonder excessieve mortaliteit in patente anastomoses in de dunne darm. Daarom hebben we de mogelijkheden van de COX2 remmer carprofen, die frequent wordt gebruikt in de veterinaire geneeskunde en bij laboratoriumdieren, onderzocht met als doel reproducerbaar naadlekkage te induceren (hoofdstuk 4). In 20 ratten werd een anastomose geconstrueerd in zowel ileum als colon en de ratten kregen carprofen of buprenorfine als pijnstelling (experiment 1). In experiment 2 werd in nog eens 20 ratten een anastomose in het ileum of het colon gemaakt en deze ratten kregen carprofen. De dieren werden na 3 dagen gedood.

In experiment 1 werd in 60% van de anastomosen in het ileum naadlekkage gezien in de carprofen-groep ten opzichte van 0% in de buprenorfine-groep (p=0.0108). De anastomosen in het colon waren niet aangedaan. In experiment 2 werd in 80% van de anastomosen in het ileum naadlekkage gezien en bij geen van de anastomosen in het colon. De barstdruk was ook verlaagd in de carprofen-groepen. Het feit dat de COX remmer carprofen zulke hevige negatieve effecten kan hebben in het ileum, hoevel niet in het distale colon, moet als een waarschuwing worden gezien dat anti-inflammatoire medicatie mogelijk het wondgenezingsproces kan verstoren. Tegelijkertijd biedt het echter de mogelijkheid een lang gezocht model voor naadlekkage te ontwikkelen, waarbij er in opzet een patente en intacte naad wordt gemaakt. Dit model voorziet in de behoefte om onderzoek te verrichten naar de effectiviteit van peroperatieve versterking van de anastomose. (zie ook hoofdstuk 7).

Hoofdstuk 5 beschrijft meerdere experimenten die zijn gericht op het verder karakteriseren van de voornoemde negatieve effecten van carprofen op de vroege genezing van de anastomose. In 159 ratten werd een anastomose geconstrueerd in het ileum. In experiment 1 werden 84 ratten verdeeld over controle en experimentele groepen welke dagelijks hetzij buprenorfine hetzij carprofen kregen toegediend en welke werden gedood op dag 1, 2 of 3 na operatie. In experiment 2 werd aan 3 groepen van 15 ratten dagelijks carprofen toegediend, te beginnen direct na operatie of 1 of 2 dagen later. De ratten werden 3 dagen na de start van toediening van carprofen gedood.

In experiment 3 werd dagelijks aan 3 groepen van 10 ratten verschillende doseringen carprofen toegediend (volledig, half of kwart) direct na operatie. Carprofen induceerde al vanaf dag 1 frequent tekenen van naadlekkage, terwijl er in de buprenorfine-groep geen naadlekkage te zien was. Als er met de toediening van carprofen 48 uur werd gewacht, was er een significante afname van naadlekkage (van 80 naar 20%; p=0.0028). In deze studie was de barstdruk het laagst in de ratten waar tekenen van naadlekkage te zien waren. De aanwezigheid van tekenen van naadlekkage ging niet gepaard met een significante afname van de hoeveelheid hydroxyproline of een verhoogde activiteit van matrix metalloproteinase. We kunnen concluderen dat carprofen de zeer vroege fase van wondgenezing in het ileum van de rat verstoorde. Als de rat hier niet aan overlijdt, inducerde dit fenomeen op zijn minst een verlaagde weerbaarheid tegen eventuele additionele negatieve postoperatieve condities.

Ondanks het gegeven dat de mechanismen die in deze experimenten verantwoordelijk zijn voor naadlekkage nog niet volledig ontrafelt zijn, dragen deze resultaten bij aan een groeiende bewijslast welke suggereert dat NSAIDs de wondgenezingcascade kunnen verstoren.

