THE INFLUENCE OF FIBRIN SEALANT ON THE HEALING COLONIC ANASTOMOSIS

an experimental study in rats

DE INVLOED VAN FIBRINE KLEEFSTOF OP DE GENEZING VAN DE COLONNAAD

een experimentele studie bij de rat

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Arie Cornelis van der Ham

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PROMOTIECOMMISSIE:

Promotor:	Prof. Dr. J. Jeekel
Co-promotor:	Dr. W.J. Kort
Overige leden:	Prof. Dr. H.A. Bruining
	Prof. Dr. J.H.P. Wilson
	Dr. Th. Hendriks

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CHAPTER I

THE PROBLEM OF COLONIC WOUND HEALING

1.1 Introduction

Resection and anastomosis of the colon is a very common procedure in abdominal surgery. Following the appendix and the gallbladder, the colon is the most frequently operated organ of the gastrointestinal tract.[1,2] Cancer, inflammatory diseases and trauma of the intestine are the major indications for resection and anastomosis. Leakage of colonic and rectal anastomosis is often seen as a major complication after surgery. The reported incidence of dehiscence varies greatly, with rates ranging from 0 to 51%, depending on the definition of leakage and the nature of the patient population.[3,4,5,6] Anastomotic leakage is associated with a significantly increased mortality and morbidity.[5] The mortality rate of patients with anastomotic dehiscence is very high, up to 25%, and one to two third of the operative mortality is caused by this complication.[7,8] In The Netherlands 6944 patients underwent a partial resection of the colon, and 2108 a resection of the recto-sigmoid, in 1990, according to information on national medical registration (SIG).[2] The clinical lethality of these patients is 6.3%.[1] In a large study on factors contributing to leakage of colonic anastomoses it was calculated by Schrock et al. that 37% of the deaths were due to anastomotic leakage.[3, 4] While considering these figures, it can be calculated that in the Netherlands aproximately 200 persons die each year because of colonic anastomotic leakage. Considering a mortality rate of 20% for patients with anastomotic leakage, it can be estimated that around 1000 persons, each year, suffer from this serious complication in our country.

Anastomotic complications are followed by a considerable increase in hospital stay. In the large multicentre study of Fielding et al. the median duration of hospital stay rose from 25.4 days for patients without anastomotic leakage to 45.7 days for those with leakage respectively.[5] Altogether these facts, illustrate the considerable financial and physical consequences of anastomotic leakage.

1.2 History

An extensive review of the history of the intestinal anastomosis was given by Mol in 1970.[9] Before the 19th century, surgery of the intestine was limited to attempts to close traumatic perforations. The oldest method to close intestinal wounds was described by the Indian physician Sushruta, 800 years before Christ, who used the jaws of ants to hold the

wound margins together. For centuries it was realised that a transverse wound of the intestine was lethal, and if the direction of the wound was longitudinal there was a small chance to survive. After the observation that perforating wounds of the intestine can heal spontaneously in case of adhesion of the opening to a neighbouring organ or the abdominal wall, it was tried to imitate this process. In the 18th century, many methods were described to adhere the wounded intestine to the abdominal wound, e.g. by Palfijn, De la Peyronie and Bell. The first circular intestinal suture in man was performed in 1730 by Ramdohr, by invagination of a gangrenous bowel segment. Through the pioneer work of Travers (1812), Lembert (1826) and Dieffenbach (1826) it became known that careful approximation of the peritoneal coating of the cut intestine provides good healing. The first report of a successful intestinal resection and anastomosis using the suture technique of Lembert, was published by Dieffenbach from Berlin in 1836.[10] Even today the non-penetrating inverting seromuscular stitch named after Lembert is utilized by many surgeons in intestinal suturing. As experience in intestinal suturing grew during the 19th century, it became clear that resection and anastomosis of the colon carried a considerable risk of leakage compared to other parts of the gastrointestinal tract. Successful surgery of the abdomen and its organs has first become possible after the discovery of general anaesthesia and the introduction of antisepsis and asepsis, after Listers publication in 1867. Still in 1884, 10 out of 20 patients who underwent intestinal resection and anastomosis in the clinic of Billroth, died because of intestinal leakage and peritonitis.[11] This led to the introduction of numerous types of sutures, anastomoses in one, two and three layers, and using different suture materials. This growth of techniques caused Bribram to state in 1920: "Im allgemeinen hat wohl jeder Chirurg seine eigene Nahtmethode".[12] Nevertheless the discussion of which type of anastomosis is the best continues up to today. The understanding of the role of colonic bacteria and the introduction of standard mechanical cleansing and the use of systemic or local antibiotics have further improved results of intestinal surgery. The introduction of staplers in recent decades have enabled to construct a safe anastomosis in places difficult to reach with conventional suture techniques. Nowadays, under normal conditions resection and anastomosis carry low risks. Nonetheless, even today conditions may be such that construction of an anastomosis has such a high risk of leakage that a (temporary) diverting stoma should be constructed. Even

today much surgical research is performed to find methods and techniques to improve the safety of colonic anastomoses, also under high-risk circumstances.

1.3.1 Wound healing

To get a better understanding of anastomotic healing it is necessary to explain the general principles of wound healing. Tissue response to injury has been divided into 3 overlapping stages.[13] The initial stage called "**lag phase**" is a period of intense cellular activity including autolysis, phagocytosis and accelerated production of mucopolysacharides. The "**fibroblastic**" or 2nd phase lasts 3 to 10 days after injury. In this proliferative phase, tensile strength of the wound increases. The final stage, "**differentiation**", is characterized by the process of rearrangement and reabsorption of unaligned fibres to develop alignment consistent with stress forces.

During the lag phase 2 processes, coagulation and inflammation are apparent. During this period of 1 to 2 days, the breaking strength of the wound decreases and depends on mechanical support of sutures. After infliction of a wound, after a transient period of vessel constriction, all small vessels dilate and bleeding occurs. Due to activation of platelets by tissue collagen, platelet aggregation occurs and activation of the clotting cascade is initiated and haemostasis is achieved. A major step in this process is the conversion of circulating fibrinogen into an insoluble matrix of fibrin under the influence of thrombin.

The fibrin network acts like a seal to protect a wound from body fluid loss, prevents contamination and provides a substrate material for cell growth; this fibrin network provides the initial wound strength which may be weakened subsequently by enzymatic degradation from inflammatory cells. Fibrin degradation products cause chemotaxis of inflammatory cells and activation of macrophages.

Immediately after the coagulation phase the **inflammatory** phase starts. The duration and intensity of the inflammatory phase depends on the amount of tissue damage and the presence of foreign material. Usually this phase subsides in 3 to 5 days.

Within hours after trauma the wound is invaded by neutrophils to reach their maximum in 48 hours. Primarily the purpose of these neutrophils is to remove cellular debris and injured tissue, by means of tissue degradation and phagocytosis. During this process many

neutrophils eventually are victim of cell lysis, while at the same time the monocytes gradually increase in number to be predominating after 4 to 5 days. The migrated monocytes change into macrophages to serve several essential functions in the healing process. It is now well accepted that the macrophage plays a major regulating role in the healing process: regulation of coagulation and fibrinolysis, elimination of cells, tissue debris and bacteria, and regulation of fibroblast activity by means of macrophage derived growth factor. If macrophages are eliminated by administration of anti macrophage serum, wound healing is severely delayed. If on the other hand prolonged activation is established by endotoxin or bacterial products, prolonged collagen production is reached to end in fibrosis.[14] Macrophage activity and migration is regulated by fibrin deposits, depending on fibrin and thrombin concentration.[15] Besides their phagocytosis activity, stimulated macrophages produce and release factors that stimulate fibroblast and endothelial cell proliferation.

This results in fibroblast infiltration and multiplication, collagen synthesis and neovascularization. This phase is also known as the **fibroblastic or cellular phase**, and by day 10 the cellular population is dominated by fibroblasts. In fact fibroplasia and collagen synthesis starts within 24 hours following trauma. Activated platelets produce "platelet derived growth factor" that stimulates fibroblasts to divide and synthesize collagen.[16]

Fibroblasts appear to use the fibrin network as a scaffolding. Endothelial cells contain a potent plasminogen activator. Thus as fibroblasts advance into the injured area, followed closely by proliferating capillaries, fibrinolysis occurs, destroying the fibrin network. The main function of fibroblasts is to synthesize and secrete collagen molecules.

1.3.2 Collagen

The quantity and quality of collagen in the submucosal layer of the intestinal wall determine the strength of the healing intestine.[17] The importance of inclusion of this layer in sutures of the intestine was first emphasized by Halstedt in 1887; "it is air-tight and watertight, and is the *skin* in which sausage meat is stuffed. It is, moreover, the coat of the intestine from which *catgut* is made".[18] The submucosal layer is almost exclusively composed of collagen fibrils. Collagen is a generic term encompassing 13 types of

molecules. All consist largely of the unique collagen triple helix and in most cases they perform a structural function. The different types of collagen have different properties and localization. Type I collagen is the major structural component of skin, tendon and bone, while type II is restricted to cartilage and type III is present in the skin in association with type I. Type IV is found mainly in the basement membranes. More detailed information about the collagen types can be found in reviews of Prockop *et al.* and Burgeson and Nimni.[19,20]

The intestinal wall contains the collagen types I, III and V. [21,22] The collagen molecule is composed of three polypeptide chains called α chains. The three helical chains are twisted around each other to form a rigid structure, the so called triple helix. The α chains contain about 1000 amino acids, of which every third amino acid is glycine, while about 100 positions are proline and about 100 are hydroxyproline. Because both proline and hydroxyproline are rigid cyclic aminoacids, they limit the rotation of the polypeptide backbone and thus contribute to the stability of the triple helix. The presence of hydroxyproline is specific for collagen since this amino acid has been found in only a few other proteins in vertebrates: elastin, the C1q subcomponent of the complement system and acetylcholinesterase. The amount of hydroxyproline is taken as a measure of the collagen content of tissues. Although anastomotic hydroxyproline concentration is a generally used biochemical parameter of wound healing in the intestine, this needs some comment. It has been stated that collagen levels may better be expressed as tissue hydroxyproline content, i.e. the amount of hydroxyproline per unit intestinal length, instead of concentration, i.e. amount of hydroxyproline per unit dry weight. Changes in non-collagenous substances in the tissue may influence the outcome of total dry weight and thus the calculated concentrations.[23] The use of collagen content as a parameter has the disadvantage that the length of the colon is rather variable, and especially the anastomotic segment will always contain more tissue per cm than normal intestine. From the above-mentioned it becomes clear that the interpretation of hydroxyproline measurements must be done with care and restraint.

Many studies on collagen metabolism in intestinal anastomoses have been performed using rat models.[24,25,26] Following the construction of an anastomosis in the colon there is a significant decrease in collagen concentration.[27,28,29] This decrease of collagen

content is highest just proximal to the anastomosis and can be detected at a distance of the anastomosis.

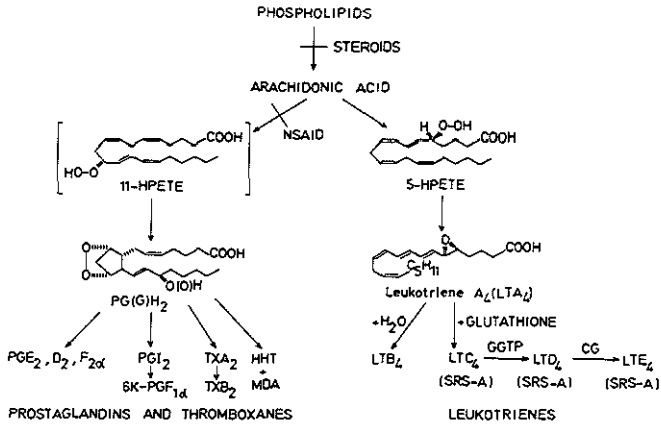
Local degradation of mature collagen might be the cause of cutting through of sutures thus causing breakdown of the anastomosis.[8,30] The suture holding capacity of colonic anastomoses, which is mainly dependent on the connective tissue in the intestinal wall may decrease by as much as 80% during the first 3 postoperative days. Comparison of net amounts of collagen to the rate of synthesis indicates increased breakdown in combination with increased synthesis of collagen. Using tritiated proline Cronin et al. observed that during the first five days destruction of old collagen is taking place in the entire colon with its maximum near the anastomosis, outweighing the formation of new collagen.[31]

Collagen is very resistant to the action of proteolytic enzymes, but breakdown is initiated by a specific enzyme, collagenase. Breakdown of damaged connective tissue or collagen is an essential part of the wound healing mechanism, in the very early phase in order to remove damaged tissue. While remodelling of the connective tissue takes place during weeks to months. The degradation of injured collagen is controlled by a variety of collagenase enzymes from granulocytes, macrophages, epidermal cells and fibroblasts. Besides providing structural support and strength to the new tissue, collagen can have a profound effect on the cells within and on its matrix. Collagen and collagen -derived peptides act as chemoattractants for fibroblasts, and collagen gels can alter the phenotype and functions of epithelial cells, endothelial cells and fibroblasts.[13, 14]

Experimental work has shown a significant increase of collagenolytic activity in the entire gastrointestinal tract after construction of an anastomosis of the colon, accompanied by a reduction of collagen content and a decrease in bursting pressure of the colonic anastomosis.[32,33] The highest collagenase activity in the gastrointestinal tract is found in the colon and possibly the high incidence of anastomotic leakage in the colon is due to the activity of this enzyme. Inhibition of the enzyme might be expected to reduce collagen breakdown and consequently improve the strength of the healing anastomosis. Experiments using protease inhiibitors (e.g. aprotinin), showed a significant increase of bursting pressure and collagen content of colonic anastomoses.[34,35,36] Factors directly influencing the activity of collagenase are bacteria and inflammation around infected anastomoses.[11,37] In addition inflammatory cells are known to produce collagenase.[38]

1.3.3 Eicosanoid mediators in inflammation

As was explained in a previous chapter, the inflammatory response is an integral part of wound healing. Many substances are now recognised as mediators of inflammation: histamine, serotonin, kinins, kallikrein, bradykinin, kallidin and eicosanoids. In the presence of kinins and the complement system, local cells produce a variety of prostaglandins. The prostaglandins appear to be the final mediators of acute inflammation, including the reversible small vessel permeability, and have a chemotactic role for fibroblasts and white cells.[39]



Afbeelding 1 Summary of the arachidonic acid cascade, showing level of interaction of NSAID and corticosteroids.

Eicosanoids are the oxygenated biologically active derivatives of the 20-carbon essential fatty acid arachidonic acid (AA). These include prostaglandins, tromboxane, prostacyclin, leukotrienes and other hydroxy fatty acids (*Figure 1*). These substances are classified as autocoids, i.e., substances which are evanescent and exert their effects in the microenvironment of the tissues where they were generated. Such effects might include regulation of vascular tone and permeability of capillaries and venules, contraction and relaxation of muscle, stimulation or inhibition of platelets, activation of leucocytes.

Eicosanoids are not stored in cells and must always be newly synthesized in response to stimulation. The precursor, AA, is present only in esterified form and must be hydrolysed prior to eicosanoid synthesis. Released AA can be processed in several ways. It may leave

the cell and become available for metabolism by another cell or it may be bound by plasma albumin. Most importantly, AA can be enzymatically oxygenated by cyclooxygenase and/or lipoxygenase into unstable intermediate compounds. In a next step either prostaglandins and tromboxanes (cyclooxygenase) or leukotrienes (lipoxygenase) are formed. Ultimately, the derivatives formed will depend on the type of converting enzymes present in the tissue.

Hydrolysis of esterified arachidonic acid from cellular phospholipid is the first rate-limiting step in the eicosanoid pathway. This step can be blocked by corticosteroids. The cyclooxygenation step can be blocked by nonsteroidal anti-inflammatory drugs (NSAIDs). The lipoxygenase pathway is found mainly in cells involved in the inflammatory response., e.g. platelets, neutrophils, eosinophils and macrophages.

Information on the role of eicosanoids in (intestinal) wound healing is scarce. Inflammatory cells e.g. macrophages are known to produce collagenase after stimulation by bacteria.[40] This collagenase production is dependent on prostaglandin E_2 (PGE_2). Rats treated with daily doses of i.p. PGE_2 showed a significantly weaker anastomosis of the colon on day 3 compared to control rats and this effect was reversed by the addition of indomethacin, a NSAID.[41]

It is commonly accepted that corticosteroids have a negative influence on wound healing, as was shown in experiments on skin healing, however information on the influence on colonic wound healing is scarce. Only few studies on the effect of corticosteroids on the healing colon were published.[42,43,44] Results of these studies are very difficult to compare because different drug regimens were used. However it seems that high doses of corticosteroids interfere with intestinal wound healing, while low doses don't. Even less information is available on the influence of NSAIDs as the effects of these products have rarely been investigated.[41,45] It was concluded by Mastboom et al. that NSAIDs may limit post-operative degradation of collagen in colonic anastomoses, but at the same time may increase susceptibility to surgical infections.

In view of the above it would be of importance to determine the changes of eicosanoid production in the healing colonic wound.

1.4.1 Risk factors in colonic anastomotic healing:

Although the direct cause of anastomotic failure may often be not clear, many factors are known to influence or impair intestinal anastomotic healing. These factors can be divided in systemic and local risk factors. Systemic risk factors are: age,[3, 4] malnutrition[46], malignancy[47], and cancer chemotherapy.[48] Other factors, known to impair wound healing in general, have not been proven to impair intestinal healing: obesity, vitamin A and C deficiency, jaundice, uraemia, diabetes mellitus, and medication with steroids.

Local factors that influence anastomotic healing are: intestinal obstruction,[47] intra-abdominal infection, faecal loading[49] and peritoneal spill[50], trauma to adjacent and remote tissues[51], decreased intestinal blood supply,[7] irradiation of the bowel.[52] Many of the mentioned risk factors are predetermined and are impossible or difficult to be influenced, nonetheless, they must be taken into account perioperatively in the decision taking process. Factors that may have a positive influence perioperatively are bowel preparation, short-term perioperative antibiotics, the choice of suture material, atraumatic surgical technique, good exposure, excellent blood supply to the bowel ends, even approximation of the bowel ends, absence of tension, meticulous haemostasis and avoidance of anastomosis in the presence of peritonitis.

1.4.2 Suture technique and material

After many decades of controversy about what is the best intestinal suture technique, the final answer is not yet given. The specific suture technique of an intestinal anastomosis may be of importance in the healing process. Inverting and everting suture techniques have been discussed in the literature extensively. Most of the reports favour the inverting technique based on both experimental[53,54,55] and clinical evidence.[56] Probably still the most common technique of intestinal suture is the technique described by Connell in 1892 with an inner row of continuous inverting sutures through all layers strengthened by an outer row of interrupted seromuscular sutures.[57] However many authors have advocated the interrupted single layer suture technique and have claimed that this provides the strongest and least obstructive anastomosis,[58,53] while others have promoted the continuous single layer suture and claim it to be safer than interrupted sutures.[6] In a comparative study in the rat interrupted sutures resulted in fewer complications and less collagen degradation

compared to continuous suture.[59] On the other hand Houdart *et al.* did not find any difference between a continuous single-layer suture and single-layer interrupted suturing in the rat colon.[60] The suture material used was a synthetic resorbing monofilament polydioxanone. It is thought that the continuous suture forming a coil while loosely adapting the two cut ends of such an anastomosis will make the anastomosis tighter upon increasing intraluminal pressure without damaging the tissue. Under the same conditions, but with interrupted sutures, stretching of the anastomosis will widen the distance between single-layer sutures, and at times will allow some degree of leakage.

Whereas older suture materials like catgut and silk are still frequently used, nowadays slowly dissolving materials have become available. Modern suture materials used for intestinal anastomoses are strong and retain their strength for an adequate time. In comparison to the quickly dissolving materials, like catgut, the polyglycolic-acid sutures produce little inflammatory reaction.[61] The use of monofilament materials reduces formation of microabscesses around the sutures. In a clinical series of 143 consecutive colonic anastomoses using 4-0 resorbable monofilament suture material not a single case of clinical leakage was found.[6] The final answer on the question which technique and what type of material is the best choice for colonic anastomoses cannot yet be given. From the above mentioned literature on experimental studies I would advise the use of monofilament material in a one-layer interrupted suture. Whether the interrupted suture is superior to a continuous single-layer suture, can only be proven by a prospective randomised clinical trial. Whatever technique or material is being used, the experience and expertise of the operating surgeon are decisive factors in the outcome of an anastomosis. In a large multicentre study it was found that the single most important factor for the outcome of the operation was the surgeon.[5]

1.4.3 Staples and compression device

During the last two decades the use of staplers in intestinal anastomotic surgery has become very fashionable. Claims have been made for a reduced incidence of leakage with the introduction of the anastomotic stapler.[62] Also the use of these instruments would enable the reduction of the number of colostomies necessary[63] and enable lower anastomoses to be made.[64] On the other hand stapled anastomoses have several technical

pitfalls that can cause leakage,[65] and are more than ten times as expensive as sutured anastomoses.[66] In spite of claims based on retrospective studies, a reduced incidence of leakage could not be proven by others in prospective investigations.[66,67]

An interesting new development is the biofragmentable bowel anastomosis ring (BAR) (Valtrac, Davis & Geck, Danbury, Conn.), that was introduced by Hardy *et al.* in 1985.[68] The principle of this device is based on Murphy's button described in 1892.[69] A small segment of bowel tissue is trapped between two opposing rings, that are locked, thus forming a sutureless, atraumatic, inverting intestinal anastomosis. When employed the BAR constructs a secure inverting anastomosis without the use of staples or stitches, except for 2 purse-string sutures. After the wound has healed, the BAR, made from polyglycolic acid and Barium sulphate, fragments and is passed with the faeces. Experiments in dogs have shown that a safe intestinal anastomosis can be constructed in a simple manner.[70] The initial clinical experience with the BAR is promising, and the benefit appears to be a more rapid and easy anastomosis construction.[71,72] Whether the use of this new device can reduce the incidence of anastomotic leakage remains to be seen.

1.4.4 Level of anastomosis

Anastomoses of the small intestine are regarded to have a lower incidence of leakage than those in the colon. Factors held responsible for this are the high bacterial content of the colon and difference in blood flow.[73] However the literature on this subject is conflicting, for instance Shikata *et al.* found no differences between local blood flow in the ileum and colon, as measured on either the mesenteric, middle or antimesenteric side.[74] Anastomoses of the rectum have a bad reputation for anastomotic disruption. Generally anastomoses in the rectum are reported to leak more frequently than more proximal parts of the colon. Goligher *et al.* reported in 1970 an incidence of radiologic leakage of 40% for high- and 69% for low anterior resection.[3] Poor blood supply and exclusion from the peritoneal cavity are factors thought to contribute to leakage of low rectal anastomoses. Hawley postulated that the high incidence of leakage from extraperitoneal anastomoses is the result of increased occurrence of infection around the rectal anastomosis. Bacteria released into the large deadspace are confined around the rectum and not dispersed throughout the

peritoneal cavity.[30] Another complicating factor is the difficulty of the technique of constructing a rectal anastomosis deep in the pelvis.

1.4.5 Infection and inflammation.

The bacterial content of the colon is greater than any other organ in the body, and during intestinal surgery there will certainly be bacterial contamination. Moreover, many operations are undertaken because of diverticulitis or peritonitis due to perforation. The peritoneal cavity has a great ability to clear bacteria. Halstedt stated already in 1887: "The absorbing power of the peritoneal surfaces is very great and, under favourable circumstances, pyogenic substances are quickly absorbed from the peritoneal cavity without causing suppurative inflammation".

Bacteria injected in the peritoneal cavity do not cause abscess formation or any other signs of local inflammation, furthermore wound healing of the colon in the rats is not affected by peritonitis after intra-peritoneal injection of live E. coli.[75]

The addition of foreign material, however, results consistently in abscess formation.[76] Deposition of powdered autoclaved rat faeces around the completed anastomosis is an established model of an infected anastomosis.[3,77] In the presence of local infection anastomoses demonstrate reduced strength.[7,37,75] This reduction correlates with a reduction of collagen content of the anastomotic zone. However 10 days postoperatively this reduction has disappeared and values of strength and collagen content are comparable to controls again. This indicates that local infection delays anastomotic healing rather than preventing it.

1.5.1 Protection of colonic anastomoses

From the early days of intestinal surgery in the 19th century, surgeons have tried to find means of protecting intestinal anastomoses and prevent leakage. An extensive historical review on this matter was given in the thesis of Mol.[9] The most important aspects are presented in the following paragraphs. Fibrin sealant in relation with anastomotic healing is dealt with in the Chapter II.

