Postoperative non-steroidal anti-inflammatory drugs and colorectal anastomotic leakage

NSAIDs and anastomotic leakage

Mads Klein

This review has been accepted as a thesis together with five previously published papers by University of Copenhagen 24.11.2011 and defended on 27.01.2012

Tutors: Ismail Gögenur & Jacob Rosenberg

Official opponents: Ingvar Syk, Claus Hovendal & Per Jess

Correspondence: Department of Surgery D, Copenhagen University Hospital Herlev, Herlev Ringvej 75, 2730 Herlev, Denmark.

E-mail: madsklein1@gmail.com

Dan Med J 2012;59(3): B4420

ARTICLES INCLUDED IN THESIS

Study I — Klein M, Andersen LPH, Harvald T, Rosenberg J, Gögenur I. Increased risk of anastomotic leakage with diclofenac treatment after laparoscopic colorectal surgery. Dig Surg 2009; 26: 27-30

Study II — Klein M, Krarup PM, Burcharth J, Agren MS, Gögenur I, Jorgensen LN, Rosenberg J. Effect of diclofenac on cyclooxygenase-2 levels and early breaking strength of experimental colonic anastomoses and skin incisions. Eur Surg Res 2011; 46: 26-31.

Study III — Klein M, Krarup PM, Kongsbak MB, Agren MS, Gögenur I, Jorgensen LN, Rosenberg J. Effect of postoperative diclofenac on anastomotic healing, skin wounds and subcutaneous collagen accumulation – a randomized blinded placebo-controlled experimental study. Eur Surg Res 2012 *in press*

Study IV — Klein M, Pommergaard HC, Gögenur I Rosenberg J. Rapidly resorbable vs. non-resorbable suture for experimental colonic anastomoses in rats - A randomized experimental study. Int J Surg 2011; 9: 332-4.

Study V — Klein M, Gögenur I, Rosenberg J. Postoperative non-steroidal anti-inflammatory drugs increase anastomotic leakage rate after colorectal resection. BMJ *submitted February 2012*

ABBREVIATIONS

AL	anastomotic leakage
NSAID	non-steroidal anti-inflammatory drug
COX	cyclooxygenase
PGE2	prostaglandin E2
TXA2	thromboxane A2
MMP	matrix metalloproteinase
ASA	American Society of Anaesthesiologists
ePTFE	expanded polytetrafluoroethylene
MIREDIF	smallest relevant difference
DCCG	Danish Colorectal Cancer Group
OR	odds ratio
RR	relative risk
HR	hazard ratio
CI	confidence interval
RCT	randomized controlled trial

INTRODUCTION

Anastomotic leakage (AL) is a serious complication following colonic or rectal resections with primary anastomosis. AL is most precisely defined as a combination of clinical findings such as pain or peritonitis; paraclinical/biochemical findings such as fever, tachycardia, radiological findings of fluid and/or gas containing collections and, finally, intraoperative findings [1,2].
AL occurs with a frequency of around 3 % after colonic resections and between 7 and 10 % after rectal resection [3]. Clinical significant leakages occur after a median of 7 days after surgery [4]. Consequences of AL include mortality rates up to 32 %, increased morbidity, length of stay, risk of permanent stoma and poor functional outcome [5-7]. The prognosis is also affected in the long term, while controversy remains whether there is an effect on oncological outcome [8,9].

Due to its clinical significance, risk factors for AL have been examined extensively and many contributing factors have been identified. These risk factors can be divided into preoperative patient-related factors, intraoperative and postoperative factors. Preoperative factors increasing leakage rate include male sex, age above 70 years, malnutrition (often described as low serum-albumin), steroid use, tobacco and excessive alcohol consumption, American Society of Anaesthesiologists (ASA) score > 3and cardiovascular disease [1,2,5,7,10-16].

During surgery it has been proven that an anastomosis close to the anal verge, prolonged operative time, bowel obstruction and dilatation, impaired blood flow to the anastomosis, pe-

rioperative blood transfusion and septic intra-abdominal conditions increase leakage rate [7,10,11,14,15,17,18].
In the postoperative period, fluid overload with excessive use of crystalloids may increase leakage rate and thus, fluid restriction regimens are recommended [19-22].
The possible effect of other factors on AL rate has also been discussed. Previously, use of drains was recommended, but Merad and collegues [23] proved that these are not beneficial. Furthermore, the risk of AL is the same after stapled or hand sewn anastomosis [24], after laparoscopic or open surgery [25] and with or without preoperative mechanical bowel preparation [3].

The effect of non-steroidal anti-inflammatory drugs (NSAIDs) on AL rate had, prior to the initiation of this Ph.D. study series, been examined in a few experimental studies [26-29]. These raised the suspicion that NSAIDs could cause damage to colorectal anastomoses. Also, suspicion was raised by Rosenberg & Harvald [30] in a short letter after a period with a high rate of AL coincided with the use of postoperative diclofenac.
Based on these suspicions, the following Ph.D. objective was defined.

Objective of Ph.D. thesis
NSAIDs are regularly used and recommended after colorectal resections as part of a multi-modal analgesic regimen[31,32]. The purpose of the studies included in this thesis was to examine whether there is an increased risk of AL after colorectal resections with primary anastomosis when NSAIDs are used for analgesia in the immediate postoperative period.
The pathogenesis of AL may be multifactorial, so to identify independent risk factors a large sample size is needed. The optimal method of investigation of the effect of NSAIDs on AL would be a randomized placebo controlled trial. However, this would be very difficult and expensive to perform requiring inclusion of many high volume centres.

Therefore, to investigate this topic, we decided to perform experimental studies to investigate pathophysiological mechanisms and retrospective studies with as robust information on NSAID consumption as possible to identify individual risk factors for AL. The studies included in the thesis are described in detail in the following sections.

PRESENTATION OF STUDIES

Study I
Increased risk of anastomotic leakage with diclofenac treatment after laparoscopic colorectal surgery.

Aim
The objective of this study was to examine if postoperative diclofenac treatment was a risk factor for AL.

Methods
This was a retrospective study based on medical chart reviews with registrations of demographical variables, surgical variables and information on postoperative AL and mortality. We included laparoscopic operations for colorectal cancer performed at Gentofte Hospital with colorectal resections and primary anastomosis in the period October 2004 – June 2007. This period was separated into two consecutive periods. In the first period, the NSAID diclofenac was a part of the postoperative analgesic regimen and in period two diclofenac was withdrawn. Anastomotic leakage was defined as clinically significant leakages requiring surgical intervention and thus all leakages were confirmed by reoperation. In the entire period, the only parameter changed was diclofenac treatment. The remaining analgesic treatment consisted of paracetamol 4g/day and i.v. morphine for rescue analgesia. In all patients a thoracic epidural blockade was performed preoperatively. All registrations were performed by two individual observers and variables registered included patient-related intraoperative and postoperative factors.

We only included laparoscopically operated patients in order to increase the homogeneity of the study population and thereby improve statistical strength. We tested for differences between the two groups of patients, receiving either postoperative diclofenac or no NSAIDs with Chi-square and Fischer's Exact test for dichotomous variables, and Mann-Whitney's U-test for continuous variables. Univariate and multivariate logistic regression analyses were performed in order to identify individual risk factors for AL and exclude any confounding factors.

Results
In this study, a total of 75 consecutive patients were included. All surgical procedures were completed laparoscopically. 33 patients received postoperative diclofenac 150 mg/day and 42 patients did not receive postoperative NSAIDs. There were significantly more AL's in the diclofenac group (7/33 versus 1/42, $p = 0.018$). The two groups were comparable with regard to all registered parameters except gender, ASA-group and resection type. There were more men in the control group; this group had a higher median ASA-score and there were more rectal anastomoses among those not receiving NSAID. Univariate logistic regression analyses were performed and only diclofenac was significantly associated with AL ($p = 0.029$). In the following multivariate logistic regression analysis, diclofenac treatment, gender, age, ASA-score, position of anastomosis, duration of surgery and weight gain on the first postoperative day were included, and again only diclofenac was significantly associated with AL.

Conclusion
In this study, diclofenac treatment was an independent risk factor for AL.

Limitations
The obvious limitation of the study was the retrospective design and limited sample size. Furthermore, there was an uneven distribution of gender, ASA-score and type of resection. However, with regard to these three factors the uneven distribution only supports our hypothesis, since more men, higher ASA-class and more rectal anastomoses in the control group would tend to increase AL rate in this group. As shown, the opposite was the case. Furthermore, the strength in multivariate logistic regression analyses lies in identification of risk factors independent of other factors registered.

Due to the intrinsic limitations in the study design, this study was hypothesis-generating.

Study II
Effect of diclofenac on cyclooxygenase-2 levels and early breaking strength of experimental colonic anastomoses and skin incisions.

Aim

In this first experimental study in the study series, the objective was to i) verify the relevant dosage of postoperative diclofenac in this experimental setting and ii) to evaluate the effect of this dose on early anastomotic and skin wound healing.

Methods

Design and setting

This was a prospective randomized experimental case control study performed on male Wistar rats (Taconic, Ejby, Denmark). The Wistar rat is an outbred albino strain originating from the *Rattus Norvegicus* species. The animals were randomized to either 4 mg/kg/day diclofenac or isotonic saline administered subcutaneously twice daily in the postoperative period. This dose was chosen after having consulted veterinarians with knowledge on dosing and metabolism of analgesics in rodents.

The study was performed at the Department of Experimental Medicine, the Panum Institute, Copenhagen, Denmark which is approved for animal experiments. Prior to the studies the animals were acclimatized for 1 week. Throughout the study period, the animals had free access to standard rat chow and tap water. The study was approved from the Danish Council of Animal Experiments before initiation (J 2008/561-1583).

Operative procedure

Anaesthesia was achieved using a combination of fentanyl and fluanisol (Hypnorm®, Janssen-Cilag EMEA, Beerse, Belgium) and midazolam (Dormicum®, F. Hoffmann-La Roche Ltd., Basel, Switzerland). This was given as a subcutaneous bolus of 0.3 ml/100 g supplemented by 0.15 ml/100 gram after 20 minutes. When anaesthesia was achieved, the rats were placed on their back, had their abdomen shaved and sterilized using ethanol and chlorhexidine and finally covered in sterile dressing. Aseptic conditions were maintained during the operative procedures. First, a 4 cm abdominal midline incision was performed and next, a rectal probe was inserted 6 cm into the large intestine to assure standardization of intestinal division. After sharp division, 1 cm of colon proximal to the division site was dissected and a hand sewn anastomosis with 8 inverted single sutures using non-resorbable 6-0 sutures (Ethilon®, Ethicon Inc., New Brunswick, NJ, USA) was performed. The muscle and fascia layers were closed using a 3-0 monofilament non-resorbable suture (Ethilon®). The skin was closed using 5 titanium clips (Appose ULC, Covidien Healthcare Group, Norwalk, Connecticut, USA).

