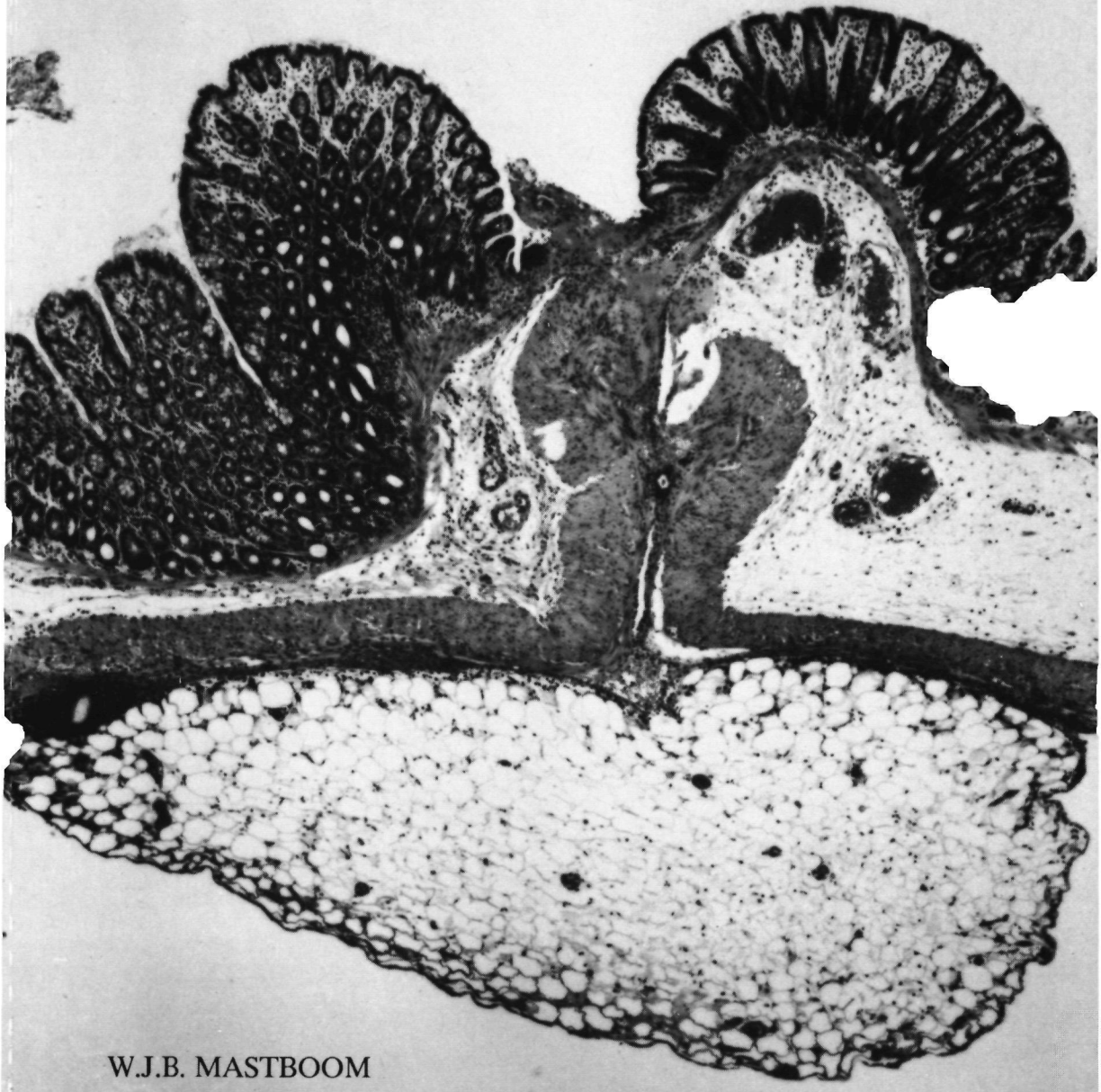
FACTORS INFLUENCING INTESTINAL ANASTOMOTIC REPAIR

An experimental study in the rat.



W.J.B. MASTBOOM

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Promotor : Prof. Dr. H.H.M. de Boer
Co-referent: Dr. Th. Hendriks

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Chapter I

INTRODUCTION

FACTORS INFLUENCING HEALING OF BOWEL ANASTOMOSES

W.J.B. Mastboom, H.H.M. de Boer, Th. Hendriks.

Department of General Surgery, St. Radboud University Hospital, Nijmegen, The Netherlands.

Summary

A multitude of factors is thought to influence the successfull healing of intestinal anastomoses. Anastomotic failure is a serious surgical complication attended with considerable morbidity and mortality. In this chapter the present opinions about circumstances which may adversely affect anastomotic healing are reviewed.

In most cases the origin of anastomotic leakage is probably multi-factorial. In some situations, such as the presence of generalized peritonitis, radiation enteritis, concurrent chemotherapy or unvital wound edges, a true contraindication exists for the construction of an intestinal anastomosis. In addition, many other factors are thought to have a negative effect on the healing process, even though conflicting reports concerning their influence can be found in the literature. A factor of considerable importance seems to be the individual surgeon who performs the operation.

It is concluded that the question "which conditions impair undisturbed healing of intestinal anastomoses" still cannot be answered unequivocally. Since the average age of the patients in hospitals is increasing and the cases offered for gastrointestinal surgery become more and more complicated, a profound knowledge about the risk-factors for the individual patient is of great importance in deciding whether to perform a primary anastomosis or to construct a stoma. In order to extend insight in this matter many more clinical and experimental studies will be necessary.

Introduction

In 1743, Ramdohr performed the first successful circular bowel anastomosis in man by invaginating and fixing a gangrenous small bowel segment [1]. The first human end-to-end anastomosis was constructed in 1836 by Diefenbach. The discovery and development of aseptic operative procedures together with the progression in anaesthesiological methods gradually improved the results of gastrointestinal surgery. Several techniques were developed for construction of anastomoses, but as yet no general agreement exists as to which one is the best. Most surgeons will still follow Czerny's advice, which dates from 1880, to construct an anastomosis of more than one layer of sutures.
In 1887 Halsted reported the submucosa to be the strongest layer of the intestinal wall and he strongly recommended to include this layer in anastomotic sutures [2]. After this discovery, other developments in medical science have contributed to decrease the complication rate of surgery on the gastro-intestinal tract. However, intestinal anastomotic dehiscence still comprises a major problem, which is attended with a high mortality. In order to prevent this complication, it must be known which factors or conditions impair wound healing.
If a bowel resection needs to be performed under conditions where the risk of anastomotic dehiscence is judged too high, a stoma, and not a primary anastomosis should be constructed. In many cases it proves to be very difficult to decide if construction of a primary anastomosis is indeed justified, since there still exists considerable controversy about the severity and importance of the various risk factors.
This chapter comprises an attempt to summarize the current knowledge concerning the most prominent factors which are believed to compromise the integrity of intestinal anastomoses.

the healing process

In order to understand mechanisms whereby anastomotic repair may be impaired, knowledge about the processes involved in undisturbed wound healing is essential. Almost all knowledge on the sequence of events during healing of soft tissues is derived from observations on skin wounds [3].

Immediately after tissue laceration reflex vasoconstriction occurs, followed shortly by dilation of the vessels and bleeding. Thrombocytes adhere to the damaged vascular wall inducing production of fibrine, resulting into formation of a haemostatic clot. Degranulation of thrombocytes increases the permeability of the vascular wall for proteins and inflammatory cells. Within three hours after the trauma polymorphonuclear granulocytes appear in the wound area. These cells are capable of phagocytosis of microorganisms and necrotic tissue, but their presence is not essential for the further healing sequence.

After 24 hours, the first macrophages are observed. Presence of these cells is essential for undisturbed healing since they can regulate the degradation and synthesis of collagen, which processes eventually result in the restoration of wound strength [4]. Macrophages also stimulate proliferation of capillaries, thereby improving oxygenation of the wound area. From the first day after laceration, epithelial cells grow from the wound edges covering the surface of the wound.

If no infection is present, most polymorphonuclear granulocytes have disappeared from the wound area within one week. At this time-point the histological picture mainly shows collagen, macrophages and fibroblasts. The macrophages gradually disappear and after several months the number of fibroblasts also decreases. After one year, most newly formed capillaries have disappeared and the scar is mainly composed of fibrotic tissue.

Similar processes occur around intestinal anastomoses [5]. In contrast with skin wounds, anatomical apposition of the different tissue layers in the intestinal wall is no purpose in intestinal surgery since an inverting anastomosis is constructed in most cases [6]. Microscopically, the non-anatomical apposition of these layers results into formation of a large fibromuscular clot, which is later covered by mucosa, newly proliferated from the wound edges [5].

Most experimental studies on intestinal wound healing measure mechanical strength and collagen levels around the anastomosis. It is generally taken for granted that effects of factors which affect anastomotic healing will be reflected by changes of these parameters [cf. chapter II].

anastomotic dehiscence

In the literature there exists no uniformity in the use of the term "anastomotic leakage". Some studies only report on the incidence of anastomotic leakage causing clinical symptoms like fever, abscess formation, peritonitis or fistula, while in others also non-symptomatic leaks, which are radiologically diagnosed, are noticed. The frequency of anastomotic insufficiency still is considerably high.
Goligher, who performed radiological examination of colorectal anastomoses, found leakage of contrast in 69% of the cases [7]. Fortunately, the number of symptomatic anastomotic leakage is a small fraction of those radiologically detected [8,9].
Since, in contrast with the large bowel, radiological control of small bowel anastomoses is not feasible, no data are available about the occurrence of asymptomatic dehiscence of small bowel anastomoses.
Though anastomotic dehiscence in the small bowel is often believed to occur seldom, actual data are rare. Hesp et al. found clinical evidence of leakage in 0.8% of 120 small bowel anastomoses in 57 patients [10]. The mortality of this complication was 18%. In several large clinical studies about healing of colonic anastomoses, overall rates of symptomatic leakage vary from 4 to 14%, while failure rates up to 25% are found for anastomoses constructed in emergency situations and for low anterior rectal resections [11-13]. Anastomotic leaks which cause clinical symptoms are associated with a mortality of approximately 25% [11-14].

suture technique and suture material

Several suture techniques have been developed for the construction of an intestinal anastomosis. Basic principles in intestinal surgery are to minimize tissue trauma and to avoid tension on the anastomosis. While constructing an anastomosis, the surgeon needs to compromise between two conflicting principles. In order to prevent vascular and tissue damage, the use of suture material should be minimized; in order to construct an anastomosis which will not leak, the use of some kind of suture material is necessary. In most Dutch hospitals a two-layer inverting anastomosis is performed for restoring continuity of the intestinal tract.

There still remains some doubt whether an inverted anastomosis is indeed superior to an everted one [15]. The main disadvantage of eversion of the wound edges is the increased formation of adhesions; on the other hand, the risk of local stenosis is reduced.
Another point at issue is the choice of suture material for the anastomosis. It is believed that use of quickly dissolving materials, like catgut, induces a severe inflammatory reaction, which might be deleterious to the healing process [16]. Application of inert materials, including staples, sometimes causes chronical inflammation and formation of fistula, which might theoretically result into induction of carcinoma. The use of monofilament wires reduces formation of microabscesses around the sutures, but these materials are more difficult to handle. In the last decennium the use of staple instruments has become very popular [8,9,17]. These instruments are especially advantageous to the patient who needs a low rectal anastomosis, since their use might prevent construction of a stoma in selected cases. However, the use of staplers does not prevent the occurrence of anastomotic dehiscence. Some authors claim that the leakage rate of hand-sewn anastomoses performed by experienced surgeons is similar to stapled ones [8,9].
In a prospective study Fielding et al. [18] have shown that the individual surgeon is quite an important factor in deciding the outcome of anastomotic healing. The leakage rates for human colonic anastomoses varied between less than 0.5% and more than 30% for the different operators. There exists no apparent relation between leakage rate and either experience or age of the surgeon.

location of the anastomosis

As already mentioned before, it is generally believed that, in contrast with large bowel, the frequency of anastomotic leakage in the small bowel is low, except for duodenal stumps after gastric resections. Several factors might contribute to this alleged superior healing of the small bowel, such as the lower concentration of micro-organisms [19], the absence of hard stools [20], or differences in local blood flow [21]. Moreover, in most cases it will be technically easier to construct an anastomosis in the small bowel than in the colon.

While reporting their results, many authors fail to describe the exact localisation of the anastomoses in the colon and only mention overall results. In studies where this differentiation has been made, dehiscence of anastomoses under the recto-peritoneal reflection occurs more frequently compared to those constructed more proximally [11-14]. Factors believed to be responsible for this difference are the anatomical limitations of the pelvis, which makes surgery technically difficult, and the absence of a serosal layer, which plays an important role in adherence of intra-abdominal intestinal anastomoses.

nutrition

A wide variety of nutritional factors is necessary for uncomplicated wound healing. In a large retrospective clinical study, Irvin noticed that anastomotic leakage occurred more frequently in patients who had reduced blood levels of albumine and proteins [11]. On the contrary, in a similar study Schrock did not find a significant increase of anastomotic leakages in malnourished patients [14].
The human body is able to synthesize most fundamental nutrients [3]. However, several essential elements should be obtained by regular oral intake. Some of these factors, e.g. certain amino acids, vitamin C, zinc and iron are essential for the healing process [22]. For many elements, the intake minimally necessary to ensure uncomplicated healing is unknown. It seems feasible that apparently underfed patients do not have deficiencies of essential factors, while well-fed patients might suffer from unknown deficiencies.
In experimental studies on rats which received a protein-free diet, a linear relation was found between the duration of the diet, the hypoproteinaemia it induced and the reduction of the mechanical strength of colonic anastomoses [23-25]. If parenteral administration of amino acids was started immediately after the operation, uncomplicated healing was observed [26].
In a randomized clinical study peri-operative parenteral nutrition did not significantly prevent anastomotic leakage in patients who underwent a colonic resection [27].

bowel preparation

Construction of an anastomosis in an unprepared colon, e.g. in emergency situations, increases the leakage rate [14,28]. Preparation of the small intestine seems less important because of the low bacterial concentration and the liquid or soft consistency of the stools [19,20]. Micro-organisms might impair anastomotic healing by production of collagenolytic enzymes and by formation of abscesses. Presence of hard faeces will threaten a new anastomosis by increasing mechanical pressure.
While it has been reported repeatedly that pre-operative decontamination of the bowel with antibiotics significantly reduces the occurrence of anastomotic dehiscence [29-31], it has also been stated that this procedure only reduces the frequency of wound infections [32].
Pre-operative lavage of the intestine seems an important factor in reducing anastomotic complications, since the frequency of leakage is much higher in unprepared colon [14,33]. Some authors have even indicated that in selected emergency cases a stoma might be avoided when lavage of the colon is performed during the operation [34,35].

oxygen supply

A sufficient oxygen level is of vital importance for the healing process. In skin wounds hypoxia leads to diminished synthesis of collagen [36] and increases the risk of wound infections [37]; so far, such data for intestinal anstomoses are lacking. Clinical and experimental studies have shown that a sudden reduction of the intestinal blood flow, such as occurs in shock, is harmful to anastomotic healing [11,38]. During an episode of shock, the oxygen supply of the bowel is affected more than in several other organs: the reduction of the blood flow is much larger while its increase occurs much later than restoration of the blood pressure [39,40].
Local ischaemia near the anastomotic area might result in insufficient repair and subsequent leakage of intestinal contents. Thus, though construction of an anastomosis of more than one layer seems to prevent leakage of intestinal contents between the sutures, it causes more tissue damage and increases the risk of local ischaemia. Another disadvantage is that in this case a

larger part of the mesenterium along the intestine should be divided, augmenting the decrease of the vascularisation in the wound edges.
The influence of anaemia on anastomotic healing is less clear. Schrock found a significantly higher number of anastomotic dehiscences when the haematocrit fell below 35% during colonic resection [14]. On the contrary, Tagart reported that the blood haemoglobin level was increased in male patients with leaking colorectal anastomoses [19].
Though atherosclerosis is a common degenerative disorder in man, nothing is known about its influence on intestinal wound healing.

peritonitis

Most surgeons are convinced that it is dangerous to construct a colonic anastomosis in patients with a generalized peritonitis [41]. It is often suggested that collagenases, produced by both bacteria and leucocytes, are responsible for the impaired healing [42]. However, clinical studies about this subject are scarce and contradictory. In contrast with Irvin [11], who did not find any difference, Schrock [14] reported that leakage of colonic anastomoses increased significantly in the presence of an abscess, a fistula or peritonitis. The different results of both studies are probably due to selection of patients, while in neither of the studies the severity of the peritonitis is mentioned.
Hinchey has composed a clinically useful classification of diverticulitis [43], which seems also applicable to resections for other bowel disorders. Stage 1 is defined as an inflammation limited to the mesentery, stage 2 is a local abscess, stage 3 is reserved for a perforation of the intestinal wall or of an abscess towards the abdominal cavity and stage 4 is a faecal peritonitis. Construction of an anastomosis is justified when the inflammation is limited to the mesentery [33]. For the other stadia, a Hartmann's procedure should be performed [44]. When the condition of the patient is reasonable it is preferable to resect the diseased bowel segment, but otherwise this procedure includes construction of a proximal stoma only. Some surgeons make a diverting stoma proximal to high-risk anastomoses [31]. This does not prevent anastomotic leakage but clinical effects are avoided in most cases [11,12,14].
Controversy exists about the treatment of traumatic perforations

of the large bowel. Most of these patients will be operated soon after the accident; therefore during operation faecal contamination of the abdominal cavity is found rather than faecal peritonitis. Post-operative septic complications frequently occur [42-44]. In an attempt to avoid these complications, some surgeons routinely construct a stoma [42,43], while others think that construction of a diverting stoma does not prevent these complications and advice primary suturing of the intestinal wound since leakage rarely occurs [44].
Few data are available on small bowel operations in the presence of peritonitis. Indications for small bowel resections are different from those for colonic resections which hampers comparison of these operations. In a retrospective study Hesp showed that presence of peritonitis significantly increased occurrence of anastomotic leakage in the small bowel [10]. Induced peritonitis in rabbits also impaired anastomotic healing in both small and large bowel [48].
In case of peritonitis, most surgeons will use the same criteria, deciding whether to construct a primary anastomosis or a stoma, for both small and large bowel.

radiotherapy

Irradiation of the abdomen causes much damage to the intestines and might induce ulceration, formation of adhesions and fistula and free perforation [49,50]. The severity of these complications is related to the dose applied. Microscopically, fibrosis and obliteration of blood vessels is seen [51], which may, even after many years, result into serious complications. It will be clear that suturing of a previously irradiated bowel segment is fundamentally wrong.
In Schrock's clinical study the number of anastomotic failures was increased if the abdomen had been irradiated at an earlier time [14]. It is likely that construction of an anastomosis shortly after irradiation is not as dangerous since the vascularisation will still be intact [52,53]: for instance, soon after radiotherapy for a rectal carcinoma, a low anterior resection can be safely performed [54]. When a laparotomy is necessary at a later stage, anastomoses should only be constructed between bowelloops which have not been irradiated [49,50,55].

corticosteroids and cytostatics

The deleterious influence of corticosteroids on skin wounds has been amply demonstrated [56-58]. These compounds inhibit the immune system, thereby increasing the risk of wound infections. They also inhibit chemotaxis and function of macrophages causing, among other things, disorders of the collagen metabolism.
Steroid medication is often suggested to be a risk factor in intestinal surgery [18,59-61], but in fact this has never been shown in clinical studies [14,62]. Experimental studies on this subject are rare [63,64].
The detrimental influence of cytostatics on anastomotic healing has been proven both in clinical an experimental studies [65,66]. It is strongly recommended to avoid intestinal surgery on patients receiving chemotherapy.

other risk factors

There are certainly several additional factors which affect the outcome of intestinal surgery. In the studies of both Irvin [11] and Schrock [14], anastomotic dehiscence occurred more frequently in patients older than 60 years. While in younger patients the frequency of leakage varied between 3 to 7%, it increased to values between 10 and 20% in older patients. It is likely that this increase is the result of a combination of several factors which are influenced by age, like malnutrition, reduction of general condition and immunity and a decrease of local vascularisation.
The effect of many factors on intestinal anastomotic healing are still unknown. Some of these factors have been proven to adversely affect healing of skin wounds, like diabetes mellitus and uraemia, but such a detrimental effect has never been demonstrated for intestinal wounds. Except for cytostatic drugs, the influence of most medicines on intestinal wound healing has hardly been investigated. In this respect, it would be interesting to examine the effect of drugs which inhibit inflammatory processes [41].

discussion

In most elective intestinal surgery it will be possible to construct a primary anastomosis, unless the bowel proves to be insufficiently prepared during the operation. On the other hand, it is not difficult to decide to refrain from anastomotic construction in the presence of a purulent peritonitis, intra-abdominally disseminated carcinoma, radiation enteritis, or in patients which are concomitantly treated with cytostatics. However, many situations occur in which the decision whether to construct a primary anastomosis or a stoma is more difficult. In most of these situations clear parameters for this decision are lacking. The consequences of the risks of both options must be considered carefully.

Clinically evident intestinal anastomotic leakage is lethal in one quarter of the cases [11-14]. However, bowel resection with construction of a stoma also leads to a considerable mortality, while morbidity and mortality after restoration of the intestinal continuity at a later stage are not to be neglected either [59,67]. Moreover, construction of a stoma is attended with lasting complications in more than one third of the patients, even when the stoma is constructed for a temporary use [68]. In our own department, 18% of the patients died after closure of a split ileostomy, which had been constructed during treatment for generalized peritonitis [10]. After this operation, occurrence of non-lethal complications, like obstruction and ileus are no rarity. Likewise, restoration of the intestinal continuity after a Hartmann's procedure is not without any risk. Some surgeons try to prevent anastomotic dehiscence by wrapping the greater omentum around high risk anastomoses, but the validity of such a procedure has never been proven.

With the increase of the average age of the western population and the progression of medical science, more complicated cases will be offered for intestinal surgery. An estimation of the risk for the individual patient will be necessary to decide if construction of a primary anastomosis is justified. Presently, relatively little is known about the processes which occur within the healing anastomosis. In this respect, further eludication is necessary in order to predict which factors will interfere with undisturbed healing. For this purpose, both fundamental research and clinical studies are necessary.

the present study

The experiments described in this thesis aim to contribute to the understanding of fundamental processes occurring during anastomotic repair. A central role in the healing sequence is played by the connective tissue protein collagen. This protein is the primary constituent of the submucosa, which layer forms the backbone of the bowel wall. As such, collagen is responsible for intestinal strength, both in uninjured and anastomosed bowel.
Investigations on factors affecting anastomotic healing often use various parameters for collagen metabolism, e.g. content or synthesis, as an index for repair (cf. chapter II). Considerable post-operative changes in collagen levels occur in the anastomotic area, probably as a result of a changing equilibrium between its degradation and synthesis. These changes may be mediated to a large extent by the inflammatory reaction which forms an intrisic part of each wound healing sequence. Thus, it is of particular interest to investigate if compounds or conditions which are expected to interfere with the inflammatory reaction also affect collagen changes around healing anastomoses.
We examine the effects of various non-steroidal anti-inflammatory drugs (chapter III-V) and one corticosteroid (chapter VI) on the healing of intestinal anastomoses. These drugs are widely used in various groups of patients and their effects on intestinal anastomoses have been hardly investigated.
The prolonged presence of suture material results in a lasting inflammatory focus which could adversely affect collagen metabolism and thus intestinal strength. Therefore, we also seek to limit inflammation by removing the sutures immediately after anastomotic construction (chapter VII).
Bacteria may contribute to post-operative collagen metabolism - particularly degradation-, either directly by producing collagenolytic enzymes, or indirectly by stimulating the activity of granulocytes, which cells comprise a rich source of collagenases. An attempt to evaluate the contribution of micro-organisms to the changes in anastomotic collagen levels is made by comparing healing in normal and germ-free rats (chapter VIII).
In all experimental animals anastomoses are constructed in both ileum and colon to allow comparison of the repair process in small and large bowel.
The experimental reports are preceded by a review on the parameters commonly used to assess anastomotic healing (chapter II).

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Chapter II

HEALING OF EXPERIMENTAL INTESTINAL ANASTOMOSES: PARAMETERS OF REPAIR

Th. Hendriks, W.J.B. Mastboom

Department of General Surgery, St. Radboud University Hospital, Nijmegen, The Netherlands.

Introduction

The healing of intestinal anastomoses remains a topic of ongoing research interest, because clinical practice shows to be disturbed rather frequently. Compromised healing may lead to dehiscence and leakage, which complication is attended by high morbidity and mortality. The occurrence of anastomotic leakage appears unavoidable in large bowel surgery, this in contrast to surgery of the small bowel where the complication has hardly been mentioned [1]. Leakage rates reported in the literature vary greatly, ranging between 0 to 35%. To a large extent, this may be due to variations in patient population and the presence of factors which increase the risk for disturbed healing [2]. Also the outcome is clearly dependent on the surgeon performing the operation [3]. Apparently, anastomoses are compromised regularly, even under optimal conditions, and a multitude of conditions may aggravate the risk considerably [4]. Thus, research on wound healing in the intestine remains much needed, in order to understand the underlying mechanisms and to find ways to decrease chances for anastomotic leakage, particularly in high risk situations. Most of the concepts on the healing process have been derived from observations on skin wounds. It appears that the basic pattern of wound healing is similar in every tissue, the process consisting of three stages: a lag phase, to get rid of debris, a second phase in which cellular elements proliferate and a maturation phase [5]. Histological observations confirm that this overall sequence also occurs in the intestinal wall [6-8]. Although one would certainly like to think that all wounds heal by common mecha-

nisms, healing being a simple phenomenon [9], various authors have expressed the necessity to exert caution in extrapolating the results from research on skin repair to the healing of other soft tissues [10-12]. In fact, it has been shown that significant metabolic differences exist between healing in the gastrointestinal tract and skin [13]. If one wants to draw conclusions regarding anastomotic healing in the intestine, it thus seems indicated to do so only on the basis of experiments performed on this particular tissue.