1.5.2 Bowel preparation

The object of bowel preparation in colorectal surgery is to empty the colon and rectum of faecal material to prevent faecal impaction proximal to the anastomosis and to avoid the risk of spilling faeces into the peritoneal cavity. If a resection with primary anastomosis is performed when faecal stagnation is present, e.g. in the emergency situation the risk of anastomotic leakage is very high.[3,4,47] The effect of faecal loading on the healing colonic anastomosis has been studied in the rat.[49] Anastomotic dehiscence occurred significantly more often when the bowel was loaded with faeces at the time of operation than when it was empty. Suture line cultures bore no relationship to the outcome of the anastomosis, in this animal model.

Conventional mechanical cleansing consists of a 3-5 day regimen of low-residue diet, purgatives and enemas. An alternative for this time consuming method has become the whole gut irrigation with large amounts of irrigation fluids administered via a nasogastric tube. The earliest attempts at colonic lavage with saline were associated with problems and potential risks due to sodium and water retention, also the use of a nasogastric tube was a serious inconvenience. These drawbacks could be avoided by the use of osmotic cathartics and the use of oral mannitol solution. During recent years the use of electrolyte-polyethylene glycol solution has become the standard regimen for mechanical bowel preparation for elective colonic surgery.

1.5.3 Antibiotics

The benefit of perioperative use of prophylactic antibiotics in reducing anastomotic leakage is not certain. Some studies show no benefit[5], while other studies claim beneficial effect of antibiotics on the healing anastomoses. In a review of the literature, overall anastomotic dehiscence was reported to be 9.5% when no antibiotics were given versus 4.5% with antibiotic prophylaxis.[78] In elective surgery the antibiotics can be given parenterally or orally. Preoperative decontamination of the bowel with antibiotics significantly reduces the incidence of anastomotic dehiscence.[79] Animal experiments have shown that antibiotics are extremely valuable in case of anastomotic ischemia.[80]

Irrespective of the effect on anastomotic healing, perioperative antibiotic prophylaxis is generally advised in order to prevent wound infection.

1.5.4 Colostomy

Staged management of colorectal lesions has gained wide acceptance. Most frequently used in emergences, e.g. left colonic obstruction, it has also been recommended in the elective treatment of many left sided lesions with a high risk of anastomotic complications.[81] Goligher recommended a protective colostomy as a routine in all low anterior resections.[82] In a randomised prospective study a protective transverse colostomy was found not to reduce the incidence of radiologic anastomotic leakage following low anterior resection and anastomosis with the EEA stapler.[83] However, clinical leakage was noted in 4% in the colostomy group and 12% in the noncolostomy group. On the other hand protective colostomy was followed by stenosis in 9 instances, compared with only 2 in the noncolostomy group.

Furthermore it must be considered that closure of a protective colostomy implies an extra operation with considerable morbidity and mortality.[84,85]

1.5.5 External cover

Some authors have described the clinical use of the pedicled omentum for protection of gastrointestinal anastomoses, particularly after rectal surgery.[86,87] From experimental studies it is known that the omentum pedicle wrap provides effective protection for a compromised intestinal anastomosis. It provides a circumferential tissue plug to prevent early leakage, and acts as a source of granulation tissue and neovasculature for later wound repair.[88] Devascularized omentum and other tissues have been previously studied for their potential to protect anastomoses. It was found that the devascularized omentum is not of benefit and actually leads to a worse anastomotic outcome.[89]

For many years investigators have searched for artificial material to 'protect' or reinforce colonic anastomoses.

Several products were used as a cover on experimental anastomoses: polyurethane foam[90], silastic gauze[91], plastic sheet[92], gelatine sponges or oxidized cellulose[93], dacron velours, marlex[89], polyglycolic acid mesh[94] and fibrin sealant. Most experiments, except for pedicled omentum and fibrin sealant, resulted in a high incidence of anastomotic disruption and peritonitis. One might conclude from these experiments that isolation of the suture line from the peritoneal surface results in impaired healing. These

findings are in accordance with the clinical finding that extraperitoneal low anterior rectal anastomoses show more disruptions of the suture line.

Additional sealing with fibrin adhesive has been advocated in normal and high-risk colonic anastomoses in human as a method to prevent anastomotic leakage.[95,96,97,98,99] This recommendation however is not supported by prospective clinical trials. Also the results of experimental studies on sealing of colonic anastomoses with fibrin sealant are conflicting, and this subject needs further investigation. This topic is elaborated in Chapter II.

1.5.6 Internal cover

Recently much attention was given to the use of intraluminal cover in protecting colonic anastomosis. In an experimental study it was shown that it is important that a tube is inside rather than outside the anastomosis.[100] This last experiment supports the idea that siting of foreign material outside an intestinal anastomosis increases the incidence of anastomotic leakage. It was suggested that the improved results of this method resulted from the tube preventing fecal contamination or fecal disruption of the anastomosis. Ravo has developed a latex tube, "Coloshield^{R}", which is positioned intraluminally and passes the anastomosis. Both experimentally and clinically good results were reported in high-risk anastomoses with this method, with few complications reported.[101,102] Although recently a case was reported of a complication of an intracolonic bypass that was found to have eroded through the colon in the early postoperative period.[103]

1.5.7 Drains

Drainage as a prophylactic measure, in the field of colonic surgery, is applied with varying conviction. It is said that drainage of the anastomotic area may avoid a peri-anastomotic haematoma or fluid collection and that these factors predispose to dehiscence. A second function of a drain is to lead faeces to the surface in case of anastomotic leakage. The clinical use of drains is not supported by experimental data. In a comparative study in rats on Latex, silicone rubber, polyvinylchloride and polytetrafluoroethylene tubes it was found that latex caused increased anastomotic leakage, while the other products were not found to interfere, but did not improve healing.[104] Earlier Crowson and Wilson had come to the conclusion that a colonic anastomosis heals best when bacterial

contamination is minimal, the anastomosis is not drained, and the anastomosis is intraperitoneally.[105] Other reports have mentioned the deleterious influence of peritoneal drainage on colonic healing.[106,107] Clinically, it was also found that drainage of intraperitoneal anastomoses is associated with increased morbidity and mortality.[108] In a prospective study in which 49 patients were randomized to have a silastic drain placed next to the anastomosis, no differences were found compared to 57 patients receiving no drain.[109] Drains may promote anastomotic leakage by preventing the formation of adhesions between the omentum and ischaemic areas of the anastomosis.

The expression of Tait, "when in doubt, drain!", is not supported by experimental and clinical data.

1.5.8 Collagenase inhibition

In anastomotic wound healing there is a significant decrease in anastomotic strength associated with reduction in collagen concentration. Collagen is lysed by a specific enzyme, collagenase (See also paragraph 1.3.2). Collagenase activity varies throughout the gastrointestinal tract. Experimental work has shown that in the gastrointestinal tract the level of activity of collagenase is lowest in the stomach and greatest in the distal colon.[29] The activity of collagenase in colonic mucosa could be effectively inhibited by aprotinin, a nonspecific proteinase inhibitor, and soy- and lima bean trypsin inhibitor.[110] It was also demonstrated that aprotinin, limits collagen degradation, ensuing increase of bursting wall tension and collagen concentration of experimental colonic anastomoses.[34,111,112] Also in prospective randomized clinical trials of aprotinin the clinical and radiologic leakage rate of colonic anastomoses was reduced.[113,114] The clinical value is not proven because the difference did not reach levels of statistical significance.

1.5.9 Fibrin sealant

Fibrin sealant is a multi-component biologic adhesive composed of concentrated human fibrinogen which can be used to establish hemostasis or as an adhesive in wound-repair. Several studies have shown that fibrin sealant contributes favourably to wound healing by producing local hemostasis and by stimulating the influx of macrophages that produce factors causing angiogenesis, fibroblast proliferation and collagen production.[115,116,117]

Furthermore, fibrin sealant contains the protease inhibitor aprotinin in order to reduce fibrin degradation. This aprotinin might inhibit the increased collagenase activity during the early phase of wound healing. It has been shown in the animal model that by sealing an intestinal anastomosis, bacteria can be confined intra-luminally thus reducing peri-anastomotic inflammation.[118] Additional sealing has been advocated in normal and high-risk colonic anastomosis in humans as a method to prevent anastomotic leakage.[99,119,120,121,122] According to the aforementioned studies this product is frequently used in clinical practice, in some European countries. However, experimental studies are conflicting and prospective randomised clinical studies are lacking.[42,123] More detailed information on the characteristics of fibrin sealant, and its use in surgery is given in Chapter II.

1.6 Concluding remarks

Colonic anastomoses are still a problem in abdominal surgery. High-risk factors, such as peritonitis, ischemia, emergency surgery, and bowel obstruction, may endanger colonic anastomotic healing. In a number of cases the operating surgeon may decide to construct a protecting diverting stoma, in order to protect the anastomosis. Closure of these temporary stomata have its own morbidity and mortality rate.

There is still a need of better methods to protect colonic anastomoses. Because the ideal way of construction of an intestinal anastomosis, is still the subject of debate, and a large amount of techniques and materials is being used. In many ways, it has been tried experimentally to protect the anastomosis, however few methods were found to be beneficial. Although already used in clinical practice, the effect of a 'protecting' fibrin seal on the colonic anastomosis needs further study, because experimental results are not consonant.

1.7 References

1. Compendium of health statistics of the Netherlands 1986. Staatsuitgeverij, 's-Gravenhage 1986: 267.
2. Jaarboek ziekenhuizen 1990; kliniek en dagverpleging. SIG informatiecentrum voor de gezondheidszorg, Utrecht, Amsterdam 1991.
3. Goligher JC, Graham NG, De Dombal FT. Anastomotic dehiscence after anterior resection of rectum and sigmoid. Br J Surg 1970;57:109-118.
4. Schrock TR, Deveney CW, Dunphy JE. Factors contributing to leakage of colonic anastomoses. Ann Surg 1973;177:513-518.
5. Fielding LP, Stewart-Brown S, Blesovsky L, Kearny G. Anastomotic integrity after operations for large-bowel cancer: a multicentre study. Br Med J 1980;281:411-414.
6. Harder F, Vogelbach P. Single-layer end-on continuous suture of colonic anastomoses. Am J Surg 1988;155:611-614.
7. Irvin TT, Goligher JC. Actiology of disruption of intestinal anastomoses. Br J Surg 1973;60:461-464.
8. Mileski WJ, Joehl RJ, Rege RV, Nahrwold DL. Treatment of anastomotic leakage following low anterior colon resection. Arch Surg 1988;123:968-971.
9. Mol PCM. Over de darmnaad. Thesis, Nijmegen 1972.
10. Dieffenbach JF. Glückliche Heilung nach Ausschneidung eines Teiles des Darms und Netzes. Wsch Ges Heilk 1836;26:401-413.
11. Hauer E. Darmresektion und Enterorraphien. Z Heilk 1884;5:83-108.
12. Pribram BO. Die innere fortlaufende Einstülpungsnaht. Zbl Chir 1920;47:422-424.
13. Clark RAF. Overview and general considerations of wound repair. In: The molecular and cellular biology of wound repair. RAF Clark, PM Henson (ed).Plenum press, New York 1988, 3-33.
14. Hunt TK, Knighton DR, Thackral KK, Goodson WH, Andres WS. Studies on inflammation and wound healing: angiogenesis and collagen synthesis stimulated in vivo by resident and activated wound macrophages. Surgery 1984;86:48-54.
15. Ciano PS, Colvin RB, Dvorak AM, McDonagh J, Dvorak HF. Macrophage migration in fibrin gel matrices. Lab Invest 1986;54:62-70.
16. Knighton DR, Hunt TK, Thakral KK, Goodson WH. Role of platelets and fibrin in the healing sequence. An in vivo study of angiogenesis and collagen synthesis. Ann Surg 1982;196:379-388.
17. Gottrup F. Healing of incisional wounds in stomach and duodenum: collagen distribution and relation to mechanical strength. Am J Surg 1981;141:222-227.
18. Halsted WS. Circular suture of the intestine - an experimental study. Am J Med Sci 1887;94:436-461.
19. Prockop DJ, Kivirikko KI, Tuderman L, Guzman NA. The biosynthesis of collagen and its disorders. N Engl J Med 1979;301:13-23.
20. Burgeson RE, Nimni ME. Collagen types; Molecular structure and tissue distribution. Clin Orth 1992;282:250-272.

21. d'Ardenne AJ, Burns J, Sykes BC, Kirkpatrick P. Comparative distribution of fibronectin and type III collagen in normal human tissue. J Pathol 1983;141:55-69.

22. Graham MF, Diegelmann RF, Elson CO, *et al.* Collagen content and types in the intestinal strictures of Crohn's disease. Gastroenterology 1988;94:257-265.

23. Hendriks Th, Mastboom WJB. Healing of experimental anastomoses: parameters of repair. Dis Colon Rectum 1990;33:891-901.

24. Jönsson K, Jiborn H, Zederfeldt B. Breaking strength of small intestinal anastomosis. Am J Surg 1983;145:800-803.

25. Jönsson K, Jiborn H, Zederfeldt B. Changes in collagen content of the small intestinal wall following anastomosis. Am J Surg 1985;150:315-317.

26. Jönsson K, Jiborn H, Zederfeldt B. Collagen metabolism in small intestinal anastomosis. Am J Surg 1987;154:288-291.

27. Wise L, McAlister W, Stein T, Schuck P. Studies on the healing of anastomoses of small and large intestines. Surg Gynecol Obstet 1975;141:190-194.

28. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomoses: III. Collagen metabolism in the colon after left colon resection. Am J Surg 1980;139:398-405.

29. Cronin K, Jackson DS, Dunphy JE. Changing bursting strength and collagen content of the healing colon. Surg Gynecol Obstet 1968;126:747-753.

30. Hawley PR. Causes and prevention of colonic anastomotic breakdown. Dis Col Rect 1973;16:272-277.

31. Cronin K, Jackson DS, Dunphy JE. Specific activity of hydroxyproline-tritium in the healing colon. Surg Gynecol Obstet 1968;126:1061-1065.

32. Hawley PR, Faulk WP, Hunt TK, Dunphy JE. Collagenase activity in the gastrointestinal tract. Br J Surg 1970;170:896-900.

33. Van der Stappen JWJ, Hendriks Th, De Boer HHM, De MAn BM, De Pont JJHHM. Collagenolytic activity in experimental intestinal anastomoses. Int J Colorect Dis 1992;7:95-101.

34. Delaney PV, Lalor D, Fitzgerald P, O'Malley E. The use of 'collagenase inhibitor' in prevention of large bowel anastomotic dehiscence. Br J Surg 1974;61:923.

35. Delaney P, Lalor D. Enzyme inhibition in colorectal surgery. Br J Surg 1976;63:23-24.

36. Högström H, Haglund U, Zederfeldt B. Beneficial effect on intestinal anastomosis of S-2441, a synthetic kallikrein-kinin antagonist: experimental studies in the rat. Am J Surg 1985;150:312-313.

37. Yamakawa T, Patin CS, Sobel S, *et al.* Healing of colonic anastomoses following resection for experimental "diverticulitis" Arch Surg 1971;103:17-20.

38. Ohlsson K. Polymorphonuclear leukocyte collagenase. In: Woolley D, Evanson J, eds. Collagenase in normal and pathologic connective tissue. Chicester, England: Wiley, 1980: 209-222.

39. Marcus AJ. The eicosanoids in biology and medicine. J Lipid Res 1984;25:1511-1516.

40. Wahl LM, Walh SM, Mergenhagen SE, Martin GR. Collagenase production by endotoxin-activated macrophages. Proc Natl Acad Sci USA 1974;71:3598-3601.

41. Brennan SS, Foster ME, Morgan A, Leaper DJ. Prostaglandins in colonic anastomotic healing. Dis Col Rect 1984;27:723-725.

42. Houston KA, Rotstein OD. Fibrin sealant in high-risk anastomoses. Arch Surg 1988;123:230-234.

43. Aszodi A, Ponsky JL. Effects of corticosteroid on the healing bowel anastomosis. Am Surg 1984;50:546-548.

44. Mastboom WJB, Hendriks T, de Man BM, de Boer HHM. Influence of methylprednisolone on the healing of intestinal anastomoses in rats. Br J Surg 1991;78:54-56.

45. Mastboom WJB, Hendriks T, van Eiteren P, de Boer HHM. The influence of NSAIDs on experimental intestinal anastomosis. Dis Col Rect 1991;34:236-243.

46. Ward MWN, Danzi M, Lewin MR, Rennie MJ, Clark CG. The effects of subclinical malnutrition and refeeding on the healing of experimental colonic anastomoses. Br J Surg 1982;69:308-310.

47. Jex RK, van Heerden JA, Wolff BG, Ready RL, Ilstrup MS. Gastrointestinal anastomoses; factors affecting early complications. Ann Surg 1987;206:138-141.

48. Ferrara JJ, Martin EW, Carey LC. Morbidity of emergency operations in patients with metastatic cancer receiving chemotherapy. Surgery 1982;92:605-609.

49. Smith SR, Connolly JC, Gilmore OJ. The effect of faecal loading on colonic anastomotic healing. Br J Surg 1983;70:49-50.

50. Hawley PJ, Hunt TK, Dunphy JE. Aetiology of colonic anastomotic leaks. Proc R Soc Med 1970;63:28-30.

51. Irvin TT, Hunt TK. The effect of trauma on colonic healing. Br J Surg 1974;61:436-438.

52. Stevens KR Jr, Fletcher WS, Allen CV. Anterior resection and primary anastomosis following high dose preoperative irradiation for adenocarcinoma of the recto-sigmoid. Cancer 1978;41:2065-2071.

53. Loeb MJ. Comparative strength of inverted, everted and end-on intestinal anastomoses. Surg Gynecol Obstet 1967;125:301-304.

54. Hamilton JE. Reappraisal of open intestinal anastomoses. Ann Surg 1967;165:917-924.

55. Trueblood HW, Nelsen TS, Kohatsu S, Oberhelman HA Jr. Wound healing in the colon: comparison of inverted and everted closures. Surgery 1969;65:919-930.

56. Goligher JC, Morris C, McAdam WAF, de Dommbal FT, Johnston D. A controlled trial of inverting versus everting intestinal suture in clinical large bowel surgery. Br J Surg 1970;57:817-822.

57. Connell ME. An experimental contribution looking to an improved technique in enterorrhaphy, whereby the number of knots is reduced to two or even one. Medical record 1892;42:335-337.

58. Gambee LP, Garnjobst W, Hardwick CE. Ten years' experience with a single layer anastomosis in colon surgery. Am J Surg 1956;92:222-225.

59. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomoses; the effect of suture technic on collagen concentration in the colonic wall. Am J Surg 1978;135:333-340.

60. Houdart R, Lavergne A, Valleur P, *et al.* Vascular evolution of single-layer end-on colonic anastomoses. Dis Colon Rectum 1985;11:97-100.

61. Clark CG, Elmasri S, Harris J, Wyllie JH, Dow J. Polyglycolic-acid sutures and catgut in colonic anastomoses. Lancet 1972;ii:1006-1007.

62. Goligher JC. Recent trends in the practice of sphincter-saving excision for rectal cancer. Ann R Coll Surg Engl 1979;61:169-176.

63. Heald RJ. Towards fewer colostomies - the impact of circular stapling devices on the surgery of rectal cancer in a district hospital. Br J Surg 1980;67:198-200.

64. Brennan SS, Pickford IR, Evans H, *et al.* Staples or sutures for colonic anastomoses - a controlled clinical trial. Br J Surg 1982;69:722-724.

65. Chassin JL, Rifkind KM, Turner JW. Errors and pitfalls in stapling gastrointestinal tract anastomoses. Surg Clin North Am 1984;64:441-459.

66. McGinn FP, Gartell PC, Clifford PC, Brunton FJ. Staples or sutures for low colorectal anastomoses: a prospective randomized trial. Br J Surg 1985;72:603-605.

67. Didolkar MS, Reed WP, Elias EG, Schnaper LA, Brown SD, Chaudhary SM. A prospective randomized study of sutured versus stapled bowel anastomoses in patients with cancer. Cancer 1986;57:456-460.

68. Hardy Jr TG, Pace WG, Maney JW, Katz AR, Kaganov AL. A biofragmentable ring for sutureless bowel anastomosis: an experimental study. Dis Colon Rectum 1985;28:484-490.

69. Murphy JB. Cholecysto-intestinal, gastro-intestinal, entero-intestinal anastomosis, and approximation without sutures (original research). Med Rec 1892;42:665-676.

70. Maney JW, Katz AR, Li LK, Pace WG, Hardy TG. Biofragmentable bowel anastomosis ring: comparative efficacy studies in dogs. Surgery 1988;103:56-62.

71. Hardy TG Jr, Aguilar PS, Stewart WRC, *et al.* Initial clinical experience with a biofragmentable ring for sutureless bowel anastomosis. Dis Colon Rectum 1987;30:55-61.

72. Cahill CJ, Betzler M, Gruwez JA, Jeekel J, Patel JC, Zederfeldt B. Sutureless large bowel anastomosis: European experience with the biofragmentable anastomosis ring. Br J Surg 1989;76:344-347.

73. Shikata J, Shida T. Effects of tension on local blood flow in experimental intestinal anastomoses. J Surg Res 1986;40:105-111.

74. Shikata J, Shida T, Furuya K, Kamiyama A. The effect of local blood flow on the healing of experimental intestinal anastomoses. Surg Gynecol Obstet 1982;154:657-661.

75. Högström H, Jiborn H, Zederfeldt B, Haglund U. Influence of intraperitoneal *Escherichia coli* with septicemia on the healing of colonic anastomoses and skin wounds. Eur Surg Res 1985;17:128-132.

76. Hesp WLEM, Hendriks T, Lubbers EJC, De Boer HHM. A reproducible model for protracted peritonitis in the rabbit. Eur Surg Res 1984;16:317-321.

77. Irvin TT. Collagen metabolism in infected colonic anastomoses. Surg Gynecol Obstet 1976;143:220-224.

78. Kusche J, Stahlknecht CE. Antibioticaprophylaxe bei colorectalen Operationen: gibt es ein Mittel der Wahl? Chirurg 1981;52:577-581.

79. Le Veen HH, Wapnick S, Falk G, Olivas O, Bhat D, Gaudre M, Patel M. Effect of prophylactic antibiotics on colonic healing. Am J Surg 1976;131:47-53.

80. Cohen SR, Cornell CN, Collins MH, Sell JE, Blanc WA, Altman RP. Healing of ischemic colonic anastomoses in the rat: Role of antibiotic preparation. Surgery 1985;97:443-446.

81. Colcock BP. Recent experience in the surgical treatment of diverticulitis. Surg Gynecol Obstet 1965;121:63-69.

82. Goligher JC. Treatment of carcinoma of the rectum. In: Surgery of the anus, rectum and colon. 3rd ed. London: Bailiére Tindall, 1975:639-813.

83. Graffner H, Fredlund P, Olsson SÅ, Oscarson J, Petersson BG. Protective colostomy in low anterior resection of the rectum using the EEA stapling instrument: a randomized study. Dis Colon Rectum 1983;26:87-90.

84. Samhouri F, Grodsinsky C. The morbidity and mortality of colostomy closure. Dis Colon Rectum 1979;22:312-314.

85. Parks SE, Hastings PR, Complications of colostomy closure. Am J Surg 1985;149:672-675.

86. Goldsmith HS. Protection of low rectal anastomosis with intact omentum. Surg Gynecol Obstet 1977;144:584-586.

87. Lanter B, Mason R. Use of omental pedicle graft to protect low anterior colonic anastomosis. Dis Colon Rectum 1979;22:448-451.

88. Adams W, Ctercteko G, Bilous M. Effect of an omental wrap on the healing and vascularity of compromised intestinal anastomoses. Dis Colon Rectum 1992;35:731-738.

89. Gulati SM, Thusoo TK, Kakar A, Iyenger B, Pandey KK. Comparative study of free omental, peritoneal, dacron velour and marlex mesh reinforcement of large bowel anastomosis. Dis Colon Rectum 1982;6:517-521.

90. Trowbridge PE, Howes EL. Reinforcement of colon anastomoses using polyurethane foam treated with neomycin: An experimental study. Am J Surg 1967;113:236-240.

91. Canalis F, Ravitch MM. Study of healing of inverting and everting anastomoses. Surg Gynecol Obstet 1968;126:109-114.

92. Rusca JA, Bornside GH, Cohn I. Everting versus inverting gastrointestinal anastomoses; bacterial leakage and anastomotic disruption. Ann Surg 1969;169:727-735.