Postoperative period

For postoperative analgesia the rats received 0.05 mg/kg/day buprenorphine (Temgesic®, Schering-Plough Europe, Brussels, Belgium) divided into 3 daily doses. Medication according to randomization was administered by animal technicians who also recorded any laboratory animal suffering or illness.

Data sampling

After 3 days the animals were sacrificed after re-anaesthesia. The titanium clips were removed and the skin on the abdomen with the midline incision placed centrally was resected and skin strips perpendicular to the incision was cut out. These were stored in isotonic saline until the subsequent breaking strength measurements.

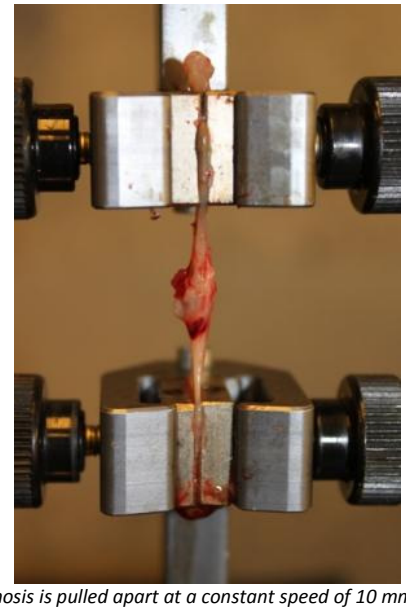
Next, the abdomen was opened, the anastomosis freed of adhesions and resected *en bloc*. Then, the breaking strength measurements of the abdominal skin strips and the anastomosis were performed using a material testing machine as described below.

After breaking, anastomoses were stored in eppendorf tubes and placed on dry ice and subsequently in freezers at – 80^{o} Celsius.

Breaking strength measurements

The tissue was placed between the claws in a material testing machine (LF*Plus*, Lloyd Instruments, Fareham, UK) equipped with an 10 Newton load cell and pulled apart at a constant speed of 10 mm/minute (figure 1 [33]). From the load-strain curve produced by the accompanying software (Nexygen®, Lloyd Instruments, Fareham, UK), breaking strength (maximal load) was derived. Furthermore, we registered where the rupture occurred, i.e. if it occurred in the anastomotic line or in adjacent healthy bowel or skin. The mean breaking strength derived from the three skin strips was used in these subsequent calculations.

Figure 1:



The anastomosis is pulled apart at a constant speed of 10 mm/min.

Cyclooxygenase-2 measurements

After being stored at -80^{o} Celsius the concentration of the COX-2 enzyme in the perianastomotic tissue was measured by a specific rat COX-2 ELISA Assay (IBL International, Hamburg, Germany). The analysis is described in detail in study II. To standardize results, total protein content of the samples was also measured and so the results were given as COX-2 per protein in nanogram/mg.

Statistics

Since COX-2 levels in perianastomotic tissue had not previously been measured, a sample size calculation based on results from the literature could not be performed. The sample size used was therefore selected based on sample sizes from similar experimental studies on colonic anastomoses in rats [26,29,34]. In the analyses, non-parametric statistics was used.

Results

A total of 32 Wistar rats were included in this study and randomized to 4 mg/kg/day diclofenac or placebo. The two groups were comparable with regard to body weight and operative time. No animals died prematurely or had to be sacrificed prematurely.

Among the rats receiving diclofenac we found a significantly reduced level of COX-2 in the perianastomotic tissue (median (range) 1.30 (0.42 - 3.31) ng/mg vs. 2.44 (0.88 - 18.94) ng/mg, p<0.001). We found no significant differences between the groups with regard to the breaking strength measurements of either the anastomoses or the skin strips. Furthermore, no significant correlations between COX-2 levels and breaking strength were found.

Conclusion

COX-2 concentration was significantly reduced at the anastomotic level by the administered dose of diclofenac. This indicates that this dose was sufficient to inhibit COX-2, and this dose is thus relevant and should be used in future studies. COX-2 inhibition did not seem to influence anastomotic or skin wound healing in the early healing phase.

Limitations

The primary limitations of experimental studies are related to difficulties of extrapolating results to the clinical setting. In this specific study, further limitations include the lack of sample size calculation and that we examined the COX-2 enzyme levels in the perianastomotic tissue and not enzyme activity. Theoretically, we have not proved that the activity of the COX-2 enzyme was decreased. However, we have not found any reports indicating that concentration of COX-2 does not equal activity of COX-2.

Furthermore, the range of results of the COX-2 levels was large. This is possibly due to the method of tissue sampling. Only a small part of the perianastomotic tissue was examined since only a small sample is needed for an ELISA analysis. Results were standardized based on the total amount of protein in the tissue sample. In spite of the deviating results, non-parametric analyses revealed a significantly decreased COX-2 level with diclofenac treatment.

Study III

Effect of postoperative diclofenac on anastomotic healing, skin wounds and subcutaneous collagen accumulation – a randomized blinded placebo-controlled experimental study.

Aim

The purpose of this study was to evaluate the effect of diclofenac on anastomotic and skin wound breaking strength 7 days postoperatively and, by introducing a new method, also evaluate the effect on subcutaneous collagen accumulation.

Methods

Design and setting was similar to study II. This was a prospective randomized experimental case control study performed in Wistar rats (Taconic, Ejby, Denmark). Again, experiments took place at the Department of Experimental Medicine, the Panum Institute.

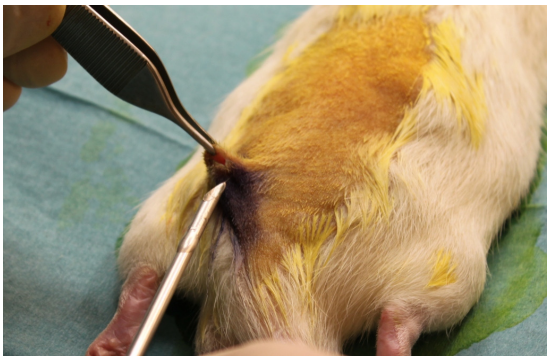
The study was approved from the Danish Council of Animal Experiments before initiation (J 2008/561-1583).

The operative procedures were performed in the same fashion as described above with the following exception: before the abdomen was opened and the anastomosis was performed, each rat had an expanded polytetrafluoroethylen (ePTFE) tube inserted in the subcutaneous space on the back. The tubes were placed at a standardized position on the left side of the back with insertion point 1 cm cranial and 1 cm lateral to the tail root (figure 2a) and propagating cranially 5 centimetres parallel to the spine. Insertion was performed through a large gauge cannula (figure 2b).

In the first 7 postoperative days, diclofenac 4 mg/kg/day or placebo was administered according to randomization. Analgesia was achieved as in study II.

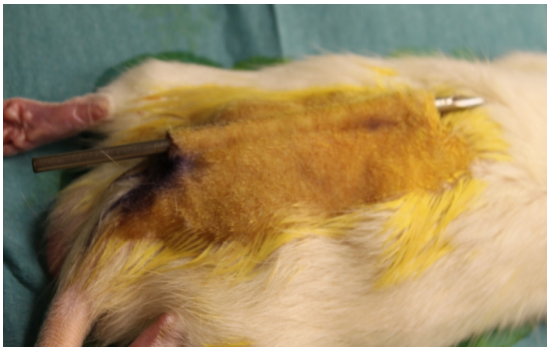
After 7 days, data were sampled. The rats were sacrificed after re-anaesthesia and skin strips and anastomoses were prepared as described above. The ePTFE tubes were removed, freed of any fibrotic capsule and stored in concentrated acetone at 5^{o} Celsius until subsequent hydroxyproline measurements.

Figure 2a:



Insertion point of ePTFE tube.

Figure 2b:



The ePTFE tube is inserted via a large gauge cannula.

Hydroxyproline measurements in ePTFE tubes

The amino acid hydroxyproline is an important part of collagen fibrils and thus collagen production can be estimated by measuring hydroxyproline levels. Hydroxyproline content in the ePTFE tubes was measured colorimetrically as described in detail in study III. Results were given as mcg hydroxyproline/mg defatted dry tissue including ePTFE tube material.

Statistics

Sample size was calculated from Ågren et al [35]. To increase likeliness of achieving a significant result, power was set at 90 %. Alpha was set at 5% and MIREDIF at 30 %. From this calculation, 22 animals in each group were needed. Based on this and the fact that a new method was introduced, we chose to include a total of 60 animals, 30 in each group. Statistical analyses were performed using non-parametric statistics and thus, Mann-Whitney's and Wilcoxon's Tests were used for intra- and inter-group differences, respectively. For correlation analyses, Spearman's tests were used. P-values equal to or below 0.05 were considered significant.

Results
For this study we used 60 Wistar rats. Eight of these died in the first postoperative hours, four from each group. Autopsies were performed and it was obvious that the deaths were due to respiratory failure and not AL or other surgical complications. Thus, the analyses were performed on the remaining 52 animals.

Unfortunately, a large proportion (29/52) of the animals had themselves removed one or more of the titanium clips closing the skin wound. Only animals (23/52) with all five titanium clips in situ were included in the breaking strength measurements of the skin strips.

We recorded no suffering; there were no premature sacrifices and the groups were comparable with regard to postoperative weight changes.

We found no differences in breaking strength measurements of either the skin strips or the anastomoses. Subcutaneous hydroxyproline accumulation was significantly decreased among the animals receiving postoperative diclofenac (median (i.q.r.) 0.29 (0.13-0.47) vs. 0.47 (0.28-0.62) mcg/mg, p = 0.03).

Correlation analyses were performed and in the placebo group increased level of subcutaneous collagen production was correlated to reduced anastomotic strength and to increased skin wound strength. These correlations were not found among the animals treated with diclofenac.

Conclusion
Diclofenac treatment reduced subcutaneous hydroxyproline accumulation and thus collagen production. Surprisingly, in the control group, we found a reverse correlation between s.c. hydroxyproline levels and anastomotic strength. On the opposite, there was direct correlation between s.c. hydroxyproline and skin wound strength. That these correlations were not found in the diclofenac group underlines the effect of the NSAIDs on collagen metabolism.