Investigating wound healing, and aiming to improve its outcome, necessitates quantification of the process. Parameters for anastomotic repair may be mechanical, biochemical or histological. Histology is not a primary tool for quantification while comparing various series of experimental anastomoses. It certainly is very useful to describe the course and eventual result of the healing sequence at tissue level [8]. Also, the successive infiltration of various cells into the wound area may be followed and obvious differences between e.g. ileal and colonic anastomoses will certainly be demonstrated this way [14]. However, the measurement of choise to evaluate anastomotic repair, and the effects of variations in surgical techniques, of administration of drugs or of any other modification to established procedures, will mostly be either mechanical or biochemical or both.

During the last two decades, a significant amount of research has been performed which involved the measurement of strength and/or the level of collagen in anastomotic segmenst. If measuring strength, one has several choises: bursting pressure, bursting wall tension or breaking strength. Likewise, if measuring collagen one could measure, for instance, concentration or content. Often, only bursting pressures or collagen concentrations have been reported. While it seems reasonable to draw conclusions from these measurements under certain conditions, they easily may be misinterpreted. The aim of this essay is to dicuss the usefullness and limitations of the parameters currently used to quantitate anastomotic healing.

Mechanical parameters

The developing mechanical strength is, without doubt, a meaningful parameter to follow while investigating anastomotic healing. For this purpose, two fundamentally different approaches can be

chosen. Firstly, the bursting strength, expressed either as bursting pressure or bursting wall tension, which is a measure for the resistance of the intestinal wall to increasing intraluminal pressure. Secondly, the breaking strength which reflects the resistance of the intestinal wall to forces exerted in a longitudinal direction. The former is generally considered to reflect the physiological strain in the intestine more accurately than the latter.

a. bursting pressure

The measurement of the bursting pressure comprises distension of a bowel wall segment with gas or liquid until a leak develops: the pressure at which disruption occurs is recorded as the bursting pressure. The technique was applied for the first time ninety years ago by Chlumsky [15]. From 1964 onwards [6], a more regular flow of data started to appear, the authors being primarily interested in comparing various suturing techniques.
It has been shown that the results of bursting pressure measurements may depend on the rate of inflation of the bowel. Nelson and Anders, using segments of small intestine from the dog, showed that inflation rates of 2 and 12 ml H_2O/min yield similar results but that a far higher rate (50 ml/sec) leads to significantly increased bursting pressures [16]. They conclude that "the longer the distension force is applied to the intestine, the lower is the pressure required for bursting or leakage". This might seem to comprise a factor which could possibly hamper comparison of the absolute data reported in various studies.

Only in a minority of the studies reported so far, bursting pressures of anastomoses are compared to control values, obtained from either unoperated control animals or from segments proximal to the anastomosis in the same animal. Mostly, the development of strength is followed in a series of animals sacrificed successively without reference to such controls. Nevertheless, it seems worthwhile to emphasize that if one wants to use unoperated intestine from the same animal as a reference value or, alternatively, if one wants to compare more than one anastomosis in the same animal [17], it should be established first that the bursting pressures of the unoperated segments in question are similar. The need for this precaution is illustrated by the fact

that bursting pressures in the intact transverse colon of rats are significantly higher than in those in the adjacent colon of the same animals [18,19].
The development of strength in healing anastomoses has been amply investigated and the pattern is well defined. During the first 3 to 4 days, the lag period, bursting pressures remain low. Thereafter, they increase fairly quickly to values well over those observed in uninjured intestine. This course is similar in the large bowel of rats [6,19-23], dogs [24,25] and rabbits [26] and also in the small bowel of the same experimental animals [24, 26-29]. While the quantitative picture seems unambiguous, the quantitative data reported in the literature show large differences.

The existence of a certain degree of variation between data from these type of experiments is not altogether unexpected, since tissue repair can be affected by a multitude of factors. At first sight, a non-uniformity in suturing techniques could be envisaged to contribute to the numerical divergence apparent from the various publications. It is regrettable that the techniques employed, and the suture materials used, are not always described fully. Evidently, these have differed widely although more recent studies are mostly performed using single-layer inverted interrupted sutures. However, it seems that a diversity in this respect could only explain the quantitative differences to a limited extent: Yale et al. [18] measured an avarage bursting pressure of 48 mm Hg in three days old inverted anastomoses as compared to 30 mmHg for everted anastomoses and Jiborn et al found no significant differences between continuous and interrupted sutures. In our opinion, the outcome of bursting pressure measurements depends strongly on the treatment of the bowel after sacrifice. In most cases, the anastomotic segment is isolated, one end is tied off and the other end connected to a nipple through which gas or liquid is infused. This procedure requires careful handling. Otherwise, adhesions involved in sealing the closures might be disturbed. It often remains unclear if adhesions are removed or left in place. Most authors only refer to excision of the anastomosis without mentioning adhesions at all. The fact that this factor is indeed of considerable influence, certainly during the first post-operative days, may be illustrated by the bursting pressures measured in anastomoses which have been left in place. Three days after operation, in situ measurement in colonic anastomoses in the rat yields bursting pressures

of 125 mm Hg [30,31], significantly higher than any measured in excised anastomotic segments. this difference appears even more explicit in one day old anastomoses, where in situ measurements yielded a bursting pressure of 115 mm Hg [32] compared to 12 [22] or 26 [Mastboom et al. unpublished results] mm Hg measured the other way.
Altogether, these data indicate that quantitative comparison of bursting pressures is only valid within one study, where one protocol is used for the various experimental groups. Comparison of quantitative data between studies is probably not meaningful: only the pattern of strength development might allow a rough comparison.

It has been argued that the bursting wall tension, the circular wall tension at the point of rupture, would be a more valid parameter for intestinal strength than the bursting pressure [16]. The bursting wall tension is calculated, according to Laplace's law, as the product of bursting pressure and internal radius. Thus, it takes into consideration the diameter of the lumen. It stands to reason that the results of comparison of strength in different intestinal segments, which may be expected to vary in diameter, will depend on the type of measurement employed. It has been reported that bursting pressures of rat large and small intestine do not differ, while the bursting wall tension is significantly higher in the large intestine [18]. Also, both parameters may yield different results if the diameter of the same intestinal segment is manipulated. Starvation of rats results in rising colonic bursting pressures, while at at the same time bursting wall tensions decline progressively since the colonic radius becomes much smaller [21]. Analysis of bursting wall tension also explains why intestinal anastomoses grow stronger than the adjacent bowel wall shortly after construction. The intestinal diameter will almost invariably be smaller in the anastomosis proper than that developed at the anastomotic site will therefore be less than that developed in the wall of the intact bowel and, at inflation, rupture will occur outside the anastomotic area as soon as the anastomosis has gained some strength.
This fact also comprises the main limitation in the application of bursting strength techniques for monitoring anastomotic healing. As soon as rupture takes place outside the immediate anastomotic area, bursting pressure values - or for that matter,

bursting wall tension - cease to relate to the process of anastomotic healing: in that case, the bursting strength measured only provides the information that anastomotic strength is greater than that of the ruptured bowel segment. It is difficult to set a general rule for the period during which bursting strength may be considered as an useful parameter for anastomotic healing. The statement of Ballantyne [7], in his review on intestinal suturing, should probably be readjusted. Ballantyne stated that "after about two weeks, bursting pressure measurements no longer reflect the strength of anastomoses". Comparing the various data, it seems more likely that this period does not exceed one week. While it is true that some authors report that seven day old anastomotic segments mostly rupture within the anastomotic area [33-35], others have found that at this time rupture occurs predominantly outside the anastomosis [16,17,19,22,26,36]. Again, this may depend on experimental variables such as animal species, intestinal segment used, suturing technique and preparation of bowel for bursting pressure measurement. Still, it seems prudent, if one aims to investigate anastomotic strength in a series of animals, to always note the bursting site and to disregard the data from those animals, which intestines do not rupture within the anastomotic area.

It is therefore regrettable that data on the bursting site is lacking in a considerable number of papers, in which bursting pressure is used as a parameter for anastomotic repair, particularly since healing is often evaluated seven days after operation. This information appears necessary in evaluating the validity of the conclusions drawn regarding the repair pocess. Moreover, it may also provide useful evidence: comparing seven days old anastomoses in rabbit ileum, we found that in animals with induced peritonitis the bursting site was in the anastomotic area in three out of nine cases. In control animals, rupture occurred outside the anastomosis in 14 out of 14 cases, a significant difference providing additional evidence for the deleterious effect of infection upon healing [37].

Bursting wall tensions have been reported in a number of studies on anastomotic healing. Its course appears to be identical to that reported for the bursting pressure, at least for the first two weeks after operation [19,25]. Apparently, the anastomotic diameter does not change within the time span. Since calculation of the bursting wall tension requires the measurement of the

internal radius of the anastomosis, which is not always easy to accomplish in a reproducible fashion, the question seems warranted if evaluation of bursting wall tensions indeed yields more information, or leads to other conclusions, than measurement of the bursting pressure alone. The data available in the literature appear to answer this question negatively. In a number of studies, in which anastomotic repair was evaluated under various conditions (e.g. changing suturing techniques and nutrition) both parameters have been measured. Invariably, differences found were reflected by both bursting pressure and bursting wall tension measurements and conclusions drawn from both sets of experimental data were the same [18,19,21,25,35,38-42]. So far, no data on anastomotic repair have been published which show qualitatively different behaviour of bursting pressure and bursting wall tension. Apparently, the internal diameter of anastomoses, constructed at a fixed site in the bowel, is quite reproducible and not easily affected by changes in experimental conditions. Unless clear indications exist that anastomoses of varying diameter are under investigation, bursting pressure seems to be an adequate measure for anastomotic strength.
Thus, in conclusion, the bursting pressure is a good parameter to monitor anastomotic repair, as long as rupture takes place within the anastomosis. This period comprises the first post-operative days where strength is low and changes for dehiscence relatively high.

b. breaking strength

The breaking strength is measured by applying an increasing force in a longitudinal direction to anastomotic segments. The peak force necessary to induce disruption is taken as the breaking strength. Nelsen and Anders [16] found this type of measurement subject to critisism because of the difficulty to apply an equally distractive force to the entire circumference of the bowel wall and because no force is exerted in the circular direction.
The technique was applied firstly to intestinal anastomoses by Hermann et al. [6]. In the earlier experiments, strips of anastomotic tissue were employed for this purpose until it was pointed out that the elasticity of the intestinal wall hampers the production of standardized strips [43]; subsequent measure-

ments have indeed consistently been conducted on the entire anastomotic segment. It stands to reason that the outcome of breaking strength measurements is equally dependent on the procedure followed for the isolation of the segment and the actual testing method applied, as the result of the measurement of bursting strength. For instance, it seems quite possible that a tensiometer which provides a constantly increasing force [e.g.43] will yield peak values which are different from those obtained by a procedure where force is increased by intervals [44]. Likewise, the preparation of the segment, in particular with respect to the removal of adhesions, may affect the actual figures.

The development of anastomotic breaking strength appears similar to that of the bursting pressure, certainly during the first post-operative week. Most data are available on rat colon. The strength of a newly constructed anastomosis, resected and measured immediately after completion, is only approximately 30% of that of intact colon [45,46]. Its strength declines even further, reaching minimal values after one or two days. Thereafter, a rapid gain in strength takes place. Seven days old anastomoses are stronger than newly constructed anastomoses, but still considerably weaker than uninjured intestine. The first authors, using breaking strength as a parameter for anastomotic strength [6], found normal strength to be approximated after 17 days, but very recently it was reported that anastomotic breaking strength 56 days after operation was only 70% of that of unoperated controls [46]. A qualitatively identical pattern exists in anastomoses of the small bowel, both in rats [47] and in rabbits [48]. Possibly, original breaking strength is restored more rapidly than in the colon, even more so in jejunum than in ileum [47]. Thus, the main difference between the course of post-operative bursting pressure and post-operative breaking strength appears to be the rate at which the values, measured in normal intestine, are approximated: bursting strength is more rapidly restored than breaking strength.

It has been pointed out in the preceding section that a major limitation of bursting strength measurements is that they reflect anastomotic strength only as long as disruption occurs within the anastomosis, which is only during the early period of healing. The fact that during breaking strength measurements breaks occurred always in the anastomotic line up until 4 weeks after

operation, was a major reason for Jiborn et al. [43] to express their preference for the use of breaking strength as a parameter while studying anastomotic repair. In this study, sutures were removed before strength was measured. In later studies from the Malmö group breaking strength is almost invariably assayed with sutures in place, in order to avoid damage to the anastomosis. During the first post-operative days strength then reflects the "suture holding capacity", the ability of the bowel wall to retain the sutures. From seven days onwards the presence of sutures does no longer affect anastomotic strength [49]. With sutures left in place, breaks still occur in the anastomotic line up until seven days [45]. However, it has not been reported what happens in a later phase, at least not in colon. Uden et al. [46] followed colonic anastomotic breaking strength up until 56 days but did not report on the disruption site. This information seems essential in order to assess the suitability of breaking strength as a valid parameter since in the small bowel disruption was reported to occur outside the anastomosis already after two weeks [47]. Arbogast et al. [48] even reported that breaks invariably occurred outside the anastomosis. This somewhat puzzling result emphasizes the notion that breaking strength, reflects anastomotic strength only for a limited period. Information about the disruption site should always be supplied, particularly for anastomoses which are older than one week.

Numerical data on breaking strength of enteric anastomoses have been reported in a number of studies which differ greatly in experimental conditions. Comparable figures have been obtained for one day old colonic anastomoses in the rat by Blomquist et al. [45] and Wilker et al. [50]. It is interesting to observe that the breaking strength of intestinal anastomoses strongly depends on the distance between the wound edge and the site at which the sutures are inserted [51,52]. No such experiments have been reported for bursting strength.

In a few cases, both breaking strength and bursting strength have been measured within the same experiment. Both Rosin et al. [53] and Young and Wheeler [54] found consistent results for the two parameters: differences between groups were equally significant using either breaking strength or bursting pressure. However, Smith et al. [55], investigating the use of different drainage materials in colonic healing, observed that breaking strength

values were similar in all groups examined while significant differences were seen between the average bursting pressures. The beneficial effect of vitamin A supplementation on bursting pressures of colonic anastomoses was reflected by the breaking strength, not in the peak values but only if the area under the breaking strength curve (which is a measure for the breaking energy) was analyzed [56].
Since breaking strength and bursting strength reflect fundamentally different properties, divergent results may be expected. Breaking strength for a somewhat more extended period than bursting strength, probably up to two weeks. However, more data on the actual site of disruption are needed since those currently available yield no consistent picture.

Biochemical parameters

The extracellular matrix consists of collagens, proteoglycans and glycoproteins. In studies on wound repair, which involves reconstitution and remodeling of the extracellular matrix, attention is mainly focused on the behaviour of collagen, presumably since this class of proteins is thought to be primarily responsible for wound strength. Still, a glycoprotein like fibronectin has many potential roles in tissue repair [57]. Also, glycosaminoglycan content and composition have been shown to change profoundly in the healing skin [58] and hyaluronic acid might be an important regulatory factor during the early healing stages [59]. So far, research on the biochemical basis of anastomotic healing in the intestine has been limited to the examination of collagen and some attention for the other extracellular matrix components in this tissue appears long overdue.
It was recognised already a century ago that the submucosa is the strongest layer in the bowel wall [60]. This connective tissue layer imposes mechanical strength and structural integrity and thus acts as the skeleton of the intestine. The submucosa is composed almost entirely of collagen fibrils and, conversely, most of the collagen in the intestinal wall is concentrated in the submucosa. The morphology of the submucosa and the lattice arrangement of its collagen fibrils have been studied in some depth [61-64]. The necessity for anchoring the sutures in the submucosa during the construction of an intestinal anastomosis [60] has been confirmed repeatedly and remains undisputed. This

being the case, the interest for post-operative collagen metabolism is easily understood. Early anastomotic strength depends on the ability of the existing -collagenous- network to retain the sutures while newly formed collagen fibrils should restore the original strength to the healing bowel. Thus, post-operative collagen degradation and synthesis are expected to affect anastomotic strength and the course of various parameters for collagen metabolism has been taken as a measure for anastomotic healing. As yet, the majority of the data reported concern quantitative aspect of collagen -concentration, content and synthesis- while its quality, in terms of e.g. solubility, crosslinking or type, has received hardly any attention at all.

a. collagen concentration and content

Quantification of collagen in enteric anastomoses has been synonymous to quantification of hydroxyproline, an amino acid unique to collagenous proteins in most tissues. The hydroxyproline level is always taken as a measure for the amount of collagen present. However, it should be emphasized that the different collagen types contain varying amounts of hydroxyproline [65]. For instance, the constituting alpha-chains of type III contain more hydroxyproline residues than the alpha-chains of type I and thus equal amounts of type III and type I collagen contain different amounts of hydroxyproline. Calculation of the exact amount of collagen from hydroxyproline levels in different tissues which may differ in terms of collagen types present (such as may very well be the case in comparing unwounded tissue with healing tissue, where the latter may be relatively rich in type III collagen [66]) and extrapolating these numbers to collagen levels may yield erroneous results. One should always bear this in mind, especially when only minor differences in hydroxyproline levels are found.

Anastomotic hydroxyproline concentrations, usually expressed on the basis of dry weight, change massively during the first post-operative period. Cronin et al. [20] were the first to report transiently lowered hydroxyproline concentrations in rats if colonic anastomoses were compared to uninjured intestine. This phenomenon was confirmed by other groups [67,68], also in the rabbit [26,69]: the hydroxyproline concentration is already sig-

nificantly reduced three hours after operation [70]. Reports on the changes observed in anastomoses of the small bowel appear less equivocal. We consistently find a similar phenomenon to occur, although to a lesser degree, in ileal anastomoses [26,29,70]. While this has also been observed by others [28,71], unchanged post-operative hydroxyproline concentrations in ileal anastomoses have been reported as well [24,72].

Changes in hydroxyproline and, by extrapolation, collagen concentrations do not necessarily represent absolute changes in the amount of collagen present in the bowel wall. A decrease in hydroxyproline concentration of an intestinal segment may indeed be caused by loss of collagen, but also by an influx of non-collagenous material or by a combination of both processes. It has been reported that the dry weight of anastomotic segments, and even of segments located immediately proximally and distally to it, can be considerably increased compared to that of equally-sized intestinal segments from unoperated animals [52,72]. This surely means that a 40 percent decrease in hydroxyproline concentration [20] does not represent degradation of an equal percentage of collagen fibrils. It remains debatable if the early decrease in hydroxyproline concentration is, at least partly, due to increased collagen lysis. The concept that post-operative collagen degradation is an integral part of the healing sequence in the intestine [73] is still widely accepted [72,74], even though conflicting quantitative data have been reported and its occurrence still needs to be demonstrated unequivocally (cf. next section). In our opinion, one should be very cautious in drawing, even qualitative, conclusions concerning this process from the post-operative course of hydroxyproline concentrations. On the other hand, the fact that anastomotic hydroxyproline concentrations start to rise again between two and four days after operation, eventually surpassing those observed in uninjured intestine, certainly must reflect an increased collagen synthesis. Here, a concomitant increase in segmental dry weight may even lead to an underestimation of the size of this effect.

Comparison of anastomotic hydroxyproline concentrations between various experimental groups has been used extensively over the last decade to study the effects of a multitude of experimental conditions and substances, such as infection [37], hypovolaemia [75], lavage [31], pectin [41], prostglandin [30], vitamin A

[56,76], aprotin [54], cytostatics [23,29] and nutrition [71]. Mostly, the position is taken that a lower anastomotic hydroxyproline concentration reflects the presence of less collagen and thus poorer healing. It follows from the foregoing discussion that this is not necessarily the case: a changed concentration may be caused by changes in non-collagenous substances and an unchanged concentration may be the result of, for instance, a loss of collagen attended by a decreased amount of non-collagenous material. One way to ascertain that differences, observed in the post-operative course of hydroxyproline concentrations between various experimental groups, are indeed attributable to a changed collagen metabolism rather than to changes in total dry weight of anastomotic segments in the groups are similar [77]. Without this type of precaution, it seems advisable to show some restraint in concluding that a certain treatment impairs or improves anastomotic repair, or anastomotic collagen metabolism. Collagen levels may also be expressed as tissue hydroxyproline content, i.e. the amount of hydroxyproline present per unit intestinal length. Changes in hydroxyproline content supposedly reflect the result of collagen degradation and synthesis more directly than changes in concentration do, since this parameter is independent of variations in other constituents of the intestinal wall. In order to describe the post-operative course of collagen content, a control value should be available which represents the collagen content at the time of operation. If the anastomotic hydroxyproline content is to be measured in an intestinal segment containing the entire anastomosis, the control cannot simply be a segment of uninjured intestine of similar length (obtained at operation from the same animal or from an unoperated control animal) since the anastomotic segment will always contain more tissue per cm than a biopsy from normal intestine. In this case, a newly constructed anastomosis should act as control. The most optimal procedure in this respect, though certainly the most laborious, is the construction of a control anastomosis, which is resected immediately after completion, followed by construction of a second anastomosis within the same animal. This way, each animal acts as its own control. Using this method, Irvin and Hunt [66] found a 26 percent decrease in the hydroxyproline content of three days old colonic anastomoses. However, other experiments where newly constructed anastomoses from control rats were used as a reference, showed a far smaller post-operative lowering of the collagen content [21,78]. Another

approach, taken by Zederfeldt's group, is to transect the anastomosis in the middle and collect the segments from either side. Using newly constructed anastomoses or unoperated intestine as controls, they never found a post-operative decrease in hydroxyproline content of the anastomotic areas but a consistent increase in hydroxyproline content within three or four days, both in ileum and colon [45,72,79]. Thus, measurements of collagen content have yielded conflicting results with regard to the quantification, or even the occurence, of the net loss of anastomotic collagen.

By far most experimentators prefer to measure collagen concentrations for the purpose of evaluating healing under various conditions. It has already be emphasized that this parameter should not be accepted as a true representation of collagen levels without due consideration. While comparison of concentrations may be allowed under certain conditions, comparison of content undoubtedly yields more direct information on the amount of collagen present at a certain time. It is therefore regrettable that quite a number of authors refer in their papers to collagen -or hydroxyproline- content of anastomoses while they only measure concentration. Clearly, both parameters yield different information and therefore both terms are not to be mixed up.

b. collagen synthesis and degradation

The anastomotic collagen content is the net result of collagen degradation and collagen syntheis and does not allow any quantitative conclusions concerning the separate processes: an elevated collagen content may be caused by a limited degradation or an increased synthesis or, for that matter, by an increased degradation coupled with an even more strongly increased synthesis. Thus, in order to draw conclusions on the course of post-operative collagen degradation and synthesis, the processes should be measured independently.
In vivo collagen synthesis in intestinal anastomoses can be quantitated after injection of radioactively labeled proline at a fixed time before sacrifice. Subsequently, the incorporation of label into hydroxyproline is determined and its specific (dpm labeled hydroxyproline per mole hydroxyproline present) or total (dpm labeled hydroxyproline per biopsy) activity is a measure for

the amount of newly synthesized collagen. This way, the occurrence of increased post-operative collagen synthesis in the anastomotic area was first demonstrated by Cronin et al. [80] in rat colon. Their results were confirmed by Jiborn et al. [81]. From two days after operation onwards, collagen synthesis in and around colonic anastomoses was increased compared to the collagen synthesis measured in the colon of unoperated control animals [82]. The same phenomenon occurs around anastomoses in the small intestine [83]. The course of anastomotic collagen synthesis may thus be followed and compared under various conditions thought to affect anastomotic repair. So far, application of this technique in the research on intestinal healing has been limited, possibly because it requires administration of large amounts of (expensive) labeled proline to living animals. This is one of the reasons why we have recently developed a system to measure collagen synthesis in short-term intestinal explants [84], which system requires only a fraction of the radioactive proline necessary for in vivo experiments. Here, collagen synthesis is quantitated by measuring incorporation of radioactive proline into collagenase-indigestible material. Synthesis in explants from anastomoses is significantly increased, with respect to the uninjured intestine, already 12 hours after operation. The post-operative course of collagen synthesis, measured in vitro around ileal and colonic anastomoses, appears very similar to that demonstrated after in vivo labeling [85]. We hope that this system will prove to be a useful and economic tool to study the influence of various conditions or compounds, which are thought to have a deleterious effect on anastomotic healing and increase the changes for anastomotic dehiscence.