93. Laufmann H, Method H. Effects of absorbable foreign substance on bowel anastomosis. Surg Gynecol Obstet 1948;86:669-673.

94. Henne-Bruns H, Kreischer HP, Schmiegelow P, Kremer B. Reinforcement of colon anastomoses with polyglycolic acid mesh: an experimental study. Eur Surg Res 1990;22-224-230.

95. Scheele J, Herzog J, Mühe E. Fibrin glue protection of digestive anastomoses. Zbl Chirurgie 1978;103:1325-1336.

96. Pointner R, Villinger R. Vorbeugung der intraperitonealen Nahtinsuffizienz durch Einsatz von Fibrin kleber. Zbl Chirurgie 1984;109:1146-1148.

97. Athanasiadis S, Kuhlgatz Ch, Girona I. Additional fibrin glueing in surgery of colon and rectum. Zbl Chirurgie 1984;109:1107-1111.

98. Waclawiczek HW, Boeckl O. Clinical experience from fibrin sealing in general and thoracic surgery. Zbl Chirurgie 1986;111:16-24.

99. Giardino R, Brulatti M, Franchini A. Colonic anastomoses protected with fibrin sealant. In: Schlag G, Redl H (eds). Fibrin sealant in operative medecine. General surgery and abdominal surgery - Vol. 6. Springer-Verlag, Heidelberg, Berlin, 1986:155-158.

100. Ross H. The effect of an intraluminal tube used as an internal drain on the healing of the rat colon. Dis Col Rect 1987;30:591-594.

101. Ravo B, Ger R. Intracolonic bypass by an intraluminal tube. An experimental study. Dis Col Rect 1984;27:360-365.

102. Ravo B, Ger R. Temporary colostomy - an outmoded procedure? Dis Col Rect 1985;28:904-907.

103. Egozi L, Sorrento JJ, Golub R, Schultz EH. Complication of the intracolonic bypass; report of a case. Dis Colon Rectum 1993;36:191-193.

104. Smith SRG, Connolly JC, Crane PW, Gilmore JA. The effect of surgical drainage materials on colonic healing. Br. J Surg 1982;69:153-155.

105. Crowson WN, Wilson CS. An experimental study of the effects of drains on colon anastomoses. Am Surg 1973;39:597-601.

106. Manz CW, La Trendesse C, Sako Y. The detrimental effect of drains on colonic anastomoses: an experimental study. Dis Colon Rectum 1970;13:17-25.

107. Berliner SD, Burson LC, Lear PE. Use and abuse of intra-peritoneal drains in colonic surgery. Arch Surg 1964;89:686-690.

108. Berliner GD, Burson LC, Lear PE. Intra-peritoneal drains in surgery of the colon. Am J Surg 1967;113:646-647.

109. Johnson CD, Lamont PM, Orr N, Lennox M. Is a drain necessary after colonic anastomosis? J Roy Soc Med 1989;82:661-664.

110. Lewin MR, Chowcat NL, Jayaraj AP, Boulos PB. Collagenase inhibition in colonic mucosa by proteinase inhibitors. Br J Exp Path 1986;67:523-526.

111. Bary S, Kotmann H, Köpcke W. Berstungsdrucke des enterotomierten Rattencolons unter Proteinaseinhition. Res Exp Med 1976;168:123-128.

112. Young HL, Wheeler MH. Collagenase inhibition in the healing colon. J Roy Soc Med 1983;76:32-36.

113. Young HL, Wheeler MH. Results of a prospective randomized double-blind trial of aprotinin in colonic surgery. World J Surg 1984;8:367-373.

114. Sheridan WG, Shandall AA, Alexander-Williams J, *et al.* A multicentre trial of the use of the proteolytic enzyme inhibitor aprotinin in colorectal surgery. Dis Colon Rectum 1989;32:505-508.

115. Banerjee SK, Glynn LE. Reactions to homologous and heterologous fibrin implants in experimental animals. Ann NY Acad Sci 1960;86:1054-1057.

116. Leibovich SJ, Ross R. A macrophage-dependent factor that stimulates the proliferation of fibroblasts in vitro. Am J Path 1976;84:501-513.

117. Pohl J, Bruhn HD, Christophers E. Thrombin and fibrin-induced growth of fibroblasts: role in wound repair and thrombus organization. Klin Wochenschrift 1979;57:273-277.

118. Petrelli NJ, Cohen HC, DeRisi D, *et al.* The application of tissue adhesives in small-bowel anastomoses. J Surg Oncol 1982;19:59-61.

119. Scheele J, Herzog J, Mühe E. Fibrin glue protection of digestive anastomoses. Zbl Chirurgie 1978;103:1325-1336.

120. Pointner R, Villinger R. Vorbeugung der intraperitonealen Nahtinsuffizienz durch Einsatz von Fibrin kleber. Zbl Chirurgie 1984;109:1146-1148.

121. Athanasiadis S, Kuhlgatz Ch, Girona I. Additional fibrin glueing in surgery of colon and rectum. Zbl Chirurgie 1984;109:1107-1111.

122. Waclawiczek HW, Boeckl O. Clinical experience from fibrin sealing in general and thoracic surgery. Zbl Chirurgie 1986;111:16-24.

123. Nordkild P, Hjortrup A, Kjærgaard J. Tissue adhesives and intestinal anastomosis. Ann Chir Gyn 1986;75:205-208.

CHAPTER II

FIBRIN SEALANT

2.1 General aspects

The therapeutic use of fibrin sealant or fibrin glue is based on the last stage of blood coagulation in which fibrinogen is converted to fibrin due to thrombin. Fibrin sealant is a multi-component biologic adhesive made of concentrated human fibrinogen which can be used to achieve haemostasis or as an adhesive in wound-repair. The product is commercially available under the trade names Tissucol^{R} (Immuno AG, Vienna, Austria) and Beriplast ^{R}(Behring AG, Marburg, BRD), or can be made locally.[1] The commercial fibrin sealant includes 2 lyophilized components:

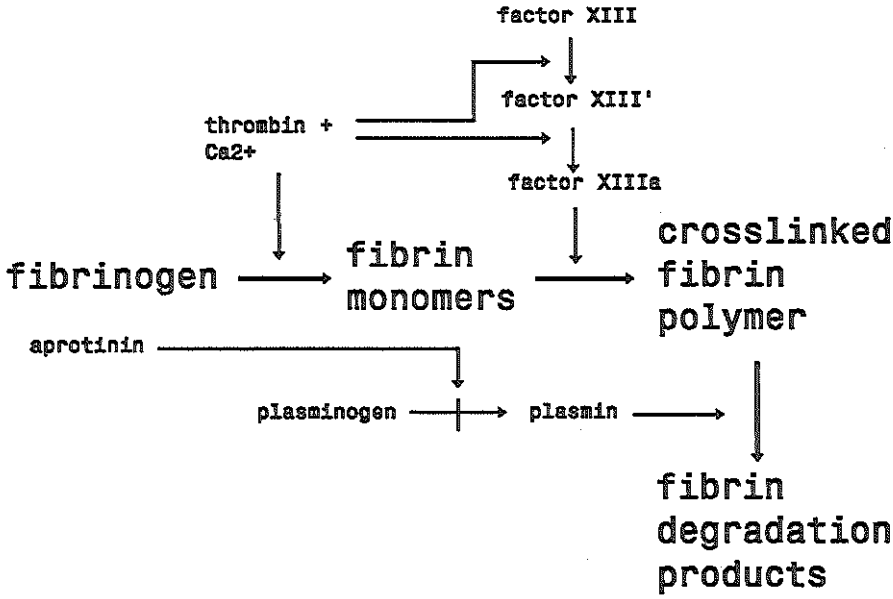
- Component I is a high concentration of human fibrinogen (75-115 mg/ml), plasma fibronectin (2-9 mg/ml), Factor XIII (10-50 U; one unit corresponds to the amount of Factor XIII contained in 1 ml of fresh normal plasma) and Plasminogen 40-120 g/ml. The lyophilized powder is reconstituted in bovine aprotinin solution (3,000 KIU/ml)
- Component II, contains Thrombin either 4 or 500 IU/ml in Calcium Chloride Solution (40 mmol $CaCl_2$/l).

On mixing the two components, the concentrated fibrinogen in the solution is converted into a solid fibrin clot, imitating the final stage of the coagulation cascade (see Figure 1). The velocity of the coagulation process depends on the concentration of the thrombin solution used. While the sealant takes 30 to 60 seconds to set with a thrombin concentration of 4 IU/ml, this process will take only a few seconds when the higher thrombin concentration of 500 IU/ml is used. Generally the higher thrombin concentration is used for haemostasis whilst the lower concentration is used for sealing of tissues.

The most important component of the sealer proteins is fibrinogen, whose molecular weight is about 340,000 dalton. The molecule consists of six polypeptide chains of three different types: α, ß and *y* which are symmetrically arranged (in pairs) in the two halves of the molecule. All chains are connected by disulphide bridges. Fibrinogen can be expressed by the formula: $(\alpha A,\beta B,y)_2$, where A and B are synonyms for the fibrinopeptides A and B. Through the action of thrombin, the fibrinopeptides A and B are split off from the α and ß-chains, in the first phase of the coagulation process. The resulting molecule is a fibrin monomer $(\alpha\beta y)_2$. These fibrin monomers aggregate loosely by means of weak hydrogen bonding and thus produce $fibrin_s$ ($_s$ stands for soluble). Factor XIII which is first activated by thrombin and Ca^{2+}, activates cross-linking of α-chains, by covalent isopeptide bridges, increasing the tensile

strength of the fibrin clot forming fibrin$_i$ ($_i$ standing for insoluble). It was found that the intrinsic tensile strength of a clot formed with fibrin seal was about 1200 g/cm^2 (157 kPa) while that of a sealed rat skin was approximately 200 g/cm^2 (17 kPa) after 10 min cross-linking at 37 C.[2] This implies that adhesion of the sealant to the tissue is the decisive factor for sealing tissue. The adhesive qualities of consolidated fibrin sealant to the tissue might be explained in terms of covalent binding between fibrin and collagen.

To achieve maximal tensile strength, cross-linking between α-chains of fibrin is necessary.



Afbeelding I A simplified diagram of the fibrin sealing system.

2.1.2 Degradation of fibrin clot:

Fibrin deposits are degraded in several ways: 1, by proteolytic enzymes produced by inflammatory cells, 2, by phagocytosis by macrophages and 3, by bacterial lysis. Aprotinin a protease inhibitor is added to the fibrin adhesive in order to prevent fibrinolysis. In vitro the resistance of fibrin sealant to urokinase- plasminogen induced lysis may be increased most efficiently by the addition of aprotinin. Nevertheless, the degradation by phagocytosis cannot

be influenced by aprotinin.[3] In vivo experiments have shown that fibrin sealant with aprotinin is more resistant to fibrinolysis than without aprotinin.

Several bacterial species are capable to lyse fibrin seal, either by the production of plasminogen activating factors or by production of direct acting fibrinolysin.[4] Among these strains are species that can be expected in the colon, e.g. *Streptococcus faecalis, Pseudomonas aeruginosa, Enterobacter cloacae, Escherichia coli* and *Staphylococcus aureus.* Especially *Streptococcus faecalis* strains are able to liquefy fibrin clots completely within 2 days.

2.1.3 Wound healing and fibrin sealant.

Several studies have shown that fibrin sealant contributes favourably to wound healing by producing local haemostasis and by stimulating the influx of macrophages that produce factors causing angiogenesis, fibroblast proliferation and collagen production.[5,6,7,8] However in this respect it is important that fibrin matrices prepared from fibrinogen that had been depleted of clotting factor XIII and/or fibronectin provided a superior matrix for macrophage migration. Also macrophage migration through fibrin gels was inversely correlated with fibrinogen concentration.[9]

After 2 days of healing, rat skin wounds treated with fibrin sealant possessed increased maximum tensile strength.[10] This increase corresponded to the initial strength of the fibrin sealed wounds (day 0 values). After 4 and 8 days of healing, no differences were found between the sealed and unsealed groups. In another study, however, it was found that mechanical properties of healing wounds depend on the concentrations of fibrinogen and thrombin. A fibrin glue with a fibrinogen concentration of 39 g/l and a thrombin concentration of 200-600 units/ml will result in wounds with significantly increased stress, energy absorption and elasticity values, when measured on day 8.[11] The authors come to the conclusion that higher fibrinogen concentrations inhibit wound healing. Furthermore, fibrin sealant contains the protease inhibitor aprotinin in order to reduce fibrin degradation. This aprotinin might inhibit the increased collagenase activity during the early phase of wound healing.

2.1.4 Adverse reactions

The use of fibrin sealant can cause serious side effects or even death. When injected in a

blood vessel the bovine thrombin component may give an anaphylactic reaction or a diffuse intravascular coagulation. Recently two serious cases, one of which was fatal, of serious reactions after use of fibrin sealant in deep hepatic wounds, were reported.[12] In fact, the manufacturer warns not to inject the sealant into the blood vessels. However, in case of deep hepatic lesions, there may be an open connection with large hepatic veins, giving access of the fibrin sealant to the circulation.

Commercial fibrin sealant is prepared from pooled human plasma. To reduce the potential risk of viral transmission only plasma units which are non-reactive in tests for HBsAg and HIV (HTLV-III/LAV) antibodies are utilised. Furthermore the product is heat treated in order to inactivate any viral contamination.

Despite generalized acceptance and use in Europe, fibrin glue is not used in clinical practice in the USA. This is due to the 1978 Food and Drug Administration ban on the sale of commercially prepared fibrinogen concentrate made from pooled donors, because of the risk of transmission of viral infection, in particular hepatitis B.[13] The present spread of HIV makes it unlikely that this policy will change in the near future.

2.2 Indications for fibrin sealant

Fibrin sealant has been used to achieve haemostasis in diffuse bleeding after surgery on parenchymatous organs, like liver, kidney and spleen,[14] thoracotomies, Dupuytrens' disease[15] mamma amputation, excision wounds in burns.[16] To achieve effective haemostasis on large areas of parenchymous organs, fibrin sealant can be applied by means of a spray or combined with a heterologous collagen fleece.[17,18]

Also it can be used to seal dacron prosthesis in thoraco vascular surgery.[19] Incisions or anastomosis of hollow viscera can be sealed water- and airtight, like the ureter, bloodvessels,[20,21] trachea[22], and bile duct.[23] In a prospective randomised study on sealing of ureters, sealing with fibrin sealant proved to be of no value.[24] Fistulae of the bronchus stump following pneumonectomy were successfully closed with fibrin sealant,[25,26] as well as vesicovaginal,[27] perineal fistulas after abdomino- perineal resection of the ano-rectum.[28]

In traumatology fibrin sealant can be used to fix osteochondral fragments,[29] or as an alternative "suture" material for repair of ruptured Achilles tendon.[30,31]

2.3.1 Fibrin sealant and intestinal anastomosis

Tissue adhesives were used in colonic anastomotic surgery based on two concepts. Firstly to prevent leakage by making an anastomosis water- and airtight by the application of a seal around an anastomosis. Secondly, the adhesive acts as a mechanical support of wound margins instead of sutures or as an extra support. Before fibrin sealant was introduced mainly cyanoacrylates were used in experimental studies and clinical practice. In the sixties cyanoacrylates were tested on intestinal anastomoses in various laboratory animals. The anastomosis enforced with cyanoacrylate was found to initially possess a strength superior to the sutured anastomosis. However, the difference diminished over the following days and there was no difference 15 days postoperatively.[32] In another animal study the mortality rate was 60 % in the sealed group and only 22 % in the control group.[33] Cyanoacrylate sealing of a conventional anastomosis is not an alternative to the conventional anastomosis as the mortality rate is higher and the anastomotic strength is not improved. Major disadvantages of cyanoacrylates, limiting their application are: (1) the tissue that is adhered must be dry; (2) the adhesive is rigid and lacks elasticity; (3) hypertrophic connective tissue reaction may occur. As a consequence of these studies cyanoacrylates were not used in humans for colonic anastomotic surgery.[34]

Fibrin sealant does not have the above mentioned disadvantages of cyanoacrylates. Several animal studies in rats, dogs and pigs with fibrin sealant have suggested its usefulness and potential clinical application in sealing intestinal anastomoses.[35,36,37,38,39,40] Leakage into the abdomen through end-to-end anastomoses of the tracer bacteria *Serratia marcescens* injected into the lumen of the small bowel of dogs, could be effectively prevented by fibrin sealant.[37] Other studies, however, could not confirm improved healing, despite initial increase of the mean bursting pressure[41,42] or even demonstrated worse healing of intestinal anastomoses.[43]

Also in humans additional sealing has been advocated and as such was applied in normal and high-risk colonic anastomosis as a method to prevent anastomotic leakage.[44,45,46] All authors of these studies claim good results and recommend fibrin sealing of intestinal anastomoses. However, these clinical studies are retrospective and lack a good control group. Sofar a prospective randomised clinical study does not exist.

2.3.2 Non-sutured anastomoses

The use of fibrin sealant enables the construction of sutureless anastomoses. Sutures may be harmful as they potentially induce tissue strangulation i.e. ischaemia, inflammatory reaction and tissue necrosis.

Good results with no morbidity or mortality were achieved after construction of a non-suture anastomosis, with fibrin sealant in the pig rectum.[47] In the pig small intestine seven-day healing of glued anastomoses was similar to a two-layer sutured anastomosis.[48] However, the crucial lag period of anastomotic healing is the period zero to four days from infliction of the wound. The glued anastomoses were weaker on the fourth postoperative day, which is alarming as this is the time when leakage is most threatening.[49]

It was proven by Mastboom *et al.* that fibrin glue is not essential to create a sutureless anastomosis.[50] They created a sutureless anastomosis in both ileum and colon in the rat by removing the sutures 30 minutes after anastomotic construction. Only on the first day after operation bursting pressures were significantly lower in both ileal and colonic anastomoses. These facts stress that experiments on sutureles anastomoses created with fibrin sealant must be judged with great caution; the control groups must be adequate.

2.4 Adhesion formation

As the number of operations grows, recurrent intestinal obstruction due to adhesions is seen more frequently nowadays. Known causes are the presence of bare serosal surfaces, ischemia, foreign bodies such as talcum powder and inflammation.[51] The pathogenesis of intra-abdominal adhesion formation has been studied histologically. Milligan and Rafferty demonstrated that on the first postoperative day, the viscera are joined by a network of fibrin containing predominantly polymorph nuclear granulocytes in addition to lymphocytes, macrophages, and plasma cells.[52] These early adhesions are from fibrin origin. By day 4, most fibrin has disappeared, and the predominant cell type seen is the macrophage with a few fibroblasts. By day 5, the adhesions are formed by fibroblasts in the presence of collagen, and the adhesions are fibrous. Since the fibrin deposition is an essential step to the formation of adhesions, one would expect an increase in adhesion formation when using fibrin sealant. However, animal studies have shown that application of fibrin glue on serosal defects actually inhibits intra-abdominal adhesion formation.[53] The inhibitory action is dependent on the

fibrinogen concentration of the fibrin glue, only a high concentration of fibrinogen prevents adhesion formation. Also the fibrin glue must be applied in a thick layer because a thin layer does not seem to be very effective.[54] These studies were done on peritoneal defects where bacteria are absent. Circumstances in anastomotic wound healing are different, and in sealing of intestinal anastomoses infection of the fibrin clot may very well occur.[43] Oka has found that sealing of rectal anastomoses reduces the formation of adhesions significantly.[40] However, in rats sealing of colonic anastomosis resulted to increased adhesion formation on day 5 postoperatively.[43] The reason for this difference is unclear, but bacterial contamination of the fibrin clot may contribute to this effect. Whether adhesion prevention by fibrin sealant does also apply to intestinal surgery needs further study.

2.5 Bacterial infection of fibrin sealant

The role of fibrin in bacterial peritonitis is not fully understood. Traditionally, fibrin has been considered a defense mechanism of the peritoneal cavity, serving to seal visceral leaks and wall off infection. Furthermore fibrin seems to trap bacteria, delays systemic sepsis and promotes abscess formation. Implantation of fibrin clots containing *E. coli* into the rat peritoneal cavity reduced the 24-hour mortality rate from 100% to 0% compared to bacteria in a similar volume of saline. However, the 10-day mortality rate was 90%, and all animals developed intraperitoneal abscesses.[55] Rotstein et al. have demonstrated that fibrin clots made of high concentrations of fibrinogen cause almost complete inhibition of the phagocytic killing capacity of neutrophils. Further it was demonstrated that the fibrinolytic agent "tissue plasminogen activator" (t-PA) prevents intra-abdominal abscess formation.[56] Also t-PA can prevent the deleterious action of an infected fibrin clot on the healing colonic anastomosis.[57] It was shown in rats that fibrin applied to intestinal anastomoses is invaded with aerobic and anaerobic bacteria, and gives rise to abscess formation. Fibrin appears to provide a site for bacterial proliferation protected from host defence, which results in abscess formation.

2.6 Concluding remarks

Fibrin sealant is a commercially available tissue adhesive, that is applied in many fields of surgery. While the use as an haemostatic agent in parenchymatous organs has been established, the benefit in intestinal surgery is doubtful. In spite of this fibrin sealant is

already being applied on colonic anastomosis clinically. The influence of fibrin glue on the healing colonic anastomosis needs further experiments, with special attention to wound healing parameters, and adhesion formation, under optimal and high-risk conditions.

2.7 References

1. Dresdale A, Rose EA, Jeevanandam V, *et al.* Preparation of fibrin glue from single-donor fresh-frozen plasma. Surgery 1985;97:750-754.
2. Redl H, Schlag G, Stanek G, Hirschl A, Seelich T. In vitro properties of mixtures of fibrin seal and antibiotics. Biomaterials 1983;4:29-32.
3. Pflüger H. Lysis and absorption of fibrin sealant. In: Schlag G, Redl H (eds). Fibrin sealant in operative medecine. General surgery and abdominal surgery - Vol. 6. Springer-Verlag, Heidelberg, Berlin, 1986, pp 39-50.
4. Hoffmann S, Moesgaard F. Bacterial lysis of fibrin seal in vitro. Eur Surg Res 1988;20:75-81.
5. Dvorak HF, Harvey VS, Strella P, *et al.* Fibrin containing gels induce angiogenesis. Lab Invest 1987;57:673-686.
6. Banerjee SK, Glynn LE. Reactions to homologous and heterologous fibrin implants in experimental animals. Ann NY Acad Sci 1960;86:1054-1057.
7. Leibovich SJ, Ross R. A macrophage-dependent factor that stimulates the proliferation of fibroblasts in vitro. Am J Path 1976;84:501-513.
8. Pohl J, Bruhn HD, Christophers E. Thrombin and fibrin-induced growth of fibroblasts: role in wound repair and thrombus organization. Klin Wochenschrift 1979;57:273-277.
9. Lanir N, Ciano PS, Van de Water L, *et al.* Macrophage migration in fibrin gel matrices. II Effects of clotting factor XIII, fibronectin, and glycosminoglycan content on cell migration. J Immunol 1988;140:2340-2349.
10. Jørgensen PH, Jensen KH, Andreassen TT. Mechanical strength in rat skin incisional wounds treated with fibrin sealant. J Surg Res 1987;42:237-241.
11. Byrne DJ, Hardy J, Wood RAB, McIntosh R, Cuschieri A. Effect of fibrin glues on the mechanical properties of healing wounds. Br J Surg 1991;78:841-843.
12. Berguer R, Staerkel RL, *et al.* Warning: fatal reaction to the use of fibrin glue in deep hepatic wounds. Case reports. J Trauma 1991;3:408-411.
13. Revocation of fibrinogen licenses. FDA Drug Bull 1978:8;15.
14. Scheele J. Erste klinische Erfahrungen mit der Fibrinklebung bei traumatischer und intraoperativer Milzverletzung. Chirurg 1981;52:531-534.
15. Eide E, Jurgeit H. Tissue adhesive in severe Dupuytren's disease. Handchirurgie 1985;17:145-146.
16. Ihara N, Suzuki K, Tanak H, *et al.* Application of fibrin glue to burns: its hemostatic and skin transplant fixation effects in the excised wound. Burns 1984;10:396-404.
17. Jakob H, Campbell CD, Stemberger A, *et al.* Combined application of heterologous collagen and fibrin sealant for liver injuries. J Surg Res 1984;36:571-577.
18. Redl H, Schlag G. Fibrin sealant and its modes of application. In: Schlag G, Redl H (eds). Fibrin sealant in operative medicine. General surgery and abdominal surgery - Vol. 6. Springer-Verlag, Heidelberg, Berlin, 1986, pp 13-26.
19. Haverich A, Walterbusch G, Borst HG. The use of fibrin glue for sealing vascular prostheses of high porosity. Thorac Cardiovasc Surg 1981;29:252-254.
20. Jacob H, Campbell CD, Qiu ZK, Pick R, Replogle RL. Evaluation of fibrin sealing for cardiovascular surgery. Circulation 1984;70 (suppl I):138-146.
21. Jacob H, Campbell CD, Qiu ZK, Pick R, Replogle RL. Use of fibrin sealant for reinforcing arterial anastomoses. J Vasc Surg 1984;1:171-180.