The opposing correlations found in the control group may be due to a heterogenic response from fibroblasts in subcutis and large bowel. It has previously been shown, that fibroblasts in the two different tissues respond different to stimulation from Interleukin 1β [36], which is produced during inflammation and differentially expressed during COX-2 inhibition [37].

Diclofenac treatment did not influence anastomotic or skin wound breaking strength, so it did not seem that the significant decrease in collagen production had a clinical impact.

Limitations
Measurements of perianastomotic hydroxyproline could have added information. However, we wished to focus solely on newly produced collagen and therefore measured the subcutaneous accumulation. Comparison between this subcutaneous accumulation and perianastomotic hydroxyproline content would not be meaningful since a measure of hydroxyproline in anastomotic tissue consists of newly produced, partly degraded and mature and immature collagen [38]. Furthermore, the reduction of sample size in the skin wound measurements due to premature removal of the titanium clips weakened the conclusions that could be made from analyses based on these data. In fact, if all animals in the control group could have been included in the correlation analysis and given a constant rho-value, the p-value would have been between 0.01 and 0.02 (figure 3) instead of 0.12 with our current sample size. Thus, the reduction in sample size resulted in a type 2 error.

Figure 3:
Formula to calculate t-value from *rho* and sample size

$$t = r\sqrt{\frac{n-2}{1-r^2}}$$

Where r denotes the rho value from the Spearman correlation test and n denotes sample size.

Study IV
Rapidly resorbable vs. non-resorbable suture for experimental colonic anastomoses in rats

Aim
When anastomoses are performed in clinical practice, resorbable sutures are used. Therefore, in order to make extrapolation from experimental studies to clinical practice easier, it was decided to investigate if these types of sutures, a resorbable suture compared to a non-resorbable suture, could produce similar and equally reproducible results.

Methods
This study was performed with the same setup as in the two previous experimental studies. The only exception was that half of the anastomoses were performed with a rapidly resorbable, braided 6-0 suture (Vicryl Rapide®, Ethicon Inc., New Brunswick, NJ, USA), the other half with a non-resorbable monofilament 6-0 suture (Ethilon®). Thus, this study was an experimental, prospective case-control study. This design was described correctly in the abstract and in the methods section in the published article, but in the title, we have mistakenly described the study as randomized. This is a regrettable error. To correct the mistake, the editor of the journal has been contacted and proposed to make a correction.

For analgesia, only buprenorphine (Temgesic®) was used. None of the rats received NSAID in the postoperative period. Seven days after surgery the anastomoses were harvested and breaking strength measurements were performed.

A sample size calculation was performed based on breaking strength data from Ågren et al [35]. In this publication, data are only presented graphically and therefore the corresponding author was contacted and raw data was provided. Based on these data (mean (SD) breaking strength 1.90 (0.67) Newton) and alpha at 0.05, beta at 0.20 and MIREDIF at 35% a total sample size of at least 32 animals was necessary. We chose to include a total of 40 animals.

For control group, the 20 latest operated animals from study III were used. This was chosen in order to avoid unnecessary use of experimental animals.
In the analyses, non-parametric statistics, including Mann-Whitney's and Wilcoxon's tests for inter- and intragroup comparisons and Spearman's test for correlation analyses, were used.

The study was approved from the Danish Council of Animal Experiments before initiation (J 2008/561-1583).

Results
A total of 40 Wistar rats were used for this study. No animal was recorded suffering or were prematurely sacrificed. The two

groups of 20 rats each were comparable with regard to postoperative weight changes. Anastomotic breaking strength was similar in the two groups. When pulled apart, the large intestine containing the anastomosis ruptured in the anastomotic region/at the anastomotic line. It was obvious that rupture occurred as sutures were pulled through the perianastomotic tissue with no difference between the two suture types.

Conclusion

The main finding of this study was that the resorbable sutures produces results similar to and as consistent as non-resorbable sutures and thus can be used for experimental studies in this setting. Thereby, daily clinical practice is mimicked to a higher degree and interpretation and extrapolation of results may be easier.

Limitations

By using methods as close to clinical practice as possible, the main limitation related to difficulty of extrapolating results to the clinical setting is reduced – but not abolished. With the use of different suture materials in this study, blinding was not possible and therefore, observer bias can theoretically not be excluded.

The fact that this study was not randomized is a limitation of the study design. However, the groups were comparable with regard to preoperative weight, postoperative weight changes, number of complications and anastomotic strength, and therefore this limitation was probably not important.

Furthermore, as no leakages occurred in this and in the previous experimental studies included in this thesis, the clinical situation of AL and thereby the pathophysiological mechanisms included therein were not evaluated. These studies only provide biomechanical data and information since the strength recorded from the breaking strength measurements of the anastomoses lie in the strength of the perianastomotic tissue. Therefore, instead of 'breaking strength' the measure achieved has previously been named 'suture holding capacity', which arguably is a more precise denotation.

However, while recognizing these and the previously mentioned limitations, the results of the experimental studies provide a contribution to the understanding of the effect of NSAIDs on anastomotic and wound healing.

Study V

Postoperative non-steroidal anti-inflammatory drugs increase anastomotic leakage rate after colorectal resection.

Aim

The objective of this study was to reach a final conclusion on whether NSAIDs increase AL rate or not. To identify individual risk factors for AL, a large sample size is needed. Therefore, the following study was conducted.

Methods

This was a database study based on data from The Danish National Colorectal Cancer Database controlled by the Danish Colorectal Cancer Group (DCCG), and on data from electronically registered medical records. We included all patients operated for colorectal cancer in eastern Denmark in the period January 1st 2006 until December 31st 2009 with colorectal resection and primary anastomosis.

From the DCCG Database we received information on patient related risk factors, intra-operative and post-operative factors and whether or not AL had occurred. AL was defined as clinically significant leakage requiring surgical intervention.

Information on NSAID consumption in the postoperative period was retrieved by individual patient by patient searches in electronically registered medical records. These records contain details of all medicine given to and taken by the patients during their hospital stay. Three observers all blinded for the presence of AL performed the searches and only NSAID doses registered as taken by the patients were included. Regular NSAID treatment was defined as ≥2 days of NSAID treatment in the first 7 postoperative days. Furthermore, the doses and types of NSAID used were registered.

From the Danish Central Person Registry (CPR registry) we retrieved information on time of death in order to report 30 days postoperative mortality.

The study was approved by the Scientific Council of The Danish Colorectal Cancer Group and by the Danish Data Protection Agency.

Data was collected as described above and in order to identify individual risk factors for AL, univariate and multivariate logistic regression analyses were performed. First, univariate analyses were performed and all factors with a p-value below 0.20 were included in the multivariate analysis. To exclude the presence of any confounding factors, tests for association between risk factors were also performed with insertion of 'association-factors' in multivariate analyses. Results were reported as odds ratios (OR) with 95% confidence intervals (CI).

Results

We received data on 3,869 patients from the DCCG. 1,103 patients were excluded since they had no electronic medical record and 10 patients were excluded since we could not retrieve information on AL. Thus, 2,756 patients were included in the final analysis. In this group 68% did not receive regular postoperative NSAID treatment and 32% did. In the NSAID group 74% received ibuprofen and 25% received diclofenac. The final percentage received other types of NSAIDs.

AL rate in the entire group was 6.5% and 30 days mortality was 3.3%. In patients with AL, 30 days mortality was 9.6%. The NSAID versus non-NSAID groups were comparable with regard to most registered parameters. The patients receiving NSAID had a significantly higher AL rate (9.5% versus 5.1%; $p<0.001$). Furthermore, there were more laparoscopic procedures and more non-smokers among the controls. Intraoperative blood loss and transfusion rates were higher among patients receiving NSAID.

The multivariate analysis showed significantly increased AL rate among patients receiving postoperative diclofenac ($p< 0.001$) and ibuprofen ($p=0.03$). Moreover, male gender, active smoking, increased blood loss, intraoperative transfusion and rectal anastomosis (versus colonic) were also associated with increased AL rate (figure 4). Intraoperative transfusion was the individual risk factor associated with the highest risk of AL (OR (95% CI) 8.1 (5.3-12.4)). The tests for possible interactions between NSAID treatment and the other variables revealed no significant interactions.

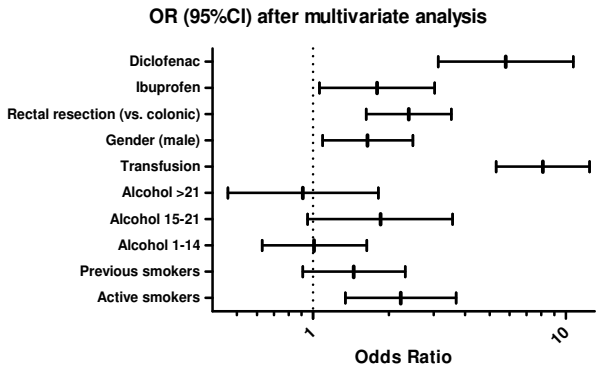
Conclusion

In this database study, both diclofenac and ibuprofen treatment in the first postoperative days were independent risk factors for AL. Other identified risk factors were consistent with previous results.

Based on these data it was recommended that all NSAIDs be abandoned after colorectal resections with primary anastomosis.

This also implies that present international recommendations[32] be changed.

Figure 4:



Punctured line indicates Odds Ratio = 1 (neither increased nor decreased risk); Odds Ratio above 1 indicates increased risk of anastomotic leakage. Intervals indicate 95% Confidence Intervals.

Limitations

The most obvious limitation in this study is the retrospective design. Furthermore, since the procedures included in the study were performed at different hospitals, it was different observers that reported whether anastomotic leakage had occurred or not. However, the difference in defining anastomotic leakage was most likely minimal since all leakages were confirmed at re-operation and the validity of the data from the DCCG database has been assessed earlier [39]. In this survey, data quality was high with identical registrations in between 84 % and 94 % of the cases.

We did not report whether the surgical procedures were performed in the acute or in the elective setting, if the operator was a specialized colorectal surgeon or had a lower degree of specialization and if the procedures performed were for palliation or with curative intent. However, it is unlikely that there should were to be an uneven distribution of these factors among patients receiving diclofenac, ibuprofen or no NSAID. Therefore, it is also unlikely that these factors have confounded our results.