While the existence of anastomotic collagen synthesis, both demonstrated by direct measurements and inferred from the development of post-operative collagen content, has been established unequivocally, there still remains some doubt about the occurrence, and certainly the magnitude, of anastomotic collagen degradation. The current view helds that a certain amount of resorption of the extracellular matrix is an integral part of tissue repair in general [5,11]. Certainly, inflammatory cells have the potential to generate enzymes which are capable of inducing collagen breakdown. Hawley et al. [69] has found an increased collagenolysis by explants from rabbit colonic anastomoses. Recently, a specific collagenase has been identified immuno-histochemically

in colonic anastomoses within 12 hours after operation [86] and we have found a transiently increased collagenolytic activity in extracts from ileal and colonic anastomoses [87]. Therefore, little doubt exists about the presence of collagenolytic capacity in the anastomotic area during the early post-operative period. However, the question as to what extent this increased potential is used, is still not answered fully. It has already been mentioned in the preceding section that conflicting data have been reported concerning the post-operative hydroxyproline content: some authors never find a decrease in hydroxyproline mass [45,72,79] while others report a net loss of hydroxyproline [67]. Another way of demonstrating collagen loss is by prelabeling intestinal collagen, preferably by repeated dosage of radioisotope from early life, and following anastomotic radioactivity. This way, a significant loss of labeled hydroxyproline from a salt- and acid-insoluble fraction, presumably representing mature collagen, has been reported in three days old colonic anastomoses in the rat [67]. However, the quantitative interpretation of such data on collagen turnover is particularly difficult because of the complex pathways of collagen degradation in vivo [88]. Although the attitude prevails that collagen degradation occurs in the early healing phase around intestinal anastomoses, this process may be too localized to detect with relatively simple procedures. This may explain why the separate process of collagen degradation, in contrast with the process of collagen synthesis, remains mostly unattended in studies on anastomotic repair. Since it appears quite possible that an enhanced collagen degradation might contribute to the development of anastomotic dehiscence in high risk situations like the presence of infection, more effort in quantitating this side of collagen metabolism is certainly warranted.

c. the quality of collagen

The concept that collagen fibrils are ultimately responsible for anastomotic strength has led to many investigations where the amount of hydroxyproline present or formed is taken as an index of healing: the presence of lowered hydroxyproline levels or decreased synthesis is thought to reflect poorer repair. While the hydroxyproline content alone may be considered as a parameter for repair, it certainly is only a relatively crude one. It is

not only the collagen mass which determines strength but also, or possibly even more so, the quality of the fibrils. In this respect, the collagen types present and the stability of the constituting crosslinks are of importance for the strength of the collagenous network.

The tensile strength and mechanical stability of the collagen fibril is based largely on the information of intermolecular crosslinks between the constituent molecules. Various pathways of crosslinking can be defined. Also, the structures mature with ageing, the number of reducible crosslinks decreasing with increasing age [89]. Considerable changes have been shown to occur in the crosslinking of granulation tissue [90], which supposedly are reflected in tensile strength. It is to be expected that collagen crosslinking in the healing anastomosis also changes with time and it is conceivable that maturation of the crosslinking could be delayed under certain conditions, thus leading to protracted periods of diminished strength. As yet, no data are available on the structure of collagen crosslinks in intestinal anastomoses.

One way to investigate the degree of collagen crosslinking is by means of its solubility. Neutral salt solutions will solubilize only the least crosslinked (newly formed) molecules present, while dilute acid extracts the more crosslinked collagen molecules. Neither of these solvents would be expected to solubilize highly crosslinked (mature) collagen. The solubility of hydroxyproline in the intestinal wall is very low. Salt- and acid-soluble hydroxyproline constitutes only 0.4 to 4 percent, respectively, of total tissue hydroxyproline from rat intestine [67,81] which impairs the measurement of post-operative changes. Solubility may be increased by sonication of the tissue and changing solubilities of hydroxyproline have indeed been demonstrated in experimental intestinal anastomoses [91]. Possibly, measurements of hydroxyproline solubility, next to hydroxyproline content, would yield a valuable parameter for the assessment of anastomotic healing.

The strength of collagenous tissue may also depend on the types of collagen present. For instance, the major collagen types in soft tissues, type I and type III, form fibrils with, respectively, large and small diameter [92]. In addition to the fact that fibril diameter plays a major part in determining its mechanical properties [93], it has also been suggested that collagen in small fibrils shows a higher turnover rate than collagen in large

fibrils [94]. Thus a changing type distribution will certainly affect tensile strength. Healing tissue shows changing patterns of collagen types: type III transiently becomes more abundant than in uninjured tissue [66,90] and an important role has also been suggested for type V [95]. While it is known that the intestinal wall contains the collagen types I, III and V [96,97], nothing has been reported about its composition after resection and anastomosis. This knowledge might be valuable in the search for disorders on the molecular level which may explain impaired anastomotic healing.

Concluding remarks

The progress of anastomotic repair may be followed by examination of mechanical and/or biochemical parameters. Both types of measurement show post-operative changes which are consistent with current views on the wound healing sequence.
The concept that the collagenous equilibrium, i.e. the balance between collagen synthesis and lysis, is critical to anastomotic repair [73], is widely accepted. A logical extension of this thought would be that a direct correlation must exist between the development of anastomotic strength and a parameter which represents "collagenous strength". It still is an open question which feature of collagen metabolism fits best in this respect. No correlations have been found between collagen concentrations and mechanical parameters. Breaking strength, but not bursting strength, data parallel collagen content "to a certain extent" [43], but it has never been shown that, within one series of experimental animals, anastomotic strength correlates with any measurement of collagen metabolism. This is quite possibly a result of the fact that research has been focussed on collagen mass rather than collagen quality.
Assuming that collagen indeed is responsible for anastomotic strength, the question arises if impairment of normal post-operative collagen metabolism may lead to decreased strength and, subsequently, to anastomotic complications. Irvin and Hunt [39] have shown that in traumatized rats the collagen content was significantly higher in intact colonic anastomoses than in anastomoses exhibiting dehiscence. However, this observation is rather unique and similar data on anastomoses, constructed under conditions where increased leakage might be expected, still have to be

reported. In this context it is noteworthy that recent studies on the healing of colon anastomoses after long-term bowel rest [98] or proximal colostomy [46], have shown retarded gain of anastomotic strength together with diminished accumulation of collagen without an increase in the complication rate. This should act as a warning that some restraint must be exercised in extrapolating the results of, relatively crude, mechanical and biochemical measurements to the progress of the intricate healing process.

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THE INFLUENCE OF NSAIDs ON EXPERIMENTAL INTESTINAL ANASTOMOSES

W.J.B. Mastboom, T. Hendriks, P. van Elteren, H.H.M. de Boer.

Department of General Surgery, St. Radboud University Hospital, Nijmegen, Department of Statistical Support, University of Nijmegen, The Netherlands.

Summary

Limiting degradation of collagen during the initial phase of wound healing is expected to improve post-operative intestinal strength and thereby decrease chances for anastomotic dehiscence. We studied the influence of four non-steroid anti-inflammatory drugs on the healing of intestinal anastomoses in rats, with special regard to changes of collagen levels around the anastomoses.

Four experimental groups of 20 rats each received daily oral doses of piroxicam, ibuprofen, aspirin or indomethacin and were compared to a control group. Animals were sacrificed 3 or 7 days after operation. Both morbidity and mortality rate in the experimental groups were high.

Collagen, measured as hydroxyproline, levels in anastomotic and adjoining 1 cm intestinal segments were compared to concentrations in control segments resected during operation. After an initial decrease on day 3 hydroxyproline concentrations increased on day 7. In colon the lowering in hydroxyproline concentrations, which was more pronounced than in ileum, was significantly reduced by administration of piroxicam and ibuprofen, both in the anastomosis and its proximal segment. On day 7, the increase of hydroxyproline concentrations in the ileum was inhibited by administration of the anti-inflammatory drugs.

It is concluded that nonsteroidal anti-inflammatory drugs may limit post-operative degradation of collagen in colonic anastomoses, but at the same time may increase the rat's susceptibility to surgical infections.

Introduction

The inflammatory reaction is an integral part of the wound healing response. However, factors which stimulate inflammation result in delayed restoration of wound strength: minimizing inflammation will result in more rapid healing [1].
The bowel wall derives its integrity and mechanical strength largely from collagen, which structural protein is concentrated mainly in its submucosal layer [2]. It is generally accepted that during the healing process collagen levels fluctuate as a result of degradation and synthesis near the wound area [3]. The first days after operation, the collagen concentration around the anastomosis decreases [4], reflecting a net loss of collagen. In this initial phase of wound healing, anastomotic strength depends on the suture holding capacity of the existing collagen fibres [5]. In a later phase, newly synthesized collagen gradually becomes of major importance in restoring pre-operative strength [6]. Limitation of collagenolysis may benefit intestinal strength during the first post-operative period.
Inflammatory cells, like polymorphonuclear leucocytes and macrophages, comprise important sources of collagenolytic enzymes [7,8]. The effects of anti-inflammatory agents on bowel healing have hardly been studied [1]. In this respect, nonsteroidal anti-inflammatory drugs (NSAIDs) may be of particular interest since they seem able, next to their common capacity to inhibit prostaglandin synthesis, to reduce cell functions e.g. migration and enzyme release from polymorphonuclear leucocytes and macrophages [9,10].
Therefore, we have studied the effects of various NSAIDs on intestinal anastomotic healing in the rat. Here, we report on complications, weight loss and the course of collagen changes in the first post-operative week.

Materials and Methods

a. animals

One hundred male Wistar rats weighing between 200 and 270 g were divided into five groups of 20 rats each. One group served as control, the others receiving one species of a NSAID. NSAIDs used were piroxicam (Pir-group), ibuprofen (Bru-group), acetylsalicylic acid (Asp-group) and indomethacin (Ind-group). The ani-

mals were fed a standard diet (Hope Farms, Woerden, The Netherlands) and allowed water ad libitum.
Within each group 10 rats were scheduled to be killed on day 3 and day 7 after operation, respectively. Weights of all rats were measured daily.

b. NSAIDs

The doses of the NSAIDs used in this study were chosen from the literature as being effective in suppressing inflammatory reactions in rats [11-14]. All drugs were administred orally, by means of a stomach-tube, twice a day in two equal portions of 1 (piroxicam) or 0.5 (others) ml. Administration was started 2 days before operation and continued till the day of sacrifiction. Piroxicam (Pfizer) was given in a dose of 2 mg/kg/day, dissolved in dilute NaOH and neutralized; ibuprofen (Boots Company) in a dose of 50 mg/kg/day, dissolved in 0.25 M $NaHCO_3$; aspirin (Bayer) in a dose of 200 mg/kg/day, dissolved in 0.25 M $NaHCO_3$; indomethacin (Merck Sharp & Dohme) in a dose of 2.5 mg/kg/day, dissolved in 0.1 M $NaHCO_3$.
A pilot study had shown these doses not to cause either reduction in weight-gain or mortality in unoperated rats, receiving the drugs for 15 days.

c. operative procedure

Operations were performed as described before [15]. Briefly, after induction of anaesthesia with sodium pentobarbital (0.1 ml/ 100g) and a median laparotomy, one cm of both ileum and colon were resected at 15 cm proximal to the ileocaecal junction and 3 cm proximal to the rectal-peritoneal reflection, respectively. An inverting one-layer end-to-end anastomosis was constructed microsurgically with 8x0 monofilament suture-material (Ethicon®). After 3 or 7 days the rats were killed by an intracardiac overdose of sodium pentobarbital. Both anastomoses were dissected and cleaned from surrounding tissue. Three one cm-samples were collected, one containing the anastomosis in the middle and the other comprising the adjacent proximal and distal segments. These post-operative samples were compared with the control segment, removed during operation. This way each animal served as its own control.
All samples were frozen immediately and stored in liquid nitrogen until further processing.
Blood samples taken from four rats of each group showed an equal

decrease of the haemoglobuline concentration and haematocrite of approximately 22% on the first post-operative day.

d. analytical procedures

The samples were pulverized in liquid nitrogen, lyophilized and kept at -30 degrees Celsius for analysis.
The dry-weights of control segments and the samples containing the anastomosis were determined.
In all samples the hydroxyproline concentration, as a measure for the collagen level, was measured as described previously, essentially according to Prockop and Udenfriend [16].
Statistical methods employed are mentioned with the results.

Results

The NSAID groups suffered from a substantial mortality (Table I) and morbidity (Table II). Two rats died immediately post-operatively, 10 others died between the second and the fifth post-operative day because of peritonitis without macroscopic evidence of anastomotic leakage into the abdominal cavity. They were excluded from further study. It appears that the complication rate was considerably higher in the NSAID groups, with the possible exception of Piroxicam, than in the control animals.
However, conclusions should be drawn with some reserve because of the fact that half of the animals in each group were killed after three days: the possibility cannot be excluded that some would have died spontaneously or developed infectious complica-

Table I. Mortality in experimental groups

group	n	overall mortality	post-operative day of death
Control	20	0	
Piroxicam	20	0	
Brufen	20	3	0, 2, 2
Aspirin	20	4	2, 3, 3, 3
Indomethacin	20	5	0, 2, 2, 4, 5

Table II. Infectious complications in experimental groups (*animals which died immediately after operation excluded*)

group	n	fascial abcess	anastom. abcess	generalized peritonitis	total
Control	20		3		3
Piroxicam	20		3	3	6
Brufen	19	2		4	6
Aspirin	20	5	1	7	13**
Indomethacin	19	2	3	7	12**

** = *Significant difference between experimental and control group (chi-squared test): $0.001<p\leq0.01$.*

tions between three and seven days if they would have been kept alive. Still, taking all groups together and considering only the mortality within three days, a nearly significant ($p=0.07$, chi-squared test) overall difference in mortality existed. Infectious complications varied from 15% in the C-group up to 65% in the NSAID group treated with aspirin and occurred significantly more often in Asp- and Ind-groups than in the C-group. Peritoni-

Table III. Loss of body weight during the first 3 days after operation
Results are expressed as mean value (g) ± SD.

group	n	pre-operative body weight	weight loss after 3 days
Control	20	242 ± 35	20.2 ± 9.6
Piroxicam	20	231 ± 26	20.2 ± 10.6
Brufen	17	246 ± 14	17.9 ± 6.3
Aspirin	16	226 ± 20	9.6 ± 7.4
Indomethacin	15	232 ± 36	17.3 ± 10.8

A highly significant difference exists between the groups in weight loss after 3 days ($p=0.007$, Kruskal-Wallis k sample test). The value for aspirin is a highly significant outlier ($p<0.0015$, Doornbos-Prins distribution free slippage test [17]).

tis without macroscopic anastomotic leakage was seen in each of the NSAID groups but never in control animals.
Following resection, all rats lost weight. Weight loss in all rats was measured on day 3 (Table III) and was in the Asp-group significantly lower than in the other groups.
For the rats which were sacrificed after seven days both maximal weight loss and the day of maximal weight loss are also given (Table IV). Maximal weight loss occurred significantly earlier in the Asp-group than in the C- and Pir-group. Also, weight loss was significantly more severe in the Pir-group than in the Asp- and Bru-group.

Table IV. Maximal loss of body weight in the first post-operative week
Results are expressed as average value ± SD.

group	n	maximal weight loss	day of max. weight loss
Control	7	23.4 ± 8.1 g	3.1 ± 1.7●
Piroxicam	10	35.4 ± 16.3 g	3.3 ± 1.9●
Brufen	9	18.9 ± 4.0 g*	1.6 ± 0.7
Aspirin	6	15.0 ± 4.7 g**	1.0 ± 0
Indomethacin	7	27.9 ± 18.4 g	2.9 ± 2.2

There is a significant difference between the groups, both in maximal weight loss (p=0.015) and in the day of maximal weight loss (p=0.011, Kruskal-Wallis k sample test).
*Significant differences (simultaneous test based on Wilcoxon's two sample test and Bonferroni's inequality) in maximal weight loss are found between the Pir-group and both the Bru- and Asp-group (*). Likewise, the day of maximal weight loss in the C-group and the Pir-group is different from that in the Asp-group (●): *, ● = 0.01<p≤0.05; **= 0.001<p≤0.01.*

The hydroxyproline concentrations of ileal and colonic control segments, resected before anastomotic construction, are shown in Table V. Obviously, there existed some differences between hydroxyproline concentrations in the various groups, since significant differences were found between averages using analysis of variance. However, these differences are of limited importance since we calculated within each rat the changes in hydroxyproline

Table V. Hydroxyproline concentrations in control-segments
Results are expressed as mean value (µg/mg dry weight) ± SD.

	day	n	ileum	colon
Control-group	3	10	7.5 ± 1.9	16.2 ± 3.9
	7	10	7.2 ± 1.4	15.4 ± 1.8
Pir-group	3	10	7.7 ± 1.7	12.7 ± 1.4
	7	10	8.8 ± 1.1	13.8 ± 1.5
Bru-group	3	8	8.8 ± 1.4	14.7 ± 2.0
	7	9	9.1 ± 1.2	15.9 ± 2.0
Asp-group	3	10	8.7 ± 1.5	14.1 ± 1.4
	7	6	8.5 ± 0.4	15.0 ± 1.4
Ind-group	3	8	8.6 ± 1.2	14.3 ± 2.3
	7	7	8.6 ± 1.0	14.2 ± 1.1

The differences between average values for rats killed at day 3 and day 7 tested within each group are not significant (Student's two sample t-test). Testing the differences between groups with one-way analysis of variance, the result is significant for ileum day 7 ($p=0.007$) and colon day 3 ($p=0.04$), and nearly significant for colon day 7 ($p=0.053$).

concentration between the control segment, removed at operation, and the segments obtained at sacrifice.

Resection of two bowel segments led to extensive changes of hydroxyproline concentrations around both ileal (Figure 1) and colonic (Figure 2) anastomoses in all groups. In the C-group, a significant decrease in the ileal anastomotic hydroxyproline concentration of approximately 20% was observed at day 3. Seven days after operation, the hydroxyproline concentration in this group was increased to a level significantly above that measured in the per-operative control segments. Hydroxyproline levels in the proximal segment followed a similar course, while in the distal segment they remained unchanged at day 3. In the colon of the C-group, lowering of the hydroxyproline concentration in anastomotic and proximal segments three days after operation was more pronounced than in ileum, while at 7 days levels still

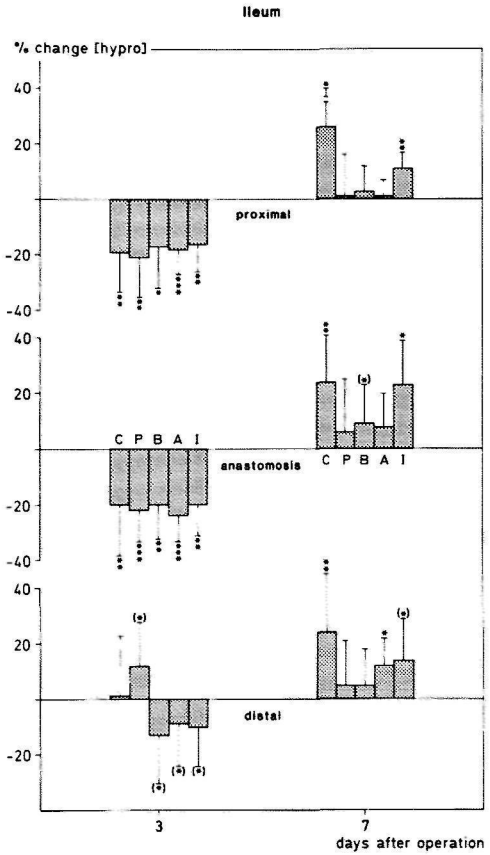

Figure 1.

*Histogram showing relative changes in hydroxyproline concentrations around ileal anastomoses. Results are expressed as average mean percentual change, calculated with respect to the pre-operative value, with standard deviation. Levels of significance for the difference between post-operative and control segments are calculated by means of a paired sample two-sided Student t-test and represented in the following way: (*)= 0.05<p≤0.1; *= 0.01<p≤0.05; **= 0.001<p≤0.01; ***= p≤0.001.*

C = C-group; P = Pir-group; B = Bru-group; A = Asp-group; I = Ind-group.

remained significantly below those measured in control segments. The changes in post-operative hydroxyproline concentrations were clearly affected by administration of NSAIDs. According to an analysis of variance there existed the following significant group effects on the post-operative changes in hydroxyproline

concentrations (Table VI): in the ileum at 3 days for the distal segments and at 7 days for the proximal segments, and in colon at 3 days for both proximal and anastomotic segments and at 7 days for the proximal segments. Further analysis of these differences was performed using a Tukey test (Table VII).

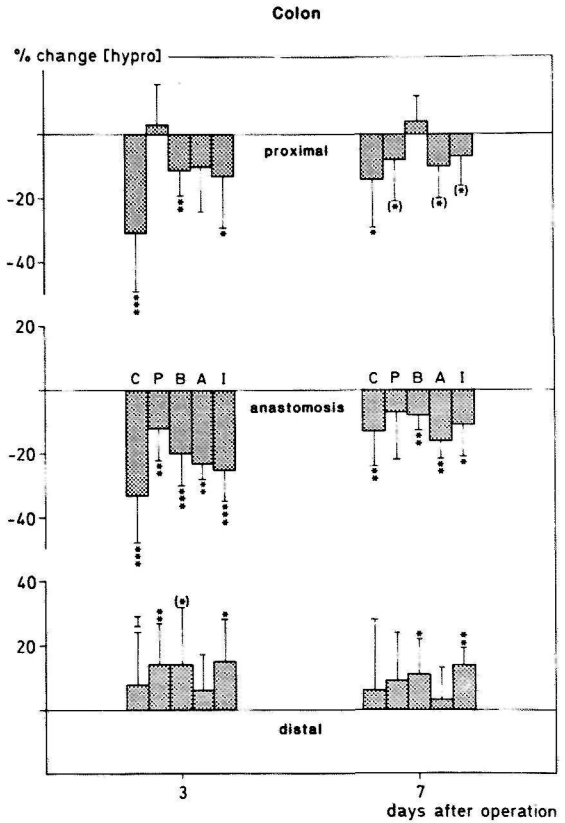

Figure 2.

*Histogram showing relative changes in hydroxyproline concentrations around colonic anastomoses. Results are expressed as average mean percentual change, calculated with respect to the pre-operative value, with standard deviation. Levels of significance for the difference between post-operative and control segments are calculated by means of a paired sample two-sided Student t-test and represented in the following way: (*)= 0.05<p≤0.1; *= 0.01<p≤0.05; **= 0.001<p≤0.01; ***= p≤0.001.*

C = C-group; P = Pir-group; B = Bru-group; A = Asp-group; I = Ind-group.

Table VI. Analysis of variance, using all five groups, to detect group effects in the post-operative changes in the hydroxyproline concentrations

p-value:	Ileum		Colon	
	3 days	7 days	3 days	7 days
proximal segment	0.93	0.0104	<0.0001	0.04
anastomosis	0.93	0.06	0.0011	0.46
distal segment	0.03	0.054	0.80	0.65

Table VII. Significant and nearly significant differences in hydroxyproline changes between various groups

	day	segment	groups compared	mean % change in [hydroxyproline]	p
Ileum	3	distal	Bru vs Pir	-13 vs 12	*
			Ind vs Pir	-10 vs 12	(*)
	7	proximal	Pir vs C	1 vs 26	*
			Asp vs C	1 vs 26	*
			Bru vs C	3 vs 26	(*)
Colon	3	proximal	C vs Pir	-31 vs 5	**
			C vs Asp	-31 vs -5	**
			C vs Bru	-31 vs -11	*
			Ind vs Pir	-14 vs 5	(*)
		anastomosis	C vs Pir	-33 vs -12	**
			C vs Bru	-33 vs -20	(*)
			Ind vs Pir	-25 vs -12	(*)
	7	proximal	C vs Bru	-14 vs 4	(*)

Significance of the differences (p) between groups is tested by means of a simultaneous Tukey-test comparing groups two by two:
*(*)= 0.05<p≤0.1; *= 0.01<p≤0.05; **= 0.001<p≤0.01.*

In ileum, no significant differences between the C-group and the experimental groups were observed in the anastomotic segments. The group effect 3 days after operation in the distal segment was mainly due to a concentration increase in the Pir-group. Seven days after operation, various NSAIDs, in particular piroxicam and ibuprofen, appeared to limit the rise in hydroxyproline concentration observed in the C-group. In the colon effects of NSAIDs were more pronounced in the proximal and anastomotic segments. At 3 days, all NSAIDs showed a reduction in the lowering of proximal hydroxyproline concentrations if compared to the C-group. A similar significance was measured in the anastomosis for piroxicam and, almost, for ibuprofen. At 7 days, no differences were found, except for the proximal segment where the Bru-group already showed an increase in hydroxyproline concentration, while levels in the other groups still remained below those in uninjured intestine.
The preceding data on hydroxyproline all refer to concentrations, i.e. μg hydroxyproline per mg dry weight. It could be argued that differences between groups are caused by different changes in biopsy weights rather than by changes in the actual amount of hydroxyproline. In order to exclude this possibility it should be demonstrated that changes in biopsy weight in the groups were similar. To this extent, we have compared the weight ratio's between post-operative (anastomotic and proximal) segments and per-operative control segments within each experimental group. No significant differences in weight increase were observed between the C-group and the NSAID-groups. Thus, the differences observed in the ratio of anastomotic and control hydroxyproline concentrations may indeed be attributed to changes in hydroxyproline levels.