22. Kram HB, Shoemaker WC, Hino ST, *et al.* Trachal repair with fibrin glue. J Thorac Cardiovasc Surg 1985;90:771-775.

23. Kram HB, Garces MA, Klein SR, Shoemaker WC. Common bile duct anastomosis using fibrin glue. Arch Surg 1985;120:1250-1256.

24. Schultz A, Christiansen LA. Fibrin adhesive sealing of ureter after ureteral stone surgery. Eur Urol 1985;11:267-268.

25. Waclawiczek HW, Chmelizek F. Endoscopic treatment of bronchus stump fistulae following pneumonectomy with fibrin sealant in domestic pigs. Thorac Cardiovasc Surg 1985;33:344-346.

26. Jessen C, Sharma P. Use of fibrin glue in thoracic surgery. Ann Thor Surg 1985;39:521-524.

27. Papadopoulos I, Schnapka B, Kelâmi A. Anwendung des Humanfibrinklebers beim Verschluss von Blasen-Scheiden-Fisteln. Urol Int 1985;40:141-144.

28. Hedelin H, Nilson AE, Teger-Nilsson AC, Thorsen G, Pettersson S. Fibrin occlusion of fistulas postoperatively. Surg Gynecol Obstet 1982;154:367-368.

29. Gaudernak T, Zifko B, Skorpik G. Clinical experiences using fibrin sealant in the treatment of osteochondral fractures. In: Schlag G, Redl H (eds). Fibrin sealant in operative medecine. Traumatology - Orhtopaedics - Vol. 7. Springer-Verlag, Heidelberg, Berlin, 1986, pp 91-102.

30. Paar O, Bernett P. Therapie der Achillessehnen-Ruptur beim Sportler. Fortschr Med 1984;102:1106-1108.

31. Winter U. Versorgung frischer Achillessehnenrupturen mit dem Fibrin-Kleber. Akt Traumatol 1985;15:219-221.

32. Jordan GL, Harrington OB, Guerriero WG, Wiemer DR, Spjut HJ. A comparative study of the use of sutures and polymerizing adhesives for intestinal anastomoses. Proc Symp Adhesive in Surgery, p. 127. Vienna Medical Academy. Vienna 1967.

33. Uroskie TW, Swank M, Wolferth CC, Matsumoto T. Effects of isobutyl cyanoacrylate tissue adhesive on small bowel anastomosis in puppies. Am Surg 1971;37:397-401.

34. Nordkild P, Hjortrup A, Kjærgaard J. Tissue adhesives and intestinal anastomosis. Ann Chir Gyn 1986;75:205-208.

35. Scheele J, Herzog J, Mühe E. Fibrin glue protection of digestive anastomoses. Zbl Chirurgie 1978;103:1325-1336.

36. Giardino R, Brulatti M, Franchini A. Colonic anastomoses protected with fibrin sealant. In: Schlag G, Redl H (eds). Fibrin sealant in operative medecine. General surgery and abdominal surgery - Vol. 6. Springer-Verlag, Heidelberg, Berlin, 1986, pp 155-158.

37. Petrelli NJ, Cohen HC, DeRisi D, *et al.* The application of tissue adhesives in small bowel anastomoses. J Surg Oncol 1982;19:59-61.

38. Kessler B, Zimmermann RE. Gewebekleber unter Berücksichtigung der Fibrinklebung. Langenbecks Arch Chir 1981;355:485-489.

39. Kessler B, Arndt M, Zimmermann RE, Wiiting Ch. Absicherung von Dickdarmanastomosen mit biogenem Klebematerial. Helv Chir Acta 1982;49:195-199.

40. Oka H, Harrison RC, Burhenne HJ. Effect of a biologic glue on the leakage rate of experimental rectal anastomoses. Am J Surg 1982;143:561-564.

41. Hulkko OA, Haukipuro KA, Laitinen ST. Fibrin glue protection of primary anastomosis in the obstructed left colon. Acta Chir Scand 1988;154:49-52.

42. Anders A, Bodemann T, Baer U, Bauknecht KJ, Lawerenz JU. Eignen sich Fibrinklebung und Kollagenvlies zu zusätzlichen Nahtsicherung von Colonanastomosen. Langenbecks Arch Chir 1981;342:175-180.

43. Houston KA, Rotstein OD. Fibrin sealant in high-risk anastomoses. Arch Surg 1988;123:230-234.

44. Pointner R, Villinger R. Vorbeugung der intraperitonealen Nahtinsuffizienz durch Einsatz von Fibrin kleber. Zbl Chirurgie 1984;109:1146-1148.

45. Athanasiadis S, Kuhlgatz Ch, Girona I. Additional fibrin glueing in surgery of colon and rectum. Zbl Chirurgie 1984;109:1107-1111.

46. Waclawiczek HW, Boeckl O. Clinical experience from fibrin sealing in general and thoracic surgery. Zbl Chirurgie 1986;111:16-24.

47. Giardino R, Ussia G, Grilli Cicilioni C, Salerno A, Feldmann D, Farruggia F, Galletti G. Colorectal anastomoses with fibrin sealant and transanal approaching device in pig. Eur Surg Res 1986;18 S1:29.

48. Hjortrup A, Nordkild P, Kiærgaard J, Sjøntoft E, Olesen HP. Fibrin adhesive versus sutured anastomosis: a comparative intraindividual study in the small intestine of pigs. Br J Surg 1986;73:760-761.

49. Haukipuro KA, Hulkko OA, Alavaikko MJ, Laitinen ST. Sutureless colon anastomosis with fibrin glue in the rat. Dis Colon Rectum 1988;31:601-604.

50. Mastboom WJB, Hendriks Th, de Boer HHM. Intestinal anastomotic healing in the absence of suture material: an experimental study in rats. Int J Colorect Dis 1991;6:33-37.

51. Ellis H. The cause and prevention of postoperative intraperitoneal adhesions. Surg Gynecol Obstet 1971;133:407-411.

52. Milligan DW, Raftery AT. Observations on the pathogenesis of peritoneal adhesions: a light and electron microscopical study. Br J Surg 1974;61:274-280.

53. deVirgillio C, Dubrow T, Sheppard BB. Fibrin glue inhibits intra-abdominal adhesion formation. Arch Surg 1990;125:1378-1382.

54. Lindenberg S, Steentoft P, Sørensen SS, Olesen HP. Studies on prevention of intra-abdominal adhesion formation by fibrin sealant. Acta Chir Scand 1985;151:525-527.

55. Ahrenholz DH, Simmons RL. Fibrin in peritonitis.I. Beneficial and adverse effects of fibrin in experimental E. coli peritonitis. Surgery 1980;88:41-47.

56. Rotstein OD, Kao J. Prevention of intra-abdominal abscesses by fibrinolysis using recombinant tissue plasminogen activator. J Ifect Dis 1988;158:766-772.

57. Houston KH, McRitchie DI, Rotstein OD. Tissue plasminogen activator reverses the deleterious effect of infection on colonic wound healing. Ann Surg 1990;211:130-135.

CHAPTER III

AIMS OF THE STUDY

3.1 Aims of the study

The experiments described in this thesis were performed in order to find methods to influence and improve colonic anastomotic wound healing. It is known that inflammation may affect the healing of colonic anastomoses. Eicosanoids are known mediators of the inflammatory process, being a part of the healing process. In order to influence the healing process anti inflammatory drugs may be used. To evaluate the possible role of eicosanoids in uncomplicated healing of colon anastomosis we decided to measure eicosanoid synthesis in colon tissue and peritoneal macrophages. Activity of peritoneal macrophages was studied based on the HPLC profile of eicosanoids. Colon tissue was studied as well in order to compare both profiles to establish which cell type is active in the healing colon (Chapter IV).

Colonic anastomoses may be covered with fibrin sealant in order to promote wound healing and prevent leakage. Presently, this is already performed in many clinical situations. However data from experimental animal studies are conflicting, and prospective clinical studies are lacking. The influence of human- and rat fibrin sealant on the mechanical strength en collagen metabolism of a sutured colonic anastomoses is investigated (Chapter V).

Ischemia of the wound margins is a high-risk condition in the healing colonic anastomosis, with a considerable chance of anastomotic dehiscence. The effect of fibrin sealant on the healing ischemic anastomosis is tested (Chapter VI). Another known risk-factor is the presence of peritonitis. The role of fibrin sealant in the presence of faecal peritonitis is described in Chapter VII. A technical imperfect anastomosis may result in leakage of intestinal contents. In order to examen the effect of fibrin sealant in such a complication, fibrin was applied in a model of "incomplete anastomosis", constructed with 4 sutures only (Chapter VIII). Due to the high bacterial load of the colon, bacterial contamination of an anastomosis as well as the applied fibrin sealant is a possible negative factor in wound healing. Bacterial contamination is a known stimulus for inflammatory reaction. Fibrin sealant can be used as a vehicle for local application of antibiotics to the anastomosis. In the last experiment the effect of antibiotics in fibrin sealant on healing anastomoses in the rat was tested (Chapter IX).

CHAPTER IV

EICOSANOID PROFILE OF HEALING COLON ANASTOMOSIS AND PERITONEAL MACROPHAGES IN THE RAT

A.C. van der Ham, W.J.Kort, A.M. Bijma, F.J. Zijlstra*, M.A. Vermeer*, J. Jeekel.

From the Departments of Experimental Surgery, and *Pharmacology, Erasmus University Rotterdam, The Netherlands.

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Abstract

Because intraperitoneal administration of prostaglandin E_2 (PGE_2) has a negative influence on the healing of colonic anastomosis, the production of eicosanoid products in the healing rat colon after resection and anastomosis was studied using high performance liquid chromatography. Normal colonic tissue metabolises small amounts of arachidonic acid into cyclo-oxygenase and lipoxygenase products. After construction of an anastomosis, however, there is increased production of lipoxygenase products, while cyclo-oxygenase activity remains low. Increased amounts of PGE_2 and other cyclo-oxygenase products are not produced after anastomosis of the colon and probably do not play a major role in the uncomplicated healing of the large intestine the rat. During the first eight days of repair in the anastomosed colonic tissue, a statistically significant increase of 12-hydroxyeicosatetraenoic acid (12-HETE) production was found compared with control colon tissue (P=0.001). At the same time peritoneal macrophages from these rats showed increased 12-HETE production. Eicosanoid synthesis of peritoneal macrophages resembled eicosanoid synthesis of anastomosed colon taken from the same rat indicating that the 12-HETE, in particular, might be of macrophage origin.

Introduction.

The leakage of colonic and rectal anastomosis is a major complication after large intestine surgery but the reported incidences vary greatly. Using routine barium enema studies, leakage was detected in up to 50% of patients who underwent anterior resection of the sigmoid colon and rectum.[1] Clinically evident anastomotic leakage is associated with increased mortality and morbidity.[2] There are many factors that contribute to either healing or leakage of an anastomosis: blood supply, oxygen tension, type of sutures, bowel preparation, surgical technique, tension on the anastomosis, patient condition.[2,3,4] All these factors influence the formation of connective tissue, the key product in wound healing.

Halsted[4] first showed the importance of the submucosal connective tissue in relation to anastomotic healing in 1887. The quantity and quality of collagen in the submucosal layer of the intestinal wall determines the strength of the intestine and the capacity to hold sutures.[5]

Many studies on collagen metabolism in intestinal anastomoses have been performed using rat models. For the first three days after intestinal anastomosis a reduction of the suture holding capacity was found.[6,7] During this early phase there was a significant decrease in the collagen concentration in the colon.[8,9] This reduction in collagen content is highest just proximal to the anastomosis and can also be detected further away. After the third day, a rapid increase in strength is observed. Collagen production is increased from the very first day after operation and is highest in the vicinity of the anastomosis.[10] Comparison of net amounts with the rate of synthesis indicates an increased breakdown of collagen.[10]

Local degradation of mature collagen may be the cause of severing of sutures resulting in breakdown of the anastomosis.[8,11] When collagenase inhibitor was given a significant increase in the bursting pressure of a colon-anastomosis was found in rabbits.[12,13,14]

Factors that directly influence the formation of collagenase are infection and inflammation. As expected high collagenase activity was found in the colonic mucosa in colitis and around infected anastomoses.[11,15] Inflammatory cells, for example macrophages, are known to produce collagenase after stimulation by bacteria.[16] This collagenase production is dependent on prostaglandin E_2 (PGE_2). Rats treated with PGE_2 showed a significantly weaker anastomosis of the colon on day three than control rats, but this finding was reversed by the addition of indomethacin, a prostaglandin synthesis inhibitor.[17]

To evaluate the possible role of prostaglandins in uncomplicated healing of colonic anastomosis we decided to measure eicosanoid synthesis in colon tissue and peritoneal macrophages. Peritoneal macrophages were studied as a possible source of the eicosanoid products.

Material and methods

Male inbred Wag/Rij rats weighing 200-300 g were used. The animals had free access to a standard diet (Hope Farms, Woerden, The Netherlands) and to water before and after surgery. Anaesthesia was induced by ether inhalation. After median laparotomy, the colon was divided 2.5 cm above the peritoneal reflection, and a standardised left colonic resection of 1 cm was carried out with end to end anastomosis. The end to end

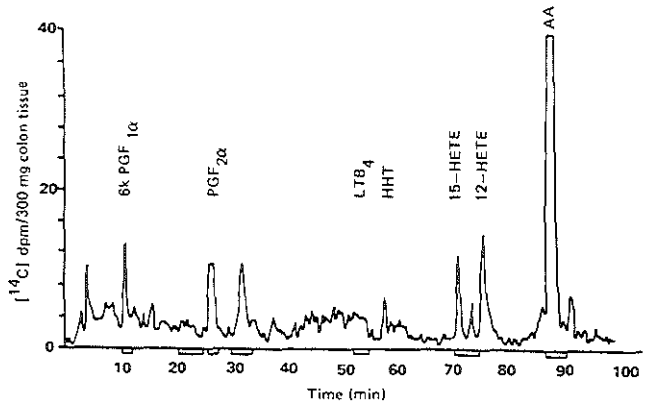

Figure 1: Reversed phase HPLC separation of eicosanoids produced by chopped normal rat colon tissue after loading with (^{14}C) arachidonic acid and Ca^{++}-ionophore A23187 challenge. Bars under time trace indicate peak width of both (^{14}C) labelled metabolites and (^{3}H) labelled standards.

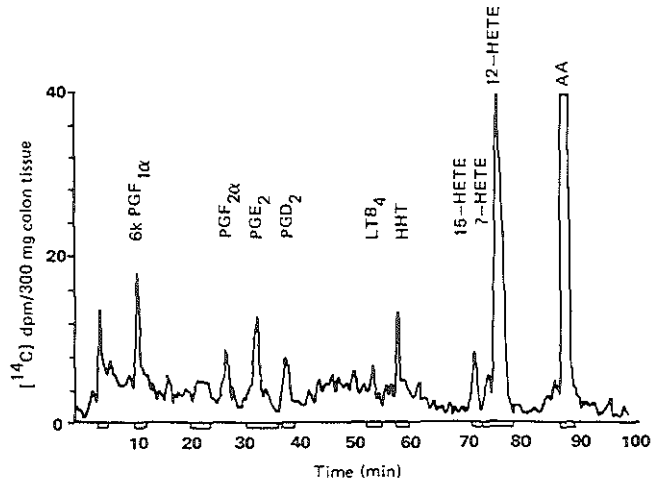

Figure 2: Reversed phase HPLC separation of eicosanoids produced by chopped rat colon tissue one day after resection and anastomosis, in response to (^{14}C) arachidonic acid and Ca^{++}-ionophore A23187. Bars under time trace indicate peak width of both (^{14}C) labelled metabolites and (^{3}H) labelled standards.

anastomosis was made with 12 interrupted inverting sutures through all layers, using 7x0 polypropylene (Prolene^{R}, Ethicon, FRG). On days 1, 2, 4, 8 and 16 the animals were sacrificed to obtain peritoneal macrophages and colonic tissue. Peritoneal macrophages and colonic tissue from rats who had not undergone surgery were used as controls.

Preparation of peritoneal macrophages

Peritoneal cells were collected during ether anesthesia by lavage with 50 ml of cold phosphate-buffered saline (PBS) (pH 7.2). The fluid containing the cells was centrifuged for 10 min at 250x g. The cells were resuspended in 5 ml cold PBS, washed once and counted. The peritoneal macrophages were isolated from granulocytes by centrifugation and sedimentation on a Percoll gradient (Pharmacia, Sweden).[18]

Preparation of colon tissue

After peritoneal lavage, the abdomen was reopened and the colon was dissected free from the mesentery. Two segments of colonic tissue, each weighing 300 mg each, were taken

from the ascending and the descending colon. The latter, which contained the anastomosis, was removed, rinsed of blood with PBS and immediately put on ice. The tissue was minced using a Mc Ilwain tissue chopper and suspended in 5 ml PBS.

Determination of eicosanoids

Peritoneal macrophages and colon tissue cells were incubated with 0.5 Ci (1-^{14}C) arachidonic acid and 2 M calcium ionophore A23187 (Sigma, USA) in 10 ml Krebs Henseleit buffer at 37 C for 10 min. After this, known amounts of [^{3}H]-labeled standards (Thromboxane B_2, Prostaglandin E_2, Leukotriene B_4 and 12-hydroxy-5,8,10,14,-eicosatetraenoic acid (12-HETE)) were added and the incubation sample was centrifuged for two min at 2800 g at 4 C. The supernatant was put in a SepPak C_{18} cartridge (Waters Associates, USA). The C_{18} cartridge was prewashed with 10 ml of absolute ethanol and 10 ml of water. The ethanol eluate was dried with a Savant Speed Vac concentrator, combined with a vacuum pump. The pellet was dissolved in 250 l of methanol and filtered through a Gelman LC3a 0.45 m filter (Gelman Sciences, USA) into a high performance liquid chromatography (HPLC) microvial. Of this sample, 100 l was injected onto a Zorbax C_{18} HPLC column (250x4.6 mm id; Dupont, USA). Reversed phase chromatography was performed with a solvent system consisting of 30% acetonitrile in water, acidified with acetic acid to pH 2.4, and a flow of 1ml/min at 37 C. After 35 minutes the acetonitrile

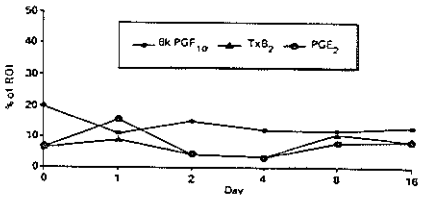

Figure 3: Synthesis of prostanoids by chopped rat colon tissue after resection and anastomosis. Values are generated from reversed phase HPLC and given as percentage of the total region of interest (ROI). Each time point represents two tissue samples in one rat. Single measurements were performed.

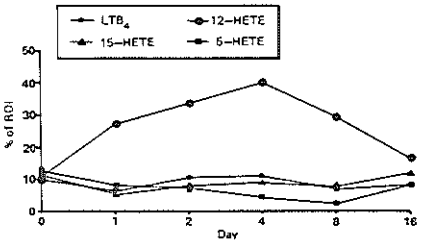

Figure 4: Synthesis of lipoxygenase products by chopped rat colon tissue after resection and anastomosis. Values are generated from reversed phase HPLC and given as percentage of the total region of interest (ROI). Each time point represents two tissue samples in one rat. Single measurements were performed.

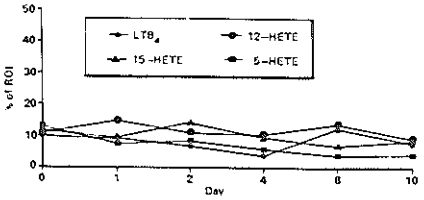

Figure 5: Synthesis of lipoxygenase products by chopped rat colon tissue proximal to colon anastomosis after resection and anastomosis of the left colon. Values are generated from reversed phase HPLC and given as percentage of the total region of interest (ROI). Each time point represents two tissue samples in one rat. Single measurements were performed.

was increased to 49% in 13 min. and maintained for 40 min. Arachidonic acid was eluted at 100% acetonitrile. HPLC was performed using a Hewlett-Packard 1084B (USA) liquid chromatograph consisting of a double head pump, a temperature-controlled column compartment and a variable volume injector. An on-line Berthold LB 506c Radioactivity monitor (Wildbad, FRG) was controlled by the HP 1084B terminal.

Statistical methods

Statistical analyses were performed using multi and univariate analysis of variance (Anova). The null hypothesis of no difference is rejected at the level of 0.05. However in case of findings by chance, Bonferroni's inequality implies that the level of significance is P=0.0125.[19]

Results

All animals operated survived the operation. The profile of eicosanoids synthesised by normal rat colon is shown in Figure 1. Normal rat colon tissue metabolises a small portion only of the exogenous arachidonic acid; approximately 15% is converted into a number of eicosanoids from which small peaks of 6 keto $PGF_{1\alpha}$, $PGF_{2\alpha}$, LTB_4, HHT (12-hydroxy-5,8,10-heptadecatrienoic acid), 15-HETE, 12-HETE and 5-HETE can be detected. Of the lipoxygenase products, the 12-HETE is synthesised most prominently (40%). On day 1 after surgery the HPLC profile of the segment of colon containing the anastomosis had changed drastically: 6 keto $PGF_{1\alpha}$, PGE_2 ,PGD_2 and HHT tended to increase, but not significantly; 15-HETE had decreased; but there was a fourfold increase in production of 12-HETE (Fig 2).

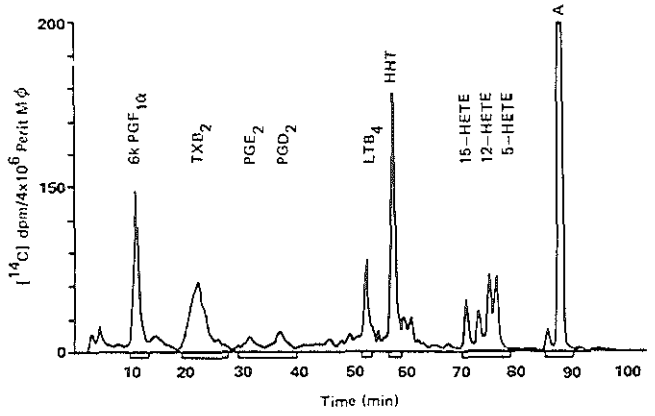

Figure 6: Reversed phase HPLC separation of eicosanoids produced by rat peritoneal macrophages obtained 1 day after colonic resection and anastomosis, in response to (^{14}C) arachidonic acid and Ca^{++}-ionophore A23187. Bars under time trace indicate peak width of both (^{14}C) labelled metabolites and (^{3}H) labelled standards.

In Figures 3 and 4 the values of the lipoxygenase products LTB_4, 15-HETE, 12-HETE and 5-HETE and prostanoids 6 keto $PGF_{1\alpha}$, TxB_2 and PGE_2 as calculated from the HPLC profiles are given as percentage of region of interest. The graph clearly shows the increased 12-HETE synthesis, reaching maximal differences from basic values at day 4 (statistically significant, $P<0.001$). On day 16, 12-HETE synthesis in colonic anastomosis tissue had returned to normal values. In the segments taken proximal to the anastomosis only minor changes in production of eicosanoids occurred (Fig 5).

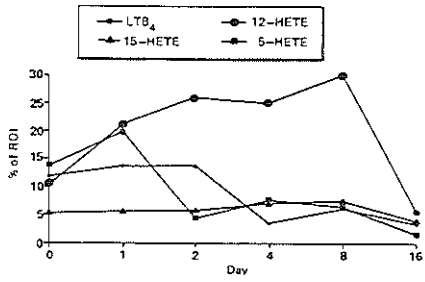

Figure 7: Synthesis of lipoxygenase products by rat peritoneal macrophages after resection and anastomosis of the left colon. Values are generated from reversed phase HPLC and given as percentage of the total region of interest (ROI). Each time point represents two tissue samples in one rat. Single measurements were performed.