A large proportion (1,103/3,869) of the primary sample population did not meet inclusion criteria, since these patients did not have an electronic medical record with registration of medicine consumption. Since this exclusion was based on whether the departments had implemented electronic medical records or not, some departments had more exclusions than others. However, all of the departments (one excluded) had similar AL rates and there are no indications that the excluded group of patients should differ from the patients included in the final analysis with regard to any of the registered variables.

In retrospective as well as in observational studies, there is always a risk that the association between a given outcome and the factor examined is subject to confounding by indication. In the present study for example, it cannot be completely excluded that patient factors (i.e. low-grade fever, abdominal pain etc.) possibly related to the development of AL have caused medical staff to administer more NSAID to this group of patients. However, the NSAID administered at the surgical departments is most often administered as part of standard analgesic regimens and therefore given to all patients with colorectal resections. For rescue analgesia, opioids are most commonly used. Furthermore, to exclude the presence of confounding by indication, multivariate analysis is normally recommended [40]. This was performed in this study.

These limitations aside, based upon the large sample size and the robust data it is with a high degree of certainty proven, that NSAIDs are harmful in the immediate postoperative period, and the recommendation that the drugs be abandoned seems justified**.**

ETHICAL CONSIDERATIONS

Experimental studies

Animal experiments are justified when they aim to reduce human morbidity and mortality. After colorectal AL, mortality rates up to 32 % have been reported [5]. Thus, this thesis' field of research fulfils this criterion and justifies the use of laboratory animals.

The experimental studies included in this thesis were all approved by the Danish Council of Animal Experiments, and guidelines concerning humane endpoint were followed. All animals were treated humanely, with sufficient analgesia and any animals that seemed to be suffering were immediately sacrificed.

Retrospective studies

The working hypothesis of this study series was that NSAIDs increase the risk of colorectal AL. As previously mentioned, the optimal method of investigation of the effect of NSAIDs on AL would be a randomized controlled trial. However, it would be logistically very difficult and expensive to perform, and there would probably be no industry support for such a study. Furthermore, it could be regarded unethical to perform randomized studies in which a treatment that potentially increase AL rate, and thereby morbidity and mortality, is used. Finally, since AL rate may be affected by many factors, a large sample size is needed to identify individual risk factors. For these reasons, it was decided to perform studies based on historical data instead of subjecting patients to a potentially harmful experiment.

DISCUSSION

In this study series it was investigated whether NSAIDs influenced anastomotic leakage rate and furthermore possible mechanisms responsible for this effect were evaluated. In experimental studies a direct effect on the anastomoses could not be shown, but the studies showed effects on collagen production and metabolism, and thus have contributed to the understanding of mechanisms involved in the healing of anastomoses.

In the final database study, the detrimental effect of the NSAIDs was proven. This can potentially have important impact on daily clinical practice.

In the following sections, the mechanisms of action and effects of the NSAIDs on the risk of thromboembolic events will be reviewed. Moreover, the methods used in the experimental and retrospective studies and possible reasons behind the effect of the NSAIDs will be discussed.

Non-steroidal anti-inflammatory drugs – mechanism of action

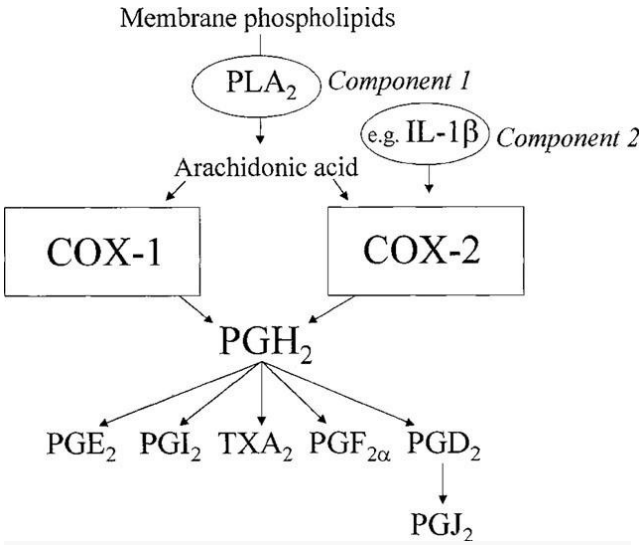
Non-steroidal anti-inflammatory drugs are recommended for analgesia treatment after most types of surgery [41]. After colorectal resections, NSAIDs are used especially after fast track surgery [31]. Thus, in the most recently updated procedure-specific recommendations, NSAIDs are part of the multi-modal analgesic regimen after colorectal resections [32].

Mechanisms of action

The NSAIDs inhibit the cyclooxygenase (COX) enzymes COX-1 and COX-2. These enzymes catalyze the rate limiting steps in the conversion of arachidonic acid to prostaglandin-H2 and the subsequent production of the prostanoides [42] (figure 5 [43]). The COX-enzymes are membrane-bound proteins of the endoplasmatic reticulum.

COX-1 is present in all tissues, and is primarily involved in homeostatic mechanisms. For this reason this isoform has been called the "housekeeping enzyme". COX-2 is, via stimulation from interleukin-2-beta (IL-2β) and other cytokines, induced in the presence of inflammation [44]. When inflammation and inflammatory cytokines are present, COX-2 is produced in excess and when this is the case, the primary prostanoid produced is prostaglandin E2 (PGE2) [43].

Figure 5:



One and two component models of prostanoid production by, respectively, cyclo-oxygenase-1 (COX-1) and cyclo-oxygenase-2 (COX-2). PLA_2, phospholipase A_2; PG, prostaglandin; TX, thromboxane. Figure from Mitchell et al [43] reused with permission.

During inflammation, the NSAIDs inhibit COX-2 and thus PGE2 production and thereby the desired analgesic and anti-inflammatory effect is achieved. When PGE2 level is decreased the nerve endings are less sensibilized and furthermore, nociception is decreased via an effect in the central nervous system. The excellent analgesic effect of the NSAIDs is illustrated by the low number needed to treat (NNT), with typical values ranging from 1.6 to 3.4 [41].

As mentioned, the primary prostanoid in the inflammatory setting is PGE2. The production of the other prostanoids depends on the presence of "downstream" enzymes and cell/tissue type. In the endothelium, prostacyklin (PGI2), which functions as a potent vasodilator and inhibits platelet aggregation, is the primary product. The production of PGI2 is dependent on the COX-2 enzyme. Conversely, in platelets, thromboxane A2 (TXA2) is preferentially produced and the production is controlled by COX-1, which is the only isoform expressed in this cell type. TXA2 promotes platelet aggregation. Prostaglandin-D2 (PGD2) and prostaglandin-F2 (PGF2) is produced in many different cell types and have various "housekeeping" effects [43].

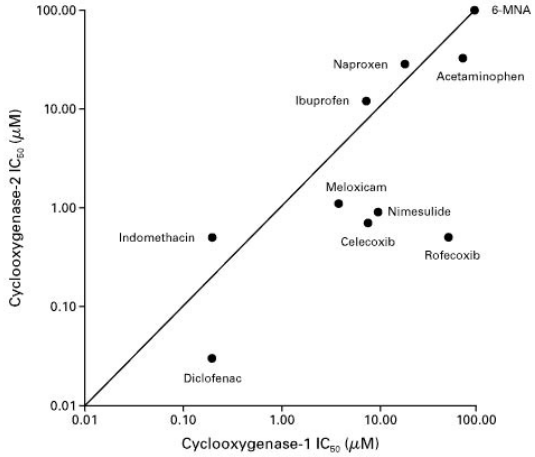
Pharmacokinetics and -dynamics of the NSAIDs

As early as in 1897, aspirin was the first NSAID to be introduced. This original NSAID became extremely popular due to its antipyretic, analgesic and anti-inflammatory effects [45]. Aspirin is along with the other traditional NSAIDs (tNSAIDs) preferentially administered orally. After oral administration, the drugs are absorbed rapidly and completely. They are bound to plasma albumin (>95%) and plasma half-life ranges from 20 minutes for aspirin, 1-2 hours for diclofenac, 2-2.5 hours for ibuprofen and up to 15 hours for naproxen [46]. The NSAIDs are metabolised in the liver by the cytochrome P450 system and excreted as conjugated metabolites in urine or bile. Renal excretion is minimal [46]. The newer, more COX-2 selective NSAIDs named the coxibs have a lower bioavailability when compared to the tNSAIDs. Bioavailability is especially low for the less lipophilic celecoxib (20-40%) whereas the two other available coxibs, etoricoxib and lumiracoxib, have higher levels (74-100%). The coxibs also circulate protein-bound and are metabolised by cytochrome P450 in the liver. Plasma half life is somewhat greater than that of the tNSAIDs; 5-8 hours for etoricoxib, 11-16 hours for celecoxib and 19-32 hours for etoricoxib [45].

The different NSAIDs, i.e. both the tNSAIDs and the coxibs, inhibit the two COX-enzymes with different potency (figure 6a [47] and 6b [48]) and are divided into subgroups according to this selectivity. The selectivity is best assessed ex vivo (not in vitro) by whole blood assays as described by Patrono et al [49].

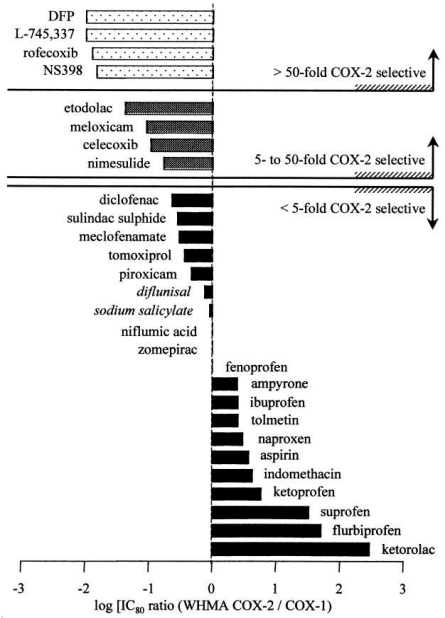
The tNSAIDs and the coxibs inhibit both COX-1 and -2 in a competitive fashion and thus, their effects are dose-dependent and reduced over time [50]. As shown in figures 5a and 5b, drugs belonging to the group of tNSAIDs, including ibuprofen, diclofenac and naproxen, have very different COX-1/COX-2 selectivity. Ibuprofen and naproxen tend to slightly favour COX-1-inhibition, whereas diclofenac has a COX-2 selectivity comparable to that of celecoxib, the first of the coxibs developed and launched [47,49]. The coxibs developed at a later stage are even more COX-2 selective, with lumiracoxib at a COX-2/COX-1 ratio above 500 being the most selective [51].