Discussion

The effect of anti-inflammatory agents on the healing of intestinal anastomotic healing has hardly been studied [1]. In general, little is known about the effects of NSAIDs on wound healing. Some data are available on indomethacin effects on the healing of skin wounds [18,19] and bone repair [20,21] where this compound is reported to be either inhibitory or without effect. Both aspirin and ibuprofen were found to exert a negative influence [19].

In our study, several differences between control and NSAID treated rats were found after construction of two intestinal anastomoses.
Anastomotic construction led to a high morbidity and a considerable mortality in the NSAID treated animals. A striking finding in the medically treated rats was that in all groups generalized peritonitis was found at autopsy or after sacrifice without evidence of free anastomotic leakage. This might be explained by the fact that per-operative spilling of bowel contents, which is harmless to normal (control) rats, would be harmful to experimental rats as result of suppression of their immune system by the administration of NSAIDs [10,22].
The post-operative metabolism of collagen is thought to be crucial for intestinal anastomotic strength. The intestinal wall derives its strength from the submucosa which is almost entirely composed of collagen [2]. The transient post-operative decrease in hydroxyproline concentrations which occur during the first week after operation, both in colon [23,24] and, to a lesser extent, in ileum [4,25] is thought to be the result of the collagenous equilibrium [3]: immediately after operation collagenolysis occurs followed by de novo collagen synthesis. During the initial phase anastomotic strength depends on the suture holding ability of the existing collagen fibres [5]. Thereafter, newly formed collagen must restore continuity and strength to the bowel wall. Thus, it stands to reason that limitation of the post-operative reduction in collagen concentration would be beneficial to anastomotic strength.
One way to accomplish this would be by limiting collagenolysis. Both granulocytes and monocytes, which are present in the anastomotic area within 3 and 24 hours after operation [4], respectively, are powerful sources of collagenolytic activity. Since NSAIDs are able to reduce migration and secretory capacity of these inflammatory cells [9,10], and more particularly their collagenase production [26,27], such compounds could possibly reduce post-operative loss of collagen. The present study shows that administration of piroxicam and ibuprofen indeed results in a significant reduction of the lowering of hydroxyproline concentrations in colonic anastomoses and their proximal segments, as measured three days after operation (Table VII). No such effect was found around the ileal anastomoses. This difference between ileum and colon remains as yet unexplained, although it should be noted that reduction of hydroxyproline concentrations,

and presumably collagenolysis, in the control group is clearly less in ileum than in colon. Recently, flurbiprofen was also reported to increase hydroxyproline concentrations in rat colonic anastomoses [28]. Also, ibuprofen administration resulted in significantly increased levels of hydroxyproline during the early healing phase of experimental myocardial infarction in the rat, which phenomenon was interpreted to have been caused by retardation of collagenolysis [29]. Thus, it is our contention that some NSAIDs indeed are usefull in limiting early post-operative degradation of collagen.

Seven days after operation hydroxyproline concentrations have risen again, as a result of collagen synthesis. This effect is far more pronounced in ileum than in colon. At 7 days, NSAIDs affect hydroxyproline levels in ileum more than in colon. Generally speaking, they limit the increase in hydroxyproline concentration, presumably as a result of inhibition of collagen synthesis. Such an effect may easily be explained by the presumed inhibition of macrophage migration and function. These cells play an important role in fibroplasia, inducing proliferation of fibroblasts, the major collagen-producing cells. A delayed collagen synthesis could be detrimental to anastomotic strength, while an early limitation of collagen degradation might be beneficial. In this respect it would be interesting to examine the effects of administration of NSAIDs only during the first two post-operative days. Such short-time use might also reduce the rate of infectious complications.

At this stage, a comparison of the efficacy of the various NSAIDs can only be tentative: one dose of each was used, chosen on the basis of its effectiveness in suppressing inflammatory reactions in rats. Still, it is noteworthy that piroxicam appears the most efficient in inhibiting early post-operative collagen degradation, at the same time being the least detrimental in terms of morbidity and mortality.

In summary, it can be stated that NSAIDs profoundly affect the collagen meatbolism around intestinal anastomoses. However, administration according to the present regimen, i.e. from two days before operation onwards, appears to increase susceptibility to surgical infections and to result in a high morbidity and mortality rate.

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EFFECTS OF NSAIDs ON THE HEALING OF EXPERIMENTAL BOWEL ANASTOMOSES: A PRELIMINARY HISTOLOGICAL STUDY

W.J.B Mastboom, P.H.M. Schillings, Th. Hendriks, H.H.M. de Boer.

Department of General Surgery and Pathology, St. Radboud University Hospital, Nijmegen, The Netherlands.

Summary

This chapter describes some histological observations on healing ileal and colonic anastomoses in rats. The study is largely descriptive: the size of the groups and the number of specimen per time point do not allow unequivocal conclusions about differences per group. Four experimental groups of 24 animals each received daily oral doses of piroxicam, ibuprofen, aspirin and indomethacin and were compared to a control group. The rats were sacrificed 3, 12 and 24 hours or 2, 3 and 7 days after operation.
The most salient findings are that both macrophages and fibroblasts are observed earlier in colon than in ileum in four of the five groups. Furthermore, in ileal slides the appearance of polymorphonuclear granulocytes seems to be retarded in the four NSAID groups, while the number of fibroblasts is initially lower both in ileal and colonic samples.
The results emphasize that semiquantitative microscopic studies only can yield a general impression of the inflammatory process on tissue level. For more detailed quantification of certain cell types special staining techniques should be used.

Introduction

In the preceding chapter the effects of a number of nonsteroidal anti-inflammatory drugs (NSAIDs) on post-operative changes of collagen concentrations in the anastomotic area have been reported. The importance of collagen for intestinal, and anastomotic

strength has also been pointed out. The main reason for investigating the effects of NSAID administration was that inflammatory cells may participate in the degradation of collagen and thus are instrumental in weakening anastomotic strength. NSAIDs reportedly suppress inflammatory reactions in rats [1-4] and we found that they indeed limited post-operative lowering of anastomotic collagen concentrations (cf. chapters III and V)[5].
Most studies on the healing of intestinal anastomoses comprise measurements of mechanical and, or biochemical parameters (cf. chapter II). Histology is often used to emphasize a particular point or to illustrate repair, but mostly in a limited way. Studies in which results of repair are specified primarily in a histological fashion remain relatively rare [6-9] and comprehensive histological descriptions of the repair sequence are even more scarce [10,11]. In view of this paucity of histological information available we decided to extend this line of research and collect some preliminary morphological data. The aim of this part of study was to describe the sequential behaviour of several histological parameters, as measured by semiquantitative methods, to demonstrate potential differences between ileal and colonic anastomoses and to examine if NSAID effects could also be demonstrated by these techniques.

Materials and Methods

a. animals

One hundred thirty-two male Wistar rats weighing between 200 and 270 g were divided into four experimental groups of 24 rats each and a control group (C-group) of 36 rats. Animals in the experimental groups received either piroxicam (Pi-group), ibuprofen (Bru-group), acetylsalicylic acid (Asp-group) or indomethacin (Ind-group). The animals were fed a standard diet (Hope Farms, Woerden, The Netherlands) and allowed water ad libitum.
Within each group 4 rats were killed at 3, 12 or 24 hours or 2, 3 or 7 days after operation, respectively. In the C-group an additional 6 rats were killed at days 3 and 7.

b. NSAIDs

The doses of the NSAIDs used in this study were chosen from the literature as being effective in suppressing inflammatory reactions in rats [1-4]. All drugs were administred orally, by means

of a stomach-tube, twice a day in two equal portions of 1 (piroxicam) or 0.5 (others) ml. Administration was started 2 days before operation and continued till the day of sacrifice. Piroxicam (Pfizer) was given in a dose of 2 mg/kg/day, dissolved in dilute NaOH and neutralized; ibuprofen (Boots Company) in a dose of 50 mg/kg/day, dissolved in 0.25 M $NaHCO_3$; aspirin (Bayer) in a dose of 200 mg/kg/day, dissolved in 0.25 M $NaHCO_3$; indomethacin (Merck Sharp & Dohme) in a dose of 2.5 mg/kg/day, dissolved in 0.1 M $NaHCO_3$.
A pilot study had shown these doses, if administered for 15 subsequent days, not to cause either reduction in weight-gain or mortality in unoperated rats.

c. operative procedure
Surgical procedures have been described elsewhere [12].
Animals were killed by an intra-cardiac overdose of sodium pentobarbital. Both ileal and colonic anastomoses were prepared free, leaving surrounding tissue adherent to the anastomoses untouched, and removed.

d. histological processing
Anastomotic segments, collected at sacrifice, were cut longitudinally at the mesenterial border, extended and fixed on paper and preserved in 4% (w/v) formaldehyde. Then, standard samples obtained from the anti-mesenterial side were dehydrated with aceton, methyl-benzoate and toluene and embedded in paraffin. Sections, 6μ thick, were cut and stained with hematoxylin-eosin.
Two slides of each sample were examined by one pathologist, who was unaware of their origin. The following histological parameters were observed and recorded semiquantitatively: the aspect of approximation of the wound edges, the degree of necrosis, mucosal repair, the presence of inflammatory cells like polymorphonuclear granulocytes and macrophages, proliferation of fibroblasts and formation of new capillaries.

Results

Altogether, six rats died prematurely: 4 died immediately after operation, 2 in the Pi-group (day 2 and day 7) and 2 in the Asp-group (both day 2); the other 2, both from the Pi-group (day 2 and day 7), died from pneumonia and peritonitis, respectively.

Five times ileal anastomoses were accidentally disrupted during dissection. These samples (3 in the C-group, day 3; one in the Asp-group, day 1 and one in the Ind-group, day 3) were excluded from further analysis. Preparation of colonic segments always succeeded without anastomotic disruption. The number of samples within each group remaining available for histological examination is given in Table I.

Table I. Number of histological samples available for analysis
In each group, numbers are given for both ileum (I) and Colon (C).

time after operation	group									
	Control		Piroxicam		Aspirin		Brufen		Indocid	
	I	C	I	C	I	C	I	C	I	C
3h	4	4	4	4	4	4	4	4	4	4
12h	4	4	4	4	4	4	4	4	4	4
24h	3	4	4	4	3	4	4	4	4	4
2d	4	4	2	2	2	2	4	4	4	4
3d	7	10	4	4	4	4	4	4	3	4
7d	10	10	2	2	4	4	4	4	4	4

For every group, the average results of each histological parameter observed at the different time-points are shown in Tables II and III. Parameters of the C- and NSAID-groups, as examined between three hours and seven days after construction of both intestinal anastomoses, are presented in table II. In the ileal anastomoses of the C-rats polymorphonuclear granulocytes were already abundantly present in the anastomotic area three hours after operation. These cells were still found after seven days. The first macrophages were seen at 24 hours, while fibroblasts and neovascularisation were observed from the second day onwards. Approximation of the ileal anastomotic sides yielded an inconstant picture: it seemed particularly poor in the animals which were killed two or three days post-operatively. Necrosis was present from the beginning and had disappeared at the seventh

Table II. Histological features in ileal and colonic anastomoses between three hours and seven days after operation

a. approximation*

	ileum					colon				
	C	Pi	Asp	Bru	Ind	C	Pi	Asp	Bru	Ind
3h	+	+	+	+	±	±	+	+	+	+
12h	±	±	+	±	+	+	±	+	+	+
24h	+	±	±	±	+	±	+	+	+	+
2d	-	±	+	±	±	±	+	+	±	+
3d	-	±	-	±	±	+	+	+	+	±
7d	±	+	+	+	±	+	+	+	+	+

b. regeneration of mucosa**

	ileum					colon				
	C	Pi	Asp	Bru	Ind	C	Pi	Asp	Bru	Ind
3h	-	-	-	-	-	-	-	-	-	-
12h	-	-	-	-	-	-	-	-	-	-
24h	-	-	-	-	-	-	-	-	-	±
2d	-	-	-	-	-	-	-	-	-	-
3d	-	-	-	±	-	±	-	±	-	-
7d	±	+	±	±	±	±	±	±	±	-

c. presence of necrosis***

	ileum					colon				
	C	Pi	Asp	Bru	Ind	C	Pi	Asp	Bru	Ind
3d	+	+	+	+	±	±	±	-	±	+
12h	+	+	+	+	±	±	+	+	±	+
24h	+	+	+	+	+	±	+	+	+	+
2d	+	+	+	+	+	±	+	±	+	+
3d	+	+	+	±	+	±	±	+	+	+
7d	-	-	-	-	±	-	-	-	-	+

d. presence of polymorphonuclear granulocytes***

	ileum					colon				
	C	Pi	Asp	Bru	Ind	C	Pi	Asp	Bru	Ind
3h	+	-	±	±	±	+	+	+	±	±
12h	+	+	+	+	+	+	+	+	+	+
24h	+	+	+	+	+	+	+	+	+	+
2d	+	+	+	+	+	+	+	+	+	+
3d	+	+	+	+	+	±	+	+	+	+
7d	±	-	±	±	±	±	+	±	±	+

e. presence of macrophages***

	ileum					colon				
	C	Pi	Asp	Bru	Ind	C	Pi	Asp	Bru	Ind
3h	-	-	-	-	-	-	-	-	-	-
12h	-	-	-	-	±	±	±	±	+	+
24h	±	+	+	±	+	+	+	+	+	+
2d	+	+	+	+	+	+	+	+	+	+
3d	+	+	+	+	+	+	+	+	+	+
7d	+	+	+	+	±	+	+	+	+	+

f. presence of fibroblasts***

	ileum					colon				
	C	Pi	Asp	Bru	Ind	C	Pi	Asp	Bru	Ind
3h	-	-	-	-	-	-	-	-	-	-
12h	-	-	-	-	-	-	-	-	-	-
24h	-	-	-	-	-	+	-	±	±	±
2d	+	±	±	±	+	+	+	+	+	±
3d	+	+	+	+	+	+	+	+	+	+
7d	+	+	+	+	+	+	+	+	+	+

g. proliferation of capillaries***

	ileum					colon				
	C	Pi	Asp	Bru	Ind	C	Pi	Asp	Bru	Ind
3h	-	-	-	-	-	-	-	-	-	-
12h	-	-	-	-	-	-	-	-	-	-
24h	-	-	-	-	-	-	-	-	-	-
2d	±	+	-	-	-	±	-	+	±	-
3d	+	±	±	±	±	±	±	+	+	+
7d	+	+	+	+	±	+	+	+	+	+

***, **, ***: the meaning of +, ± and -:**

	+	±	-
* :	fair	reasonable	poor
** :	no epithelial contact	one layer of mucosa covering the anastomotic gap	complete mucosal regeneration
*** :	abundantly present	occasionally present	absent

Table III. Histological features of 3 and 7 days old ileal and colonic anastomoses in control rats

	3 days						7 days					
	ileum			**colon**			**ileum**			**colon**		
	n = 7			n = 10			n = 10			n = 10		
	-	±	+	-	±	+	-	±	+	-	±	+
approximation	6	0	1	2	0	8	3	0	7	0	0	10
mucosa	5	2	0	5	5	0	4	2	4	1	8	1
necrosis	1	1	5	4	0	6	10	0	0	9	1	0
granulocytes	0	2	5	0	4	6	0	5	5	0	4	6
macrophages	0	0	7	0	0	10	0	0	10	0	2	8
fibroblasts	0	0	7	0	0	10	0	0	10	0	0	10
capillaries	0	1	6	0	6	4	0	0	10	0	0	10

The meaning of -, ±, and + for the various parameters is the same as described in Table II.

post-operative day. At this time-point a thin mucosal layer had bridged the luminal side of the anastomotic gap.
Generally speaking, similar features were noticed in the ileal slides of the NSAID-groups, although some differences among the various groups were apparent. Comparing C- and NSAID-groups, the number of granulocytes three hours after anastomotic construction was much smaller in all NSAID-groups and these cells were even completely absent in the Pi-group. Moreover, in this latter group disappearance of these leucocytes seemed to occur earlier than in the other groups. Macrophages were already observed at 12 hours in the Ind-animals, as opposed to the other groups here they were noticed only after 24 hour. Proliferation of capillaries was seen from day two in the C- and Pi-group and from day three in the other groups. At several time-points, tissue necrosis in samples from the Ind-group seemed less pronounced than in the other groups. However, in contrast with the other groups necrosis was still present at day 7. In all groups, reepithelialisation was observed from the beginning as a one cell thick epithelial layer growing over the anastomotic wound. In the Bru-group, this single epithelial layer had already covered the anastomotic gap three days after operation. In

Figure 1. *Twenty-four hours old colonic anastomosis of a control rat. There is only a mild inflammatory reaction and slight necrosis of the muscular layers. At the bottom adherent omental tissue covers the anastomosis.*
(haematoxylin and eosin stained - magnification 40x)

Figure 1.

Figure 2. *A properly adapted, 12 hours old, ileal anastomosis in an Asp-rat. Inflammatory cells have entered the wound area. Tissue necrosis is present on the luminal side of the inverted anastomosis.*
(haematoxylin and eosin stained - magnification 40x)

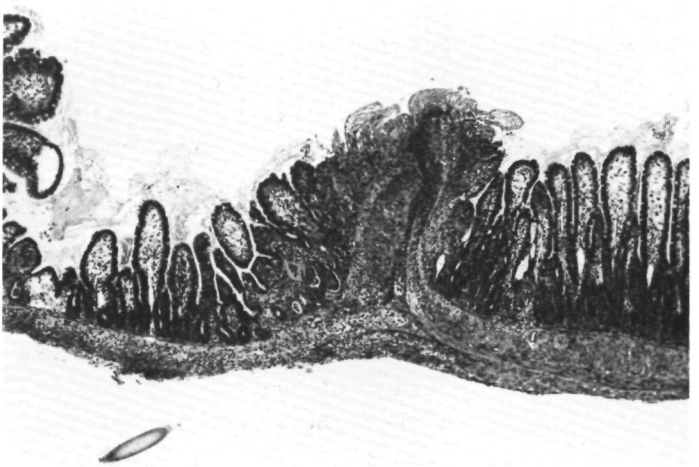

Figure 2.

Figure 3.

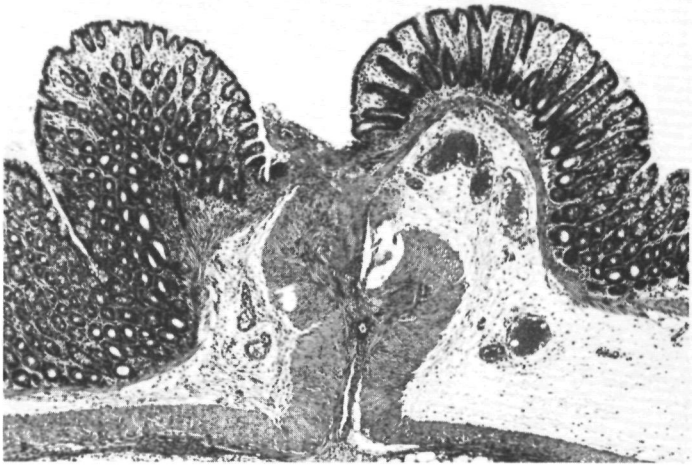

Figure 3. *A 12 hours old colonic anastomosis in a rat treated with aspirin. There is considerable edema of the submucosal layer. A mild cellular infiltrate is present. Omental fat has covered the serosal side of the anastomosis. (haematoxylin and eosin stained - magnification 40x)*

Figure 4.

Figure 4. *A 2 days old colonic anastomosis of a rat from the Asp-group. The inflammatory reaction is judged to be moderate and only little necrosis is present. Regeneration of the mucosal layer from the wound edges is clearly visible. (haematoxylin and eosin stained - magnification 40x)*

Figure 5. *An anastomotic abscess, with a large infiltrate in a 2 days old ileal anastomosis of an animal treated with indomethacin. Except for the mucosa, anatomical layers are all absent.*
(haematoxylin and eosin stained - magnification 40x)

Figure 5.

Figure 6. *Anastomotic repair with a large fibro-muscular mass between all muscular layers and the submucosa in a 7 days old ileal anastomosis of a rat from the Asp-group. An superficial mucosal layer is covering the anastomotic defect.*
(haematoxylin and eosin stained - magnification 40x)

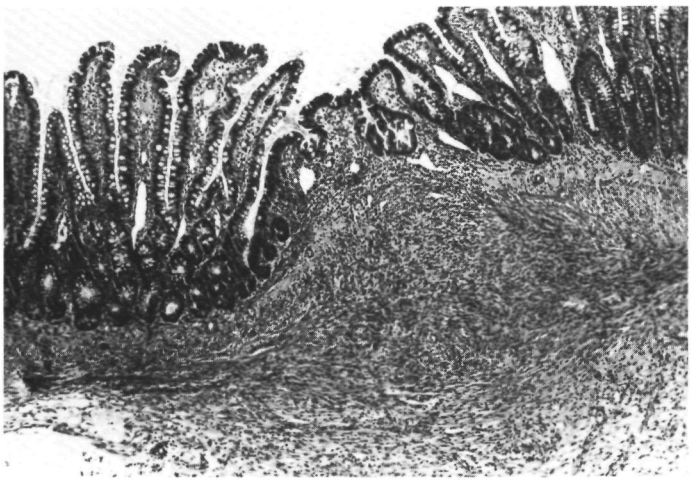

Figure 6.

the two Pi-rats a full thickness mucosal layer had been restored over the ileal anastomotic defect at day 7.
Altogether, histological features of healing colonic anastomoses in C-rats resemble those of ileal anastomoses. The first granulocytes, macrophages and fibroblasts were observed at respectively 3, 12 and 24 hours after operation. First neovascularisation was seen at day two. Necrosis, present from the beginning, had been cleared away at day seven. Complete one-layer epithelial bridging was noticed at day 3, but had not been completed at day 7. Approximation of the anastomotic ends was judged "fair" to "good".
Some differences between C- and NSAID-groups were also found in colon. The first fibroblasts appeared 24 hours after operation in all groups, except for the Pi-rats where these cells were only noticed at day 2. Both at 3 hours and at day 7, there existed gradual differences in the number of granulocytes observed. Marginal differences were also seen between the time-point at which the first macrophages, fibroblasts and capillaries appeared in the wound area.
Approximation was judged to be somewhat better in the NSAID-groups than in the C-group. Tissue necrosis was most extensive in the Ind-animals. A complete single epithelial layer covering the anastomosis is first seen at day three in C- and Asp-groups. The epithelial covering observed at 24 hours in the Ind-group must be some temporary effect, since even at day 7 no complete contact was noticed.
In Table III histological features of 3 and 7 days old anastomoses in control rats are presented. Except for the approximation and neovascularisation at day 3, no obvious differences appear to exist between anastomoses in small and large bowel.

Discussion

The inflammatory reaction is considered to be essential to initiate and regulate the sequence of events necessary for healing of wounds [13]. At the moment of -surgical- trauma, blood leaves the damaged vessels and enters into the wound area. Thrombocytes, clotting to the cut edges of blood vessels, activate thrombin, a protein which in turn activates formation of fibrin. Fibrin is a weak sealant of the wound and also stimulates chemotaxis of granulocytes. At the same time, the permeability of the intact capillaries near the wound area increases,

allowing exsudation of proteins and leucocytes. In the initial phase, polymorphonuclear granulocytes migrate into the wound area. Their main functions are phagocytosis of micro-organisms and removal of debris, but their presence has been proven not to be essential for uncomplicated healing [14]. Somewhat later monocytes migrate from the blood circulation into the wound. In contrast with polymorphonuclear leucocytes, their presence is essential to the healing sequence. Macrophages not only have a phagocytic function but are involved in the metabolism of collagen as well [15,16]. They may induce degradation of collagen fibrils by secretion of collagenolytic enzymes, while, on the other hand, they stimulate migration and collagen synthesis of fibroblasts. Early repair of collagen favours a quick restoration of intestinal strength after anastomotic construction. Also, macrophages induce proliferation of capillaries.

In general, the cellular features described above were also observed in the present study (Tables II and III), which was designed as a pilot and thus limited in size and ment only to be descriptive. The relatively small number of slides examined in each group does not allow any conclusions about the significance of the differences tentatively observed between groups.

When ileal and colonic anastomoses are compared, some events seem to occur at different times, particularly the appearance of both macrophages and fibroblasts, which was noticed to occur earlier in colon than in ileum in four of the five groups.

In contrast with histological studies on healing of intestinal anastomoses in rabbits, where all necrosis had been cleared away at day 7 in the ileum while it was still present in most samples of the colon [9], in our study necrosis was absent at that time in both anastomotic specimen of C-rats (table III). At day 7, the anastomotic gap from serosa to mucosa is often filled with a fibro-muscular plug, which observation has also been described by other authors [10,11,18]. Houdart et al. [11] postulated three forms of anastomotic healing from the seventh post-operative day: the most common way is formation of a fibro-muscular mass between the different muscular intestinal layers, the second is a musculair mass without fibrosis and the third is separate repair of all different layers, which is rarely observed.

In most of the 7 day samples, cuboidal epithelial cells will grow from the intact wound edges over the anastomotic plug. Later, this layer will regenerate to full mucosal thickness. Houdart

observed superficial epithelial contact in 27 of 40 rat colonic anastomoses at day 7 and complete mucosal repair in all at day 14. The presence of a necrotic plug seems to prevent complete epithelial contact.