Eicosanoid synthesis by peritoneal macrophages shows a shift from cyclo-oxygenase to lipoxygenase products on day 1 compared with day 0. An example of an HPLC profile of eicosanoids synthesised by peritoneal macrophages is shown in Figure 6. Like colonic tissue at the site of the anastomosis, peritoneal macrophages from the same rat show a noticeable lipoxygenase activity, starting on day 1 (Fig 7). On the 8th day the 12-HETE production is maximal. The other lipoxygenase products show minor variation.

Discussion

This study shows that normal rat colonic tissue metabolises a small portion only of exogenous arachidonic acid into cyclo-oxygenase and lipoxygenase products. The 6 keto $PGF_{1\alpha}$ was the major cyclo-oxygenase product, but 12-HETE was the major lipoxygenase product detected. These findings agree with reports showing that the mucosa of normal rat intestine metabolises a very small portion only of exogenous arachidonic acid into cyclo-oxygenase products.[20] Although the proportion metabolised is still small, the profile of products changes after resection and anastomosis of the colon: our results show a sharp increase of 12-HETE production, whereas cyclo-oxygenase products show only minor increase.

PGE_2 administered intraperitoneally has been reported to have a negative influence on the healing of colonic anastomosis in the rat.[17] Our results show, however, that PGE_2 and other cyclo-oxygenase products are not produced in excess after anastomosis of the colon and probably do not play a major role in uncomplicated healing of the large intestine the rat.

The origin and possible role of 12-HETE in wound healing are interesting issues. After tissue injury such as surgery, phospholipase A_2 is activated.[21] This phospholipase releases arachidonic acid from the phospholipid stores in cell membranes. The liberated arachidonic acid is the substrate of eicosanoids. The type of eicosanoids synthesised may vary greatly between different cell types.[22] During the first 24 hours after the construction of an anastomosis, the colonic wound is infiltrated by neutrophil granulocytes, after which eosinophils and macrophages gradually appear.[23] 5-Lipoxygenase products, LTC_4 and LTB_4 are produced in high amounts by all cell types mentioned, however, platelets synthesise mainly 12-HETE.[24] This means that basically any of these cell types may be responsible for the observed alteration of eicosanoid synthesis in our study.

The primary influx of granulocytes, which produce, in particular, the chemotactic leukotriene B_4, could be responsible for the LTB_4 formation. During the early phase of wound healing, however, we did not observe a significant increase in this product. Moreover, the pattern of the lipoxygenase arachidonic acid metabolites did not resemble that of stimulated peripheral blood neutrophils. It is possible that thrombocyte contamination of the sample tissue occurred, but in this case one would also expect high TxB_2 and HHT values and these were not noted in our study.

The results of this study show that normal colonic tissue does not itself have a significant lipoxygenase activity. Using a rat colitis model LTB_4 and 5-HETE were found in the mucosa of the large intestine, whereas in normal rat colon mucosa no lipoxygenase products were found.[20] The enhanced intestinal prostanoid synthesis in inflammatory bowel disease is thought to come from stimulated local mononuclear cells.[25]

This leads us to our final candidate, the macrophage. Macrophages are a known source of lipoxygenase metabolites, 12-HETE being the major one produced. This strongly suggests that the increase in 12-HETE found in our study is of macrophage origin and that macrophages play an important role in colonic anastomosis healing as early as 1 day after

surgery. This is in contrast with the results of other investigators who found that in rat colonic anastomosis monocytes are not seen for 48 hours.[26] To learn more about the presence of macrophages in the first few days after anastomosis more advance studies, for example, using monoclonal antibodies against macrophages, are needed.

There is substantial evidence that some arachidonic acid metabolites cause or enhance the signs of inflammation, the first phase of wound healing.[22] Compared with other eicosanoids, there is only little information on the function of 12-HETE. Although less active than LTB_4, 12-HETE also has chemotactic activity on inflammatory cells.[27] The products of the lipoxygenase pathway stimulate proliferation of epidermal cells and lymphocytes.[28] Topical administration of LTB_4 and 12-HETE stimulates epidermal proliferation.[29] In addition, it was found that monoHETEs, including 12-HETE exert modulatory actions on arachidonic acid metabolism in peritoneal macrophages.[30] Interestingly, 12-HETE inhibited synthesis of PGE_2. The mentioned properties of 12-HETE may indicate a possible role in wound healing. From this information, however, we cannot deduce whether 12-HETE plays a significant role in colon healing or whether it is a rather aspecific finding. As macrophages are a known source of collagenase, their activity in the healing colon may cause collagen breakdown in the early phase of wound repair.

It is evident that the raised lipoxygenase activity after the construction of a colonic anastomosis needs further study, to determine whether other species and humans show the same phenomena.

References

1. Goligher JC, Graham NG, De Dombal FT. Anastomotic dehiscence after anterior resection of rectum and sigmoid. Br J Surg 1970;57:109-118.
2. Fielding LP, Stewart-Brown S, Blesovsky L, Kearny G. Anastomotic integrity after operations for large-bowel cancer: a multicentre study. Br Med J 1980;281:411-414.
3. Shandall A, Lowndes R, Young HL. Colonic anastomotic healing and oxygen tension. Br J Surg 1985;72:606-609.
4. Halsted WS. Circular suture of the intestine - an experimental study. Am J Med Sci 1887;94:436-461.
5. Gottrup F. Healing of incisional wounds in stomach and duodenum: collagen distribution and relation to mechanical strength. Am J Surg 1981;141:222-227.
6. Jönsson K, Jiborn H, Zederfeldt B. Breaking strength of small intestinal anastomosis. Am J Surg 1983;145:800-803.

7. Jönsson K, Jiborn H, Zederfeldt B. Changes in collagen content of the small intestinal wall following anastomosis. Am J Surg 1985;150:315-317.

8. Wise L, McAlister W, Stein T, Schuck P. Studies on the healing of anastomoses of small and large intestines. Surg Gynecol Obstet 1975;141:190-194.

9. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomoses: III. Collagen metabolism in the colon after left colon resection. Am J Surg 1980;139:398-405.

10. Jönsson K, Jiborn H, Zederfeldt B. Collagen metabolism in small intestinal anastomosis. Am J Surg 1987;154:288-291.

11. Hawley PR. Causes and prevention of colonic anastomotic breakdown. Dis Col Rect 1973;16:272-277.

12. Delaney PV, Lalor D, Fitzgerald P, O'Malley E. The use of 'collagenase inhibitor' in prevention of large bowel anastomotic dehiscence. Br J Surg 1974;61:923.

13. Delaney P, Lalor D. Enzyme inhibition in colorectal surgery. Br J Surg 1976;63:23-24.

14. Högström H, Haglund U, Zederfeldt B. Beneficial effect on intestinal anastomosis of S-2441, a synthetic kallikrein-kinin antagonist: experimental studies in the rat. Am J Surg 1985;150:312-313.

15. Yamakawa T, Patin CS, Sobel S, et al. Healing of colonic anastomoses following resection for experimental "diverticulitis" Arch Surg 1971;103:17-20.

16. Wahl LM, Walh SM, Mergenhagen SE, Martin GR. Collagenase production by endotoxin-activated macrophages. Proc Natl Acad Sci USA 1974;71:3598-3601.

17. Brennan SS, Foster ME, Morgan A, Leaper DJ. Prostaglandins in colonic anastomotic healing. Dis Col Rect 1984;27:723-725.

18. Ouwendijk RJTh, Zijlstra FJ, Wilson JHP, Vincent JE, Bonta IL. Production of leukotrienes and prostaglandins by human ascites cells. Eur J Clin Invest 1985;15:327-331.

19. Ingelfinger JA, Mosteller F, Thibodeau LA, Ware JM. Biostatistics in clinical medicine. New York: MacMillan Publishing co., inc.; 1987.

20. Sharon P. Inflammatory bowel disease: Treatment modalities and mucosal prostaglandin/leukotriene formation. In: Domschke W, Dammann HG. (Eds.) Prostaglandins and leukotrienes in gastro- intestinal diseases. Berlin: Springer-Verlag, 1988:273-283.

21. Piper P, Vane JR. The release of prostaglandins from the lungs and other tissues. Ann NY Acad Sci 1971;180:363-385.

22. Williams KI, Higgs GA. Eicosanoids and inflammation. J Pathol 1988;156:101-110.

23. Hesp WLEM, Hendriks Th, Schillings PHM, Lubbers EJC, de Boer HHM. Histologic features of wound repair: a comparison between experimental ileal and colonic anastomoses. Br J Exp Path 1985;66:511-518.

24. Lorenzet R, Niemetz J, Marcus AJ, Broekman MJ. Enhancement of mononuclear procoagulant activity by platelet 12-hydroxy-eicosatetraenic acid. J Clin Invest 1986;78:418-423.

25. Zifroni A, Treves AJ, Sachar DB, RachmilewitzD. Prostanoid synthesis by cultured intestinal epithelial and mononuclear cells in inflammatory bowel disease. Gut 1983;24:659-664.

26. Högström H, Bondeson L, Haglund. Neutrophil-induced decrease in wound margin strength after intestinal anastomosis; influence on collagen and mechanisms of granulocyte action. Eur Surg Res 1988;20:260-266.

27. Goetzl EJ, Woods JM, Gorman RR. Stimulation of human eosinophil and neutrophil polymorphonuclear leukocyte chemotaxis and random migration by 12-l-hydroxy-5,8,10,14-eicosatetraenoic acid. J Clin Invest 1977;59:179-183.

28. Gualde N, Atluru D, Goodwin JS. Effect of lipoxygenase metabolites of arachidonic acid on proliferation of human T cells and T cell subsets. J Immunol 1985;134:1125-1129.

29. Chan CC, Duhamel L, Ford-Hutchinson A. Leukotriene B_4 and 12-hydroxyeicosatetraenoic acid stimulate epidermal proliferation in vivo in the guinea pig. J Invest Dermatol 1985;85:333-334.

30. Chang J, Lamb B, Marinari L, Kreft AF, Lewis AJ. Modulation by hydroxyeicosatetraenoic acids (HETEs) of arachidonic metabolism in mouse resident peritoneal macrophages. Eur J Pharmacol 1985;107:215-222.

CHAPTER V

THE EFFECT OF FIBRIN SEALANT ON THE HEALING COLONIC ANASTOMOSIS IN THE RAT

A.C. van der Ham, W.J. Kort, I.M. Weijma, H.F.G.M. van den Ingh*, J.Jeekel.

From the Laboratory for Experimental Surgery, Erasmus University and *Department of Pathology, St Clara Ziekenhuis, Rotterdam, The Netherlands.

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Abstract

Fibrin adhesives have been advocated as a protective seal in colonic anastomosis, in order to prevent leakage. In order to assess the effect of fibrin glue sealing we compared the healing of sutured colonic anastomosis in the rat (group 1) with the addition of human-derived fibrin sealant (group 2). As a control for a possible reaction to foreign protein, in group 3 the sutured anastomosis was sealed with specially prepared rat fibrin adhesive. On days 2, 4 and 7, ten animals in each group were killed. Adhesion formation was scored and the in-situ bursting pressure was measured. The collagen concentration, and degradation were estimated by measuring hydroxyproline. Adhesion formation was significantly increased in groups 2 and 3 compared with the control group. On days 2 and 7 the bursting pressure was not different between the groups. On day 4 the bursting pressure in groups 2 and 3 was significantly lower than in Group 1 ($P<0.001$). These findings correspond with the results of collagen measurements. On day 4 the concentration of hydroxyproline was significantly reduced in groups 2 and 3. Histological examination showed infiltration of neutrophilic granulocytes into the sealant on days 2 and 4, on day 7 the sealant had vanished. From these results it is concluded that fibrin sealing of the colonic anastomosis in the rat does not improve the healing, as demonstrated by bursting pressure and hydroxyproline concentration. On the contrary, it seems to have a negative influence.

Introduction

Leakage from a colonic and rectal anastomosis is a major complication after surgery with a reported incidence of up to 50%.[1] It is associated with a significantly increased mortality and morbidity.[2] Many factors are reported to contribute to the healing of or leakage from an anastomosis such as blood supply, oxygen tension, tension-free anastomosis, type of sutures or staples, surgical technique and bowel preparation.[2,3,4] All these factors have in common the fact that they influence the synthesis of new or degradation of old collagen the key product in wound healing.[4]

The quantity and quality of collagen in the submucosal layer of the intestinal wall determines the strength of the healing intestine.[5] Many studies on collagen metabolism in intestinal anastomosis have been performed using a rat model.[6,7,8] Following the

construction of an anastomosis in the colon there is a significant decrease in collagen.[9,10,11] This decrease in collagen content is highest just proximal to the anastomosis. During the early phase of healing there is increased breakdown of mature collagen in combination with increased synthesis of new collagen.[11] Local degradation of mature collagen might be the cause of cutting through of sutures thus causing breakdown of the anastomosis.[8,12] Using tritiated proline Cronin *et al.* observed that, during the first 5 days, destruction of old collagen takes place in the entire colon, with its maximum near the anastomosis outweighing the formation of new collagen.[13]

Collagen is very resistent to the action of proteolytic enzymes, but breakdown is initiated by a specific enzyme collagenase. A significant increase of collagenase was found in the gastrointestinal tract after anastomosis in the colon.[14] An experiment using aprotinin, a protease inhibitor, showed a significant increase in bursting pressure of colonic anastomoses in rabbits.[15,16,17] Factors directly influencing the activity of collagenase are bacteria and inflammation around infected anastomoses.[11,18] In addition, inflammatory cells are known to produce collagenase.[19]

Fibrin sealant is a multicomponent biologic adhesive made of concentrated human fibrinogen which can be used to establish hemostasis or as an adhesive in woundrepair. Several studies have shown that fibrin sealant contributes favorably to wound healing by producing local haemostasis and by stimulating the influx of macrophages that produce factors causing angiogenesis, fibroblast proliferation and collagen production.[20,21,22] It has been shown that by sealing an intestinal anastomosis, bacteria can be confined intra-luminally, thus reducing perianastomotic inflammation.[23] Furthermore, fibrin sealant contains the protease inhibitor aprotinin in order to reduce fibrin degradation. Additional sealing has been advocated in normal and high-risk colonic anastomosis in humans as a method of preventing anastomotic leakage.[24,25,26,27,28] However, experimental studies are conflicting and prospective randomised clinical studies are lacking.[29,30] The aim of the present study was to determine the effect of fibrin sealing on healing of normal anastomoses in the rat.

Materials and methods

Ninety male Wag/Rij rats, weighing 180-230 g, were randomly allocated to three treatment groups and were allowed water (acidified, pH 3.0) and food (AM II, Hope Farms, Woerden, The Netherlands) *ad libitum* before and after operation. Operations were performed using ether anesthesia. Through a midline incision, 1 cm of the left colon, 3 cm proximal to the peritoneal reflection, was resected. A single layer end-to-end anastomosis was performed with 12 interrupted, inverting 7/0 polypropylene (Prolene^{R}, Ethicon, F.R.G) stitches in a standard fashion. The resected segment of colon was frozen and kept at -80 C until analysis to serve as an individual control.

The study comprised the following groups: group 1, control sutured anastomosis; group 2, sutured anastomosis plus human fibrin sealant; group 3, sutured anastomosis plus rat fibrin sealant. The human fibrin sealant (Tissucol^{R}, Immuno AG, Vienna, Austria) and rat fibrin sealant (Immuno AG, Vienna, Austria) were prepared according to the manufacturers instructions. Fibrin sealant is a multicomponent adhesive consisting of a freeze-dried protein concentrate of human fibrinogen (120 mg/ml) which is reconstituted in a solution of aprotinin (3000 kallidinogenase inactivator units/ml) and a solution of thrombin (500 units/ml) and calcium chloride (40 mmol/l). After preheating to 37 C the components are reconstituted in their solutions and drawn up in separate syringes. These syringes fit in a specially designed syringe holder making it possible to mix and apply both components simultaneously. On mixing and application the fibrinogen was activated to form fibrin and the solutions were transformed into a rubber-like adhesive mass.

For one anastomosis 0.2 ml of fibrin sealant was used. On day 2, 4 and 7, ten rats from each group underwent relaparotomy. Following ether anesthesia the laparotomy incision was reopened and the degree of adhesion formation was assessed. We used the following scale: 0 no adhesions; 1+ minimal adhesions, mainly between anastomosis and omentum; 2+ moderate adhesions, i.e. between omentum and the anastomotic site and between the anastomosis and a loop of small bowel; 3+ severe and extensive adhesions, including abscess formation.[30] Thereafter the rectum was cannulated and the left colon was ligated twice, 1 cm proximal and 1 cm distal to the anastomosis, the latter around the rectal cannula. Care was taken not to disturb the anastomosis and its adhesions. The rectal cannula was connected to an infusion pump (Hospal^{R}, Italy), with a side arm to a pressure

transducer connected to a recorder. The *in vivo* anastomotic bursting pressure was measured by infusing normal saline at a constant rate of 1 ml/min, and was marked by abrupt loss of pressure. After measuring the bursting pressure the animal was killed by intrathoracic bleeding. The left colon including the anastomosis was excised and dissected free of mesenteric fat tissue. Three rings of tissue measuring 0.5 cm wide were cut: one ring of tissue containing the anastomosis, one ring just proximal and one ring distal to the anastomosis. For histologic examination, a small segment of the anastomotic ring was cut and put in 4 per cent (w/v) formaldehyde. The remaining tissue samples were immediately frozen and kept at -80 C for hydroxyproline assay.

Hydroxyproline as a measure of collagen was determined using a modified version of the colorimetric assay of Stegemann.[31,32] Collagen concentrations were calculated in g/mg dry weight. The collagen concentration of the segment of colon resected at the initial operation served as an individual control, and was used to calculate any change in collagen content.

A segment of each anastomotic ring was cut off for histological examination and fixed in 4 per cent (w/v) formaldehyde. The samples for histology were dehydrated and embedded in paraffin. From all paraffin blocks 5 m sections were cut and staining was performed with haematoxylin and eosin. In addition a periodic acid Schiff (PAS), a Gomori and a Weigert-van Gieson stain for assessment of the tissue reaction were performed. The inflammatory infiltrate (neutrophils, eosinophils, macrophages) and tissue organization (fibroblast and capillary

Table 1 *Adhesions following colonic anastomosis in the rat*

	Day 2		Day 4			Day 7	
Group	0–1	2–3	0–1	2–3		0–1	2–3
1 (control)	6	4	9	1	* †	6	4
2 (fibrin seal)	5	5	4	6		6	4
3 (rat fibrin)	2	8	1	9		4	6

Adhesions were graded as follows: 0–1, no to minimal adhesions; 2–3 moderate to severe adhesions; * $P = 0{\cdot}02$, † $P < 0{\cdot}001$, Fisher's exact test

Table 2 *Anastomotic bursting pressure of colonic anastomosis in the rat*

Group	Day 2	Day 4		Day 7
1 (control)	51(13)	123(12)	*	193(8)
2 (fibrin seal)	72(10)	65(6)		195(6)
3 (rat fibrin)	41(8)	84(10)		176(8)

Results are given as mean(s.e.m.) values in mmHg; * $P = 0{\cdot}001$, Wilcoxon rank sum test

Table 3 *Point of rupture on testing anastomotic bursting pressure*

	Day 2			Day 4			Day 7		
Group	P	A	D	P	A	D	P	A	D
1 (control)	0	10	0	0	8	2	8	0	2
2 (fibrin seal)	0	10	0	0	10	0	7	1	2
3 (rat fibrin)	0	10	0	0	10	0	9	1	0

P, proximal to anastomosis; A, anastomosis; D, distal to anastomosis

proliferation) were scored semiquantitatively as follows: +, numerous; , occasionally present; -, absent.

For statistical analysis of the results, the mean values, the standard deviation and the standard error of the mean were calculated. Results were compared using the Wilcoxon rank sum test, X^2 test and Fisher's exact test when appropriate.

Results

All animals survived the operation. Not a single animal showed macroscopic anastomotic leakage from the anastomosis at the time of reoperation. Adhesion formation was more extensive in groups 2 and 3 than in group 1 by day 4, when comparing adhesions >1 (P=0.02) (*Table* 1) In groups 2 and 3 the adhesion score was highest on day 4; on day 7 fewer adhesions were noted. The maximal adhesion score was 2+ and not a single animal showed abscess formation.

Table 4 *Mean concentration (μg mg dry weight) and relative change (per cent) of hydroxyproline in colonic anastomosis in the rat*

		Day 2		Day 4		Day 7	
	Group	Concentration (μg/mg)	Relative change (%)	Concentration (μg.mg)	Relative change (%)	Concentration (μg/mg)	Relative change (%)
Proximal to anastomosis	1	91(8·2)	−49	109(7·4)	−29 *	158(11·8)	−7·4 †
	2	82(5·4)	−50	85(8·8)	−47	102(3·7)	−38
	3	96(6·7)	−28	101(5·4)	−30	124(2·9)	−11
Anastomosis	1	97(5·5)	−44	113(5·7)	−26 *	152(7·4)	−11
	2	77(5·9)	−52	81(9·2)	−49	139(3·4)	−15
	3	61(4·3)	−54	79(6·4)	−45	131(4·4)	−6·2
Distal to anastomosis	1	135(10·1)	−22	146(7·0)	−5·7 *	157(11·2)	−7·8
	2	131(5·5)	−19	130(4·8)	−19	144(3·2)	−11
	3	114(5·7)	−15	108(4·8)	−24	140(4·8)	+1·0

Values are mean(s.e.m.); relative change is percentage of individual control value; * $P<0·05$, † $P<0·001$, Wilcoxon rank sum test

The results of anastomotic bursting pressure measurements are presented in *Table* 2. On day 2 no significant differences were noted, but there was a slight increase of bursting pressure with fibrin sealant. However on day 4, the anastomosis in the group with protection of fibrin sealant was significantly weaker compared with the control group (P<0.001). Also in group 3 anastomotic bursting pressure was significantly lower (P=0.001) on day 4 compared with the control group. On day 7 the difference in anastomotic bursting pressure had disappeared in both groups.

On day 2 bursting invariably occurred at the anastomosis (*Table* 3). On day 4 in the control group 80 per cent of bursting occurred at the anastomosis; in groups 2 and 3 all bursting occurred at the anastomosis. On day 7 in group 1 the bursting invariably occurred outside the anastomosis; in each of groups 2 and 3 the anastomosis was the place of rupture in one animal. Bursting was most likely to take place proximal to the anastomosis in 80 per cent, 70 per cent and 90 per cent in groups 1, 2 and 3 respectively. On day 7 the colon wall was significantly weaker proximal to the anastomosis compared with the distal colon ($P<0.01$; Wilcoxon rank sum test).

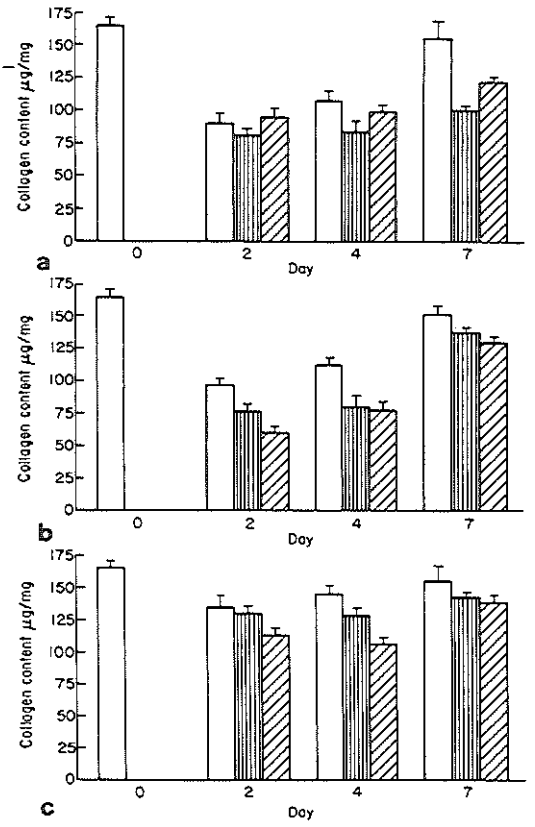

Figure 1 *Collagen content (µg hydroxyproline/mg dry weight)* **a** *proximal to colonic anastomosis,* **b** *in anastomotic area,* **c** *distal to colonic anastomosis. The column on day 0 represents normal colon tissue;* □, *control;* ▥, *fibrin glue;* ▨, *rat fibrin*

The values of hydroxyproline concentration around the anastomosis and relative change as compared with individual normal values are presented in *Table* 4. Hydroxyproline concentration was lowest on day 2 in all groups (*Figure* 1). The relative loss of collagen was prominent in all groups except for group 3 distal to the anastomosis on day 7. Proximal to the anastomosis on day 4 and day 7, collagen decrease in Group 2 was significantly higher than in group 1 (*Figure 1a*). In all groups and on all days, collagen loss was more marked proximally than distally.