Figure 6a:



Each point is the mean of three or four values. Drugs plotted below the diagonal line indicating equivalence are more potent inhibitors of cyclooxygenase-2 than drugs plotted on or above the line. 6-MNA denotes 6-methoxy-2-naphthylacetic acid. Figure from Fitzgerald et al [47] resused with permission.

Figure 6b:



Determinable log [IC_{80} ratio (WBA-COX-1/COX-2)] for all agents assayed. The "0 line" indicates equipotency, i.e., an IC_{80} ratio of 1. Italics indicate compounds with very low potency. Figure from Warner et al. [48] reused with permission.

Aspirin is in a subgroup of its own. This is due to its high level of COX-1 selectivity and its mechanism of action. Aspirin does not inhibit COX-1 competitively, but in a permanent fashion by irreversibly acetylating a serine residue at position 529 [47]. Thereby, in platelets, TXA2 production is irreversibly inhibited and this lays the ground for the drugs' anticoagulant effect, which again is the reason why the drug is recommended for primary and secondary prophylaxis in patients in risk of thromboembolic cardiovascular events [52].

Mechanisms to explain effect on the anastomosis

In the early healing process, an anastomosis is dependent upon regulation of connective tissue breakdown and production. Furthermore, blood supply to the anastomosis is crucial. Thus, factors influencing these dependables may also influence anastomotic leakage rate.

Collagen metabolism

During the first few days after surgery and anastomosis, in the inflammatory phase of wound healing, collagen is the degraded faster than new collagen is produced [38,53]. This effect is believed to be due to the activity of specific collagenases named the matrix metalloproteinases (MMPs). The MMPs are zinc-dependent endopeptidases [54] and are increased after tissue injury by inflammatory mediators such as PGE2 [38,55]. Four of the MMPs are primarily responsible for the degradation of collagen in this early phase of wound healing. MMP-1 and MMP-13 cleaves fibrillar collagen I and III, whereas MMP-2 and MMP-9 are responsible for the further degradation [54,56]. It has been shown that collagen concentration correlates inversely with MMP activity [57] and indeed, that the inhibition of the MMPs increase anastomotic breaking strength [55] but did not in this study affect collagen concentration in the anastomotic area nor the production of new collagen.

In the later proliferative phase, collagen production is predominant and is dependent on activation of fibroblasts. The fibroblasts are activated by inflammatory cytokines and prostaglandins [58]. Four days postoperatively, collagen synthesis predominates over collagenolysis and after 7 days, a net increase in collagen concentration can be measured [38].

Most studies evaluating perianastomotic collagen content have measured hydroxyproline concentration in the tissue since this is a major part of collagen fibrils. Thus, hydroxyproline reflects the total amount of collagen present [27-29,55,58]. However, it is important to bear in mind that different types of collagen fibrils contain different amounts of hydroxyproline [53,58] and that the strength of the perianastomotic tissue lie not only in the amount, but rather in the type of collagen present and the quality of the same collagen, i.e. the level of crosslinkages formed [38,53,58]. Solubility assays can be used to differentiate between immature collagen without crosslinkages, more mature fibrils with a few crosslinkages, and mature, stable collagen (type I) with many crosslinkages´[53,58]. This method should be used to a higher degree in future studies on collagen metabolism in perianastomotic tissue.

Given the anti-inflammatory effect of the NSAIDs, it would be logical to assume that these drugs could inhibit the MMPs (that are activated by inflammatory cytokines) and thereby reduce the early degradation of collagen. This effect was indeed shown by Mastboom et al. [58], but has been contradicted in subsequent studies showing unaltered [27,28] and even decreased [29] hydroxyproline levels in the early anastomotic healing phases with NSAID treatment.

As mentioned above, NSAIDs inhibit collagen synthesis via a direct effect on fibroblasts in the proliferative phase [58] and as described in study III, we have shown that collagen production was significantly decreased in the subcutaneous space as a result of NSAID treatment.

Previous experimental studies have investigated the effect of NASIDs on colonic anastomotic strength. Results have been conflicting. As mentioned, a study has proposed that anastomoses benefit from the effect of the NSAIDs [58], others that NSAIDs may be harmful [26,28,29] and others again that these drugs does not influence anastomotic healing [59,60]. In the studies presented in this thesis, we did not find a detrimental effect on anastomotic breaking strength with postoperative NSAID treatment.

Interestingly, in one of the only studies on this subject performed in humans, Stumpf et al. [54] showed a lower level of type I and III collagen and a more often expression of MMP-13 in the colon of patients with AL compared to patients without AL. This indicates that AL risk may increase with predisposing patient-related factors that influence collagen metabolism.

In summary, the effect of the NSAIDs on collagen metabolism is possibly bimodal with a reduced degradation in the early healing phase and a reduced production in later phases. Whether the net effect increases or decreases total collagen content and anastomotic wound strength is still unknown.

Although not all mechanisms are known and data are only provided from experimental studies, it is possible that the effect of NSAIDs on anastomotic leakage rate is at least partly due to an effect on connective tissue metabolism. This topic should be examined further, preferentially in clinical studies or studies on healthy volunteers and studies should focus on the types of connective tissue constituents and the quality of same constituents.

Blood supply

For sufficient healing blood supply is a cornerstone. As described above, the NSAIDs influence the coagulatory system via regulation of thromboxane A2, prostacyklin (PGI2) and other prostanoids. As reviewed briefly below, the (longterm) use of NSAIDs is associated with adverse cardiovascular events and this effect has been attributed to the aforementioned mechanisms of action.

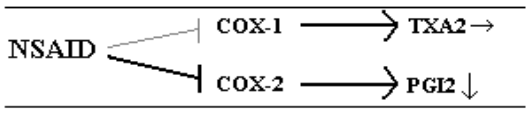
In the late 1990's the different COX-2 selective drugs named the coxibs were developed based on the hypothesis that they could produce the desired analgesic and anti-inflammatory effect without producing gastrointestinal side effects. This hypothesis was based on the knowledge that the increased risk of formation of peptic ulcers with NSAID treatment were – and are – due to COX-1 inhibition [61]. COX-1 inhibition in gastric and duodenal mucosa leads to insufficient levels of tissue-protective prostaglandins (preferentially PGD2 and PGF2) [62].

Celecoxib and rofecoxib were the first coxibs to be developed. The VIGOR study published in 2000 was a randomized controlled trial (RCT) designed to compare rofecoxib 50 mg/d to naproxen 500 mg bidaily with regards to gastrointestinal side effects [63]. This study found a reduced incidence of gastrointestinal perforation, bleeding and symptomatic peptic ulcers with rofecoxib treatment, but the risk of myocardial infarction was markedly lower among those treated with naproxen (RR (95% CI) 0.2 (0.1-0.7)). A few years later, in 2004, the APPROVe study [64] confirmed the results of the VIGOR study. On that basis, rofecoxib was withdrawn from the market [51]. An increased risk of myocardial infarction was also found with valdecoxib [65], which was withdrawn in 2005. In the same year, the APC study revealed a markedly increased risk of adverse cardiovascular events (HR (95% CI) 3.4 (1.4-7.8)) with high-dose celecoxib treatment [66]. However, in 2006, the MEDAL study comparing etoricoxib and diclofenac found no difference in rate of major cardiovascular events [67]. All of these studies were not designed to investigate the cardiovascular risk of the tNSAIDs or the coxibs, and the results on this issue were based on *post hoc* analyses. However, the studies together revealed a possible connection between NSAID treatment and adverse cardiovascular events and raised a clear warning sign. Therefore, metaanalyses including both data from RCT's and observational studies were performed. Kearney et al. [68] showed in 2006 that the coxibs and the tNSAIDs diclofenac and ibuprofen carried an increased risk of adverse cardiovascular events and myocardial infarction, when compared to placebo. Risks were similar when coxibs were compared to tNSAIDs with the exception of naproxen, which seemed to be protective [68]. Another metaanalysis found increased risk with rofecoxib and diclofenac, but not with ibuprofen, naproxen and low-dose celecoxib [69]. Moreover, in a more recent study by it was shown that the risk of MI increased with COX-2 selectivity in a dose dependent manner [70], which again strengthens the hypothesis that COX-2 inhibition may increase cardiovascular risk. Finally, it has recently been shown that the risk also is present in young and healthy individuals [71] and after short (<14 days) duration of treatment [72].

In summary, there is compelling evidence that the NSAIDs increase the risk of adverse cardiovascular events. The risk seems to increase with COX-2 selectivity, but some studies have found similar adverse cardiovascular event rates among coxibs and tNSAIDs, and therefore it cannot be rejected that the non-selective NSAIDs poses a risk similar to that of the coxibs. Importantly, as concluded in a recent review [50], the NSAIDs are a cluster of compounds that are heterogeneous with respect to pharmacodynamic and pharmacokinetic features and thus, cardiovascular risk should be assessed for each of these compounds individually. Similarly, the risk of other postoperative complications should be investigated for the individual NSAID.

The leading hypothesis with regard to the biological basis for the cardiovascular consequences of COX-inhibition is that the balance between pro-thrombotic COX-1-stimulated TXA2 production in platelets and vasodilating, antithrombotic COX-2 controlled PGI2 production by the endothelium is 'tipped' towards generation of thromboses when COX-2 is inhibited [50,73]. Although the NSAIDs inhibit both COX-1 and COX-2, the TXA2 production is not sufficiently inhibited unless inhibition is above 90% and is sustained [50]. This is illustrated in figure 7, and therefore, also non-selective tNSAIDs can cause adverse cardiovascular events [74,75].

Figure 7:



Typically, NSAIDs inhibit COX-2 fully, but COX-1 only partially. This results in decreased (cardioprotective) PGI2 production and maintained (prothrombotic) TXA2 production.

In the majority of studies revealing increased cardiovascular risk with NSAID treatment, the adverse events typically occur after long term use [63,64]. This may contradict the hypothesis that the NSAIDs are harmful after only a few days use for postoperative analgesia. However, as recently shown, cardiovascular risk may also be evident after less than 14 days of treatment [72]. Furthermore, in studies evaluating the safety and efficacy of the coxib valdecoxib (and its intravenous analogue parecoxib) after coronary artery bypass surgery (CABG) an increased number of adverse cardiovascular events were reported [65,76]. In the studies, valdecoxib/parecoxib was administered in the first 10 postoperative days and when data from these studies were combined by Furberg et al [77], relative risk for cardiovascular events was significantly increased (RR (95% CI) 3.08 (1.20-7.78), p = 0.019) with coxib treatment. The earliest thromboembolic events recorded occurred after only a few days of NSAID exposure.