In theorie lijkt het logisch een soort van afdichting rond de anastomose aan te brengen om lekkage te voorkomen. Zeer recent is het wederom benadrukt dat de potentiele voordelen van een dergelijke versterking van de anastomose nader onderzoek behoeven. Het concept dat fibrinellijn gebruikt kan worden om de anastomose te versterken is niet nieuw. Eerder onderzoek heeft echter nog geen consistent en betrouwbare data opgeleverd welke de effectiviteit van fibrinelijn
In experiment 1 verschilde de frequentie van naadlekkage en ileus en de wond op dag 3 en de ratten in experiment 2B op dag 4 na operatie. Die elk uit 15 dieren bestonden. De ratten in experiment 1 en 2A werden gedood in de periode rond de anastomose in het colon de wondsterkte negatief kan beïnvloeden. Daarom hebben we bestudeerd in de controle met een indicatie voor het gebruik in de kliniek mogelijk maken.

In experiment 2 werden 90 ratten verdeeld over 2 groepen welke of carprofen (1.25 mg/kg/dag) kregen toegediend vanaf de operatie. Zowel groep 2A als groep 2B kregen carprofen (5mg/kg/dag) vanaf de operatie. Alle ratten kregen carprofen (5mg/kg/dag) vanaf de operatie. In experiment 1 werden 20 ratten verdeeld over een controle en fibrinelijm-groep. Alle ratten kregen carprofen (5mg/kg/dag) vanaf de operatie. De barstdruk bleef laag op dag 1 en 3, zonder verschillen tussen de groepen. In beide groepen nam de barstdruk fors toe (p<0.001) tussen dag 3 en dag 5, zonder significante verschillen tussen de groepen. Op dag 5 was de barstdruk van de fibrinelijm-groep lager dan die van de controle-groep, maar dit was alleen significant (p=0.0138) in het ileum. Op dag 5 bevatte de anastomosen in de fibrinelijm-groep minder collagen dan in de controle-groep. Dit verschil was op dag 7 verdwenen. De histologische architectuur was niet verschillend in beide groepen. Ondanks een vertraging in herstel van wondsterkte, lijkt de wondsterkte in de eerste 3 dagen niet aangedaan. Het beschikbare bewijs lijkt niet aan te kunnen tonen dat toepassing van fibrinelijm of een fibrine gecoate matrix rond de anastomose wordt aangebracht. Deze maatregelen veroorzaken zelfs ileus. De resultaten suggereren dat het gebruik van dergelijke methoden met zorg moeten worden afgewogen.

Toekomstige onderzoek

In de wetenschap is het vaak zo dat de uitkomsten van experimenten leiden tot nieuwe vragen en experimenten. Nieuwe data zorgen altijd voor nieuwe vragen. Dit geldt ook voor de experimenten en resultaten die hier zijn beschreven. Alle data zijn verzameld uit preclinische experimenten en in hoeverre ze ook gelden in de klinische situatie zal nog moeten blijken. Over het algemeen zal klinische validatie van de meest opvallende bevindingen zeker onderdeel moeten zijn van toekomstig onderzoek. Daarnaast zijn er ook resultaten die verder onderzoek in de preclinische setting vragen.

Onze data gebieden dat voorzichtigheid is geboden bij het overmatig toedienen van NSAIDs in de periode rond de operatie. Bij drie verschillende NSAIDs wordt in meer of mindere mate verstoring van de genezing van de darmanastomose aangetoond. Met name diclofenac lijkt dit proces te verstoren, terwijl dit middel steeds vaker in de kliniek gebruikt wordt na (snel herstel) operaties. In de laatste jaren zijn er incidentele retrospectieve studies verschenen die het optreden van naadlekkage lijken te correleren aan het gebruik van NSAIDs. De vraag of perioperatief gebruik van NSAIDs inderdaad een risicofactor is voor naadlekkage kan alleen worden beantwoord door een gerandomiseerd klinisch onderzoek. Misschien zou een dergelijke studie vooraf moeten worden gegaan door experimentele studies welke zich concentreren op onze hypothese dat de COX-2 selectiviteit van verschillende medicijnen samenhangt met de mate waarin wondgenezing van de anastomose wordt verstoord. Naast diclofenac en naproxen zullen ook andere frequent gebruikte NSAIDs met een variatie in COX-1 en COX-2 selectiviteit moeten worden onderzocht. De rol van COX2 in de vroege wondgenezing en de expressie ervan gedurende genezing moeten eveneens onderwerp zijn van verder onderzoek. Het feit dat in ratten alleen het ileum is aangedaan en
niet het distale colon vraagt om verdere bestudering van de basale en geïnduceerde expressie van (COX-1 en) COX-2 in de tractus gastrointestinalis. Uiteindelijk zou het zo kunnen zijn dat deze onderzoekslijn genoeg aanwijzingen geeft om ons huidige gebruik van postoperatieve pijnstilling te heroverwegen en aan te passen.