Collagen loss at the anastomosis was maximal on day 2 measuring 52 per cent and 54 per cent in groups 2 and 3 respectively. On day 4 collagen decrease was higher in groups 2

and 3 than in group 1 ($P<0.05$) (*Figure 1b*). On day 7 this difference had disappeared again.

Distal to the anastomosis, collagen change was less pronounced compared with the anastomosis and proximal part on all days (*Figure 1c*). However, also in this part on day 4 collagen decrease in groups 2 and 3 was significantly higher compared to group 1. Overall there were slight differences in relative collagen loss between groups 2 and 3; however, the differences between these did not reach statistical significance.

Histological examination showed remnants of fibrin glue outside the anastomosis on days 2 and 4 (*Figure 2*); on day 7, however, this fibrin was no longer demonstrated. Invariably on days 2 and 4 the fibrin seal was heavily infiltrated and surrounded by neutrophilic graulocytes (*Figure 3*). The intestinal wall showed influx of neutrophilic and eosinophilic granulocytes and macrophages on day 2 (*Table 5*). On day 2 fibroblasts were only occasionally present. On days 4 and 7 macrophages and fibroblasts were abundant in all groups while granulocytes were again reduced in number. On day 7, however, the presence of macrophages had increased in group 2 compared with group 1 ($P<0.05$). No differences were noted in the number of fibroblasts and capillary proliferation.

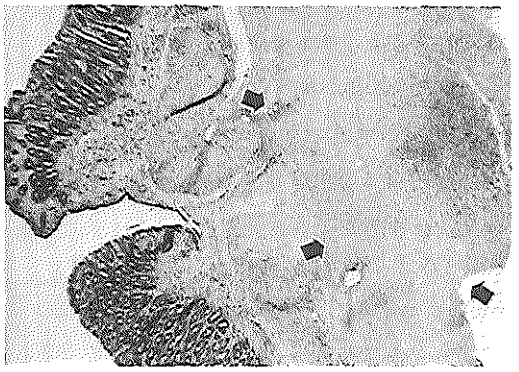

Figure 2 *Four-day-old colonic anastomosis showing fibrin seal on the peritoneal side (arrows). The submucosa shows slight oedema and cellular infiltrate. Massive cellular infiltrate is present in the fibrin seal. Haematoxylin and eosin stain. Original magnification ×50*

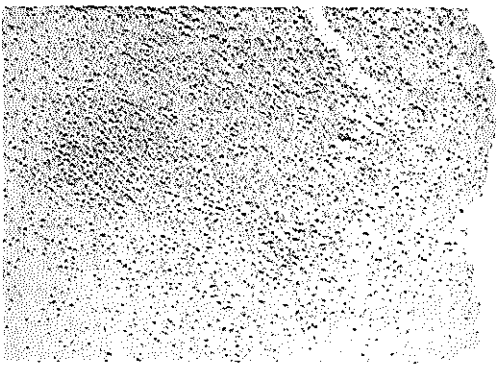

Figure 3 *Fibrin seal of 4-day-old colonic anastomosis, showing infiltration of neutrophilic granulocytes. Haematoxylin and eosin stain. Original magnification ×160*

Table 5 *Presence of different cell types in the healing colonic anastomosis in the rat*

Cell type	Group	Day 2			Day 4			Day 7		
		+	±	−	+	±	−	+	±	−
Neutrophils	1	2	4	1	0	5	3	1	1	7
	2	8 *	0	0	8 *	0	0	2	3	3
	3	9	0	0	7	0	0	2	3	2
Eosinophils	1	6	1	0	1	6	1	4	3	2
	2	5	1	2	5	1	2	1	4	3
	3	4	5	0	3	4	0	0	5	2
Macrophages	1	3	4	0	4	4	0	4 †	5	0
	2	4	3	1	7	1	0	8	0	0
	3	2	6	1	3	4	0	4	3	0
Fibroblasts	1	0	4	3	8	0	0	8	1	0
	2	3	2	3	6	2	2	7	1	0
	3	0	3	6	4	3	0	7	0	0

+,Numerous; ±, occasionally present; −, absent; * $P<0{\cdot}01$, † $P=0{\cdot}02$, χ^2 test

Discussion

Additional sealing has been advocated in normal and high-risk colonic anastomoses in patients as a method to prevent anastomotic leakage.[24-28] However, experimental studies are conflicting and prospective randomised clinical studies are lacking.[29,30] We performed the present study in order to investigate the influence of fibrin sealant on the healing colonic anastomosis in the rat, with regard to intraperitoneal adhesion formation, mechanical strength, collagen metabolism and histological appearance. The application of fibrin sealant (human as well as rat fibrinogen) resulted in increased adhesion formation around the anastomosis on day 4. On day 7 this difference was not prominent. We did not find increased abscess formation as reported by Houston and Rotstein.[30] In fact in the present series no abscesses at all were found, while these authors report abscess formation in 20 per cent in the control group and nearly 100 per cent following fibrin sealing. An important factor accounting for these differences with in results might be the use of silk suture material in their study, while in the present study monofilament polypropylene was used. Quality of technique may also account for this difference. In the authors' opinion fibrin sealant *per se* cannot be held liable for abscess formation in colonic anastomosis.

Application of human as well as rat fibrinogen did not result in an elevation of anastomotic bursting pressure during the first 7 days of healing, contrary to expectations. Surprisingly a statistically significant lower bursting pressure was observed on day 4. This effect seems to be only temporary and had disappeared by day 7. As a consequence the

sealed anastomosis can be considered weak during the critical period of healing. This negative effect on the mechanical strength of colonic anastomoses corresponds with one other rat study.[30] The mechanical weakness of the sealed 4-day anastomosis is especially alarming as this is the time when leakage threatens.

After sealing, collagen concentration within the anastomosis and proximal and distal to the anastomosis was lower on day 4. On day 7 this difference was still apparent, but exclusively proximal to the anastomosis. The significantly lower bursting pressure on day 4 in the sealed anastomosis in groups 2 and 3 corresponds with a statistically significant decrease in collagen concentration. This decreased collagen concentration can account for the lower bursting pressure because the bursting pressure is mainly dependent on submucosal collagen.[11] The finding of asymmetrical collagen changes around the anastomosis has been reported in other studies.[11,33] We also were able to demonstrate that collagen changes are more marked proximally than distally. This may explain why rupture took place almost exclusively proximal to the anastomosis on testing the bursting pressure on day 7. No good explanation has so far been given for the cause of this phenomenon.

Histological examination showed excessive accumulation of neutrophil granulocytes in the fibrin seal covering the anastomosis on days 2 and 4. Although no abscesses or pus were noted, both human and rat fibrinogen gave rise to infiltration of granulocytes. This finding suggests that local fibrin deposition predisposes to local infection and inflammation. Under normal conditions deposition of fibrin is a normal phenomenon of wound healing and within 3 h the wound is infiltrated by granulocytes, followed by macrophages during the first 24 h.[34] The presence of foreign material, e.g. fibrin or bacteria, can prolong the inflammatory phase. It may be that the application of fibrin sealant provides a site for bacterial proliferation. Using germ-free rats a significant reduction in the lowering of hydroxyproline concentration around the anastomosis was observed by Mastboom *et al.*[35]

Inside the intestinal wall macrophages were more abundant near the anastomosis on day 7 following application of fibrinogen; however, these cells were not very prominent on days 2 and 4. Neutrophil granulocytes, macrophages and bacteria are known sources of proteolytic enzymes including collagenase and might cause increased collagen breakdown in the intestinal wall. The postoperative decrease of breaking strength of colonic anastomoses is prevented in rats with neutropenia.[36] It seems likely that in the present

model the increased number of neutrophils in direct contact with the anastomosis was responsible for the increased loss of hydroxyproline in the colonic wall after the addition of fibrin seal.

Another possible explanation may be the formation of a physical barrier around the anastomoses by fibrin sealant. Wrapping of colonic anastomoses in Silastic® (Dow Corning, Reading, UK) sheeting results in leakage and high mortality because of separation of the anastomosis from the peritoneal defences.[37] Fibrin sealing might separate the anastomoses from the defence mechanisms of the peritoneum analogous to the wrapping in Silastic.

The present results do not show enhanced healing of colonic anastomosis following sealing with fibrin glue, contrary to other studies.[24-28] Our findings give support to the conclusion of Houston and Rotstein that the application of fibrin on colonic anastomosis may lead to delayed healing.[30] These authors postulated that the negative effect of human fibrin sealant is caused by a reaction to foreign protein. In order to evaluate this mechanism we used sealant made of rat fibrinogen in a distinct control group. Using rat fibrinogen the same impeding effect as with human material was observed.

From these results we may conclude that fibrin sealing of normal colonic anastomoses in the rat does not improve the healing process; on the contrary, it has a negative influence during the first week. As a consequence, the routine use of fibrin sealant in non-complicated intestinal anastomoses in the clinical situation does not seem justified. Whether this is also true for high-risk anastomoses, e.g. those associated with peritonitis and ischemia, is subject to further study.

References

1. Goligher JC, Graham NG, De Dombal FT. Anastomotic dehiscence after anterior resection of rectum and sigmoid. Br J Surg 1970;57:109-118.

2. Fielding LP, Stewart-Brown S, Blesovsky L, Kearny G. Anastomotic integrity after operations for large-bowel cancer: a multicentre study. Br Med J 1980;281:411-414.

3. Shandall A, Lowndes R, Young HL. Colonic anastomotic healing and oxygen tension. Br J Surg 1985;72:606-609.

4. Hunt TK, Hawley PR, Hale J, Goodson W, Thakral KK. Colon repair: the collagenous equilibrium. In: Hunt TK, ed. Wound healing and Wound infection. Theory and surgical practice. New York: Appleton-Century-Crofts,1980:153-159.

5. Gottrup F. Healing of incisional wounds in stomach and duodenum: collagen distribution and relation to mechanical strength. Am J Surg 1981;141:222-227.

6. Jönsson K, Jiborn H, Zederfeldt B. Breaking strength of small intestinal anastomosis. Am J Surg 1983;145:800-803.

7. Jönsson K, Jiborn H, Zederfeldt B. Changes in collagen content of the small intestinal wall following anastomosis. Am J Surg 1985;150:315-317.

8. Jönsson K, Jiborn H, Zederfeldt B. Collagen metabolism in small intestinal anastomosis. Am J Surg 1987;154:288-291.

9. Wise L, McAlister W, Stein T, Schuck P. Studies on the healing of anastomoses of small and large intestines. Surg Gynecol Obstet 1975;141:190-194.

10. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomoses: III. Collagen metabolism in the colon after left colon resection. Am J Surg 1980;139:398-405.

11. Cronin K, Jackson DS, Dunphy JE. Changing bursting strength and collagen content of the healing colon. Surg Gynec Obstet 1968;126:747-753.

12. Hawley PR. Causes and prevention of colonic anastomotic breakdown. Dis Col Rect 1973;16:272-277.

13. Cronin K, Jackson DS, Dunphy JE. Specific activity of hydroxyproline-tritium in the healing colon. Surg Gynecol Obstet 1968;126:1061-1065.

14. Hawley PR, Faulk WP, Hunt TK, Dunphy JE. Collagenase activity in the gastrointestinal tract. Br J Surg 1970;170:896-900.

15. Delaney PV, Lalor D, Fitzgerald P, O'Malley E. The use of 'collagenase inhibitor' in prevention of large bowel anastomotic dehiscence. Br J Surg 1974;61:923.

16. Delaney P, Lalor D. Enzyme inhibition in colorectal surgery. Br J Surg 1976;63:23-24.

17. Högström H, Haglund U, Zederfeldt B. Beneficial effect on intestinal anastomosis of S-2441, a synthetic kallikrein-kinin antagonist: experimental studies in the rat. Am J Surg 1985;150:312-313.

18. Yamakawa T, Patin CS, Sobel S, et al. Healing of colonic anastomoses following resection for experimental "diverticulitis" Arch Surg 1971;103:17-20.

19. Ohlsson K. Polymorphonuclear leukocyte collagenase. In Woolley D, Evanson J, editors. Collagenase in normal and pathologic connective tissue. Chicester, England, 1980, Wiley, pp 209-222.

20. Banerjee SK, Glynn LE. Reactions to homologous and heterologous fibrin implants in experimental animals. Ann NY Acad Sci 1960;86:1054-1057.

21. Leibovich SJ, Ross R. A macrophage-dependent factor that stimulates the proliferation of fibroblasts in vitro. Am J Path 1976;84:501-513.

22. Pohl J, Bruhn HD, Christophers E. Thrombin and fibrin-induced growth of fibroblasts: role in wound repair and thrombus organization. Klin Wochenschrift 1979;57:273-277.

23. Petrelli NJ, Cohen HC, DeRisi D, et al. The application of tissue adhesives in small-bowel anastomoses. J Surg Oncol 1982;19:59-61.

24. Scheele J, Herzog J, Mühe E. Fibrin glue protection of digestive anastomoses. Zbl Chirurgie 1978;103:1325-1336.

25. Pointner R, Villinger R. Vorbeugung der intraperitonealen Nahtinsuffizienz durch Einsatz von Fibrin kleber. Zbl Chirurgie 1984;109:1146-1148.

26. Athanasiadis S, Kuhlgatz Ch, Girona I. Additional fibrin gluing in surgery of colon and rectum. Zbl Chirurgie 1984;109:1107-1111.

27. Waclawiczek HW, Boeckl O. Clinical experience from fibrin sealing in general and thoracic surgery. Zbl Chirurgie 1986;111:16-24.

28. Giardino R, Brulatti M, Franchini A. Colonic anastomoses protected with fibrin sealant. In: Schlag G, Redl H (eds). Fibrin sealant in operative medicine. General surgery and abdominal surgery - Vol. 6. Springer-Verlag, Heidelberg, Berlin, 1986, pp 155-158.

29. Nordkild P, Hjortrup A, Kjærgaard J. Tissue adhesives and intestinal anastomosis. Ann Chir Gyn 1986;75:205-208.

30. Houston KA, Rotstein OD. Fibrin sealant in high-risk anastomoses. Arch Surg 1988;123:230-234.

31. Stegemann H, Stalder K. Determination of hydroxyproline. Clin Chim Acta 1967;18:267-273.

32. Grant RA. Estimation of hydroxyproline by the AutoAnalyser. J Clin Path 1964;17:685-686.

33. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomosis; the effect of suture technic on collagen concentration in the colonic wall. Am J Surg 1978;135:333-340.

34. Hesp WLEM, Hendriks Th, Schillings PHM, Lubbers EJC, de Boer HHM. Histologic features of wound repair: a comparison between experimental ileal and colonic anastomoses. Br J Exp Path 1985;66:511-518.

35. Mastboom W.J.B, Hendriks T, de Boer H.H.M. Collagen changes around intestinal anastomoses in germ-free rats. Br J Surg 1989;76:797-801.

36. Högström H, Haglund U. Neutropenia prevents decrease in strength of rat intestinal anastomosis: partial effect of oxygen free radical scavengers and allopurinol. Surgery 1986;99:716-720.

37. Canalis F, Ravitch MM. Study of healing of inverting and everting intestinal anastomoses. Surg Gynecol Obstet 1968;126:109-114.

CHAPTER VI

HEALING OF ISCHEMIC COLONIC ANASTOMOSIS: FIBRIN SEALANT DOES NOT IMPROVE WOUND HEALING

A.C. van der Ham, W.J. Kort, I.M. Weijma, H.F.G.M. van den Ingh*, J. Jeekel.

From the Laboratory for Experimental Surgery, Erasmus University *Department of Pathology, St. Clara Ziekenhuis, Rotterdam, The Netherlands.

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Abstract

Fibrin adhesives have been advocated as a protective sealant in high-risk colonic anastomoses, in order to prevent leakage. In order to assess the effect of fibrin glue sealing on the healing ischemic anastomosis, we compared the healing of sutured colonic anastomoses in the rat, with and without fibrin adhesive (Groups IA and IB), and ischemic anastomoses with and without fibrin adhesive (Groups IIA and IIB). On days two, four and seven, 10 animals in each group were sacrificed. Adhesion formation was scored and the *in situ* bursting pressure was measured. The collagen concentration and degradation were estimated by measuring hydroxyproline. Adhesion formation was more prominent in Groups IB, IIA and IIB on day four only; abscesses were noted in the ischemic group in four rats. Anastomotic bursting pressure was significantly lower in sealed (IB) and ischemic anastomoses (IIA) than in normal anastomoses (IA) on day four. Sealing of ischemic anastomoses did not change bursting pressures on days two, four and seven. The relative decrease of collagen in the sealed anastomoses is significantly higher on day four only. It is concluded that sealing of normal colonic anastomoses in the rat has a negative effect on wound healing. Ischemia at the anastomotic site results in weaker anastomotic strength on day four postoperatively. Also in ischemic anastomoses, fibrin sealant does not improve wound healing during the first seven days. Adhesion formation on ischemic intestinal anastomoses was not prevented by fibrin sealing.

Introduction

Leakage of colonic and rectal anastomoses is a major complication after surgery, with a reported incidence of up to 50 percent[1], associated with a significantly increased mortality and morbidity.[2] Many factors are reported to contribute to the healing or leakage of an anastomosis such as tension-free anastomosis, type of suture or staples, surgical technique and bowel preparation.[2,3] A good blood supply is regarded as having paramount importance in anastomotic healing because synthesis of collagen and resistance to infection depend on adequate tissue oxygenation.[4] An explanation for the high incidence of leakage of low colorectal anastomoses has been suggested to have a vascular etiology.[5] Also the increased incidence of anastomotic dehiscence in the elderly may result from poor blood supply.[6,7]

Fibrin sealant is a multicomponent biologic adhesive made of concentrated human fibrinogen which can be used to establish hemostasis or as an adhesive in wound repair. Several studies have shown that fibrin sealant contributes favorably to wound healing by producing local hemostasis and by stimulating the influx of macrophages that produce factors causing angiogenesis, fibroblast proliferation and collagen production.[8,9,10] It has been shown that by sealing an intestinal anastomosis, bacteria can be confined intra-luminally, thus reducing perianastomotic inflammation.[11] Additional sealing has been advocated in normal and high-risk colonic anastomoses in humans as a method to prevent anastomotic leakage.[12,13,14,15,16] However, results of experimental studies are conflicting, and prospective, randomised clinical studies are lacking.[17,18,19,20]

A study was designed to evaluate the effect of fibrin sealant on the healing colonic anastomoses in the rat. To mimic a high-risk situation, in one group the colon was made ischemic at the anastomotic site.

Material and methods

One hundred twenty male Wag/Rij rats, weighing 180 to 240 g, were randomly allocated to four treatment groups and were allowed water (acidified, pH 3.0) and food (AM II, Hope Farms, Woerden, the Netherlands) *ad libitum* preoperatively and post-operatively. Operations were performed using ether anesthesia. Through a midline incision, 1 cm of the left colon, 3 cm proximal to the peritoneal reflection, was resected. A single-layer end-to-end anastomosis was performed with 12 interrupted, inverting 7-0 polypropylene (Prolene^{R}, Ethicon, F.R.G) stitches. The resected segment of colon was frozen and kept at -80 C until analysis to serve as an individual control. The study comprised the following groups: IA, control sutured anastomosis; IB sutured anastomosis plus fibrin sealant; IIA, ischemic anastomosis; IIB, ischemic anastomosis plus fibrin sealant.

Fibrin sealant

The human fibrin sealant (Tissucol^{R}; Immuno AG, Vienna, Austria) was prepared according to the enclosed instructions. Fibrin sealant is a multicomponent adhesive consisting of 1) a freeze-dried protein concentrate of human fibrinogen (120 mg/ml) that is reconstituted in a solution of aprotinin (3000 KIU/ml) and 2) a solution of thrombin (500 IU/ml) and calcium chloride (40 mmol/liter). After preheating to 37 C, the components are

reconstituted with its solutions and drawn up in separate syringes. These syringes fit in a specially designed syringe holder (Duploject®; Immuno AG, Vienna, Austria), enabling mixing and application of the two components simultaneously. On mixing and application, the fibrinogen is activated to form fibrin and the solution is transformed into a rubberlike adhesive mass. For one anastomosis, 0.2 ml of fibrin sealant was used.

Ischemic anastomosis

Colonic ischemia was induced by bipolar coagulation and division of the mesenteric blood vessels for 2 cm on either side of the anastomosis. In this way the blood supply to the left colon is exclusively intramural. The effect of this model of ischemia was illustrated in other studies and has been demonstrated by means of a laser Doppler flowmeter study.[21,22] Ischemia of this magnitude resulted in profound morphologic changes but was not lethal.[23]

On days two, four and seven, 10 rats from each group underwent relaparotomy. Following ether anesthesia, the laparotomy incision was reopened and the degree of adhesion formation was assessed. The following scale was used: 0, no adhesions; 1+, minimal adhesions, mainly between the anastomosis and the omentum; 2+, moderate adhesions, *i.e.*, between the omentum and the anastomotic site and between the anastomosis and a loop of small bowel; 3+, severe and extensive adhesions, including abscess formation.[18] Thereafter, the rectum was cannulated and the left colon was ligated twice: 1 cm proximal and 1 cm distal to the anastomosis, the latter around the rectal cannula. Care was taken not to disturb the anastomosis and its adhesions. The rectal cannula was connected to an infusionpump (Hospal®; Dasco Spa, Medolla, Italy), with a side arm to a pressure transducer, in connection with a recorder. The *in vivo* anastomotic bursting pressure was measured by infusing normal saline at a constant rate of 1 ml per minute and was marked by abrupt loss of pressure. After measuring of bursting pressure, the animal was sacrificed by intrathoracic bleeding. The left colon including the anastomosis was excised and dissected free of mesenteric fat tissue. Three rings of tissue measuring 0.5 cm wide were cut: one ring of tissue containing the anastomosis, one ring just proximal, and one ring distal to the anastomosis. The tissue samples were immediately frozen and kept at -80 C for later hydroxyproline assay.

Hydroxyproline as a measure of collagen was determined using a modified version of the colorimetric assay of Stegemann.[24,25]

Collagen concentrations were calculated in g/mg dry weight. The collagen concentration of the segment of colon resected at the initial operation served as the individual control and was used to calculate any changes in collagen content.

A segment of each anastomotic ring was cut off for histologic examination and fixed in 4 percent (w/v) formaldehyde. The samples for histology were dehydrated and embedded in paraffin. From all paraffin blocks 5- m sections were cut and staining was performed with hematoxylin and eosin. In addition a periodic acid-Schiff stain, a Gomori's stain and a Weigert-Von Gieson's stain for assessment of the tissue reaction were performed. The inflammatory infiltrate (neutrophils, eosinophils, macrophages) and tissue organization (fibroblast and capillary proliferation) were scored semiquantitatively as follows: +, numerous; , occasionally present; -, absent.

For statistical analysis, the mean values and SEM were calculated. Results were compared using one-way analysis of variance with the Student-Newman-Keuls test, the chi-squared test and Fisher's exact test when appropriate. The null hypothesis of no difference was rejected at the P level of 0.05.

Results

All animals survived the operation and postoperative period until sacrifice. The average body weight loss in group I and II was maximal on day two. On day four, body weight loss was lowest in Group IA, but differences did not reach the level of statistical significance (Fig. 1).

There were no overt signs of anastomotic leakage or dehiscence in the control or test groups. The adhesion score is shown in Table 1. On day four, adhesion formation in Group IB was significantly raised following fibrin sealing (Table 1). Ischemia (Group IIA) also resulted in increased adhesion formation on day four (P=0.001, chi-squared test). On day seven, however, no differences were noted anymore in this respect. Abscesses were not noted in Group I and in one and three cases in Groups IIA and IIB respectively. Adhesion formation was not different with or without fibrin sealing in ischemic anastomoses.