A common finding in both the C- and nearly all NSAID-groups, is the generally "fair" to "poor" qualification of ileal anastomotic approximation, especially from 12 hours till 3 days. It is unlikely that this phenomenon can be ascribed to better surgery performed on animals predestined for sacrifice at 3 hours and 7 days. It is suggested that degenerating tissue adjacent to the wound interferes with the assessment of the approximation. In the microscopical slides, lysis of smooth muscles and mucosa near the wound is seen [10]. The resulting tissue gap might be interpretated as "poor" approximation, while the "good results" at day 7 are due to removal of necrosis and formation of a fibromuscular granuloma and regeneration of mucosa.

Collagen studies on intestinal anastomotic healing within one week after operation show considerable change during the first week after operation [19-22]. At the third post-operative day, reduction of the hydroxyproline concentration [a measure for the collagen level] is maximal. This effect is larger in colon than in ileum. Seven days after operation, the original hydroxyproline level has been nearly restored in colonic anastomoses, while in ileal anastomoses the pre-operative level is exceeded with circa 20%. Lysis and synthesis of collagen are thought to be, at least partly, influenced by polymorphonuclear granulocytes [23] and macrophages [15]. However, in histological slides of 3 and 7 days old anastomoses no differences between ileum and colon are observed in the distibution of inflammatory cells between ileum and colon (Table III). From day 3, both macrophages and fibroblasts are abundantly present in intestinal segments. In this respect, it is remarkable that in nearly all groups fibroblasts seem to appear earlier in colon than in ileum.

Other investigators have found that NSAIDs inhibit migration and functions of several leucocytes [1-4]. In our study, some obvious differences were observed between experimental and control animals, especially concerning the appearance of polymorphonuclear granulocytes and fibroblasts. The influence of anti-flogistics on leucocytes is observed most clearly in ileum, where 3 hours after operation the number of polymorphonuclear granulocytes seems to be much smaller in the four experimental groups than in the C-group. This effect is most striking in the Pi-

group, where at that time granulocytes are still absent. Other reports have shown that all NSAIDs used in our study, are potential inhibitors of the chemotaxis of granulocytes; among them piroxicam has proven to be the most effective [2,3].
A similar observation was made regarding the time that the first fibroblasts appeared in the wound area. Their number was much smaller in most of the experimental groups than in the C-group. So far, no direct relation of NSAIDs on chemotaxis of fibroblasts is known. Probably, the retarded presence of fibroblasts is caused by the inhibitory effect of NSAIDs on macrophages, which cells regulate fibroblast proliferation [16]. A similar explanation is proposed for the somewhat retarded proliferation of capillaries in most anastomoses in the experimental groups. The disadvantage of a qualitative histological study like the present is that, although various cells types can be differentiated, their number can only be roughly estimated and one can only speculate about their functions.
In other studies we investigated the influence of the same NSAIDs in similar doses on the changing collagen concentrations around ileal and colonic anastomoses 3 and 7 days after operation (chapter III and V)[5]. Among these NSAIDs, piroxicam was shown to be the most effective. Administration of this drug reduced the decrease of collagen concentrations in the colon 3 days after operation, but also inhibited the commonly increase observed in ileal anastomoses at day 7. We suggest these effects to be caused by inhibition of leucocytes, migration and/or function, which hypothesis appears to find support in the histological slides showing retarded appearance of these cells. A reduced number of collagenase containing leucocytes might result into a decrease of the reduction of collagen concentrations, but also in retarded repair of collagen, as is seen in ileal anastomoses at day 7, since stimulation of fibroblasts by macrophages will also be inhibited.
Despite the small number of observations in each group, some notable differences were observed between ileal and colonic anastomoses during the first week after anastomotic construction. Administration of NSAIDs resulted in some changes on the sequence and extent of the inflammatory reactions, but these findings do not fully explain the changes found in biochemical studies about anastomotic changes in collagen concentrations.

Our results emphasize the fact that semiquantitative microscopic studies only yield an impression of the inflammatory process on tissue level, allowing only tentative conclusions. For more detailed quantification of certain cell types special staining techniques should be used.

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PIROXICAM AFFECTS COLLAGEN CHANGES AROUND EXPERIMENTAL INTESTINAL ANASTOMOSES

W. J.B. Mastboom, T. Hendriks, P. van Elteren, H. H.M. de Boer.

Department of General Surgery, St. Radboud University Hospital, Nijmegen, and Department of Statistical Support, University of Nijmegen, The Netherlands.

Summary

The effects of piroxicam on post-operative changes of collagen - measured as hydroxyproline - concentrations were measured around intestinal anastomoses in rats. Piroxicam, in a dose of 2 mg/kg/day, significantly reduced the decrease of hydroxyproline concentrations around colonic anastomoses during the first three days after operation but also reduced the increase of hydroxyproline concentrations observed at day 7 around ileal anastomoses in the control group. Ten mg piroxicam/kg/day resulted in a 100% lethal peritonitis after the fifth post-operative day. We suggest that Piroxicam affects collagen metabolism by inhibiting granulocyte functions.

Introduction

The healing of intestinal anastomoses remains a subject of ongoing research interest, because of the frequency of anastomotic leakage and the resulting high morbidity and mortality.
Since the developing strength of the sutured bowel wall depends to a large extent on collagen fibrils, the post-operative collagen metabolism is crucial to anastomotic repair [1]. It is generally accepted that in the initial phase after anastomotic construction collagen degradation occurs. Since collagen degradation deteriorates the quality, and thus the suture holding capacity of the submucosal connective tissue [2], limitation of this process might improve undisturbed anastomotic healing. Inflammatory cells like polymorphonuclear leucocytes and macrophages are capable of producing proteolytic enzymes, amongst which

true collagenases [3,4], which are expected to contribute to post-operative collagen degradation. Thus, inhibition of inflammation might be beneficial in this respect. One way to modify the inflammatory response is by means of nonsteroid anti-inflammatory drugs (NSAIDs).
We have investigated the effects of piroxicam (Feldene®), a structurally novel and potent NSAID [5], on collagen changes around large and small bowel anastomoses in the rat.

Methods

a. animals

One hundred and twenty male Wistar rats, weighing between 180 and 300 g were divided into three groups of 40 rats each. Two groups received different doses of piroxicam, 2 mg/kg/day (Pi2) and 10 mg/kg/day (Pi10), respectively, the third group served as a control. The animals were fed a standard diet (Hope Farms, Woerden, The Netherlands) and allowed water ad libitum.
Within each group 10 rats were ment to be sacrificed 1, 2, 3 and 7 days after operation. However, in the Pi2 group two rats died immediatly post-operatively. In the Pi10 group three rats died, on day 1, 2 and 3, respectively, from pneumonia (one) and generalized peritonitis (two). Furthermore, all 10 animals which would have been sacrificed 7 days after operation died spontaneously of generalized peritonitis between day 3 and day 5. Thus, the Pi10 group only concerns animals sacrificed 1, 2 and 3 days post-operatively (9 at each time point).

b. piroxicam

The lower dose of piroxicam, 2 mg/kg/day, was chosen from the literature as being effective in suppressing inflammatory reactions in rats [6]. The 10 mg/kg/day dose was chosen after a pilot-study had established it as the highest dose which resulted in 100% survival in unoperated rats over a period of 15 days. Since the half-life of piroxicam in rats is relatively low [7], the drug (1ml) was administered twice a day directly into the stomach (dissolved in dilute NaOH and subsequently neutralized). Dosage was started two days before operation and maintained until the day the animals were killed. Post-operatively, this regimen led to levels of circulating piroxicam, measured in

heparinized plasma two hours after oral administration, between 4.6 and 8.5 μg/ml in the Pi2 group and between 14.6 and 24.3 μg/ml in the Pi10 group [8].

c. operative procedure

Operations were performed as described before [9]. Briefly, after induction of anaesthesia with sodium pentobarbital and a median laparotomy, 1 cm of both ileum and colon were resected at 15 cm proximal to the ileocaecal junction and 3 cm proximal to the rectal-peritoneal reflection, respectively. An inverting one-layer end-to-end anastomosis was constructed microsurgically with monofilament suture-material (8x0 Ethicon®). After 1, 2, 3 or 7 days the rats were sacrificed by an intra-cardiac overdose of sodium pentobarbital. Both anastomoses were prepared free and cleaned from surrounding tissue. Three 1 cm-samples were collected, one containing the anastomosis in the middle and the other comprising the adjacent proximal and distal segments. These post-operative samples were compared with the control segment, removed during operation. This way each animal served as its own control.

All samples were frozen immediately and stored in liquid nitrogen until further processing.

d. analytical procedures

The samples were pulverized in liquid nitrogen, lyophilized and kept at -30 degrees Celsius for analysis.

The dry-weights of control segments and the samples containing the anastomosis were determined.

In all samples the hydroxyproline concentration, as a measure for the collagen level, was measured as described previously [10]. Hydroxyproline concentrations are expressed per milligram dry weight.

In order to calculate post-operative changes in hydroxyproline content (μg/cm) in the anastomosis of a particular rat, both hydroxyproline concentration and the weight per cm should be known of the control segment and the anastomotic segment. However, the weight of the control segment as such is not directly comparable to the weight of the anastomosis, because construction of an anastomosis will increase the weight/cm. In a separate experiment the relation between the weight/cm of a colonic segment containing an anastomosis and collected immediatly after anastomotic construction, and the weight/cm of the

control segment removed during operation was investigated. It was found that, although the weight/cm of the anastomotic segment was indeed always greater than that of the control segment, the correlation between the two was very weak. As a result, no reliable equation could be derived to estimate in the present series the weight/cm anastomotic segment, as present immediately after operation, from the control segment collected during operation. Thus, results are presented as changes in hydroxyproline concentrations rather than as changes in hydroxyproline content. Statistical methods employed are mentioned with the results.

Results

All animals lost weight after operation. There was no significant difference in weight loss after one or three days between the control, the Pi2 and the Pi10 groups (Kruskal-Wallis three-sample test). Average maximal weight loss, reached three to four days after operation, was 7.7% ± (SD) 3.8% for the control group and 14.8% ± (SD) 6.7% for the Pi2 group. This difference is significant (Wilcoxon two sample test: p=0.014). The maximal weight loss could not be computed for the P10 group because of the mortality in this group. At sacrifice after seven days the weight of control animals averaged 98.4% ± 6.9% and that of Pi2 rats 93.2% ± 11.2% of their pre-operative weight.
The hydroxyproline concentrations in control segments, resected before anastomotic construction, are given in Table I. Obviously, hydroxyproline concentrations were always higher in the

Table I. Hydroxyproline concentrations in control segments

	n	ileum	colon
Control group	40	7.2 ± 1.6	15.6 ± 2.9
Pi2 group	38	8.9 ± 1.8**	13.5 ± 1.8**
Pi10 group	27	8.5 ± 1.3**	13.4 ± 1.6**

*Results are expressed as average value (μg/mg dry weight) ± SD. Levels of significance for the differences between piroxicam treated groups and C group were calculated by means of Tukey's multiple comparison test: **: $p \leq 0.01$.*

colon than in the ileum. Significant differences were found between rats in the control group and those constituting the Pi2 and Pi10 groups. In the latter, concentrations were higher in the ileum and lower in the colon, as compared to the control group. Anastomotic construction led to profound changes in hydroxypoline concentrations near the wound area, both in ileum (Figure 1) and in colon (Figure 2). In the control group, hydroxyproline concentrations were significantly reduced at all times in both proximal and anastomotic segments in the colon. In ileum, a significant reduction was only found after three days. The size of this effect was considerably less in ileum than in colon. Seven days after operation hydroxyproline concentrations were significantly increased in these ileal segments and also in the area distal to the anastomosis. In the latter segment, no lowered hydroxyproline concentrations were observed, either in ileum or in colon.

Treatment with piroxicam had substantial effects on the post-operative hydroxyproline changes. In the ileum the significant differences between control and piroxicam groups were limited to the distal segment at day 1 and all segments at day 7 (Table II). Here, the increase in hydroxyproline concentrations, observed in the control group, was significantly reduced. In the colon, the effects were much more pronounced. Both in the anastomosis and its proximal segment the post-operative reduction in hydroxyproline concentrations was limited by piroxicam, particularly one and three days after operation. Comparison of the hydroxyproline changes, observed during the first three post-operative days, in the Pi2 and Pi10 groups yielded no significant differences.

The preceding data pertain to changes in hydroxyproline concentrations, expressed per mg dry weight. In order to preclude the possibility that the differences in changes of hydroxyproline concentration, observed between control and piroxicam groups, were caused by differences in weight changes rather than differences in hydroxyproline changes, we have compared the changes in dry weight of the anastomotic segments. Table III gives the average weight ratio between the colonic anastomotic segment, obtained at sacrifice, and the control segment, removed at operation.

A 1 cm segment containing an anastomosis is always increased in weight as compared to 1 cm uninjured intestine, mainly because it contains an additional -inverted- segment of the bowel wall.

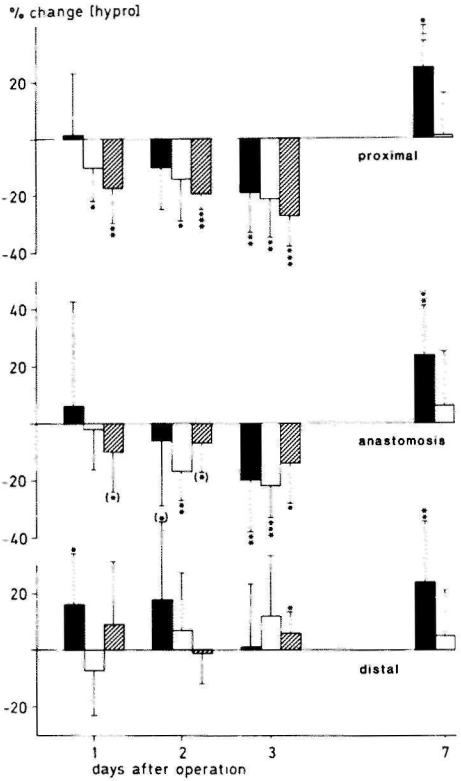

Figure 1: ileum

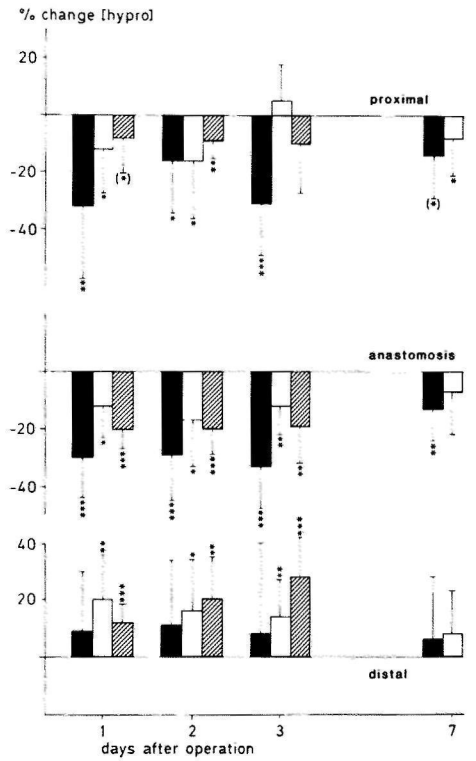

Figure 2: colon

Figure 1 and 2:

*Relative changes in hydroxyproline concentrations around ileal (Figure 1) and colonic (Figure 2) anastomoses. Within each animal the hydroxyproline concentrations in the various segments obtained after sacrifice were compared to that measured in the control segment removed at operation. For each group, the average percentual change, with respect to the control segment representing uninjured intestine, is given together with the standard deviation. Levels of significance for this difference within each group were calculated by means of a one sample, two-sided Student's t-test and represented as follows: (*): 0.05<p≤0.1; *: 0.01<p≤0.05; **: 0.001<p≤0.01; ***: p≤0.001.*

■ = Control group; □ = Pi2 group; ▨ = Pi10 group.

Table II. Significant and nearly-significant differences in hydroxyproline changes between groups

	segment	day	groups compared	average % change in hydroxyproline concentration	p-value
Ileum	proximal	1	C vs Pi10	1 vs -17	(*)
		7	C vs Pi2	26 vs 1	*
	anastomotic	7	C vs Pi2	24 vs 6	*
	distal	1	C vs Pi2	16 vs -7	*
		7	C vs Pi2	24 vs 5	*
Colon	proximal	1	C vs Pi2	-32 vs -12	(*)
			C vs Pi10	-32 vs -8	*
		3	C vs Pi2	-31 vs 5	**
			C vs Pi10	-31 vs -10	*
	anastomotic	1	C vs Pi2	-30 vs -12	**
		3	C vs Pi2	-33 vs -12	**
			C vs Pi10	-33 vs -19	(*)

significance (p-value) of differences between groups was tested by means of a Tukey's multiple comparison test:
*(*): $0.05<p\leq0.1$; *: $0.01<p\leq0.05$; **: $p\leq0.01$.*

Table III. Ratio between weights of colonic anastomotic and control segments

group	days after operation 1	2	3
Control	2.3 ± 0.7 (10)	2.2 ± 1.0 (10)	2.7 ± 0.7 (10)
Pi2	2.9 ± 1.3 (9)	2.0 ± 1.3 (9)	2.6 ± 1.4 (10)
Pi10	2.1 ± 1.0 (9)	2.0 ± 0.9 (9)	1.9 ± 0.9 (9)

Results are expressed as average ± SD and, in brackets, number of animals. Differences between Control and Piroxicam rats, sacrificed at the same day, were tested for significance by means of a Wilcoxon two-sided unpaired test but no significances were found.

Although the variations within groups were considerable, mainly due to the difficulty of obtaining reproducible 1 cm segments of living gut, the average increase in weight of the anastomotic segment was similar in control and experimental groups. The same goes for colonic proximal segments and ileal proximal and anastomotic segments (results not shown). Thus, the significant variations in post-operative changes in hydroxyproline concentrations (i.e. hydroxyproline concentration in the post-operative segment compared to that measured in the per-operative control segment), which were found to exist between control and piroxicam groups, may be attributed to a different metabolism of hydroxyproline.

Discussion

The intestinal wall derives its strength mainly from the submucosa [11,12]. This layer, which is composed almost entirely of collagen fibres, plays a central role in anastomotic healing [13] and changes in collagen are expected to affect intestinal strength [1,14-16]. During the first period after construction of an anastomosis, its strength largely depends on the suture holding capacity of the existing collagen fibres [2]. Thus, the occurrence of collagen degradation might impair this capacity and enhance the risk of anastomotic failure.

The transient lowering of collagen concentrations around experimental intestinal anastomoses, reported before [17,18] and also evident from the present data (Figures 1 and 2), is generally taken as evidence for post-operative collagen loss from the wound area. This phenomenon is more pronounced in colon than in ileum [10,19].

The first step in the degradation of interstitial collagen is mediated by the enzyme collagenase [20]. Recently, the presence of a specific collagenase around everted colonic anastomoses in the rabbit has been demonstrated immunohistochemically [16]. In addition we have found a transient post-operative increase in collagenolytic activity extractable from rat intestinal anastomoses [21]. A histological comparison of ileal and colonic anastomoses has shown that, next to a more extensive lowering of collagen concentrations, the delayed healing of colonic anastomoses is attended with a sustained presence of granulocytes [22]. These inflammatory cells comprise a major source for collagenolytic activity [3] and might thus contribute heavily to anastomo-

tic collagen degradation. Induced absence of granulocytes has no detrimental effect on wound healing [23]. Therefore, administration of compounds which suppress granulocyte function might limit loss of collagen without impairing the healing sequence, and thereby improve anastomotic integrity. One such class of compounds are the NSAIDs, of which piroxicam is a relatively new and potent representative [5].

Piroxicam interferes with the function of polymorphonuclear leucocytes at different levels: it has been shown to inhibit chemotaxis [24,25], superoxide anion generation [24-26] and release of granulo-associated enzymes [24,27]. Administration of piroxicam to our experimental animals in a dose of 2 mg/kg/day, which suppresses the inflammatory reaction in rats [6], indeed significantly affects anastomotic collagen concentrations, particularly in the colon (Figure 2 and table II). It limits the drop in collagen concentrations, observed in the anastomotic and proximal segments during the first three post-operative days. Since the changes in segment weight are similar in control and piroxicam groups, this must mean that piroxicam treatment actually limits the net loss of collagen.

At any given time, collagen content of tissues is the net result of collagen degradation and collagen synthesis. Thus, the fact that piroxicam reduces the post-operative loss of collagen may be explained by either inhibition of collagen degradation or stimulation of synthesis. It is our contention that piroxicam limits collagen degradation by interfering with granulocyte functions. As mentioned above, this effect of piroxicam is amply documented in the literature. Post-operative collagen synthesis is presumably a task for fibroblasts. No stimulatory effects of piroxicam on fibroblast function have been described. Also the fact that in ileal anastomotic segments seven days after operation, when in control rats collagen concentrations are much increased in comparison with the per-operative value, piroxicam significantly reduces this increase, argues against a stimulatory effect on collagen synthesis. On the contrary, since fibroblast proliferation in the wounded area is regulated by macrophages [4] and piroxicam is also reported to reduce monocyte function [6], it might even slow down collagen synthesis.

It is clear that piroxicam may not be given without restriction to operated animals. While in rats the LD50 (after a single oral dose) is 255 mg/kg [28] and in our pilot experiment unoperated rats tolerated sustained doses of 10 mg/kg/day relativey well, mortality increased dramatically in operated animals given this dose. In the Pi10 group, no animals survived the fifth post-operative day. In 12 out of 13 rats succumbing prematurely in these groups, peritonitis was found without signs of defective anastomoses. Possibly, per-operative bacterial spillage which is harmless in control animals, induces peritonitis in the Pi10 group as a result of suppression of the immune system [29] and defective granulocyte function.

It is a somewhat unexpected finding that there existed no significant differences in the post-operative changes of collagen concentrations between the Pi2 and Pi10 groups during the first three post-operative days, since animals in the Pi10 groups probably suffered from (sub)clinical peritonitis. In an earlier study from our laboratory anastomotic collagen concentrations were found to be lowered to a greater extent in rabbits with induced peritonitis than in control animals [30]. However, in this experiment peritonitis was induced immediately after anastomotic construction by implantation of human faeces next to the anastomosis, while in the present study it seems likely that peritonitis developed gradually since mortality only increased from three days after operation onwards.

In conclusion, our data suggest that piroxicam, in a dose of 2 mg/kg/day, is able to limit early post-operative collagen loss from colonic anastomoses. However, in view of the fatal results observed after administration of the higher dose, the efficacy of doses lower than 2 mg/kg/day should be investigated. The potential benefit of reduced anastomotic collagen loss for early post-operative intestinal strength, certainly merits further research into the effects of piroxicam on anastomotic healing.

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THE INFLUENCE OF METHYLPREDNISOLONE ON THE HEALING OF INTESTINAL ANASTOMOSES IN RATS

W.J.B. Mastboom, Th. Hendriks, B.M. de Man, H.H.M. de Boer.

Department of General Surgery, St. Radboud University Hospital, Nijmegen, The Netherlands.

Summary

Although steroids are generally expected to impair intestinal anastomotic healing, this effect has never been proven unequivocally in either clinical or experimental studies.
We have investigated the influence of methylprednisolone, which was given in either of two doses (2.5 or 10 mg/kg/day) from two days before operation onwards, on 3 and 7 days old ileal and colonic anastomoses in rats.
Anastomotic abscesses occurred more frequently in the ileum, but not in the colon, after steroid medication. However, methylprednisolone did not affect anastomotic bursting pressures in either of the bowel segments.
Comparison of the hydroxyproline content of the anastomotic segment yielded no difference between control and methylprednisolone groups in either small or large bowel. If the hydroxyproline content was expressed as a ratio between anastomotic and [per-operatively obtained] control segments, a significant decrease was found only 3 days after operation in the ileum of animals receiving the high dose of methylprednisolone.
Thus, healing of experimental colonic anastomoses remains unaffected by this corticosteroid; in the ileum, anastomotic hydroxyproline formation may be slightly impaired without affecting anastomotic strength.

Introduction

Healing of intestinal anastomoses and the factors influencing this process remain a worthwhile subject for experimental and clinical study, since in man anastomotic dehiscence is associated with a high mortality-rate. In general, steroid medication is

believed to be one of the factors which increase the risk of anastomotic leakage [1-2]. Still, in two large retrospective clinical studies the negative influence of corticosteroid medication on anastomotic healing could not be estimated [3,4].
The idea that steroids are detrimental to anastomotic repair is mainly based on experimental studies on skin wounds, where administration of steroids has invariably resulted in retarded healing [5-7]. However, it has been emphasized by several authors that it must not be taken for granted that wounds in other tissues react similarly [8,9].
Few investigations have been reported on the influence of steroids on intestinal healing. The data available on the alimentary tract indicate that administration of steroids impairs healing of gastric and duodenal incisional wounds [10] and suggest a negative influence on mechanical strength of anastomoses in jejunum [11] and colon [12].
If steroid medication indeed affects healing of intestinal anastomoses, one would expect this effect to be reflected in both anastomotic strength and the post-operative collagen metabolism around the anastomosis.
In the present study, we have investigated the influence of methylprednisolone on mechanical strength and hydroxyproline content of anastomoses in small and large bowel of rats.