Hence, in the postoperative setting with pre-existing intense haemostatic activation [78], suppression of COX-2 derived PGI2 can cause thrombosis formation. Also, it has been shown that after CABG, there is, for unknown reasons, an apparent 'aspirin resistance' [79,80]. This implies that risk of thromboses is high even when aspirin is administered, and this may also be the case after colorectal resections. With these factors present, the balance may more easily shift towards thrombosis formation in the small vessels supplying blood flow to the anastomosis, which again can lead to insufficient anastomotic blood supply.

In the light of these findings, it is reasonable to hypothesize that the formation of microembolies or- thromboses and thereby limited anastomotic blood supply is an important mechanism behind the harmful effects of the NSAIDs, especially the ones with high COX-2 affinity.

Extrapolation on experimental studies to clinical practice

Extrapolation of results achieved in the experimental setting to the clinical setting is difficult and not always possible. As mentioned previously, this is the primary limitation of experimental studies. In the experimental studies in this study series effort was made to mimic the clinical setting to as high a degree as possible.

Firstly, prior to performing the studies the principle investigator performed 52 anastomoses with the same technique as in the studies. This was done to eliminate any confounding due to a learning curve phenomenon. As there was no literature on learning curve in this setting available, operative time and the standard deviation of anastomotic breaking strength were used as surrogate markers for learning curve. Operative time was slightly reduced during the study periods, but this reduction was due to more effective shifts between each animal, faster anaesthesia, shaving and disinfection. Actual surgical time remained constant. Standard deviation was also constant throughout the studies. This showed that the number of anastomoses performed prior to the studies was sufficient.

Secondly, since previous experimental studies have used varying NSAID-doses [26,28,29,58] we wished to use a dose comparable to daily clinical practice. Thus, calculations adjusted for weight, metabolic rate and analgesic effect were performed and a comparable dose was found. This dose was tested in study II; proved to be relevant and was therefore used in subsequent studies.

Thirdly, we examined in study IV whether resorbable suture as used in the clinical practice could give reproducible results in the experimental setting. When the resorbable suture was used the results remained constant when compared to the non-resorbable suture.

Sutures with a more coarse surface than the one on monofilament sutures have previously been shown to increase the inflammatory reaction [81,82] and, based on evaluation of scanning electron microscopic pictures, produce greater intestinal damage [83]. However, these differences do not seem to produce differences in tensile strength [84] and the differences registered are not likely to be of any clinical significance [81].

Another potential problem with the use of resorbable suture could be inconsistent results due to heterogeneous degradation of the resorbable suture. Studies on the subject do not address this problem and as noted in a review on this type of research [38], no suture material has been shown to be consistently superior. Therefore, to mimic daily clinical practice, we recommend that resorbable sutures are used in this experimental setting.

Finally, we chose to measure breaking strength and not bursting pressure, which also previously has been used to assess anastomotic healing. These two methods have been evaluated and compared previously [38,85,86].

Breaking strength is widely used and as described in study IV, when breaking strength measurements are performed, disruption occurs when the sutures are pulled through the perianastomotic tissue. Thereby, a measure of perianastomotic tissue strength is provided and not necessarily the actual strength of the anastomosis line. However, the method has been criticized for its lack of ability to apply an equal amount of force to the entire bowel wall and the lack of force applied in the circumferential direction [38]. Moreover, Månsson et al. compared bursting pressure and breaking strength in the very early – inflammatory – healing phase (<3 days after surgery). The authors concluded that breaking strength is not sensitive enough to record changes in this phase of anastomotic healing since breaking strength was constant throughout the period whereas bursting pressure increased in an almost linear fashion [86]. Conversely, when the two methods were compared by Ikeuchi et al. in both the early and the later – proliferative – healing phase, tensile strength was best suited for assessing anastomotic healing [85].

As described by Koruda et al [38], bursting pressure depends on bowel radius and thus, bursting pressure itself may not be relevant. Instead, bursting wall tension should be calculated (figure 8 [38]).

Bowel radius is not easily measured precisely, and furthermore, since the radius of bowel adjacent to the anastomosis is normally larger than at the anastomosis itself, bowel disruption tends to occur outside the anastomosis when bursting pressure measurements are performed [87]. Furthermore, bursting pressure is dependent on different methodological factors as well. These include whether the sutures are removed prior to the measurement or are left *in situ*; if the burst is performed with the anastomosis *in situ* or resected; how fast the bowel is distended and if the bowel radius is measured before or after bursting [38].

Figure 8:

Bursting Wall Tension

In the longitudinal direction:	$BWT_{long} = K \times BP \times R/2$
In the circular direction:	$BWT_{circ} = K \times BP \times R$

Note. BWT, bursting wall tension (dynes/cm); BP, bursting pressure (mm Hg); $K = 1.33 \times 10^3$ (dyne/cm^2/mm Hg); and R, the principle radius of curvature (cm).

Table from Koruda et al. [38] reused with permission.

Based on the arguments presented above, breaking strength (or 'suture holding capacity') may provide a better measure for anastomotic healing while bursting pressure can evaluate overall anastomotic integrity [85]. Since the studies included in this thesis aimed to measure differences in anastomotic and skin wound healing, breaking strength was chosen as the method of use.

Although effort is made to mimic clinical practice, the clinical situation of AL is not reproduced in experimental studies in rats. Therefore, the studies are not able to evaluate the pathophysiological mechanisms that occur with AL and must therefore focus on healing capacity.

Other animal species has previously been used to examine AL and factors that influence its rate. The use of these different animals has been reviewed recently [88]. Herein, the authors conclude that studies on rats have their advantages when assessing wound healing, but when assessing the clinical implications of AL, studies on mice or pigs may be more suitable. Especially mice are recommended [89] since they, as rats, are cheap, easy to handle and have a gastrointestinal system similar to humans [88]. Pigs may be an alternative, but there is a lack of established clinically relevant models [88].

In retrospect, more clinically relevant outcomes could perhaps have been found if mice or pigs were chosen for the experimental studies included in this thesis. However, the studies have contributed to the understanding of healing processes in the presence of NSAID treatment. Thus, the results obtained can form the basis for further investigations on the effect of NSAIDs on anastomotic and general wound healing.

Extrapolation of retrospective studies

In study V and again in this thesis, the recommendation that NSAID be abandoned after colorectal resections with primary anastomosis is made based on retrospective data and previous knowledge of the drugs' effect on other organ systems. The recommendation is made while recognizing the inborn limitations of these studies, as discussed above.

This may seem overly enthusiastic or premature. Indeed, it would strengthen the message significantly if randomized controlled trials could verify the results. However, similar recommendations have previously been made based on sub-level I evidence data. For example, the increased cardiovascular risk with tNSAID treatment was established by observational studies [68,69,71] and based on these findings the recommendation that tNSAIDs are used with caution in patients in risk of adverse cardiovascular events [71]. Thus, the extrapolation of retrospective data to clinical recommendations can be justified when there is compelling retrospective evidence; the seriousness of the illness/complication prompts safe and meticulous medication choices and alternatives to the possibly harmful treatment are available. Since this is the case with regard to NSAIDs and the risk of AL, our recommendation seems justified.

CONCLUSION

The studies included in this thesis have provided new knowledge concerning the effect of the NSAIDs on the risk of AL after colorectal resections with primary anastomosis.

The experimental studies performed on rats showed that NSAIDs inhibits COX-2 at the anastomotic level; that NSAIDs inhibits subcutaneous collagen formation and that experimental studies can be optimized in order to make these studies more relevant to daily clinical life. The experimental studies did not, however, show a direct effect on colonic anastomotic strength with NSAID treatment.

The retrospective studies presented herein have, respectively, raised a warning sign and shown that NSAID consumption in the postoperative period increases AL rate after colorectal resections with primary anastomosis.

This detrimental effect is most likely due to an effect on collagen metabolism resulting in weakened connective tissue surrounding the anastomosis and/or an effect on the risk of thrombosis formation in small vessels supplying the anastomosis, resulting in decreased blood flow, ischemia, necrosis and leakage.

While recognising the above mentioned limitations of the studies it is, based on the findings in the studies presented herein and knowledge from previous studies, recommended that NSAIDs be abandoned after colorectal resections with primary anastomosis. This also implies that the current international procedure specific recommendations [32] be changed.

FUTURE PERSPECTIVES

In future studies it should be examined in detail how the NSAIDs exert their detrimental effect on the anastomosis. This implicates that the effect on collagen metabolism (with emphasis on qualitative collagen analyses) and thrombosis formation be examined in depth. Researchers should thrive to develop models capable of assessing both microscopic circulation (perfusion measurements) and connective tissue production. Also, it should be investigated if the increased risk of AL with NSAID treatment is dependent on patient genotype (i.e. individual differences in drug metabolism and/or connective tissue properties).

Furthermore, it is crucial that the effect of NSAIDs is investigated with regard to complications after other types of surgery where early wound healing is cornerstone. Thus, is should be investigated if there is a similar effect on anastomoses in the upper gastrointestinal tract and after other types of surgery. There are most likely surgical procedures where NSAIDs do not increase the risk of complications or indeed are helpful, but this should be examined as well. It is therefore significant to emphasize the importance of, and further encourage the ongoing development towards procedure specific analgesic regimens.

Possibly, these procedure specific regimens should include new promising analgesic methods such as the transabdominal plane (TAP) block [90] and/or gabapentin usage [91]. However, before any new methods are introduced, their effect (and possible side-effects) should be examined further in randomized controlled trials and on a procedure specific level.

SUMMARY

Anastomotic leakage (AL) is the most important and one of the most serious complications after colorectal resections with primary anastomosis. Any factors that contribute to increase the risk of AL should be identified and – if possible – eliminated. Non-steroidal anti-inflammatory drugs (NSAIDs) are often used for treating pain after surgical procedures, among these also colorectal resections.

The objective of this Ph.d. thesis was to investigate whether the use of NSAIDs in the postoperative period increases the risk of AL, and investigate the effect on pathophysiological mechanisms. In order to achieve this, the following studies were performed.