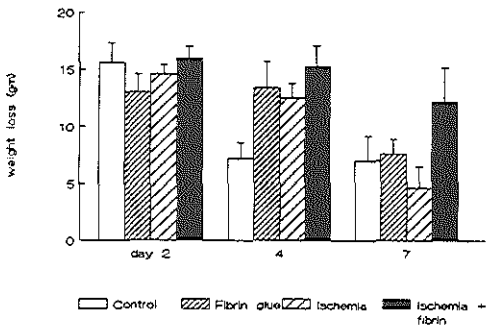

Figure 1. Loss of body weight following resection and anastomosis of the left colon in rats. n = 10.

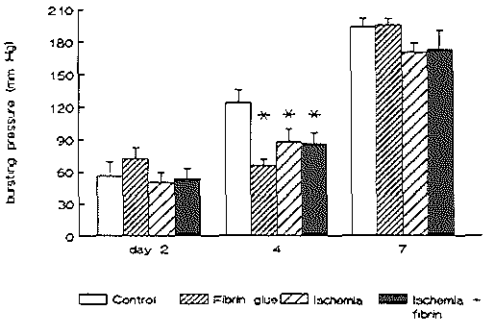

Figure 2. Colonic bursting pressure on days two, four, and seven following creation of end-to-end anastomosis. *Statistically significant ($P < 0.05$). n = 10.

Table 1.
Adhesion Score Following Colonic Anastomosis in the Rat

Group	n	Day 2				Day 4				Day 7			
		0	1+	2+	3+	0	1+	2+	3+	0	1+	2+	3+
IA, control	10	0	6	4	0	0	9	1	0	0	6	4	0
IB, + fibrin sealant	10	2	4	4	0	1	3	6	0	0	6	4	0
IIA, ischemia	10	1	5	4	0	0	1	8	1	0	6	4	0
IIB, + fibrin sealant	10	1	7	2	0	1	0	6	3	0	2	7	1

* Statistically significant ($P < 0.05$).

The bursting pressures measured in this study are shown in Table 2. On day two, the highest bursting pressure was measured in Group IB (Fig. 2). There were no statistical differences in either one of the experimental groups in anastomotic bursting pressure on day two and seven. However, on day four, in Group IB the mean bursting pressure (65 mm Hg) was significantly lower than Group IA (123 mm Hg). The ischemic anastomosis in Group IIA showed a lower bursting pressure (87 mm Hg) on day four compared with group IA ($P<0.01$). On days two and seven, the differences were not significant. There was no difference in bursting pressure noted between Groups IIA and IIB on all occasions.

On day two, the point of disruption during the test of anastomotic bursting pressure was invariably the site of the anastomosis (Table 3). On day four few anastomoses ruptured adjacent to the anastomosis. On day seven, in the control and ischemic anastomoses without fibrin (Groups IA and IIA), in all cases the anastomosis was stronger than the adjacent colon. On day seven, the most likely place of rupture was proximal to the

Table 2.
Anastomotic Bursting Pressure of Colonic Anastomosis in the Rat

Group	n	Day 2	Day 4	Day 7
IA, control	10	56 ± 13	123 ± 12	193 ± 8
IB, + fibrin sealant	10	72 ± 10	65 ± 6*	195 ± 6
IIA, ischemia	10	50 ± 9.5	87 ± 12*	170 ± 8.5
IIB, + fibrin sealant	10	54 ± 8.5	86 ± 10	173 ± 17

Results are given as mean values ± SEM in mm Hg.
* Statistically significant ($P < 0.05$).

Table 3.
Point of Disruption on Testing Anastomotic Bursting Pressure

Group	Day 2			Day 4			Day 7		
	P	A	D	P	A	D	P	A	D
IA, control	0	10	0	0	8	2	8*	0	2
IB, + fibrin sealant	0	10	0	0	10	0	7	1	2
IIA, ischemia	0	10	0	0	8	2	7	0	3
IIB, + fibrin sealant	0	10	0	0	10	0	6	2	2

P = proximal to anastomosis, A = anastomosis, D = distal to anastomosis.
* Statistically significantly different, proximal *vs.* distal ($P < 0.05$).

Table 4.
Mean Concentration and Relative Change of Collagen Proximal to Colonic Anastomosis in the Rat

Group	Day 2	Day 4	Day 7
IA, control	91 ± 7 (−48%) *	108 ± 7 (−29%) *	158 ± 11 (−7%) *
IB, + fibrin sealant	82 ± 6 (−50%)	85 ± 8 (−47%)	102 ± 3 (−37%)
IIA, ischemia	102 ± 6 (−34%)	109 ± 9 (−31%) *	112 ± 6 (−34%)
IIB, + fibrin sealant	70 ± 2 (−53%)	67 ± 4 (−57%)	119 ± 10 (−22%)

Values given are mean values ± SEM in μg/mg dry weight; brackets indicate the relative change compared with the individual control value.
* Statistically significant ($P < 0.05$).

Table 5.
Mean Concentration and Relative Change of Collagen in Colonic Anastomosis in the Rat

Group	Day 2	Day 4	Day 7
IA, control	97 ± 6 (−44%)	113 ± 6 (−26%) *	152 ± 7 (−11%)
IB, + fibrin sealant	77 ± 6 (−52%)	81 ± 5 (−49%)	139 ± 3 (−15%)
IIA, ischemia	95 ± 6 (−39%)	97 ± 7 (−37%)	135 ± 6 (−19%)
IIB, + fibrin sealant	83 ± 8 (−44%)	90 ± 6 (−43%)	123 ± 10 (−17%)

Values given are mean values ± SEM in μg/mg dry weight; brackets indicate the relative change compared with the individual control value.
* Statistically significant ($P < 0.05$).

Table 6.
Mean Concentration and Relative Change of Collagen Distal to Colonic Anastomosis in the Rat

Group	Day 2	Day 4	Day 7
IA, control	135 ± 10 (−22%)	146 ± 7 (−6%) *	157 ± 11 (−8%)
IB, + fibrin sealant	131 ± 5 (−19%)	130 ± 5 (−19%)	144 ± 3 (−11%)
IIA, ischemia	135 ± 10 (−13%)	126 ± 9 (−20%)	129 ± 7 (−23%)
IIB, + fibrin sealant	109 ± 6 (−25%)	121 ± 11 (−22%)	131 ± 7 (−13%)

Values given are mean values ± SEM in μg/mg dry weight; brackets indicate the relative change compared with the individual control value.
* Statistically significant ($P < 0.05$).

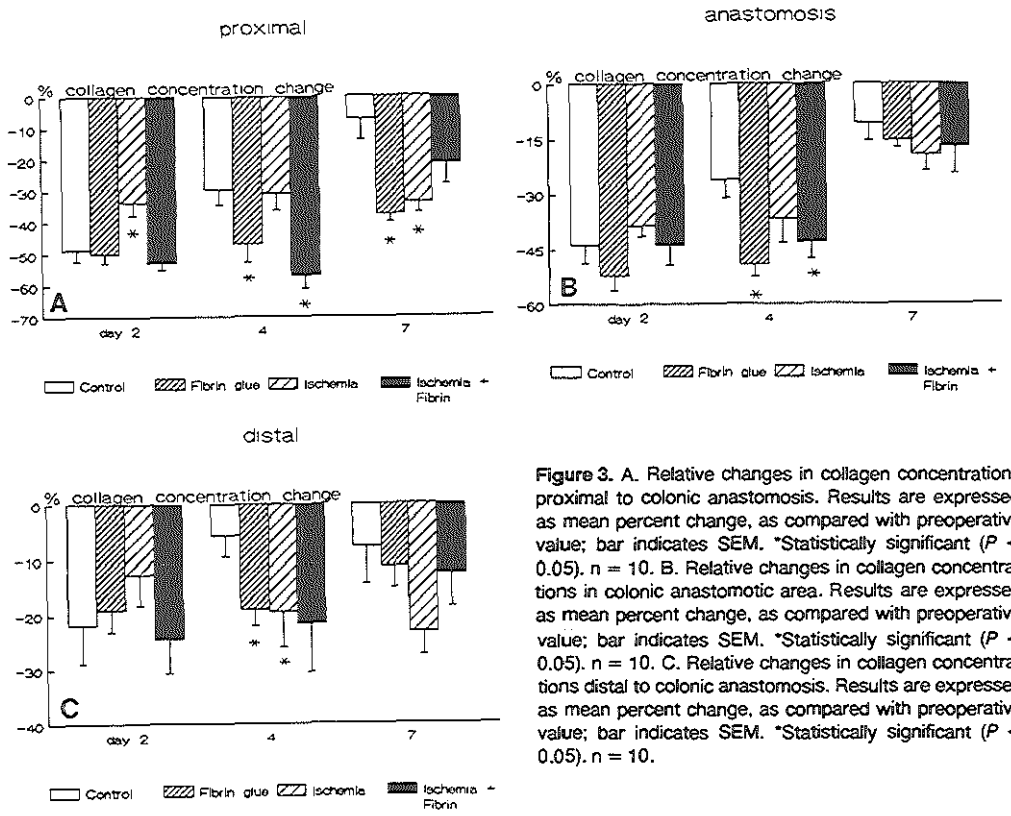

Figure 3. A. Relative changes in collagen concentrations proximal to colonic anastomosis. Results are expressed as mean percent change, as compared with preoperative value; bar indicates SEM. *Statistically significant ($P < 0.05$). n = 10. B. Relative changes in collagen concentrations in colonic anastomotic area. Results are expressed as mean percent change, as compared with preoperative value; bar indicates SEM. *Statistically significant ($P < 0.05$). n = 10. C. Relative changes in collagen concentrations distal to colonic anastomosis. Results are expressed as mean percent change, as compared with preoperative value; bar indicates SEM. *Statistically significant ($P < 0.05$). n = 10.

anastomosis.

Collagen proximal to the anastomosis

Ischemia of the colon resulted in lower collagen loss proximal to the anastomosis on day two ($P<0.05$) (Table 4). Fibrin sealing, however, caused increased relative collagen loss in Group IIB on day two (Fig. 3A). On day four, fibrin sealing of normal anastomoses (Group IB) resulted in higher relative collagen loss. Also, in the sealed ischemic anastomosis, collagen concentration is lower than in the ischemic anstomosis without sealing. On day seven, fibrin in Group IB sealing caused increased collagen breakdown. In Group II, collagen loss was higher than in the control group.

Collagen at the anastomotic site

Collagen concentration in colonic anastomoses did not differ significantly between the treatment groups at day two (Table 5). On this occasion the relative loss of collagen was

maximal, reaching values of >50 percent (Fig. 3B). On day four, the addition of fibrin sealant resulted in increased collagen loss in Group IB (P=0.002). Also the addition of fibrin sealant on the ischemic anastomosis (Group IIB) resulted in increased collagen degradation. On day seven, no statistically significant difference in collagen loss was noted.

Collagen distal to the anastomosis

Distal to the anastomoses, collagen decrease was less pronounced than proximally and at the suture line (Table 6). On day two, fibrin sealing generated increased relative collagen loss distal to the ischemic anastomosis (Fig. 3C). On day four, relative collagen loss was significantly higher in Groups IB, IIA and IIB. The relative loss of collagen in Group II on day seven was not statistically different.

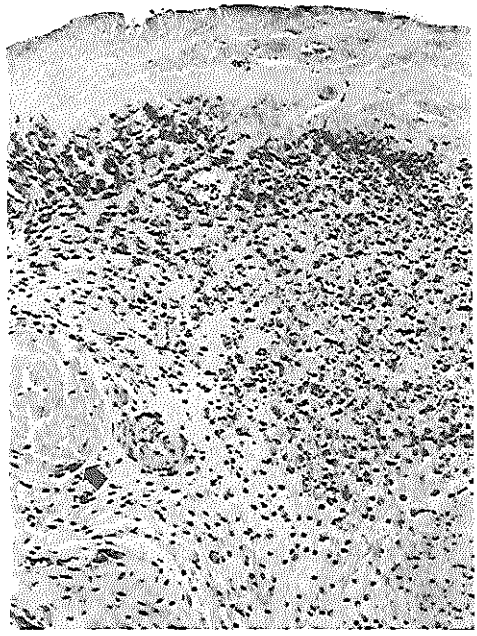

Figure 4. Postoperative day four histologic section of ischemic colonic tissue reveals extensive necrotic mucosa with granulocytic infiltrate. Submucosal vessels show fibrinous thrombosis (arrow; hematoxylin and eosin stain, ×100).

Histologic examination of the ischemic anastomotic area showed, in addition to an increased neutrophilic granulocytic infiltrate, a more extensive mucosal necrosis and fibrin thrombi (Fig. 4). Also, collagen bridging and mucosal regeneration was inhibited compared with the control group. The fibrin sealant was visible on days two and four; however, after seven days it had disappeared. Instead, increased collagen fibrosis and inflammatory infiltrate were visible on day seven in Groups IB and IIB. Neutrophilic granulocytes were more abundant in the anastomotic area on days two and four in Group IB (Table 7).

Table 7.
Presence of Different Cell Types in the Healing Colon Anastomosis in the Rat

Cell Type	Group	Day 2			Day 4			Day 7		
		+	±	−	+	±	−	+	±	−
Neutrophils	IA	2	4	1	0	5	3	1	1	7
	IB	8	0	0*	8	0	0*	2	3	3
	IIA	7	0	3	3	3	3	1	4	4
	IIB	3	3	4	3	4	1	2	3	2
Eosinophils	IA	6	1	0	1	6	1	4	3	2
	IB	5	1	2	5	1	2	1	4	3
	IIA	1	8	1	2	6	1	0	9	0
	IIB	0	9	1	2	6	0	2	3	2
Macrophages	IA	3	4	0	4	4	0	4	5	0
	IB	4	3	1	7	1	0	8	0	0
	IIA	0	7	3	2	5	2	2	7	0
	IIB	1	9	0	1	6	1	4	3	0
Fibroblasts	IA	0	4	3	8	0	0	8	1	0
	IB	3	2	3	6	2	2	7	1	0
	IIA	0	9	1	6	3	0	7	2	0
	IIB	6	4	0	4	4	0	6	1	0

+, numerous; ±, occasionally present; −, absent.
IA, control; IB, control + fibrin; IIA, ischemia; IIB, ischemia + fibrin.
* Statistically significant ($P < 0.05$).

Discussion

Additional sealing has been advocated in normal and high-risk colonic anastomoses in patients as a method to prevent anastomotic leakage.[12-16] However, the results of experimental studies are conflicting, and prospective, randomized clinical studies are lacking.[17-19] We have reported recently that, in the rat model, application of human and rat fibrinogen has a negative influence on the healing (noncompromised) colonic anastomosis.[20] Ischemia or hypoxia is a known risk factor in colonic anastomotic healing.[6,26] The poor vascularity of the resected bowel ends of low colorectal anastomoses may explain anastomotic failure. Good blood supply is essential for wound healing since synthesis of collagen depends on adequate tissue oxygenation.[4] The quantity and quality of collagen in the submucosal layer of the intestinal wall determine the strength of the healing intestine.[27] Following the construction of an anastomosis in the colon there is a significant decrease in collagen.[28,29,30] The results we present confirm the finding shown earlier by other investigators that the decrease in collagen content is highest just proximal to the anastomosis.[11] The reduced collagen concentration proximal to the anastomosis may have accounted for the disruptions taking place almost exclusively in this area on testing bursting pressure on day seven.

It was suggested that local degradation of mature collagen is the reason why sutures cut through the tissue, thus causing breakdown of the anastomosis.[8,31] Collagen is very resistant to the action of most proteolytic enzymes, but breakdown is initiated by a specific enzyme: collagenase. A significant increase of collagenase was found in the gastrointestinal tract after construction of an anastomosis in the colon.[32] The highest collagenase activity is found in the colon, and the high incidence of anastomotic leakage in the colon is possibly due to this enzyme. It was surprising that collagen loss was not very pronounced in the ischemic anastomosis; in fact, during the early phase of healing, collagen breakdown was even less than the controls. This may be explained by a low local tissue collagenase activity, which was found to be present in ischemic colonic tissue.[5] From our results it seems that in ischemic anastomoses the breakdown of collagen is not increased, but on the other hand it takes more time for the tissue to regain normal collagen concentration. It seems that, in ischemic colonic tissue, both breakdown and synthesis of collagen are low. From studies on skin wounds, it was demonstrated that hypoxia leads to diminished collagen synthesis.[4]

Our findings support the hypothesis that tissue ischemia is an important stimulus in postoperative adhesion formation.[33] Previous studies have suggested that fibrin adhesive may decrease intra-abdominal adhesion formation.[34,35,36] Contrary to this, we found increased adhesion formation following fibrin sealing of colonic anastomosis on day four. It is not known from our results whether these adhesions are long-lasting or transient. A possible explanation of this difference may be the likely bacterial infection of the fibrin clot on the anastomosis. Also, in the ischemic anastomoses, adhesion formation was not reduced by fibrin sealant.

Conclusion

From our results, it is concluded that sealing of normal colonic anastomosis in the rat has a negative effect on the healing colon. Ischemia at the site of the anastomosis results in weaker anastomotic strength on day four postoperatively. Also, in a high-risk situation of ischemia, fibrin sealant does not improve wound healing during the first seven days. Fibrin sealant does not prevent adhesion formation in control and ischemic anastomoses. As a

consequence of this, it seems to us that the clinical use of fibrin sealant on ischemic colonic anastomoses has to be reconsidered.

References:

1. Goligher JC, Graham NG, De Dombal FT. Anastomotic dehiscence after anterior resection of rectum and sigmoid. Br J Surg 1970;57:109-118.

2. Fielding LP, Stewart-Brown S, Blesovsky L, Kearny G. Anastomotic integrity after operations for large-bowel cancer: a multicentre study. Br Med J 1980;281:411-414.

3. Shandall A, Lowndes R, Young HL. Colonic anastomotic healing and oxygen tension. Br J Surg 1985;72:606-609.

4. Hunt TK, Pai MP. The effect of varying ambient oxygen tensions on wound metabolism and collagen synthesis. Surg Gynecol Obstet 1972;135:561-567.

5. Foster ME, Lancaster J, Leaper DJ. Leakage of low rectal anastomoses - an anatomic explanation. Dis Col Rect 1984;27:157-158.

6. Schrock TR, Deveney CW, Dunphy JE. Factors contributing to leakage of colonic anastomoses. Ann Surg 1973;127:513-518.

7. Tagart REB. Colorectal anastomosis: factors influencing success. J Roy Soc Med 1981;74:111-118.

8. Banerjee SK, Glynn LE. Reactions to homologous and heterologous fibrin implants in experimental animals. Ann NY Acad Sci 1960;86:1054-1057.

9. Leibovich SJ, Ross R. A macrophage-dependent factor that stimulates the proliferation of fibroblasts in vitro. Am J Path 1976;84:501-513.

10. Pohl J, Bruhn HD, Christophers E. Thrombin and fibrin-induced growth of fibroblasts: role in wound repair and thrombus organization. Klin Wochenschrift 1979;57:273-277.

11. Petrelli NJ, Cohen HC, DeRisi D, et al. The application of tissue adhesives in small-bowel anastomoses. J Surg Oncol 1982;19:59-61.

12. Scheele J, Herzog J, Mühe E. Fibrin glue protection of digestive anastomoses. Zbl Chirurgie 1978;103:1325-1336.

13. Pointner R, Villinger R. Vorbeugung der intraperitonealen Nahtinsuffizienz durch Einsatz von Fibrin kleber. Zbl Chirurgie 1984;109:1146-1148.

14. Athanasiadis S, Kuhlgatz Ch, Girona I. Additional fibrin glueing in surgery of colon and rectum. Zbl Chirurgie 1984;109:1107-1111.

15. Waclawiczek HW, Boeckl O. Clinical experience from fibrin sealing in general and thoracic surgery. Zbl Chirurgie 1986;111:16-24.

16. Giardino R, Brulatti M, Franchini A. Colonic anastomoses protected with fibrin sealant. In: Schlag G, Redl H (eds). Fibrin sealant in operative medecine. General surgery and abdominal surgery - Vol. 6. Springer-Verlag, Heidelberg, Berlin, 1986, pp 155-158.

17. Nordkild P, Hjortrup A, Kjærgaard J. Tissue adhesives and intestinal anastomosis. Ann Chir Gyn 1986;75:205-208.

18. Houston KA, Rotstein OD. Fibrin sealant in high-risk anastomoses. Arch Surg 1988;123:230-234.

19. Hulkko OA, Haukipuro KA, Laitinen ST. Fibrin glue protection of primary anastomosis in the obstructed left colon; an experimental study on the rat. Acta Chir Scand 1988;154:49-52.

20. Van der Ham AC, Kort WJ, Weijma IM, Van den Ingh HFGM, Jeekel J. The effect of fibrin sealant on the healing colonic anastomosis in the rat. Br J Surg 1991;78:49-53.

21. Foster ME, Brennan SS, Morgan A, Leaper DJ. Colonic ischaemia and anstomotic healing. Eur Surg Res 1985;17:133-139.

22. Kirk D, Irvin TT. The role of oxygen therapy in the healing of experimental skin wounds and colonic anastomosis. Br J Surg 1977;64:100-103.

23. Cohen SR, Cornell CN, Collins MH, Sell JE, Blanc WA, Altman RP. Healing of ischemic colonic anastomoses in the rat: Role of antibiotic preparation. Surgery 1985;97:443-446.

24. Stegemann H. Stalder K. Determination of hydroxyproline. Clin Chim Acta 1967;18:267-273.

25. Grant RA. Estimation of hydroxyproline by the AutoAnalyser. J Clin Path 1964;17:685-686.

26. Kirk D, Irvin TT. The role of oxygen therapy in the healing of experimental skin wounds and colonic anastomoses. Br J Surg 1977;64:100-103.

27. Gottrup F. Healing of incisional wounds in stomach and duodenum: collagen distribution and relation to mechanical strength. Am J Surg 1981;141:222-227.

28. Wise L, McAlister W, Stein T, Schuck P. Studies on the healing of anastomoses of small and large intestines. Surg Gynecol Obstet 1975;141:190-194.

29. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomoses: III. Collagen metabolism in the colon after left colon resection. Am J Surg 1980;139:398-405.

30. Cronin K, Jackson DS, Dunphy JE. Changing bursting strength and collagen content of the healing colon. Surg Gynec Obstet 1968;126:747-753.

31. Hawley PR. Causes and prevention of colonic anastomotic breakdown.
Dis Col Rect 1973;16:272-277.

32. Hawley PR, Faulk WP, Hunt TK, Dunphy JE. Collagenase activity in the gastrointestinal tract. Br J Surg 1970;170:896-900.

33. Ellis H. The cause and prevention of postoperative intraperitoneal adhesions. Surg Gynecol Obstet 1971;133:407-511.

34. Brands W, Jung M, Manegold C, Liedgens P, König M, Stock M. Prophylaxis and therapy of intra-abdominal adhesions with highly concentrated human fibrinogen: experimental and clinical results. in: Waclawiczek HW. (Ed.) Progress in fibrin sealing. Springer-Verlag, Berlin Heidelberg New York, 1989, pp 141-145.

35. Lindenberg S, Steentoft P, Sørensen SS, Olesen HP. Studies on prevention of intra-abdominal adhesion formation by fibrin sealant: an experimental study in rats. Acta Chir Scand. 1985;151:525-527.

36. DeVirgillio C, Dubrow T, Sheppard BB, et al. Fibrin glue inhibits intr-abdominal adhesion formation. Arch Surg 1990;125:1378-1382.

CHAPTER VII

EFFECT OF FIBRIN SEALANT ON THE INTEGRITY OF COLONIC ANASTOMOSES IN RATS WITH FAECAL PERITONITIS

A.C. van der Ham, W.J. Kort, I.M. Weijma, H.F.G.M. van den Ingh*, J. Jeekel.

From the Laboratory for Experimental Surgery, Erasmus University, and the Department of Pathology*, St. Clara Ziekenhuis, Rotterdam, The Netherlands.

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Abstract

Objective: To assess the influence of fibrin adhesive on the healing of colonic anastomoses in rats with and without faecal peritonitis.

Design: Controlled study.

Setting: Laboratory for experimental surgery, Erasmus University Rotterdam, The Netherlands.

Material: 120 male Wag/Rij rats.

Interventions: All rats had a single layer end-to-end anastomosis fashioned with 7/0 polypropylene. Faecal peritonitis was then induced in half of the rats by placement of 200 mg powdered autoclaved rat faeces in the peritoneal cavity near the anastomosis. Rats were allocated to one of four groups (n=30 in each): 1-control; 2-additional sealing with fibrin glue; 3 -peritonitis alone; and 4-peritonitis with fibrin glue.