Materials and Methods

a. animals

Sixty male Wistar rats weighing between 200 and 270 g were divided into three equal groups. The two medically treated groups received, respectively, 2.5 mg methylprednisolone/kg/day (MP-2.5 group) and 10 mg methylprednisolone/kg/day (MP-10 group), and the remaining group served as control (C group), receiving an equal dose of isotonic saline. The animals were fed a standard diet (Hope Farms, Woerden, The Netherlands) and allowed water ad libitum.

b. methylprednisolone

Doses of methylprednisolone, 2.5 mg/kg/day and 10 mg/kg/day, were chosen from doses used in previous studies [5-7,11,13-16] where they are believed to effectively suppress inflammatory reactions in rats. In a pilot-study, it was established that

neither dose caused mortality in unoperated rats over a period of 15 days. Daily placebo and medication, dissolved into 0.1 ml isotonic saline, were administred intra-muscularly into one of the back tighs. Dosage was started two days before operation and maintained until the day of sacrifice.

c. operative procedure

Operations were performed as described before [17]. Briefly, after induction of anaesthesia with sodium pentobarbital and a median laparotomy, one cm of both ileum and colon were resected at 15 cm proximal to the ileocaecal junction and 3 cm proximal to the rectal-peritoneal reflection, respectively. An inverting one-layer end-to-end anastomosis was constructed with monofilament nylon (8x0 Ethicon®) using a microsurgical technique. After 3 or 7 days the rats were killed by an intra-cardiac overdose of sodium pentobarbital. Circa 5 cm intestinal segment containing the anastomosis was prepared free, leaving surrounding tissue untouched, in order to measure the bursting pressure. Subsequently, the anastomotic proper was prepared free and an 1 cm segment, with the anastomosis in the middle, was collected to determine the collagen content.
All samples were frozen immediately and stored in liquid nitrogen until further processing.

d. bursting pressure

After careful removal of faeces, the bowel segment was attached to an infusion pump, which was filled with methylene blue-stained saline solution (0.9%). During pressure registration, the pressure was raised with an infusion rate of 2.5 ml/min. Leakage was recorded immediately by loss of pressure. The site of the lesion, indicated by effusion of methylene blue into the water, was noted.

e. analytical procedures

The samples were pulverized in liquid nitrogen, lyophilized and kept at -30 degrees Celsius for analysis.
The dry-weights of control segments and the samples containing the anastomosis were determined. In all samples the hydroxyproline content, as a measure for the collagen level, was measured as described previously [18].
Statistical methods employed are mentioned with the results.

Results

Neither free anastomotic perforation nor premature mortality was observed among the experimental groups. However, there occurred a significantly higher number of ileal anastomotic abscesses in both corticosteroid-treated groups (Table I) in comparison with the C goup, while abscesses around colonic anastomoses were only found once.

Table I. Abscess formation around ileal anastomoses

group	*n*	day 3	day 7	total
Control	20	1	0	1
MP-2.5	20	4	3	7*
MP-10	20	5	5	10**

*Differences between C and MP groups were tested for significance using Fisher's exact test, and are represented as follows: *: $P<0.05$; **: $p<0.01$.*

The course of post-operative body weight development was similar in all groups. After an average loss of circa 12% between the second and fourth day after operation, body weight increased up to the pre-operative level on the seventh day.

During collection of the anastomotic segments after sacrifice three days post-operatively, dehiscense of some anastomoses occurred: as a consequence their bursting pressure could not be measured. Bursting pressures of the remaining anastomotic segments are presented in Table II. At day 3 both ileal and colonic segments bursted within the anastomosis, without significant differences between the groups. At day 7 all perforations occurred away from the anastomotic area. However, significant differences were found for colonic segments, where bursting pressures in the MP groups were significantly higher than in the C group.

Tables III and IV give the hydroxyproline content of the anastomotic and control segments, together with their ratio, in ileum and colon, respectively. Figure 1 illustrates the post-operative course of the anastomotic hydroxyproline content.

No differences between groups were found in, pre-operatively

Table II. Bursting pressures of ileal and colonic anastomoses

Bursting pressures (BP) are given as average mm Hg ± SD.

	group	n	day 3	n	day 7
Ileum	Control	6	63 ± 14	10	201 ± 62
	MP-2.5	8	72 ± 26	10	227 ± 60
	MP-10	10	57 ± 22	10	209 ± 51
Colon	Control	9	109 ± 54	10	149 ± 21
	MP-2.5	10	90 ± 40	10	178 ± 36*
	MP-10	8	99 ± 39	10	187 ± 24**

Levels of significance for the differences between the groups are calculated by means of a Wilcoxon two-sided unpaired test.
*Significant differences between C and MP groups are only found in colon at day 7 and are represented in the folowing way: *: P<0.05; **: p<0.01.*

Table III. Hydroxyproline content in ileal anastomotic segments

Results are are expressed (µg/cm) as average value ± SD. The last column represents the ratio between anastomotic and control segments.

group	day after operation	control segment	anastomotic segment	ratio
Control	3	94 ± 27	166 ± 27	1.8 ± 0.4
	7	86 ± 31	235 ± 70	2.8 ± 0.6
MP-2.5	3	89 ± 39	143 ± 43	1.7 ± 0.4
	7	87 ± 31	270 ± 58	2.9 ± 0.7
MP-10	3	112 ± 26	161 ± 38	1.4 ± 0.2*
	7	79 ± 30	223 ± 52	2.8 ± 0.9

*Presence of significant differences between control and both MP-groups is calculated by means of a Kruskal-Wallis K-sample test. Levels of significance between the individual groups are calculated using a Wilcoxon two-sided unpaired test: *: ratio : MP-10 versus C : p<0.05*
MP-10 versus MP-2.5 : p<0.05

Table IV. Hydroxyproline content in colonic anastomotic segments
Results are are expressed (µg/cm) as average value ± SD. The last column represents the ratio between anastomotic and control segments.

group	day after operation	control segment	anastomotic segment	ratio
Control	3	124 ± 36	252 ± 45	2.2 ± 0.6
	7	114 ± 15	264 ± 54	2.4 ± 0.7
MP-2.5	3	122 ± 45	216 ± 42	2.3 ± 1.0
	7	128 ± 49	240 ± 52	2.0 ± 0.6
MP-10	3	135 ± 23	216 ± 34	1.6 ± 0.3
	7	125 ± 33	233 ± 39	2.0 ± 0.1

No significant differences between control and MP-groups were found using a Kruskal-Wallis K-sample test.

obtained, control segments either in ileum or colon. The average hydroxyproline content in uninjured colon was 30% higher than in uninjured ileum. In ileum, a significant rise in anastomotic hydroxyproline level was observed between 3 and 7 days after operation. This occurred in all three groups and no effect of methylprednisolone was apparent, with one exception: comparison of the ratio's between anastomotic and control hydroxyproline content showed a significantly lower ratio in the high-dose

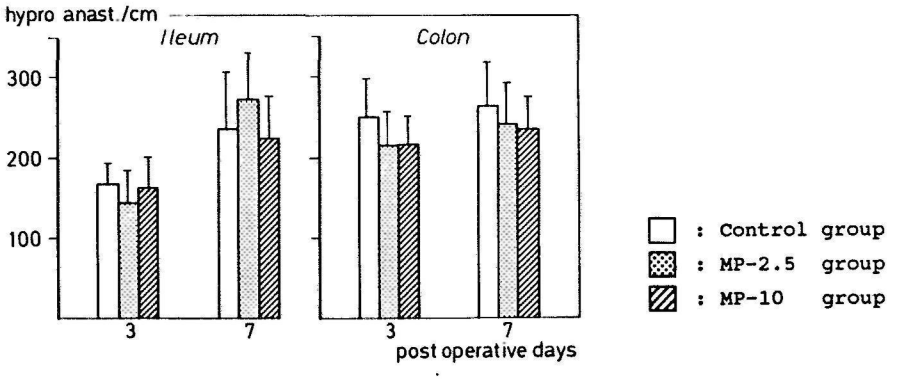

Figure 1. Anastomotic hydroxyproline content
Values are expressed as mean ± SD in µg/cm.

methylprednisolone group 3 days after operation (Table III). In colon (Table IV), the hydroxyproline content of 7 days old anastomoses was not elevated with respect to that in 3 days old anastomoses. Here, no significant differences were found between control and methylprednisolone groups at all.

Discussion

Experimental studies have shown that corticosteroids impair wound healing [5-7,16,19]. Mechanisms thought to be responsible for the retarded skin healing during steroid administration are inhibition of granulocyte and macrophage function [20-23], fibroplasia [14,15] and angioneogenesis [14], resulting ultimately in delayed connective tissue repair and epithelial regeneration. Comparison of the results from the various studies is hampered by the fact that they differ in many respects, like compound used, dose and time and method of administration. For the present study we have chosen methylprednisolone in a dose (10 mg/kg/day) which inhibits development of granulation tissue in rats [13,14,16]. Neither this dose, nor the lower dose used, resulted in a loss of strength of anastomotic segments in either ileum or colon (Table II).

Very few data are available on the effect of steroids on anastomotic repair. Aszodi and Ponsky found a significant reduction in bursting pressure of 4-, 5- and 7-days old jejunal anastomoses in rats which had been treated with hydrocortisone (5 mg/kg/day) from 14 days pre-operatively onwards [11]. However at the time of operation these animals had lost 15% of their body weight, which implies a considerable degree of malnutrition compared to their controls. This raises the question whether the delay in recovery of strength was the result of malnutrition rather than a direct effect of corticosteroid action. Very recently, Houston and Rotstein reported that administration of methylprednisolone significantly reduced colonic strength 5 days after operation [12]. The dose of methylprednisolone used in their study, 25 mg/kg/day, appears rather high, more than twice as high as the highest dose we used. Their steroid treatment also increased the incidence of peri-anastomotic abscess formation from 20 to 58%. We find only one case of abscess formation in the 60 colonic anastomoses constructed in the various groups. In contrast to these findings, steroid administration led to a significant rise

in the number of abscesses in ileal anastomoses (Table I). We can only speculate on the cause of this phenomenon. The rats in our study have been operated under clean but unsterile conditions, which usually does not lead to signs of intra-abdominal infections. It is likely that this is the result of a strongly developed immune system. However, it might be proposed that administration of corticosteroids, which are well known suppressors of the immunic system, inhibit the antibacterial functions of leucocytes [22-24], resulting into an increased bacterial growth, leading to enhanced abscess formation. However, this leaves unexplained why the incidence of colonic abscesses was not increased, unless one assumes that defence mechanisms are more highly developed in the colon, e.g. because of its greater intrinsic bacterial population.

Anastomotic collagen content is frequently used as a parameter for repair. Since collagen provides structure and strength to the intestinal wall, the post-operative collagen metabolism is generally thought to be crucial to the development of anastomotic strength [25]. During the first days after operation, anastomotic strength mainly depends on the "stuture holding capacity" of the remaining intact collagen fibrils [26]. Subsequently, newly synthesized collagen bridges the anastomotic gap and provides for its strength.

The inflammatory process dominates the first stage of the wound healing sequence. Inflammatory cells may strongly affect collagen metabolism. For instance, polymorpho nuclear granulocytes and macrophages contain collagenolytic enzymes [27,28] while macrophages also regulate fibroplasia [22,23]. Thus, corticosteroids, which are potent suppressors of migration of function of granulocytes and macrophages, would be expected to be able to affect degradation and synthesis of collagen. Direct effects of glucocorticoids on collagen synthesis have been amply demonstrated [29]. Indeed, methylprednisolone, used in doses similar to ours [12,13,15], significantly lowered accumulation of collagen in granulation tissue of rats. However, the effects we measured on anastomotic hydroxyproline content are very limited.

Most studies on intestinal healing purporting to measure collagen as a parameter, supply data on hydroxyproline concentrations. A decrease of these concentrations is generally thought to reflect collagen degradation, while an increase is interpreted as a net synthesis of collagen. However, it is likely that during the healing process the amount of non-collagenous material also

changes considerably. Thus, a decrease of hydroxyproline concentrations might be the result of loss of collagen, but might also be caused by an increase in dry weight. In order to avoid this difficulty, we have measured the hydroxyproline content per cm intestine. However, this method induces an additional experimental error since it is difficult to obtain a reproducible segment of 1 cm of vital bowel. This is reflected in the relatively high standard deviations observed in hydroxyproline content (Tables III and IV). The hydroxyproline content of anastomotic segments is always higher than that of control segments of equal length, since they contain a greater length of intestinal wall because of the inversion used to construct the anastomosis. Methylprednisolone did not affect the amount of hydroxyproline present in colonic anastomoses, neither if the content in the anastomotic segment was compared nor if the ratio between anastomotic and control segments was used as a parameter. In the ileum, there was only a reduction in the ratio between anastomotic and control segments at 3 days in the high-dose group. However, this effect might possibly be explained by the relatively high control values measured in this group. Altogether, the effects of methylprednisolone administration on anastomotic collagen content in the intestine appear to be of limited significance and have no consequence for anastomotic strength in the rat.

Thus, it is apparent that administration of corticosteroids, in doses which have been shown to affect healing in other tissues, does not impair the development of intestinal anastomotic strength. Colonic repair does not appear to suffer at all, while in ileum only abscess formation increases. It remains to be demonstrated if such abscesses eventually may result in anastomotic dehiscence. Still, we conclude that the premises that steroid medication is essentially detrimental to (colonic) anastomotic repair should not be taken for granted.

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INTESTINAL ANASTOMOTIC HEALING IN THE ABSENCE OF SUTURE MATERIAL: AN EXPERIMENTAL STUDY IN RATS

W.J.B. Mastboom, Th. Hendriks, H.H.M. de Boer.

Department of General Surgery, St. Radboud University Hospital, Nijmegen, The Netherlands.

Summary

In order to investigate the influence of sutures on intestinal anastomotic healing, 48 rats underwent both ileal and colonic resection. In 24 rats all intestinal sutures were removed 30 minutes after anastomotic construction, while in the remaining animals sutures were left in place.
One, 3 and 7 days after operation, bursting pressures and collagen (hydroxyproline) levels in anastomotic segments were measured.
Two lethal ileal dehiscences and 9 anastomotic abcesses (5 ileal and 4 colonic) occurred in the experimental group, versus 3 ileal anastomotic abcesses in the control group. Only the first day after operation bursting pressures were significantly lower in both sutureless ileal and colonic anastomoses. The post-operative course in changes of collagen concentrations in ileal and colonic segments did not differ between the groups.
Apparently, in the rat sutures are only essential for anastomotic strength during the very first post-operative period, but seem not to affect the collagen metabolism in the healing anastomotic wound.
The present results on the sutureless anastomoses are very similar to those reported for the healing of experimental sutureless anastomoses constructed by application of a glue.

Introduction

At tissue level, sutures locally induce an inflammatory reaction, ischaemia and tissue necrosis. Presence of suture material also

increases the risc of wound infection. Thus, an ideal anastomosis should at the same time manage without use of foreign body material and still ensure complete apposition of the various tissue layers which compose the bowel wall [1].

In an attempt to avoid some adverse effects of suture material, several authors have examined the influence of fibrin-glue [2,3] and non-biologic glues on the healing of non-sutured intestinal anastomoses [4-6]. Others have shown that the time sutures are actually needed in place is very short [7-9]. In their studies uncomplicated healing of experimental colonic anastomoses occurred, when only a half till one hour after anastomotic construction, all intestinal sutures were removed.

In studies on the healing of intestinal wounds, the behaviour of collagen is often investigated. This protein, which is mainly responsible for the structural integrity of the intestinal wall, is thought to play an important role during the healing of intestinal anastomoses: initially it provides a matrix for anchoring the suture and subsequently newly formed collagen fibrils must bridge the defect and restore pre-operative strength. The first days after anastomotic construction, collagen concentrations near the wound decrease; later, increased concentrations are found. The collagen level measured is the net result of lysis and synthesis [10-13]. The initial decrease is considered to be mainly the result of degradation of collagen, while the subsequent increase is thought to reflect synthesis. The occurrence of collagen degradation around intestinal anastomoses has been confirmed recently by the demonstration of increased levels of tissue collagenase [14] and collagenolytic activity [15]. Since immediate post-operative intestinal strength largely depends on the intact collagen fibres, collagenlysis in the anastomotic area must essentially be detrimental to anastomotic strength.

Bowel resection induces a chain of inflammatory reactions which, among other things, induce degradation of collagen. The use and continued presence of sutures might prolong inflammation and result in excessive loss of collagen.

In order to investigate this aspect on anastomotic healing, we have studied bursting pressures and collagen concentrations around sutureless anastomoses in rats.

Materials and Methods

a. animals

Fourty-eight male Wistar rats with an avarage weight of 271 ± 15 (SD) g were used. All animals received both an ileal and a colonic anastomosis. They were divided into two equal groups: in 24 rats, representing the "sutureless anastomosis" (SLA) group, all intestinal suture material was removed half an hour after anastomotic construction, while the other animals comprised the control (C) group, where sutures were left in place. The rats were fed a standard diet (Hope Farms, Woerden, The Netherlands) and allowed water ad libitum.
In both groups, eight animals were predestined to be killed 1, 3 and 7 days after operation. Body weights of all rats were daily measured.

b. operative procedure

Operations were performed as described before [16]. Briefly, after induction of anaesthesia with sodium pentobarbital and a median laparotomy, one cm of both ileum and colon were resected at 15 cm proximal to the ileocaecal junction and 3 cm proximal to the rectal peritoneal reflection, respectively. An inverting one-layer end-to-end anastomosis was constructed microsurgically with monofilament nylon (Ethicon® 8xO). In SLA rats, all anastomotic suture material was removed -with utmost caution to prevent anastomotic dehiscense- half an hour after resection of the intestinal segments. C rats were kept under similar narcotic conditions for an equal time period and abdominal closure was performed more than half an hour after construction of the second intestinal anastomosis. Immediately after operation two rats of the C-group died, probably as a result of the long duration of the operative and anaesthetic period. Animals were killed by an overdose of sodium pentobarbital 1, 3 or 7 days after operation. Bowel segments of circa 5 cm, containing the anastomoses, were prepared free, leaving adherent tissue intact, for measuring bursting pressures. Thereafter, the anastomosis was cleaned from all adherent tissue and 1 cm-segments, with the anastomosis in the middle, were frozen and stored in liquid nitrogen until further processing. These post-operative samples were compared with the control segments, removed during operation. This way, each animal served as its own control.

c. bursting pressure

After removal of fecal material, the bowel segment was attached to an infusion pump and a pressure-gauge recording intra-luminal pressure. Subsequently, pressure was increased by infusion of saline solution (0.9%), coloured with methylene blue, at a rate of 2.5 ml/min into the bowel. Meanwhile, the intestine was kept under water. Leakage was immediatly recorded by loss of pressure. The site of the lesion, indicated by effusion of methylene blue into the water, was noted.

d. analytical procedures

The samples were pulverized in liquid nitrogen, lyophilized and kept at -30 degrees Celsius for analysis.

The dry-weights of both control and anastomotic samples were determined.

In all samples the hydroxyproline content, as a measure for the collagen level, was measured as described previously [17], essentially according to Prockop and Udenfriend [18].

Statistical methods employed are mentioned with the results.

Results

One day after operation two SLA-animals died from dehiscence of the ileal anastomosis. They were excluded from the study. Figure 1 depicts the weight loss in both the C- and SLA- 7 day-groups. The avearge initial weight was 276 ± 11 (SD, n=7) g in the C-group and 265 ± 14 (SD, n=7) g in the SLA-group. Maximal weight loss was 13.8 ± 2.7% for the C-group and 15.2 ± 3.9% for the SLA-group. Although SLA rats appeared to lose slightly more weight, this effect remained non-significant.

Ileal anastomotic abscesses occurred in both groups (Table I): in the C-group three times versus five times in the SLA-group. Colonic abscesses were not found in the C-group and four times in the SLA-group. These differences between groups were not significant (chi-squared test). Since the parameters measured in the animals, which displayed anastomotic abscesses, did not differ from those measured in the other animals; rats with abscesses were not excluded from the study.

The bursting pressures measured are given in Table I. With one exception, a one day ileal anastomosis in the SLA-group, leakage of all anastomotic segments obtained for one or three days after

operation occured within the anastomotic area. All seven days old anastomoses were stronger than the uninjured intestine: leakage occurred outside the anastomotic area. On the first day after operation bursting pressures in the SLA-group were significantly lower than those observed in the C-group, both in ileum and in colon. This difference had already disappeared after three days.

The hydroxyproline concentrations in the control segments, resected at operation, did not differ between the various C- and SLA-groups. Average values were 8.7 ± 1.4 (SD, n=44) µg/mg dry weight in ileum and 16.0 ± 1.7 µg/mg dry weight in colon. After anastomotic construction the hydroxyproline concentrations changed considerably in the anastomotic segment: Tables II and III give the ratios between hydroxyproline concentrations in anastomotic and control segments, for ileum and colon, respectively.

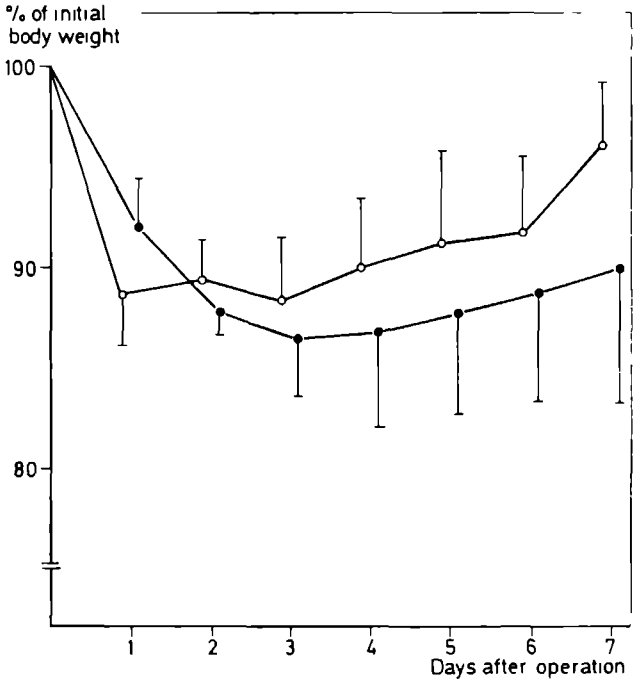

Figure 1. Average percentual change of body weight in both the C- (O) and SLA- (●) group.
Values are given with SD and are derived from animals killed seven days after operation (n=7).

Table I. Bursting pressure and abscess formation

Results of bursting pressure (BP) are given as average mm Hg ± SD.

		C-group			SLA-group		
	day	n	abscess	BP	n	abscess	BP
Ileum	1	7	0	14 ± 9	8	0	4 ± 7*
	3	8	2	27 ± 25	7	3	29 ± 26
	7	7	1	168 ± 62	7	2	164 ± 80
Colon	1	7	0	26 ± 14	8	0	3 ± 8**
	3	8	0	60 ± 54	7	2	29 ± 32
	7	7	0	186 ± 15	7	2	181 ± 28

*Differences between BP of C- and SLA-groups were tested for significance using a Wilcoxon 2-sided unpaired test: *: p=0.044; **: p=0.006.*

Table II. Changes of hydroxyproline concentrations ([hydroxyproline]) and dry weights in ileal anastomotic segments

Values in anastomotic segments were compared to those in control segments removed at operation and resulting mean ratios ± SD are given. Data pertain to 7 animals except in the SLA-1 day group and in the C-3 day group, which both comprise 8 rats.

	[hydroxyproline] anastomosis / [hydroxyproline] control segment		dry weight/cm anastomosis / dry weight/cm control segment	
day	C	SLA	C	SLA
1	0.93 ± 0.11	0.84 ± 0.12*	1.81 ± 0.91	2.32 ± 0.83
3	0.77 ± 0.10*	0.82 ± 0.10*	2.12 ± 0.80	1.99 ± 0.42
7	1.17 ± 0.12*	1.19 ± 0.30	2.87 ± 0.66	3.34 ± 2.01

*Changes in hydroxyproline concentrations were tested for significance using a signed rank test: *: 0.01 < p ≤0.05.*

Using a Wilcoxon 2-sided unpaired test, no significant differences, either in weight or in hydroxyproline change, were found between C- and SLA-groups.

Table III. Changes of hydroxyproline concentrations ([hydroxyproline]) in dry weights in colonic anastomotic segments

Values in anastomotic segments were compared to those in control segments removed at operation and resulting mean ratios ± SD are given. Data pertain to 7 animals except in the SLA-1 day group and in the C-3 day group which both comprise 8 rats.

day	[hydroxyproline] anastomosis / [hydroxyproline] control segment		dry weight/cm anastomosis / dry weight/cm control segment	
	C	SLA	C	SLA
1	0.77 ± 0.06*	0.75 ± 0.12*	2.46 ± 0.85	2.58 ± 0.56
3	0.76 ± 0.08*	0.75 ± 0.14*	3.10 ± 1.18	2.46 ± 0.40
7	0.90 ± 0.07*	0.87 ± 0.14*	2.30 ± 0.61	2.30 ± 0.84

*Changes in hydroxyproline concentrations were tested for significance using a signed rank test: *: $0.01 < p \leq 0.05$.*

Using a Wilcoxon 2-sided unpaired test, no significant differences, either in weight or in hydroxyproline change, were found between C- and SLA-groups.