Study I was a retrospective, case-control study in 75 patients undergoing laparoscopic colorectal resection for colorectal cancer. 33 of these patients received the NSAID diclofenac in the postoperative period; the remaining 42 did not receive any NSAID. There were significantly more ALs among the patients receiving diclofenac (7/33 vs. 1/42, p=0.018). In uni- and multivariate logistic regression analyses, diclofenac was the only factor associated with increased AL rate. This study functioned as a hypothesis generating study and laid the ground for the subsequent studies.

Study II was an experimental, randomized, case-control study in 32 Wistar rats. The rats had a colonic anastomosis performed and were randomized to diclofenac or placebo treatment. After three days, the rats were sacrificed and the anastomoses were harvested. First, the anastomotic strengths were tested by longitudinal; subsequently, the levels of the enzyme cyclooxygenase-2 (COX-2) in the anastomotic tissues were measured. There was no difference among the groups with regard to anastomotic strength, but the animals treated with diclofenac had significantly lower COX-2 levels (median (range) 1.30 (0.42 - 3.31) ng/mg vs. 2.44 (0.88 - 18.94) ng/mg, p<0.001). This study showed that the used dose of diclofenac was sufficient and relevant, but did not show a direct damaging effect on the anastomoses due to NSAID treatment.

Study III was also an experimental, randomized, case-control study. This time round, 60 Wistar rats were included. Again, colonic anastomoses were performed and the rats were randomized to diclofenac or placebo. Also, expanded polytetrafluoruethylene (ePTFE) tubes were placed under the skin of the rats. In this material, substituents of connective tissue accumulate and the amount of accumulation can be measured. After 7 days, the rats were sacrificed and, again, anastomotic strengths were measured along with collagen content in the ePTFE tubes. Anastomotic strength

was similar in the two groups while collagen accumulation was significantly decreased among the rats treated with diclofenac (median (i.q.r.) 0.29 (0.13-0.47) vs. 0.47 (0.28-0.62) mcg/mg, p = 0.03). This study for the first time showed that NSAID inhibit subcutaneous collagen formation and that this formation is reversely correlated to anastomotic strength. This information can be used in further studies in this subject.

Study IV was the final experimental case-control study in 40 Wistar rats. This time, in order to more easily extrapolate experimental results to daily clinical life, the colonic anastomoses were sutured with the same type of suture material as used in the clinical setting. Thus, half the anastomoses was performed with resorbable suture; the other half with non-resorbable suture. None of the rats received NSAID. The breaking strength was compared and found similar in the two groups. This study showed that experimental studies can be optimized in order to make comparisons and extrapolations to the clinical setting easier.

Study V was a database study based on data from the Danish Colorectal Cancer Group's (DCCG) prospective database and electronically registered medical records. From the database information on demographic, surgical and postoperative variables (including AL) were provided. Information on NSAID consumption was retrieved by individual searches in the patients' medical records. Based on these data, uni- and multivariate logistic regression analyses were performed. These analyses identified NSAID treatment in the postoperative period as an individual risk factor for AL. Other risk factors identified were consistent with the available literature. The detrimental effect of the NSAIDs are possibly due to an effect on collagen metabolism leading to weakened tissue around the anastomosis and/or on the risk of thrombosis formation leading to more thromboses in the vessels supplying the anastomosis, thereby limiting anastomotic blood flow.

In conclusion, the studies included in this thesis have elucidated some of the physiological and pathophysiological mechanisms involved in anastomotic healing and leakage, and furthermore have shown that the use of NSAIDs in the postoperative period increase the risk of AL in patients undergoing colorectal surgery with primary anastomosis. Based on the findings in these studies, and based on existing knowledge, it is recommended that NSAIDs be abandoned after colorectal resection with primary anastomosis.

It should be investigated whether the NSAIDs are also harmful to other types of anastomoses and after other surgical procedures where early tissue healing is crucial.

REFERENCES

1. Law WI, Chu KW, Ho JW et al. Risk factors for anastomotic leakage after low anterior resection with total mesorectal excision. Am J Surg 2000; 179: 92-6.
2. Lipska MA, Bissett IP, Parry BR et al. Anastomotic leakage after lower gastrointestinal anastomosis: men are at a higher risk. ANZ J Surg 2006; 76: 579-85.
3. Guenaga KK, Matos D, Wille-Jorgensen P. Mechanical bowel preparation for elective colorectal surgery. Cochrane Database Syst Rev 2009; CD001544.
4. Kingham TP, Pachter HL. Colonic anastomotic leak: risk factors, diagnosis, and treatment. J Am Coll Surg 2009; 208: 269-78.
5. Choi HK, Law WL, Ho JW. Leakage after resection and intraperitoneal anastomosis for colorectal malignancy: analysis of risk factors. Dis Colon Rectum 2006; 49: 1719-25.
6. Hallbook O, Sjodahl R. Comparison between the colonic J pouch-anal anastomosis and healthy rectum: clinical and physiological function. Br J Surg 1997; 84: 1437-41.
7. Rullier E, Laurent C, Garrelon JL et al. Risk factors for anastomotic leakage after resection of rectal cancer. Br J Surg 1998; 85: 355-8.
8. den DM, Marijnen CA, Collette L et al. Multicentre analysis of oncological and survival outcomes following anastomotic leakage after rectal cancer surgery. Br J Surg 2009; 96: 1066-75.
9. Mirnezami A, Mirnezami R, Chandrakumaran K et al. Increased local recurrence and reduced survival from colorectal cancer following anastomotic leak: systematic review and meta-analysis. Ann Surg 2011; 253: 890-9.
10. Alberts JC, Parvaiz A, Moran BJ. Predicting risk and diminishing the consequences of anastomotic dehiscence following rectal resection. Colorectal Dis 2003; 5: 478482.
11. Alves A, Panis Y, Pocard M et al. Management of anastomotic leakage after nondiverted large bowel resection. J Am Coll Surg 1999; 189: 554-9.
12. Golub R, Golub RW, Cantu R, Jr. et al. A multivariate analysis of factors contributing to leakage of intestinal anastomoses. J Am Coll Surg 1997; 184: 364-72.
13. Iversen LH, Bulow S, Christensen IJ et al. Postoperative medical complications are the main cause of early death after emergency surgery for colonic cancer. Br J Surg 2008; 95: 1012-9.
14. Konishi T, Watanabe T, Kishimoto J et al. Risk factors for anastomotic leakage after surgery for colorectal cancer: results of prospective surveillance. J Am Coll Surg 2006; 202: 439-44.
15. Makela JT, Kiviniemi H, Laitinen S. Risk factors for anastomotic leakage after left-sided colorectal resection with rectal anastomosis. Dis Colon Rectum 2003; 46: 653-60.
16. Sorensen LT, Jorgensen T, Kirkeby LT et al. Smoking and alcohol abuse are major risk factors for anastomotic leakage in colorectal surgery. Br J Surg 1999; 86: 927-31.
17. Platell C, Barwood N, Dorfmann G et al. The incidence of anastomotic leaks in patients undergoing colorectal surgery. Colorectal Dis 2007; 9: 71-9.
18. Vignali A, Fazio VW, Lavery IC et al. Factors associated with the occurrence of leaks in stapled rectal anastomoses: a review of 1,014 patients. J Am Coll Surg 1997; 185: 105-13.
19. Marjanovic G, Villain C, Juettner E et al. Impact of different crystalloid volume regimes on intestinal anastomotic stability. Ann Surg 2009; 249: 181-5.
20. Muller S, Zalunardo MP, Hubner M et al. A fast-track program reduces complications and length of hospital stay after open colonic surgery. Gastroenterology 2009; 136: 842-7.
21. Schnuriger B, Inaba K, Wu T et al. Crystalloids after primary colon resection and anastomosis at initial trauma laparotomy: excessive volumes are associated with anastomotic leakage. J Trauma 2011; 70: 603-10.
22. Schricker T, Carvalho G. Effects of intravenous fluid restriction on postoperative complications: comparison of two perioperative fluid regimens. Ann Surg 2005; 241: 194.
23. Merad F, Yahchouchi E, Hay JM et al. Prophylactic abdominal drainage after elective colonic resection and suprapromontory anastomosis: a multicenter study controlled by randomization. French Associations for Surgical Research. Arch Surg 1998; 133: 309-14.