Het is duidelijk en vaak benoemd dat naadlekkage een probleem blijft dat verdere bestudering behoeft. De frequentie waarmee deze complicatie optreedt, zeker onder hoog risico-omstandigheden, lijkt onacceptabel en de consequenties kunnen desastreus zijn. In de laatste tientallen jaren is vaak geponeerd dat, om naadlekkage in de preklinische setting adequaat te kunnen onderzoeken, er een (dier)model beschikbaar moet zijn waar lekkage de primaire uitkomstmaat is. Daarbij moet worden opgemerkt dat ook wondsterkte als een goede maat kan worden gezien voor het risico van het optreden voor naadlekkage. Tot op heden wordt deze inderdaad vrijwel altijd gebruikt als primaire uitkomstparameter. Indien er echter een model beschikbaar zou zijn waar een patente, intacte anastomose in de loop van de tijd toch gaat lekken zou dit een gewenst en bruikbaar model zijn. De resultaten van de experimenten naar de effecten van NSAIDs, in het bijzonder carprofen, laten zien dat het toedienen van dit medicijn dit gewenste effect zou kunnen bewerkstelligen.

Een doel van toekomstig onderzoek op korte termijn zou het vaststellen van het mechanisme en het optimaliseren van het model in termen van lekkage en andere parameters kunnen zijn. Dan zou het model gebruikt kunnen worden om methoden te bestuderen die (de consequenties van) naadlekkage kunnen minimaliseren, uitstellen of voorkomen. In dit kader zal het versterken van de anastomose door (fibrine)lijm of chirurgische tape ook nader moeten worden onderzocht.
Curriculum Vitae
Curriculum Vitae

Rozemarijn van der Vijver was born on the 6th of July 1982 in Tiel, the Netherlands. She grew up in Brabant as the oldest daughter of two. After finishing the Atheneum in 2001 she attended the first year of medical school at VU University of Amsterdam and she completed medical school at the Radboud University in Nijmegen. During this study she fulfilled a foreign internship (Cardiology) at the Liverpool Hospital in Sydney (Australia). After obtaining her medical degree in 2007 she started working as a surgical resident in Jeroen Bosch Medical Centre in Den Bosch and subsequently at the Radboud University Nijmegen Medical Centre. In 2010 and 2011 the experiments described in this thesis were performed at the Surgical Research Laboratory and Central Animal Laboratory of the Radboud University of Nijmegen. January 2012 she started her formal surgical training in the Nijmegen region, where she is currently working in the Department of Surgery (prof. dr. CJHM van Laarhoven) of the Radboud University Nijmegen Medical Centre. From January 2014 she will continue her surgical training in the Rijnstate Hospital in Arnhem (dr. MMPJ Reijnen). Rozemarijn lives in Nijmegen and loves to travel.

List of Publications
Publications

Related to this thesis

van der Vijver RJ, van Laarhoven CJHM, Lomme RMLM, Hendriks T. The effect of sealing a high-risk anastomosis in the rat ileum with fibrin glue or a fibrin coated collagen patch. To be submitted.


Other publications


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Dankwoord

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