In the ileal anastomoses, an initial decrease of the hydroxyproline concentrations during the first three days after operation, is followed by an extensive increase, leading to values significantly exceeding the original level, on the seventh day.
After an initial reduction, anastomotic hydroxyproline concentrations in the colon also increased on the seventh post-operative day, but at that time they still remained significantly lower than those measured in uninjured intestine.
The post-operative changes in hydroxyproline concentrations were very similar in C- and the SLA-groups, both in ileum and in colon: no indications were found for a difference between these groups at any of the time points measured.
Anastomotic construction will inevitably lead to an increase in weight/cm intestine; therefore the weight of 1 cm anastomotic segement is always greater than that of 1 cm control segment. In a separate experiment (results not shown) it could be estimated that 1 cm ileal segment containing an anastomosis was, immediately after anastomotic construction, on average 1.60 times heavier than the 1 cm control segment removed at operation. For

colon, this figure was 1.95. Tables II and III show that the relative changes in dry weight were similar in C- and SLA-groups. Thus, the comparison between these groups of hydroxyproline concentrations, which were calculated on the basis of dry weight, indeed allows conclusions regarding the similar behaviour of hydroxyproline (and thus collagen) content in C- and SLA- groups.

Discussion

Removal of the intestinal sutures half an hour after anastomotic construction led to lethal disruption of two ileal anastomoses and to a considerable number of anastomotic abscesses. Similar complications were found by authors who tested non-biological glues for construction of sutureless intestinal anastomoses [4-6]. During the first week, use of human fibrin-glue led to significant weaker colonic anastomoses in rats only at the fourth day after operation but complications were rare [2]. However, in rats treated with corticosteroids fibrin sealant, applied to sutured colonic anastomoses, enhanced abscess formation and reduced the bursting pressures [19].
In our study, twenty-four hours after operation bursting pressures in the SLA-group were significantly lower than in the C-group. After three and seven days, bursting pressures did not differ between the groups. Wilker reported the same phenomenon in colonic anastomoses, but the difference at day 1 was not significant, probably due to the small number of animals used in his study [8]. In another study by the same group a significantly reduced breaking strength during the first 24 hours was found [9]. Thus, if we consider the bursting pressure as a measure for the risc of anastomotic disruption, sutures might be helpful in preventing anastomotic dehiscense only during the immediate post-operative period, i.e. one or possibly two days.
The connective tissue protein collagen provides the mechanical strength and ensures the integrity of the intestinal wall: consequently, it plays an important role in the healing process of anastomoses [13]. Morphologic studies have shown that suture material damages intestial tissue, and that also the submucosal layer, where collagen is mainly stored, is affected [20]. Since sutures act as foreign boy material, their lasting presence

might prolong the inflammatory reaction. Inflammatory cells like macrophages and polymorphonuclear granulocytes contain collagenase activity, and thus promote collagen degradation [21-22]. Consequently, a sustained inflammatory reaction, elicted by sutures and resulting in the lasting presence of inflammatory cells in the wound area, might increase collagen degradation. Since post-operative loss of collagen could be detrimental to anastomotic strength, restriction of this process would benefit undisturbed healing. However, comparison of the course of post-operative changes in hydroxyproline concentrations around anastomoses in both ileum and colon did reveal no differences between C- and SLA-groups.

Since hydroxyproline concentrations were calculated on the basis of dry weight we also compared the differences in dry weight between anastomotic and control segments in both groups. Dry weight changes were similar in C- and SLA-groups. This means that the comparison of the hydroxyproline concentrations indeed signifies that anastomoses in C- and SLA-groups contain equal amounts of collagen at the time points measured.

Thus, the early removal of sutures does not have a measurable effect on the quantity of collagen present in the anastomoses. Apparently, transection of the bowel wall must be the major cause of post-operative changes in collagen concentrations. Still, we believe that the influence of suture material on collagen degradation is theoretically very likely, but that this effect is too small to be measured with our present methods. Another possibility would be that the continued presence of sutures stimulates both degradation and synthesis of collagen. In such a case the net result, in terms of hydroxyproline content, could be the same, but the quality of the collagen present would be different. Separate measurements of collagen degradation and synthesis are needed to investigate the existence of such an effect.

The differences between the C- and SLA- group in our study are very similar to those reported by others, who have examined healing of glued non-sutured anastomoses [2-6]. We suggest that the phenomena observed in the healing of the glued non-sutured anastomoses should not be ascribed to properties of the different sealants applied, but to the natural healing process of sutureless anastomoses.

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COLLAGEN CHANGES AROUND INTESTINAL ANASTOMOSES IN GERM-FREE RATS

W.J.B. Mastboom, T. Hendriks, H.H.M. de Boer.

Department of General Surgery, St. Radboud University Hospital, Nijmegen, The Netherlands.

Summary

The changes in collagen, measured as hydroxyproline, concentration around both ileal and colonic anastomoses in germ-free (GF) and control (C) rats have been investigated and compared with each other. The GF rats were raised, operated and maintained under completely pyrogen free conditions. Animals were killed 2, 3 or 7 days after operation. There was a significant reduction in the lowering of hydroxyproline concentrations around the colonic anastomosis of GF rats compared with C rats both in the anastomosis at day 2 and 3 ($p=0.04$ and $p=0.006$ respectively) and in the proximal segment at day 3 ($p=0.03$). In ileal anastomoses, significant differences between C and GF rats were only found at 7 days. Here, the increase in hydroxyproline concentration observed in C rats was significantly reduced in GF rats.
These data are taken to support the hypothesis that bacteria affect colonic anastomotic healing by contributing to postoperative collagen degradation.

Introduction

Dehiscence of large bowel anastomoses is a frequent complication in colonic surgery and is associated with a considerable mortality rate [1]. On the other hand, the leakage rate of ileal anastomoses is low [2]. In order to devise measures to prevent dehiscence, the crucial steps in anastomotic healing should be defined. Comparison of the healing sequence in small and large bowel might be helpful in this respect.
The structural protein collagen is of major importance in main-

taining the strength of the intestinal wall. Investigations into the healing of intestinal anastomoses in various animals have shown that massive changes in collagen concentrations occur during the first week after operation [3-5], which changes are more extensive in colon than in ileum [6,7]. It is generally believed that immediately after operation collagen degradation occurs, followed by de novo synthesis. This "collagenous equilibrium" is thought to be of paramount importance to undisturbed healing [8].
An obvious difference between small and large bowel is the increased presence of micro-organisms in the latter. Since bacteria are capable of producing collagenolytic enzymes [9], they might enhance post-operative collagen degradation and consequently affect anastomotic healing. Thus, it is of interest to examine the behaviour of collagen under conditions where bacteria are completely absent during operation and subsequent healing.
For this purpose, we have compared changes in collagen concentrations around intestinal anastomoses in germ-free rats to those in normal rats.

Materials and methods

a. animals

Two groups of male Wistar rats were used: 30 control (C) rats (average weight 244g) and 45 germ-free (GF) rats of similar age and weight. They were fed a standard diet (Hope Farms, Woerden, The Netherlands) and allowed water ad libitum.
The GF rats were born and raised under sterile conditions [10]. Immediatly before operation the animals were moved, taking full precautions against bacterial contamination, from quarantine to a cage in a horizontal laminar flow workstation, where the operation was performed under pyrogen-free conditions. The animals remained in the same place until they were killed. Before operation and before killing, faecal samples were taken for culture. All pre-operative samples were negative. However, 20 of the 45 faecal samples taken before killing the rats showed bacterial contamination. These animals were excluded from the series. Thus 25 GF rats remained which were killed 2 (n=7), 3 (n=11) and 7 (n=7) days after operation. The three comparable control groups, operated on under non-sterile conditions, consisted of 10 animals each.

b. operative procedure

Operations were performed as described before [11]. Briefly, anaesthesia was induced with sodium pentobarbital, a median laparotomy was performed and 1cm of both the ileum and the colon were resected at 15 cm proximal to the ileocaecal junction and three cm proximal to the rectal peritoneal reflection respectively. An inverting one-layer end-to-end anastomosis was constructed using a microsurgical technique. After 2, 3 or 7 days the rats were killed by an intra-cardiac overdose of sodium pentobarbital. Both anastomoses were dissected out and cleaned from surrounding tissue. Three 1cm-samples were collected, one containing the anastomosis in the middle and the others comprising the adjacent proximal and distal segments, respectively. These postoperative sections were compared with the control segment removed during operation. In this way each animal served as its own control.

All samples were frozen immediately and stored in liquid nitrogen until further processing.

c. analytical procedures

The samples were pulverized in liquid nitrogen, lyophilized, weighed and kept at -30 degrees Celcius for analysis.

In all samples the hydroxyproline content, as a measure for the collagen level, was measured as described previously, essentially according to Prockop and Udenfriend [12]. Total protein was assayed by the Lowry method [13], using bovine serum albumin as a standard. Statistical methods employed are mentioned with the results.

Results

The hydroxyproline concentrations in uninjured intestine, as represented by the control segments removed at operation, are given in Table I. Concentrations were always higher in colon than in ileum. While in the ileum of GF rats hydroxyproline levels were significantly higher than those measured in C rats, the colon of GF rats contained significantly less hydoxyproline than the colon of C rats.

Table I. Hydroxyproline concentrations in control-segments

	day	n	ileum	colon
Control rats	2	10	7.3 ± 1.7	15.3 ± 2.6
	3	10	7.5 ± 1.9	16.2 ± 3.9
	7	10	7.2 ± 1.4	15.4 ± 1.8
		30	7.4 ± 1.6	15.7 ± 2.8
Germ-free rats	2	7	9.3 ± 2.1	12.9 ± 2.9
	3	11	7.9 ± 0.9	12.1 ± 1.8
	7	7	9.3 ± 0.9	15.0 ± 1.9
		25	8.7 ± 1.5	13.1 ± 2.5
Control versus Germ-free			p = 0.0045	p = 0.0015

Results are expressed as avarage value (µg/mg dry weight) ± SD.
The levels of significance for the differences observed between GF and C rats are calculated by the Kruskal-Wallis K-sample test.

Construction of an anastomosis strongly affects hydroxyproline concentrations around the operative area, both in ileum (Figure 1) and in colon (Figure 2). In C rats, the post-operative decrease of hydroxyproline levels was most marked in the colon, where a significant difference with uninjured intestine was found in both the anastomosis proper and its proximal segment at all three time points investigated. A similar phenomenon was also seen in ileum, where the effect was less marked and where pre-operative levels were restored more rapidly. No lowering of hydroxyproline concentrations was seen in the area distal to the anastomosis. The transient reduction of hydroxyproline concentrations in proximal and anastomotic segments was also observed in GF rats. However, there were significant quantative differences between both species (Table II). In the colon, the reduction of hydroxyproline levels was less marked in GF than in C rats, particularly in the anastomosis at 2 and 3 days and in the proximal segment at 3 days after operation. In the ileum restoration of pre-operative hydroxyproline concentrations appeared retarded in GF rats: at 7 days levels had only returned back to pre-operative values while at this time they were significantly enhanced in C rats.

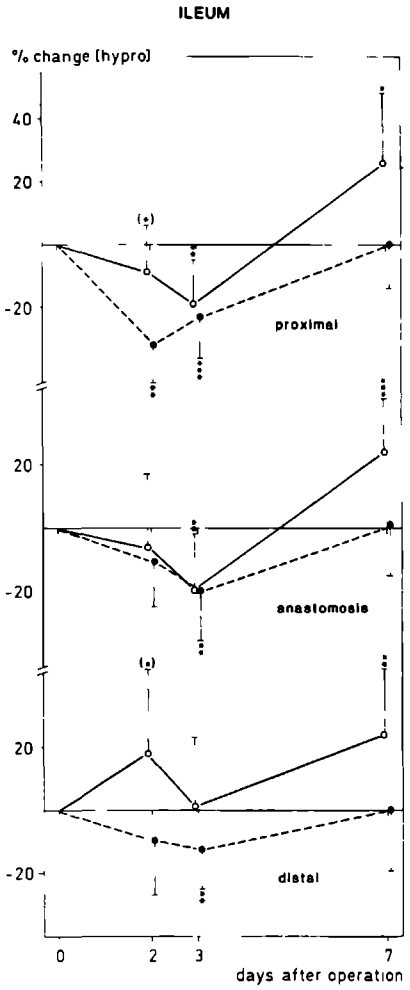

Figure 1.

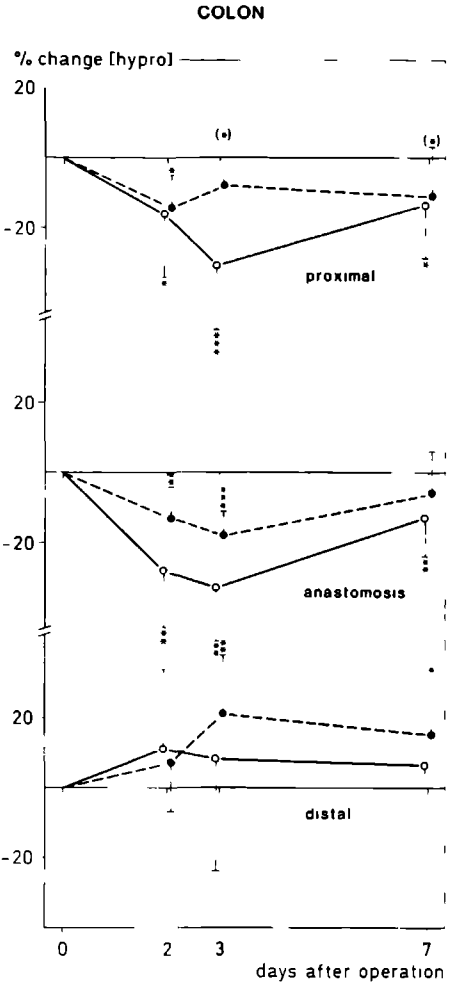

Figure 2.

Figure 1 and 2:

*Relative changes in hydroxyproline concentrations around ileal (Figure 1) and colonic (Figure 2) anastomoses. Results are expressed as average percentual change, calculated with respect to the pre-operative value, with standard deviation, both for C (O) and GF (●) rats. Levels of significance for the difference between post-operative and control segments are calculated by means of a paired sample 2-sided Student's t-test and represented as follows: (*)= 0.05<p≤0.1; *= 0.01<p≤0.05; **= 0.001<p≤0.01; ***= p≤0.001.*

Table II. Significant differences in post-operative hydroxyproline changes between Control and Germ-free rats

	day	segment	average % change in hydroxyproline concentration		p-value difference
			C	GF	C vs GF
Ileum	2	proximal	- 9	-32	0.0047
		distal	18	-10	0.0295
	7	proximal	26	0	0.0330
		anastomosis	24	1	0.0142
		distal	24	1	0.0387
Colon	2	anastomosis	-28	-13	0.0423
	3	proximal	-31	- 8	0.0033
		anastomosis	-33	-18	0.0063

Results are expressed as mean percentual change, calculated with respect to the pre-operative value. Levels of significance for the differences between GF and C rats are calculated by means of a Wilcoxon two-sided unpaired test.

As the changes in the immediate wound area are probably most relevant to the healing sequence, the apparent differences between GF and C rats in the anastomotic segment are of most interest. Hydroxyproline changes have been expressed in terms of concentrations (μg/mg dry weight). To determine whether the differences in post-operative hydroxyproline concentration changes may be attributed to differences in hydroxyproline content rather than to differences in dry weight (per cm anastomotic segment), we have compared the changes in dry weight of the anastomotic segments, both for ileum (Table III) and for colon (Table IV).

The weight of uninjured intestine was higher in C rats than in GF rats: 1cm ileum weighed on average 11.7 (SD 3.7) mg in C rats (n=30) and 7.3 (SD 2.7) mg in GF rats (n=25) ($p<0.0001$); values for colon were 9.4 ± 3.0 and 7.8 ± 2.1 ($p=0.02$), respectively. In ileum, the weight of the anastomotic segment increased with time, while it remained constant in colon. The increase in weight relative to that of the control segment was similar in C and GF

Table III. Weight of ileal control and anastomotic segments

	day	n	per-operative control segment	post-operative anastomotic segment	anastomotic segment control segment
C rats	2	10	13.4 ± 2.9	18.7 ± 3.6	1.46 ± 0.38
	3	10	12.0 ± 4.3	28.5 ± 8.6	2.73 ± 1.46
	7	10	9.6 ± 2.9	35.6 ± 7.0	4.03 ± 1.52
GF rats	2	7	6.6 ± 2.5	15.6 ± 2.4	2.65 ± 0.87
	3	11	6.5 ± 3.0	17.5 ± 5.6	3.03 ± 1.23
	7	7	9.3 ± 1.6	38.3 ± 7.1	4.22 ± 0.98
C2 vs GF2			p=0.0012	p=0.0185	p=0.0038
C3 vs GF3			p=0.0146	p=0.0083	n.s.
C7 vs GF7			n.s.	n.s.	n.s.

Results are expressed as average weight ± SD (mg) per cm segment. Differences between GF and C (Control rats day 2 = C2 id.) rats are tested for significance by means of a Wilcoxon two-sided unpaired test. n.s.= not significant.

Table IV. Weight of colonic control and anastomotic segments

	day	n	per-operative control segment	post-operative anastomotic segm.	anastomotic segm. control segment
C rats	2	10	10.2 ± 3.8	22.3 ± 6.3	2.22 ± 0.99
	3	10	10.0 ± 3.0	25.8 ± 6.9	2.69 ± 0.70
	7	10	7.9 ± 1.5	20.4 ± 4.4	2.69 ± 0.87
GF rats	2	7	8.0 ± 1.4	18.9 ± 3.8	2.42 ± 0.57
	3	11	7.5 ± 2.5	19.1 ± 5.1	2.69 ± 0.78
	7	7	8.1 ± 2.0	22.7 ± 3.5	2.88 ± 0.62
C2 vs GF2			n.s.	n.s.	n.s.
C3 vs GF3			p=0.0699	p=0.0317	n.s.
C7 vs GF7			n.s.	n.s.	n.s.

Results are expressed as average weight ± SD (mg) per cm segment. Differences between GF and C (Control rats day 2 = C2 id.) rats are tested for significance by means of a Wilcoxon two-sided unpaired test. n.s.= not significant.

rats, exept in ileum 2 days after operation. Likewise, there were no significant differences in weight change of the proximal segment in GF and C rats (results not shown), except - again - in the ileum after 2 days, where the weight gain was significant higher in the GF rats.

Tabel V. Protein concentrations in control and anastomotic segments 2 and 3 days after operation of the colon

		n	control segment	anastomotic segment	anastomosis / control segment
day 2:	C	9	695 ± 80	686 ± 54	0.99 ± 0.07
	GF	7	719 ± 51	666 ± 37	0.93 ± 0.06
day 3:	C	10	686 ± 45	666 ± 41	0.97 ± 0.07
	GF	11	691 ± 78	675 ± 36	0.99 ± 0.11

Results are expressed as average value (µg/mg dry weight) ± SD.

Finally, total protein concentrations were measured in those segments where the reduction in hydroxyproline levels was most pronounced (i.e. in the colon anastomoses 2 and 3 days after operation). Table V shows that no significant decrease of protein concentration was found in any of the groups investigated.

Discussion

The negative influence of micro-organisms on anastomotic healing in the large bowel has often been suggested [14-15]. Indeed, bowel preparation with antibiotics and irrigation appears to be beneficial to healing of experimental and human colonic anastomoses [16-20]. The mechanism whereby intraluminal bacteria affect wound healing remains a matter of speculation. One way could be by interfering with anastomotic collagen metabolism. Therefore, it is of interest to investigate the behaviour of collagen concentrations in animals whose digestive tract is completely free from bacterial contamination. In the present study, opera-

tions on germ-free rats kept in a horizontal laminar flow workstation yielded a relatively high contamination rate (44%) but provided enough uncontaminated rats for analysis.

The importance of collagen in maintaining and restoring the structural integrity of the intestinal wall seems undisputed. The concept that the "collagenous equilibrium" is crucial for undisturbed healing is widely accepted [8]. Both collagen degradation and collagen synthesis are inherent to the healing sequence. Investigations into the healing process of intestinal anastomoses frequently comprise measurements of hydroxyproline concentration (per mg dry weight) or hydroxyproline content (per cm). Neither of the two allows quantitative conclusions regarding the separate processes of collagen degradation and synthesis, as a given level at a certain time only reflects the net result. However, the post-operative decrease in hydroxyproline concentration is taken to support the assumption that collagen degradation occurs. Comparison of the course of hydroxyproline concentrations during the first post-operative days in various groups of animals is meaningful if it can be shown that eventual differences are the result of changes in collagen content, rather than changes in total dry weight. Thus, if we want to compare changes in hydroxyproline concentrations around anastomoses in C and GF rats, it appears pertinent to show that the development of the increase in dry weight in the various segments is similar in both types of rat. This is shown in tables III and IV for the anastomotic segment, where the events, obviously, must have the most direct effect on wound strength. While the absolute weight of segments from uninjured intestine from GF rats was smaller than that from C rats, it can be seen that, with one exception in the ileum, the relative increase in anastomotic weight was identical in both species. Therefore, differences in hydroxyproline concentrations may be attributed to differences in collagen metabolism. In C rats, the post-operative decrease in hydroxyproline concentration was most pronounced in the colonic anastomosis. Total protein did not change. In GF rats, reduction of hydroxyproline levels still occured but was consistently and significantly less pronounced. The same difference between C and GF rats was found in the proximal segment, particularly after 3 days. These results are taken as evidence that in the colon bacteria contribute to the post-operative degradation of collagen in the anastomotic area. This seems not to be the case in the ileum. Here, the increase in

hydroxyproline observed after 7 days in C rats was significantly reduced in GF rats. This could mean that bacteria also affect, in a negative way, collagen synthesis.

As a result of the absence of exposure to bacteria, GF rats differ in many respects from normal Wistar rats [21,22]: their cardiac output is 30% lower, the regional blood flow in many organs is reduced and they do not possess a stimulated immune-response system. Differences are also found in the intestinal tract: the small intestine appears somewhat thin walled (cf. table III), the caecum is much larger and the large bowel content is more liquid than in conventional experimental rats. Despite these differences, it is our contention that our data support the hypothesis that colonic flora may be detrimental to colonic healing by affecting collagen metabolism. One result of construction of an anastomosis seems to be increased collagenolytic activity [23]. As bacteria contain collagenase activity they might directly enhance post-operative collagen breakdown [9]. Alternatively, their presence could strengthen the inflammatory response and thus the activity and enzyme production of granulocytes, which are also a rich source of collagenolytic activity [24]. Collagen synthesis by fibroblasts also is an integral part of the healing sequence. Bacteria could also interfere with anastomotic healing by parasitizing available oxygen from tissue fibroblasts and thus decreasing their synthesizing capabilities [25].

The present results allow no conclusions about which of these possibilities are true. They only support the idea that bacteria do indeed affect post-operative collagen levels in the intestinal wall. Further confirmation of their role should come from studies of the effect of bacteria on the separate processes of collagen degradation and synthesis.

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Although intestinal anastomoses have been constructed in man for more than a century, there still exist conflicting opinions regarding the question which factors actually influence the healing process. Despite the fact that progression in medical science has reduced the frequency of post-operative complications, the occurrence of dehiscence of intestinal anstomoses is still no rarity in the surgical department. When anastomotic leakage leads to clinical symptoms, it is associated with a mortality rate of approximately 30%. In order to prevent this complication, it is necessary to understand the pathophysiological principles of anastomotic healing, and it is essential to know which factors can affect this process. However, little is known about these mechanisms and therefore both clinical and experimental investigations on this subject should be encouraged.

In **chapter I** a review of the literature concerning the factors which may influence anastomotic healing is given. It is often thought that factors, which have been proven to adversely affect healing of skin wounds, also have a detrimental effect on intestinal wounds. However, the healing process of skin wounds has been shown to differ in several respects from that in other tissues, so that phenomena observed in the healing skin may not be extrapolated to the healing of intestinal anastomoses. In clinical studies only a few factors have been proven to affect the healing process of intestinal wounds. One of the most important factors is the individual surgeon who performs the operation. The percentage of colonic anastomotic dehiscence varies between surgeons from less than 1 to more than 30. Surgical experience, type of suture material and technique do not influence the outcome. Other established risk-factors are: an age over 60 years, the presence of radiation enteritis, generalized peritonitis or shock and the administration of cytostatics.

Several parameters are used to quantify healing of bowel anastomoses. At this moment, the rat is the animal most frequently used for experimental studies on intestinal wounds. In the present study we also used this animal. However, since in rats the occurrence of anastomotic leakage is rare, this is no useful parameter

for the healing process. In most of the studies, mechanical strength or collagen levels are measured. Several methods have been described to measure these parameters, but they all have their limitations disadvantages, which are further explained in **chapter II.**

The presence of collagen is essential for the strength of both the intact and the anastomosed bowel. This protein is mainly located in the submucosal layer of the bowel wall. After anastomotic construction, degradation and synthesis of collagen occur near the wound area, resulting in a tranasient decrease of the anastomotic collagen concentration, since lysis initially is greater than synthesis. The decrease is maximal around the third post-operative day. Degradation of collagen in colon is greater than in ileum, while restoration of pre-operative levels occurs more rapidly in ileum. Since collagen metabolism is probably regulated to a large extent by inflammatory cells, factors influencing the inflammation, e.g. antiflogistics, might affect anastomotic healing.
We have studied the influence of one steroidal and four non-steroidal anti-inflammatory drugs (NSAIDs) on ileal and colonic anastomoses in rats during the first post-operative week.