24. Docherty JG, McGregor JR, Akyol AM et al. Comparison of manually constructed and stapled anastomoses in colorectal surgery. West of Scotland and Highland Anastomosis Study Group. Ann Surg 1995; 221: 176-84.
25. Chapman AE, Levitt MD, Hewett P et al. Laparoscopic-assisted resection of colorectal malignancies: a systematic review. Ann Surg 2001; 234: 590-606.
26. Cahill RA, Sheehan KM, Scanlon RW et al. Effects of a selective cyclo-oxygenase 2 inhibitor on colonic anastomotic and skin wound integrity. Br J Surg 2004; 91: 1613-8.
27. de Hingh IH, van GH, de Man BM et al. Selective cyclo-oxygenase 2 inhibition affects ileal but not colonic anastomotic healing in the early postoperative period. Br J Surg 2006; 93: 489-97.
28. de Sousa JB, Soares EG, Aprilli F. Effects of diclofenac sodium on intestinal anastomotic healing. Experimental study on the small intestine of rabbits. Dis Colon Rectum 1991; 34: 613-7.
29. Inan A, Koca C, Sen M. Effects of diclofenac sodium on bursting pressures of anastomoses and hydroxyproline contents of perianastomotic tissues in a laboratory study. Int J Surg 2006; 4: 222-7.
30. Rosenberg J, Harvald T. Severe complications with diclofenac after colonic resection. Dis Colon Rectum 2007; 50: 685.
31. Kehlet H, Wilmore DW. Evidence-based surgical care and the evolution of fast-track surgery. Ann Surg 2008; 248: 189-98.
32. PROSPECT working group. PROSPECT - procedure specific postoperative pain management. Accessed at www.postoppain.org, September 14th 2011
33. Klein M, Krarup PM, Burcharth J et al. Effect of Diclofenac on Cyclooxygenase-2 Levels and Early Breaking Strength of Experimental Colonic Anastomoses and Skin Incisions. Eur Surg Res 2010; 46: 26-31.
34. Kuper MA, Scholzl N, Traub F et al. Everolimus Interferes with the Inflammatory Phase of Healing in Experimental Colonic Anastomoses. J Surg Res 2011; 167: 158-65.
35. Agren MS, Andersen L, Heegaard AM et al. Effect of parenteral zinc sulfate on colon anastomosis repair in the rat. Int J Colorectal Dis 2008; 23: 857-61.
36. Martens MF, Huyben CM, Hendriks T. Collagen synthesis in fibroblasts from human colon: regulatory aspects and differences with skin fibroblasts. Gut 1992; 33: 1664-70.
37. Coon KD, Inge LJ, Swetel K et al. Genomic characterization of the inflammatory response initiated by surgical intervention and the effect of perioperative cyclooxygenase 2 blockade. J Thorac Cardiovasc Surg 2010; 139: 1253-60.
38. Koruda MJ, Rolandelli RH. Experimental studies on the healing of colonic anastomoses. J Surg Res 1990; 48: 504-15.
39. Nickelsen TN, Harling H, Kronborg O et al. [The completeness and quality of the Danish Colorectal Cancer clinical database on colorectal cancer]. Ugeskr Laeger 2004; 166: 3092-5.
40. Grobbee DE, Hoes AW. Confounding and indication for treatment in evaluation of drug treatment for hypertension. BMJ 1997; 315: 1151-4.
41. Reuben SS. Update on the role of nonsteroidal anti-inflammatory drugs and coxibs in the management of acute pain. Curr Opin Anaesthesiol 2007; 20: 440-50.
42. Antman EM, Bennett JS, Daugherty A et al. Use of nonsteroidal antiinflammatory drugs: an update for clinicians: a scientific statement from the American Heart Association. Circulation 2007; 115: 1634-42.
43. Mitchell JA, Warner TD. Cyclo-oxygenase-2: pharmacology, physiology, biochemistry and relevance to NSAID therapy. Br J Pharmacol 1999; 128: 1121-32.
44. Khan AA, Iadarola M, Yang HY et al. Expression of COX-1 and COX-2 in a clinical model of acute inflammation. J Pain 2007; 8: 349-54.
45. Shi S, Klotz U. Clinical use and pharmacological properties of selective COX-2 inhibitors. Eur J Clin Pharmacol 2008; 64: 233-52.
46. Davies NM, Skjodt NM. Choosing the right nonsteroidal anti-inflammatory drug for the right patient: a pharmacokinetic approach. Clin Pharmacokinet 2000; 38: 377-92.
47. FitzGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. N Engl J Med 2001; 345: 433-42.
48. Warner TD, Giuliano F, Vojnovic I et al. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. Proc Natl Acad Sci U S A 1999; 96: 7563-8.
49. Patrono C, Patrignani P, Garcia Rodriguez LA. Cyclooxygenase-selective inhibition of prostanoid formation: transducing biochemical selectivity into clinical read-outs. J Clin Invest 2001; 108: 7-13.
50. Capone ML, Tacconelli S, Di FL et al. Pharmacodynamic of cyclooxygenase inhibitors in humans. Prostaglandins Other Lipid Mediat 2007; 82: 85-94.
51. Rao P, Knaus EE. Evolution of nonsteroidal anti-inflammatory drugs (NSAIDs): cyclooxygenase (COX) inhibition and beyond. J Pharm Pharm Sci 2008; 11: 81s-110s.
52. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ 2002; 324: 71-86.
53. Hendriks T, Mastboom WJ. Healing of experimental intestinal anastomoses. Parameters for repair. Dis Colon Rectum 1990; 33: 891-901.
54. Stumpf M, Cao W, Klinge U et al. Collagen distribution and expression of matrix metalloproteinases 1 and 13 in patients with anastomotic leakage after large-bowel surgery. Langenbecks Arch Surg 2002; 386: 502-6.
55. Syk I, Agren MS, Adawi D et al. Inhibition of matrix metalloproteinases enhances breaking strength of colonic anastomoses in an experimental model. Br J Surg 2001; 88: 228-34.
56. Binnebosel M, Grommes J, Koenen B et al. Zinc deficiency impairs wound healing of colon anastomosis in rats. Int J Colorectal Dis 2010; 25: 251-7.
57. Syk I, Mirastschijski U, Jeppsson BW et al. Experimental colonic obstruction increases collagen degradation by matrix metalloproteinases in the bowel wall. Dis Colon Rectum 2003; 46: 1251-9.
58. Mastboom WJ, Hendriks T, van EP et al. The influence of NSAIDs on experimental intestinal anastomoses. Dis Colon Rectum 1991; 34: 236-43.
59. Benjamin B, Hazut O, Shaashua L et al. Effect of beta blocker combined with COX-2 inhibitor on colonic anastomosis in rats. Int J Colorectal Dis 2010; 25: 1459-64.
60. Neuss H, Raue W, Muller V et al. Effects of cyclooxygenase inhibition on anastomotic healing following large bowel resection in a rabbit model--a randomized, blinded, placebo-controlled trial. Int J Colorectal Dis 2009; 24: 551-7.
61. Garcia Rodriguez LA, Cattaruzzi C, Troncon MG et al. Risk of hospitalization for upper gastrointestinal tract bleeding associated with ketorolac, other nonsteroidal anti-inflammatory drugs, calcium antagonists, and other antihypertensive drugs. Arch Intern Med 1998; 158: 33-9.

62. Graham DY. Nonsteroidal anti-inflammatory drugs, Helicobacter pylori, and ulcers: where we stand. Am J Gastroenterol 1996; 91: 2080-6.
63. Bombardier C, Laine L, Reicin A et al. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. N Engl J Med 2000; 343: 1520-8.
64. Bresalier RS, Sandler RS, Quan H et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med 2005; 352: 1092-1102.
65. Nussmeier NA, Whelton AA, Brown MT et al. Complications of the COX-2 inhibitors parecoxib and valdecoxib after cardiac surgery. N Engl J Med 2005; 352: 1081-91.
66. Solomon SD, McMurray JJ, Pfeffer MA et al. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. N Engl J Med 2005; 352: 1071-80.
67. Cannon CP, Curtis SP, FitzGerald GA et al. Cardiovascular outcomes with etoricoxib and diclofenac in patients with osteoarthritis and rheumatoid arthritis in the Multinational Etoricoxib and Diclofenac Arthritis Long-term (MEDAL) programme: a randomised comparison. Lancet 2006; 368: 1771-81.
68. Kearney PM, Baigent C, Godwin J et al. Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials. BMJ 2006; 332: 1302-8.
69. McGettigan P, Henry D. Cardiovascular risk and inhibition of cyclooxygenase: a systematic review of the observational studies of selective and nonselective inhibitors of cyclooxygenase 2. JAMA 2006; 296: 1633-44.
70. Garcia Rodriguez LA, Tacconelli S, Patrignani P. Role of dose potency in the prediction of risk of myocardial infarction associated with nonsteroidal anti-inflammatory drugs in the general population. J Am Coll Cardiol 2008; 52: 1628-36.
71. Fosbol EL, Kober L, Torp-Pedersen C et al. Cardiovascular safety of non-steroidal anti-inflammatory drugs among healthy individuals. Expert Opin Drug Saf 2010; 9: 893-903.
72. Schjerning Olsen AM, Fosbol EL, Lindhardsen J et al. Duration of treatment with nonsteroidal anti-inflammatory drugs and impact on risk of death and recurrent myocardial infarction in patients with prior myocardial infarction: a nationwide cohort study. Circulation 2011; 123: 2226-35.
73. Grosser T, Fries S, Fitzgerald GA. Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. J Clin Invest 2006; 116: 4-15.
74. Patrono C, Baigent C. Low-dose aspirin, coxibs, and other NSAIDS: a clinical mosaic emerges. Mol Interv 2009; 9: 31-9.
75. Hermann M, Ruschitzka F. Cardiovascular risk of cyclooxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs. Ann Med 2007; 39: 18-27.
76. Ott E, Nussmeier NA, Duke PC et al. Efficacy and safety of the cyclooxygenase 2 inhibitors parecoxib and valdecoxib in patients undergoing coronary artery bypass surgery. J Thorac Cardiovasc Surg 2003; 125: 1481-92.
77. Furberg CD, Psaty BM, FitzGerald GA. Parecoxib, valdecoxib, and cardiovascular risk. Circulation 2005; 111: 249.
78. Tanaka KA, Key NS, Levy JH. Blood coagulation: hemostasis and thrombin regulation. Anesth Analg 2009; 108: 1433-46.
79. Schror K, Weber AA, Hohlfeld T. Aspirin "resistance". Blood Cells Mol Dis 2006; 36: 171-6.
80. Zimmermann N, Wenk A, Kim U et al. Functional and biochemical evaluation of platelet aspirin resistance after coronary artery bypass surgery. Circulation 2003; 108: 542-7.
81. Hastings JC, Winkle WV, Barker E et al. Effect of suture materials on healing wounds of the stomach and colon. Surg Gynecol Obstet 1975; 140: 701-7.
82. Trimpi HD, Khubchandani IT, Sheets JA et al. Advances in intestinal anastomosis: experimental study and an analysis of 984 patients. Dis Colon Rectum 1977; 20: 107-17.
83. Lord MG, Broughton AC, Williams HT. A morphologic study on the effect of suturing the submucosa of the large intestine. Surg Gynecol Obstet 1978; 146: 211-16.
84. Munday C, McGinn FP. A comparison of polyglycolic acid and catgut sutures in rat colonic anastomoses. Br J Surg 1976; 63: 870-2.
85. Ikeuchi D, Onodera H, Aung T et al. Correlation of tensile strength with bursting pressure in the evaluation of intestinal anastomosis. Dig Surg 1999; 16: 478-85.
86. Mansson P, Zhang XW, Jeppsson B et al. Anastomotic healing in the rat colon: comparison between a radiological method, breaking strength and bursting pressure. Int J Colorectal Dis 2002; 17: 420-5.
87. Nelsen TS, Anders CJ. Dynamic aspects of small intestinal rupture with special consideration of anastomotic strength. Arch Surg 1966; 93: 309-14.
88. Pommergaard HC, Rosenberg J, Schumacher-Petersen C et al. Choosing the best animal species to mimic clinical colon anastomotic leakage in humans: a qualitative systematic review. Eur Surg Res 2011 in press.
89. Komen N, van der Wal HC, Ditzel M et al. Colorectal anastomotic leakage: a new experimental model. J Surg Res 2009; 155: 7-12.
90. El-Dawlatly AA, Turkistani A, Kettner SC et al. Ultrasound-guided transversus abdominis plane block: description of a new technique and comparison with conventional systemic analgesia during laparoscopic cholecystectomy. Br J Anaesth 2009; 102: 763-7.
91. Ho KY, Gan TJ, Habib AS. Gabapentin and postoperative pain--a systematic review of randomized controlled trials. Pain 2006; 126: 91-101.