The study on the effect of the four NSAIDs, piroxicam, aspirin, ibuprofen and indomethacin, on the collagen changes around 3 and 7 days old ileal and colonic anstomoses is described in **chapter III.** Both mortality- and infection-rate were increased in the NSAID groups. Overall, administration of NSAIDs reduced the decrease of the hydroxyproline concentration around colonic anastomoses, which is, at least partly, the result of collagen lysis. This effect was significant in rats who received piroxicam and nearly significant in animals treated with ibuprofen. In ileum, where 7 days after anastomotic construction hydroxyproline concentrations significantly exceeded the pre-operative level, this increase was reduced by administration of piroxicam, aspirin and ibuprofen.
NSAIDs differ from each other in the degree to which they inhibit chemotaxis and functions of inflammatory cells, like polymorphonuclear granulocytes and macrophages. Both macrophages and polymorphonuclear granulocytes contain collagenases. Thus, inhibition of the cellular activity by the different NSAIDs may be the cause of the reduced collagen lysis in 3 days old colonic

anastomoses. Collagen is primarily synthesized by fibroblasts. Proliferation of these cells is controlled by macrophages. Inhibition of macrophage functions might indirectly result in a decrease of the collagen synthesis, which phenomenon was observed around 7 days old ileal anastomoses of rats treated with NSAIDs.

Chapter IV reports on a histological pilot-study on the influence of the four NSAIDs on healing of ileal and colonic anastomoses. Three, 12, 24 hours, and 2, 3 and 7 days after operation the anastomoses were examined semiquantitatively on the presence of polymorphonuclear granulocytes, macrophages, fibroblasts, neovascularisation and necrotic tissue, and on the quality of the approximation and the regeneration of the mucosa.
Three hours after resection, polymorphonuclear granulocytes were observed around nearly all anastomoses. Both macrophages and fibroblasts appeared at an earlier time point in colon than in ileum. In colonic samples, macrophages were first seen 12 hours after operation, and fibroblasts within 24 hours, while in ileum these cells were observed after respectively 24 hours and 2 days.
Administration of NSAIDs appears to reduce the number of polymorphonuclear granulocytes slightly. The relatively small number of data in this pilot-study, which has a descriptive character, does not allow any definite conclusions. For a more detailed histological quantification, we recommend the use of specific staining techniques for identifying certain cell types.

During the first days after operation, anastomotic strength is believed to depend mainly on the sutures, and thus on the structure of the tissue layer in which they are attached. Degradation of submucosal collagen might therefore impair anastomotic strength. Thus, reduction of post-operative collagen lysis, measured as a decrease of the hydroxyproline concentration, could have a positive effect on anastomotic strength. Of the four NSAIDs investigated (chapter III), piroxicam appeared to be the most effective in reducing the decrease of the hydroxyproline concentration around colonic anastomoses; therefore, the effects of this drug were further investigated (**chapter V**).
Piroxicam was given in two doses of 2 mg/kg/day and 10 mg/kg/day respectively. Animals were sacrificed 1, 2, 3 or 7 days after

operation. The higher dose of piroxicam resulted in a 100% lethal peritonitis before the sixth post-operative day.
One and three days after anastomotic construction, both doses of piroxicam significantly reduced the decrease of the hydroxyproline concentration, indicating a reduction of the collagen degradation. However, the increased risk of infection, especially in animals treated with the high dose of piroxicam, suggests that NSAIDs have a suppressive effect on the immunic system.

Another group of agents which inhibit inflammatory processes are corticosteroids. Although pertinent data are scarce, administration of this agent is often suggested to increase the risk of anastomotic failure. In two large clinical studies, no deleterious effect of steroids on ileorectal or colonic anastomoses was found.
We have studied the influence of two doses of methylprednisolone (2.5 and 10 mg/kg/day) on the healing of 3 and 7 days old anastomoses (**chapter VI**). There was a significant increase in the number of abscesses in the ileal anastomoses of both experimental groups. However, administration of steroids did not affect either anastomotic bursting pressure or collagen level. Although collagen metabolism and anastomotic strength remained unaffected by methylprednisolone, its administration increased the risk of anastomotic abscesses, probably as the result of immune suppression.

Since the early days of intestinal anastomotic construction, surgeons have been searching for the ideal suture material. Sutures act as foreign bodies and induce local inflammation. Enhancing the inflammatory reaction around an intestinal anastomosis might impair the healing process, e.g. by an increase in the number of collagenase producing leucocytes. In **chapter VII** we report on the influence of the presence of suture material on the healing of intestinal anastomoses. Other investigators had already proven that uncomplicated healing was possible in experimental colonic anastomoses if all suture material was removed one hour after operation. As a sequel to these studies, we have measured the bursting pressures and the changes in hydroxyproline concentration in 1, 3 and 7 days old ileal and colonic anastomoses, where all suture material was removed 30 minutes after construction . The number of anastomotic abscesses was higher in the experimental group than in the control group, but

this difference was not significant.
The first day after surgery, the bursting pressure of both ileal and colonic segments was significantly lower in the experimental animals, but on the subsequent days there existed no difference with the control rats. The hydroxyproline concentrations did not differ between groups on any of the days studied.
Similar results have been published on intestinal anastomoses constructed with the help of different glues. In these studies the uncomplicated healing of these anastomoses is ascribed to properties of the glues investigated. We suggest that the phenomena described in these studies are not due to the glues applied, but are the result of the natural healing process of the intestine.

One of the causes of the lower incidence of anastomotic failure in small bowel, as compared to the large bowel, is assumed to be the higher concentration of micro-organisms and the presence of more solid faeces in the latter. Clinical studies have shown that pre-operative preparation of the bowel by antibiotics or lavage of faeces reduces the anastomotic failure rate. However, complete sterilization of the bowel will never be accomplished by these measures.
It has been proven to be possible to operate germ-free rats in a laminar flow workstation and to keep the animals sterile thereafter, even when they were left there for more than 7 days.
In order to study the influence of micro-organisms on intestinal anastomotic healing, we have compared the changes in hydroxyproline concentrations around ileal and colonic anastomoses in germ-free rats, which were operated under sterile conditions, to those observed in control animals (**chapter VIII**).
Animals were killed on the second, third or seventh day after operation.
Faecal cultures, sampled at sacrifice showed bacterial contamination in 47% of the germ-free animals, which were consequently excluded from the study. In the remaining rats, the post-operative reduction of the hydroxyproline concentration around the colonic anastomoses was significantly reduced in the germ-free group, indicating a decrease of collagen lysis. However, the increase of the hydroxyproline concentration which is usualy found in in 7 days old ileal anastomoses was significantly reduced in the germ-free animals, which suggests a reduction of

collagen synthesis. Since germ-free animals differ in many respects from normal rats no definite conclusions might be drawn on these observations. However, the present study supports the idea that bacteria have a deleterious influence on the healing of intestinal anastomoses.

The mechanisms which regulate the healing of wounds, particularly those in the intestine, are still incompletely understood. Factors which affect inflammation, a process essential for undisturbed healing, induce a variety of reactions. These reactions may result in changes of the collagen metabolism and thereby in a changed anastomotic strength. Our investigations show that factors which inhibit the inflammatory reaction often result in an unpredictable effect on parameters for anastomotic repair.

Hoewel darmanastomoses reeds meer dan 100 jaar bij de mens worden geconstrueerd, bestaan er nog vele controversen over de vraag welke factoren het genezingsproces ervan kunnen beïnvloeden. Vele ontwikkelingen in de geneeskunde hebben bijgedragen tot een vermindering van de post-operatieve complicaties, maar het optreden van naadlekkage is nog steeds geen zeldzaamheid in de heelkundige kliniek. Indien naadlekkage klinische symptomen veroorzaakt gaat deze complicatie gepaard met een mortaliteit van ongeveer 30%. Om dit te kunnen voorkomen is kennis in de patho-fysiologie van darmnaad genezing, evenals inzicht in factoren die dit proces beïnvloeden, van groot belang. Omdat hierover nog relatief weinig bekend is moeten klinisch en experimenteel onderzoek op dit gebied worden aangemoedigd.

In **hoofdstuk I** wordt een literatuur overzicht gegeven van factoren waarvan verondersteld wordt dat zij een effect hebben op het genezingsproces van darmnaden. Vaak wordt van factoren, die een nadelig effect hebben op de genezing van huidwonden, aangenomen dat zij tevens de kans op lekkage van darmnaden zullen vergroten. Gebleken is echter dat effecten bij wonden van de huid niet automatisch mogen worden geextrapoleerd naar de darm. Het blijkt dat van slechts enkele factoren - door middel van klinische studies - bewezen is dat zij van invloed zijn op het genezingsproces van darmnaden. Een zeer voorname factor is de chirurg die de operatie verricht. Het percentage dehiscenties van colonnaden varieert sterk per operateur: van minder dan 1 tot meer dan 30. Hierbij blijken ervaring, het soort hechtmateriaal en de naadtechniek geen rol te spelen. Aantoonbare risicofactoren voor het genezen van een darmanastomose zijn: een leeftijd boven de 60 jaar, het bestaan van een bestralingsenteritis, een gegeneraliseerde purulente peritonitis of shock en het gebruik van cytostatica.

Er zijn verscheidene parameters bruikbaar om de genezing van darmanastomoses in maat en getal vast te leggen. In de meeste recente experimenten dienaangaande wordt de rat als proefdier gebruikt; dit was ook het geval in ons onderzoek. Bij dit

dier treedt naadlekkage echter zelden op, zodat de frequentie ervan geen geschikt criterium vormt om de kwaliteit van het genezingsproces aan af te meten. In deze studies worden meestal de mechanische sterkte of het collageen gehalte van de naad als maat gebruikt. Voor het bepalen van deze parameters zijn verschillende methodes beschreven, maar deze hebben elk hun eigen nadelen en onzuiverheden, die in **hoofdstuk II** nader worden toegelicht.

Collageen is van eminent belang voor de sterkte van zowel de intacte als de geanastomoseerde darm. De submucosa is de laag welke verantwoordelijk is voor de sterkte en de integriteit van de darmwand. Deze bindweefsellaag bestaat voornamelijk uit collageen; omgekeerd bevindt vrijwel al het collageen zich in de submucosa. Post-operatief vindt rond de darmnaad zowel afbraak als synthese van collageen plaats, resulterend in een tijdelijke afname van de collageen concentratie in het wondgebied omdat in eerste instantie de afbraak het grootst is. Deze afname is maximaal rond de derde dag; nadien krijgt de synthese duidelijk de overhand. De afbraak van collageen in het colon is groter dan in het ileum, terwijl herstel sneller optreedt in het ileum. Doordat het collageenmetabolisme in een wond mede wordt gereguleerd door de ontstekingscellen, zouden factoren die het ontstekingsproces beïnvloeden, zoals antiflogistica, effect kunnen hebben op darmnaadgenezing.
Wij hebben het effect van één steroid en vier niet-steroide antiflogistica (NSAIDs) op ileum- en colonnaden in de eerste post-operatieve week bestudeerd en de resultaten vergeleken met die van controle ratten.

Het onderzoek naar de invloed van een viertal NSAIDs, te weten piroxicam, aspirine, ibuprofen en indomethacine, op het collageen metabolisme van 3 en 7 dagen oude ileum- en colonanastomoses wordt beschreven in **hoofdstuk III**. Zowel de mortaliteit als de frequentie waarmee infectieuze complicaties optraden, waren verhoogd in de NSAID groepen. In het algemeen gesproken, verminderde toediening van deze NSAIDs de daling van de hydroxyproline concentratie rond colonnaden, welke normaal optreedt ten gevolge van collageen afbraak. Dit effect was significant voor ratten die werden behandeld met piroxicam en bijna significant voor met ibuprofen behandelde dieren. In ileumnaden, waar ten gevolge van een sterk toegenomen collageensynthese 7 dagen na operatie de

hydroxyproline concentratie in de controle groep tot ruim boven de pre-operatieve waarde was gestegen, werd deze toename geremd door piroxicam, aspirine en brufen.
NSAIDs onderscheiden zich onderling door de verschillende mate waarin zij de chemotaxis en functies van ontstekingscellen, zoals polymorphonucleaire granulocyten en macrofagen, remmen. Zowel macrofagen als polymorphonucleaire granulocyten bevatten collagenases. Remming van de cellulaire activiteit door de verschillende NSAIDs zou derhalve een rechtstreekse oorzaak kunnen zijn van de verminderde collageen afbraak, gemeten rond de 3 dagen oude colonnaden. De collageensynthese vindt primair plaats in de fibroblasten. Omdat proliferatie van deze cellen onder controle staat van de macrofagen, zal remming van de macrofaagfunctie indirect kunnen leiden tot de verminderde collageensynthese, zoals die werd gezien rond 7 dagen oude ileumnaden van de met NSAIDs behandelde ratten.

Hoofdstuk IV beschrijft een histologische pilot-studie naar de invloed van dezelfde vier NSAIDs op genezing van ileum- en colonnaden. Op 3, 12 en 24 uur, en 2, 3 en 7 dagen na de operatie werden de anastomoses semikwantitatief beoordeeld op de aanwezigheid van polymorphonucleaire granulocyten, macrofagen, fibroblasten, neovascularisatie en necrotisch weefsel, op de kwaliteit van de approximering en op de mate van herstel van de mucosa. Drie uur na de ingreep werden in vrijwel alle groepen segmentkernige granulocyten in de naadpreparaten aangetroffen. Bij vergelijking van ileum- en colonnnaden bleken macrofagen en fibroblasten eerder in het colon te worden waargenomen dan in het ileum. De eerste macrofagen waren in het colon reeds binnen 12 uur na de resectie aanwezig, en fibroblasten binnen 24 uur; voor het ileum waren deze tijdstippen respectievelijk 24 uur en 2 dagen.
Toediening van NSAIDs lijkt de hoeveelheid polymorphonucleaire granulocyten in eerste instantie enigzins te remmen. De effecten zijn echter weinig uitgesproken. Het relatief kleine aantal gegevens in deze pilot-studie, welke veeleer een beschrijvend karakter heeft, laat niet toe dat er definitieve conclusies kunnen worden getrokken. Het verdient aanbeveling om in een vervolgstudie specifieke kleuringen voor de te onderscheiden celtypen te gebruiken, hetgeen het aantonen van relatief kleine kwantitatieve verschillen mogelijk kan maken.

Gedurende de eerste dagen na de operatie wordt de sterkte van de naad bepaald door de hechtingen, welke hun stevigheid ontlenen aan de struktuur van het bindweefsel waarin zij zijn gehecht. Afbraak van collageen kan dus de sterkte van de hechtingen ongunstig beïnvloeden. Beperking van de post-operatieve afbraak van collageen, gemeten aan de daling van de hydroxyproline concentratie rond de naad, zou een positief effect kunnen hebben op de sterkte van de naad.
Omdat piroxicam van de vier gebruikte NSAIDs het grootste - remmende - effect bleek te hebben op de verlaging van de hydroxyproline concentratie rond colonnaden, is de invloed van dit medicament nader bestudeerd (**hoofdstuk V**).
Naast de eerder gebruikte dosering (2 mg/kg/dag) werd een hogere dosering (10 mg/kg/dag) toegediend. De ratten werden opgeofferd 1, 2, 3 of 7 dagen na operatie. De hoge dosering piroxicam leidde tot peritonitis en 100% mortaliteit voor de zesde post-operatieve dag in de groep met de hoge medicatie dosis. Beide doseringen beperkten de verlaging van de hydroxyproline concentratie, gemeten op 1 en 3 dagen na operatie rond colonnaden, in dezelfde -significante- mate, hetgeen wijst op een reductie in de afbraak van collageen.
De verhoogde infectie kans, met name van de dieren die de hoge dosering piroxicam kregen toegediend, doch ook in de experimentele groepen beschreven in de voorgaande hoofdstukken, is een aanwijzing dat NSAIDs een remmend effect hebben op het immuun systeem.

Een andere groep medicamenten welke ontstekingsprocessen remt wordt gevormd door de corticosteroiden. Hoewel gebruik van deze geneesmiddelen vaak als één van de factoren wordt genoemd welke de kans op het optreden van naadlekkage kan vergroten, blijkt hiernaar weinig onderzoek te zijn verricht. In twee grote klinische studies kon geen nadelig effect van steroiden op ileorectale of colonnaden worden aangetoond.
Wij hebben de invloed van methylprednisolon in twee doseringen (2,5 en 10 mg/kg/dag) nagegaan op de genezing van 3 en 7 dagen oude ileum en colon anastomoses (**hoofdstuk VI**). Wanneer de medicatie of het placebo vanaf twee dagen voor de operatie tot aan de dag van opoffering werd toegediend trad een significante toename op van het aantal naadabcessen in het ileum van de beide experimentele groepen. Toediening van methylprednisolon had geen gevolgen voor de bursting pressure of het collageengehalte van

de naden. Hoewel methylprednisolon het collageen metabolisme en de sterkte van de darmnaad niet lijkt te beïnvloeden, vergroot het wel de kans op infectieuze processen rond de naad. Misschien dat hierbij remming van het immuun systeem door het corticosteroid een rol speelt.

Vanaf het moment dat darmnaden worden geconstrueerd is men op zoek naar het ideale hechtmateriaal. Hechtingen werken als een corpus alienum en induceren locaal ontstekingsreacties. Verergering van een ontstekingsreactie rond een darmnaad zou het genezingsproces nadelig kunnen beïnvloeden, bijvoorbeeld door locale toename van het aantal collagenase producerende leucocyten. In **hoofdstuk VII** hebben wij het effect van de aanwezigheid van hechtmateriaal op de genezing van darmnaden nagegaan. Andere onderzoekers hadden reeds aangetoond dat ongecompliceerde genezing mogelijk is van experimentele colonnaden waarin één uur na de operatie al het hechtmateriaal wordt verwijderd. Naar aanleiding hiervan hebben wij de bursting pressure en veranderingen van de hydroxyprolinegehaltes gemeten van 1, 3 en 7 dagen oude ileum- en colonnaden, waaruit een half uur na het anastomoseren alle hechtingen waren verwijderd. Het aantal naadabcessen bleek zowel in het ileum als in het colon groter in de experimentele groep dan in de controle groep, maar dit verschil was niet significant. De eerste dag na de operatie was de bursting pressure van beide naden in de experimentele groep verlaagd, doch in de daaropvolgende dagen bestond geen verschil meer met controle dieren. Op geen der onderzochte dagen werd een verschil tussen de groepen in de veranderingen van de hydroxyproline concentraties gevonden.
Soortgelijke resultaten zijn beschreven over naden die werden geconstrueerd met behulp van verschillende soorten lijmstoffen. In deze studies wordt het gunstige beloop van de naadgenezing toegeschreven aan eigenschappen van deze lijmstoffen. Op grond van ons onderzoek veronderstellen wij dat de resultaten van deze studies niet berusten op eigenschappen van de lijmen, maar een gevolg zijn van het natuurlijke genezingsproces van de naden.

Als oorzaak voor het minder frequent optreden van naadlekkage in de dunne darm ten opzicht van de dikke darm, wordt vaak de hogere concentratie bacteriën en de aanwezigheid van vastere faeces in het colon genoemd. Klinische studies wijzen erop dat het pre-operatief voorbereiden van de darmen door middel van

antibiotica of door schoonspoelen een reductie geven in het aantal naadcomplicaties. Ondanks deze maatregelen kan een volledig steriele darm op deze wijze nooit worden bereikt.
Het blijkt mogelijk te zijn om ratten die zijn opgegroeid onder volledig steriele omstandigheden, in een "laminar flow kast" onder te brengen, te opereren en gedurende meer dan zeven dagen vrij van micro-organismen te houden.
Wij hebben het beloop van de veranderingen van de hydroxyproline concentraties in ileum- en colonnaden vergeleken tussen kiemvrije ratten, die op bovengenoemde wijze werden geopereerd, en controle ratten (**hoofdstuk VIII**). Controle ratten ondergingen dezelfde operatie, doch zonder speciale steriele voorzorgsmaatregelen. De dieren werden opgeofferd op de tweede, derde of zevende dag na de operatie.
Bij het kweken van de faeces verkregen na opoffering, bleek contaminatie te zijn opgetreden bij 47% van de kiemvrije ratten, welke derhalve werden uitgesloten van verder onderzoek. In de overgebleven steriele dieren trad een significante reductie op van de post-operatieve daling van de hydroxyproline concentratie in de colonnaadsegmenten op dag 2 en dag 3. De gebruikelijke toename van de hydroxyproline concentratie in het ileum op de zevende post-operatieve dag was echter significant gereduceerd in de kiemvrije dieren. Omdat kiemvrije ratten in vele opzichten verschillen van normale ratten moet men voorzichtig zijn met het trekken van conclusies. De gegevens uit dit onderzoek steunen wel het idee dat bacteriën het genezingsproces van darmnaden nadelig kunnen beïnvloeden.

Het genezingsproces van wonden, en van darmanastomoses in het bijzonder, herbergt nog vele raadsels. Het beïnvloeden van de ontstekingsreactie, die essentieel is voor de voortgang van de genezing, heeft waarschijnlijk een groot scala van reacties tot gevolg. Deze reacties kunnen resulteren in veranderingen in het collageenmetabolisme en zich manifesteren door veranderingen van de sterkte van de naad. Uit ons onderzoek blijkt echter dat factoren die remmend werken op het ontstekingsproces vaak een onvoorspelbaar effect hebben op de parameters voor de naadgenezing.

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CURRICULUM VITAE

Walter Mastboom werd geboren op 26-10-'57 te Rotterdam. In 1976 behaalde hij aan het Huygens Lyceum te Voorburg het eindexamen Atheneum-B. Aansluitend studeerde hij geneeskunde aan de Rijks Universiteit te Leiden. In de periode 1978-1980 was hij als student-assistent werkzaam op de afdeling Gastro-enterologie (toenmalig hoofd Prof.Dr. A.J.Ch. Haex). Het arts-examen werd afgelegd op 27 februari 1983. Hierna werd de militaire dienstplicht vervuld als arts bij het Korps Commando Troepen te Roosendaal, alwaar hij als "hobbyist" de groene baret behaalde. Gedurende dezelfde periode was hij tevens werkzaam als arts op de eerste hulp afdeling van het ziekenhuis "Lievensberg" te Bergen op Zoom.
Via de landelijke selectieprocedure van de Nederlandse Vereniging van Heelkunde werd op 1 juli 1984 begonnen met de opleiding tot algemeen chirurg in de Heelkundige Kliniek van het St. Radboud Ziekenhuis te Nijmegen (opleiders Prof.Dr. H.H.M. de Boer en Prof.Dr. R.J.A. Goris).

Stellingen

behorende bij het proefschrift

Factors influencing intestinal anastomotic repair
- an experimental study in the rat -

1. Het is onjuist te veronderstellen dat factoren die een ongunstig effect hebben op genezing van huidwonden ook altijd het genezingsproces van darmnaden nadelig beinvloeden.
(*dit proefschrift*)

2. Evaluatie van de genezing van experimentele darmnaden door het meten van de "bursting pressure" is slechts zinvol zolang de perforatie in de naad plaats vindt.
(*dit proefschrift*)

3. Medicamenten die remmend werken op ontstekingsreacties hebben vaak een onvoorspelbaar effect op de parameters voor darmnaadgenezing.
(*dit proefschrift*)

4. Het is mogelijk om kiemvrije ratten te opereren en langer dan 7 dagen kiemvrij te houden in een laminar-flow kast.
(*dit proefschrift*)

5. Voor een aantal factoren, waarvan aangenomen wordt dat zij de genezing van darmnaden nadelige beinvloeden, geldt dat ieder wetenschappelijk bewijs hiertoe ontbreekt.
(*dit proefschrift*)

6. Bij patienten met een mammatumor, welke klinisch en radiologisch suspect maligne is en waarin bij cytologisch onderzoek maligne cellen worden aangetroffen is het verantwoord om zonder een histologische diagnose, een radicale operatie te verrichten.
(*Ned Tijdschr Geneesk 1988, 132: 206-8*)

7. De histologische diagnose "rheumatoide nodulus" gesteld op een subcutane tumor bij kinderen, die geen verschijnselen van een gewrichtsaandoening hebben, heeft klinisch geen betekenis.
(*Arch Dis Childhood 1988, 63: 662-4*)

8. Het optreden van niet-iatrogene darmperforaties bij patienten met een gegeneraliseerde peritonitis, die behandeld worden door het open laten van de buik, dient te worden beschouwd als een complicatie van deze therapie; de etiologie ervan is echter onbekend.
(*Arch Surg 1989, 124: 689-92*)

9. De "minitracheotomie" is een waardevol hulpmiddel ter preventie en bestrijding van post-operatieve pulmonale complicaties ten gevolge van sputumretentie.

10. Het inbrengen van een "minitracheotomiecanule", voor het verrichten van bronchiaal toilet, kan detubatie van geselecteerde patienten op een intensive care afdeling bespoedigen.

11. Het pre-operatief verrichten van CT-scan onderzoek om lymfekliermetastasen van een cardia- of een oesofaguscarcinoom op te sporen heeft geen waarde.

12. Het feit dat in Nederland jaarlijks meer papier wordt "gerecycled" lijkt een logisch gevolg van de aldoor toenemende aantallen reclamefolders die wekelijks de brievenbussen vullen.

13. Indien de Inca-cultuur niet door westerse invloeden te gronde was gericht zouden Peru en Bolivia nu waarschijnlijk geen "ontwikkelingslanden" zijn.

14. Een veel voorkomende vorm van discriminatie in winkels is dat een telefonische bestelling onmiddelijk wordt verwerkt ondanks de lijfelijke aanwezigheid van wachtende clienten.