Main outcome measures: Body weight, adhesion formation, anastomotic bursting pressure and collagen concentration around the anastomosis on days 2, 4, and 7 in 10 rats from each group.

Results: 11 rats died of peritonitis before the experiment was completed. Peritonitis caused increased formation of adhesions and abscesses, with or without fibrin sealant. Bursting pressure at the anastomosis was significantly reduced in peritonitis compared with controls on days 4 and 7, and this was not prevented by fibrin. Sealing of anastomoses resulted in lower bursting pressures on day 4 in control animals. Collagen concentration was significantly reduced in peritonitis with or without fibrin sealant on days 4 and 7, and after fibrin sealing of control anastomoses.

Conclusion: Faecal peritonitis reduced mechanical strength and collagen concentration of colonic anastomoses, and this was not prevented by additional sealing of the anastomosis with fibrin sealant.

Introduction

Leakage from the anastomosis is a major complication after colonic and rectal resection[8], and is associated with a high mortality and morbidity.[5] Many factors contribute to the healing and integrity of an anastomosis including adequate blood supply, absence of tension, degree of inflammation, type of suture material or staples, quality of bowel prep-

aration and age of the patient.[5,6,12,28,30] The presence of an abscess or peritonitis is likely to increase the leak rate significantly,[28] so under such circumstances many surgeons will avoid construction of an anastomosis by doing a Hartmann's procedure, while others make a diverting stoma proximal to a high risk anastomosis.[23,34]

Additional sealing with fibrin adhesive has been advocated in both normal and high risk colonic anastomoses in humans to prevent anastomotic leakage.[2,7,26,27,32] and this was said to be particularly beneficial in the presence of peritonitis or abscesses. Fibrin sealant is a biologic adhesive made of concentrated human fibrinogen which can be used to establish haemostasis or as an adhesive in wound repair. Several studies have shown that fibrin sealant aids wound healing by producing local haemostasis and by stimulating the influx of macrophages that produce factors that cause angiogenesis, fibroblast proliferation, and collagen production.[3,21,25] By sealing an intestinal anastomosis, bacteria may be confined within the lumen, thus reducing inflammation around the anastomosis[24] Results of experimental studies on sealing of colonic anastomoses are conflicting however, and we know of no prospective randomised clinical studies.[15,16,22,31] We therefore designed a study to test the effect of fibrin sealing on the healing of colonic anastomoses in a peritonitis model in rats.

Material and methods

120 Male Wag/Rij rats that weighed 180-230 g, were randomly assigned to four treatment groups (n=30 in each). They were allowed free access to water (acidified, pH 3.0) and food (AM II, Hope Farms, Woerden, the Netherlands) before and after operation. Ether anaesthesia was used, and through a midline incision 1 cm of the left colon was resected, 3 cm proximal to the peritoneal reflection. All animals had a single layer, end-to-end anas-

Table I. *Mean (SEM) loss of body weight (g) after colonic resection and anastomosis in 120 rats*
There were 30 rats in each group on day 2; the corresponding figures for days 4 and 7 were 30, 30, 27 and 22, respectively

Group	Day 2	Day 4	$p < 0.05$	Day 7	$p < 0.05$
1 Control	15.6 (1.7)	7.2 (1.5)	]]	7.0 (2.2)	]]
2 Control with fibrin seal	13.0 (1.6)	13.4 (2.4)		7.6 (1.3)	
3 Peritonitis	18.4 (1.2)	29.8 (2.2)	]]	31.4 (4.8)	
4 Peritonitis with fibrin seal	17.7 (1.0)	25.0 (4.4)		26.6 (4.2)	

Table II. *Incidence of adhesions after colonic resection and anastomosis in rats*

Group	Day 2					Day 4				
	None	Mininal	Moderate	Severe	$p < 0.05$	None	Mininal	Moderate	Severe	$p < 0.05$
1 Control	0	6	4	0	]	0	9	1	0	]
2 Control with fibrin seal	2	4	4	0	]	0	4	6	0	]
3 Peritonitis	0	0	3	7	]	0	0	0	8	]
4 Peritonitis with fibrin seal	0	0	0	10	]	0	0	0	4	]

Table III. *Bursting pressures (mm Hg) of colonic anastomoses in rats*

Group	Day 2		Day 4			Day 7		
	No of rats	Mean (SEM)	No of rats	Mean (SEM)	$p < 0.05$	No of rats	Mean (SEM)	$p < 0.05$
1 Control	10	56 (13)	10	123 (12)	]	10	193 (8)	]
2 Control with fibrin seal	10	72 (10)	10	65 (6)	]	10	195 (6)	]
3 Peritonitis	10	71 (9)	8	41 (15)	]	9	147 (13)	
4 Peritonitis with fibrin seal	10	96 (11)	4	40 (20)	]	8	123 (15)	

Table IV. *Point of disruption of colon during test of anastomotic bursting pressure*

Figures are number of rats

Group	Day 2			Day 4			Day 7		
	Proximal	At anastomosis	Distal	Proximal	At anastomosis	Distal	Proximal	At anastomosis	Distal
1 Control	0	10	0	0	8	2	8	0	2
2 Control with fibrin seal	0	10	0	0	10	0	7	1	2
3 Peritonitis	0	10	0	0	7	1	1	7*	1
4 Peritonitis with fibrin seal	0	10	0	0	3	1	5	3	0

* $p = 0.0007$ compared with control.

tomosis fashioned with 12 interrupted, inverting 7/0 polypropylene (Prolene®, Ethicon, FRG) stitches. The resected segment was frozen and kept at -80 C until analysis to serve as an individual control. The four groups studied were: 1) control sutured anastomoses; 2) sutured anastomosis with fibrin sealant; 3) sutured anastomosis with faecal peritonitis; and 4) sutured anastomosis with faecal peritonitis and fibrin sealant.

Fibrin sealant

The human fibrin sealant (Tissucol®, Immuno Co, Vienna, Austria) was prepared according to the manufacturer's instructions. It consists of a freeze-dried protein concentrate of human fibrinogen (120 mg/ml) which is reconstituted in a solution of aprotinin

CONTINUATION OF TABLE II.

Day 7				
None	Mininal	Moderate	Severe	$p < 0.05$
0	6	4	0	
0	6	4	0	
0	0	3	6	
0	0	1	7	

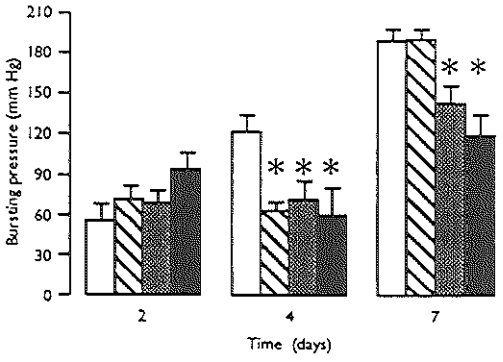

Fig. 1. Mean (SEM) colonic bursting pressures on days 2, 4 and 7 after the fashioning of end-to-end colonic anastomoses. The open bars indicate control rats, the crossed bars control rats with additional fibrin glue, the hatched bars rats with peritonitis, and the solid bars rats with peritonitis and fibrin glue. There were 10 rats in each group on day 2; the corresponding figures for day 4 were 10, 10, 8 and 4, and for day 7 this was 10, 10, 9, and 8 respectively. * $p < 0.05$ compared with the control group.

(3000 KIU/ml), and a solution of thrombin (500 IU/ml) and calcium chloride (40 mmol/l). After preheating to 37 C the components were reconstituted with their solutions and drawn up in separate syringes. These syringes fit in a specially designed syringe holder (Duplo-ject[R]) which enables the two components to be mixed and applied simultaneously. Mixing and application activated the fibrinogen to form fibrin and the solutions were transformed into a rubber like adhesive mass. One anastomosis required 0.2 ml of fibrin sealant.

Faecal peritonitis (groups 3 and 4)

On completion of the colonic anastomosis, we placed 200 mg of powdered autoclaved rat faeces in the peritoneal cavity near the anastomosis.

Measurements

On day 2, 4 and 7, 10 rats from each group were designated to undergo relaparotomy. Body weight was measured beforehand and compared with the individual body weight at the time of the first operation. After anaesthesia with ether we reopened the laparotomy incision and assessed the degree of adhesion formation: none; minimal (mainly between the anastomosis and the omentum); moderate (between omentum and the anastomotic site and between the anastomosis and a loop of small bowel); and severe and extensive (including abscess formation). The rectum was then cannulated and the left colon was

Table V. *Mean (SEM) concentration, and percentage change compared with individual values, of collagen (μg/mg dry weight) proximal to colonic anastomoses in rats*

Group	Day 2				Day 4				Day 7			
	No of rats	Mean (SEM)	Percentage change	$p < 0.05$	No of rats	Mean (SEM)	Percentage change	$p < 0.05$	No of rats	Mean (SEM)	Percentage change	$p < 0.05$
1 Control	10	91 (7)	−49		10	109 (7)	−29		10	158 (11)	−7	
2 Control with fibrin seal	10	82 (6)	−50		10	85 (8)	−47		10	102 (3)	−38	
3 Peritonitis	10	84 (7)	−43		8	61 (7)	−56		9	102 (7)	−26	
4 Peritonitis with fibrin seal	10	59 (7)	−62		4	49 (6)	−62		8	92 (6)	−38	

Table VI. *Mean (SEM) concentration, and percentage change compared with individual values, of collagen (μg/mg dry weight) in colonic anastomoses in rats*

Group	Day 2				Day 4				Day 7			
	No of rats	Mean (SEM)	Percentage change	$p < 0.05$	No of rats	Mean (SEM)	Percentage change	$p < 0.05$	No of rats	Mean (SEM)	Percentage change	$p < 0.05$
1 Control	10	97 (6)	−44		10	113 (6)	−26		10	152 (7)	−11	
2 Control with fibrin seal	10	77 (6)	−52		10	81 (5)	−49		10	139 (3)	−15	
3 Peritonitis	10	99 (8)	−33		8	68 (6)	−50		9	105 (6)	−23	
4 Peritonitis with fibrin seal	10	67 (10)	−56		4	65 (9)	−50		8	110 (9)	−26	

Table VII. *Mean (SEM) concentration, and percentage change compared with individual values, of collagen (μg/mg dry weight) distal to colonic anastomoses in rats*

Group	Day 2				Day 4				Day 7			
	No of rats	Mean (SEM)	Percentage change	$p < 0.05$	No of rats	Mean (SEM)	Percentage change	$p < 0.05$	No of rats	Mean (SEM)	Percentage change	$p < 0.05$
1 Control	10	135 (10)	−22		10	146 (7)	−6		10	157 (11)	−8	
2 Control with fibrin seal	10	131 (5)	−19		10	130 (5)	−19		10	145 (3)	−11	
3 Peritonitis	10	101 (8)	−32		8	83 (7)	−39		9	115 (3)	−15	
4 Peritonitis with fibrin seal	10	83 (10)	−46		4	67 (6)	−47		8	110 (10)	−26	

ligated twice, 1 cm proximal and 1 cm distal to the anastomosis, the latter around the rectal cannula. We took care not to disturb the anastomosis and its adhesions. The rectal cannula was connected to an infusion-pump (Hospal^{R} ,Italy), with a side arm to a pressure transducer, which was connected to a recorder. The *in vivo* anastomotic bursting pressure was measured by infusing normal saline at a constant rate of 1 ml/minute, and was marked by abrupt loss of pressure. After we had measured the bursting pressure, the animal was sacrificed by exsanguination. The left colon including the anastomosis was excised and dissected free of mesenteric fat tissue, and three rings of tissue each measuring 0.5 cm were cut. One ring contained the anastomosis, and one ring was just proximal,

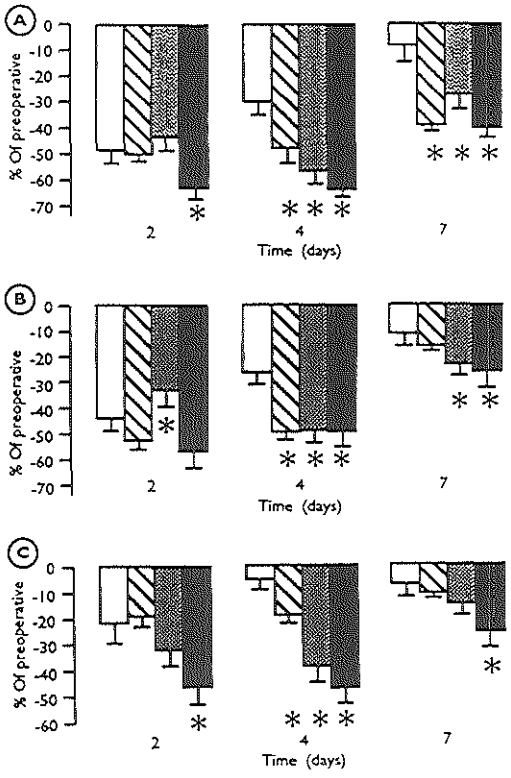

Fig. 2. Mean (SEM) percentage changes in hydroxyproline concentrations on days 2, 4 and 7 compared with the preoperative values (A) proximal to colonic anastomosis; (B) in the area of the anastomosis; (C) distal to the anastomosis. The *open bars* indicate control rats, the *crossed bars* control rats with additional fibrin glue, the *hatched bars* rats with peritonitis, and the *solid bars* rats with peritonitis and fibrin glue. There were 10 rats in each group on day 2; the corresponding figures for day 4 were 10, 10, 8 and 4, and for day 7 this was 10, 10, 9, and 8 respectively. * $p < 0.05$ compared with the control group.

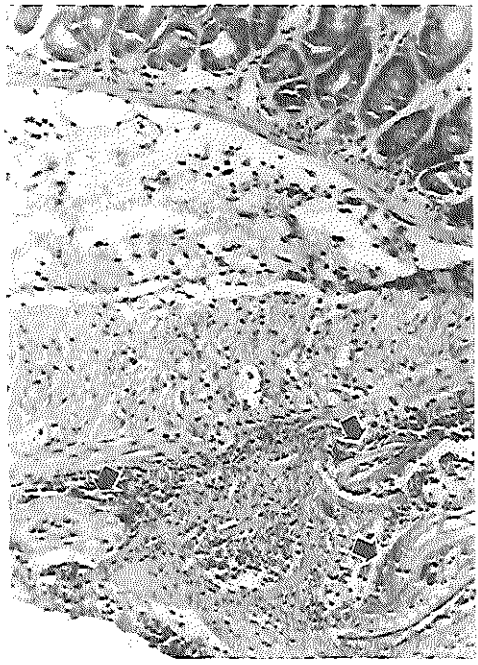

Fig. 3. Histological slide of colonic anastomosis with peritonitis on day 4 showing fibrin and inflammatory cells around faecal material (*arrows*). Haematoxylin and eosin, original magnification × 100.

and one ring just distal to the anastomosis. The tissue samples were immediately frozen and kept at -80 C for later assay of hydroxyproline concentration. The hydroxyproline concentration (as a measure of collagen) was measured with a modified version of the colorimetric assay described by Stegemann and Stalder.[10,29] Collagen concentrations were calculated in g/mg dry weight. The collagen concentration of the segment of colon resected at the initial operation served as the individual control, and was used to calculate

Table VIII. *Histopathologic signs in healing colonic anastomoses in rats*

Variable and group	Day 2				Day 4			
	Numerous	Few	Absent	$p < 0.05$	Numerous	Few	Absent	$p < 0.05$
Inflammatory cells								
Control	2	4	1		0	5	3	]]
Control with fibrin seal	8	0	0		8	0	0	
Peritonitis	7	2	0		8	1	0	
Peritonitis with fibrin seal	2	6	0		4	0	0	
Fibroblasts								
Control	0	4	3		8	0	0	
Control with fibrin seal	3	2	3		6	2	2	
Peritonitis	0	9	0		5	4	0	
Peritonitis with fibrin seal	0	1	7		3	1	0	
Fibrin								
Control	1	6	0	]]	0	8	0	]]
Control with fibrin seal	8	0	0		9	1	0	
Peritonitis	9	0	0		8	1	0	
Peritonitis with fibrin seal	8	0	0		4	0	0	
Capillary proliferation								
Control	0	4	3		8	0	0	
Control with fibrin seal	3	2	3		6	2	2	
Peritonitis	0	7	2		1	8	0	
Peritonitis with fibrin seal	0	1	7		2	2	0	

any changes in collagen content. A segment of each anastomotic ring was cut off for histological examination and fixed in 4% (w/v) formaldehyde; it was then dehydrated and embedded in paraffin. Sections 5 m thick were cut from all paraffin blocks and stained with haematoxylin and eosin. In addition, periodic-acid-Schiff (PAS), Gomori, and Weigert-Van Gieson stains were applied for assessment of the tissue-reaction. The inflammatory cellular infiltrate (neutrophils, eosinophils, lymphocytes and macrophages) and tissue organization (fibroblast and capillary proliferation) were scored semi-quantitatively as: numerous, few, or absent.

For presentation of the results, we calculated the mean values and the standard error of the mean (SEM). Results were compared using one-way analysis of variance (ANOVA) with the Student-Newman-Keuls test, Chi-square test and Fisher's exact test when appropriate. A probability of less than 0.05 was accepted as significant.

Results

No animals died in groups 1 and 2, but three died in group 3 (10%) and eight in group 4 (27%), all with symptoms of peritonitis (Table I). The difference in mortality between groups 3 and 4 is not significant ($p = 0.09$), but that between groups 1 and 2, and 3 and 4,

CONTINUATION OF TABLE VIII.

Day 7			
Numerous	Few	Absent	$p < 0.05$
1	1	7	]]
2	3	3	
4	4	0	
7	0	0	
8	1	0	
7	1	0	
6	2	0	
7	0	0	
0	0	9	
0	0	8	
2	2	4	
1	1	5	
8	1	0	
7	1	0	
6	2	0	
7	0	0	

is (p = 0.001). All the animals that died did so after the assessment on the second day.

The mean loss of body weight is shown in Table I. There were no overt signs of anastomotic leakage or dehiscence in the rats in groups 1 and 2; as all the rats with peritonitis (groups 3 and 4) developed severe adhesions, inspection of their anastomoses was unreliable. The adhesion scores are shown in Table II. Intraperitoneal abscesses developed around the faecal material in all animals in groups 3 and 4.

The bursting pressures are shown in Table III and Fig. 1, and the points of disruption during the tests of bursting pressure in Table IV.

The concentration of collagen proximal to the anastomosis is shown in Table V, and the relative changes in collagen concentrations in Fig. 2A. The corresponding results figures for collagen at the anastomotic site and distal to the anastomosis are shown in Table VI and Fig. 2B, and Table VII and Fig. 2C, respectively.

Histologic examination confirmed the macroscopic findings of peritonitis in groups 3 and 4 (Fig. 3), and the detailed findings are shown in Table VIII.

Discussion

The presence of infection is a critical prognostic factor in resection of the left colon in humans,[28] and additional sealing has been advocated to prevent anastomotic leakage.[2,7,26,32] Some authors indicate that fibrin sealing of colonic anastomosis is particularly beneficial in case of infected anastomoses.[27,33] Wound healing of the colon in rats is not affected by peritonitis caused by intraperitoneal injection of live Escherichia coli.[14] The peritoneal cavity has a well developed ability to clear bacteria, and bacteria by themselves

are relatively harmless. The addition of foreign material, however, results consistently in the formation of abscesses,[13] and deposition of powdered autoclaved rat faeces around the completed anastomosis is an established method of infecting an anastomosis.[12,17] Eleven of our 60 animals with faecal peritonitis died, giving a mortality of 18%, compared with 0 in the uninfected rats. The increase in weight loss is only a rough indication of the poor condition of the animals with peritonitis. The severe peritoneal reaction, in combination with a moderate mortality, show that this model is suitable for studies on the influence of peritonitis on colonic wound healing during the first postoperative week.

These results show that there was a significant reduction in mechanical strength of the control (uninfected) anastomoses after fibrin glue sealing by day 4. This inhibition cannot be attributed to the fact that human fibrinogen was used, as in a previous study we showed that rat fibrinogen had the same effect.[31] Faecal peritonitis did not result in a lowering of the anastomotic bursting pressure on the second postoperative day, but on days 4 and 7 it was significantly lower, which confirms previous reports[12]. We were unable to prevent this reduction by the addition of fibrin sealant. The weakness of the infected colonic anastomoses was also reflected by the fact that on day 7 the anastomosis itself was still the predelected point of disruption while in the control groups it was the bowel wall outside the anastomosis. The quantity and quality of collagen in the submucosal layer of the intestinal wall determines the strength of the healing intestine.[9] After the construction of an anastomosis in the colon there is a significant decrease in collagen.[4,19,35] Our results confirm that the reduction in collagen content is highest just proximal to the anastomosis.[18] The reduced collagen concentration proximal to the anastomosis may indicate why disruption took place almost exclusively in this area when bursting pressures were tested on day 7.

Faecal peritonitis caused significant reductions in collagen concentrations around the anastomoses on days 4 and 7; in control anastomoses collagen concentrations were least on day 2, and in the case of faecal peritonitis on day 4, which suggests that in cases of peritonitis colonic wound healing is slowed down. As with the bursting pressure, the collagen loss could not be prevented by additional sealing. On day 2 the reduction in the collagen concentration was significantly greater after fibrin sealing of the infected anastomosis, but on days 4 and 7 the differences were not significant.

Collagen is resistant to the action of proteolytic enzymes, but breakdown is initiated by a specific enzyme: collagenase. A significant increase in collagenase has been reported in the gastrointestinal tract after anastomosis in the colon.[2] Collagenase activity is found to be highest in the colon and possibly the high incidence of anastomotic leakage in the colon is the result of the activity of this enzyme. Local degradation of mature collagen might be the cause of cutting through of sutures thus causing breakdown of the anastomosis.[12,16] We have previously shown that fibrin applied to a colonic anastomosis is infiltrated by inflammatory cells,[31] which are a known source of collagenase and other proteolytic enzymes. The significant reduction of collagen concentrations on day 4 was probably the result of increased collagen breakdown by these inflammatory cells. Implantation of fibrin clots containing *E coli* in the peritoneal cavities of rats, can cause the formation of intraperitoneal abscesses.[8] The present results show that fibrin sealing of control anastomosis had the same detrimental effect on mechanical strength and collagen concentration as faecal peritonitis. It has been suggested that the reduction of collagen concentration in the presence of infection is not the result of increased breakdown of old collagen, but of reduced synthesis of new collagen.[17] Whatever the actual mechanism, the lower concentration of collagen was associated with reduced mechanical strength. The impaired healing of colonic anastomoses after faecal soiling of the peritoneal cavity, that we found, once again stresses the necessity for meticulous washing of the peritoneum in case of faecal spill, during laparotomy.

We conclude that faecal peritonitis impairs the healing of colonic anastomoses, and that in the case of faecal peritonitis fibrin sealant does not improve healing of colonic anastomoses in rats.

References

1. Ahrenholz DH, Simmons RL. Fibrin in peritonitis.I. Beneficial and adverse effects of fibrin in experimental E. coli peritonitis. Surgery 1980;88:41-47.

2. Athanasiadis S, Kuhlgatz Ch, Girona I. Additional fibrin gluing in surgery of colon and rectum. Zbl Chirurgie 1984;109:1107-1111.

3. Banerjee SK, Glynn LE. Reactions to homologous and heterologous fibrin implants in experimental animals. Ann NY Acad Sci 1960;86:1054-1057.

4. Cronin K, Jackson DS, Dunphy JE. Changing bursting strength and collagen content of the healing colon. Surg Gynec Obstet 1968;126:747-753.

5. Fielding LP, Stewart-Brown S, Blesovsky L, Kearny G. Anastomotic integrity after operations for large-bowel cancer: a multicentre study. Br Med J 1980;281:411-414.

6. Foster ME, Lancaster J, Leaper DJ. Leakage of low rectal anastomoses - an anatomic explanation. Dis Col Rect 1984;27:157-158.

7. Giardino R, Brulatti M, Franchini A. Colonic anastomoses protected with fibrin sealant. In: Schlag G, Redl H (eds). Fibrin sealant in operative medicine. General surgery and abdominal surgery - Vol. 6. Springer-Verlag, Heidelberg, Berlin, 1986, pp 155-158.

8. Goligher JC, Graham NG, De Dombal FT. Anastomotic dehiscence after anterior resection of rectum and sigmoid. Br J Surg 1970;57:109-118.

9. Gottrup F. Healing of incisional wounds in stomach and duodenum: collagen distribution and relation to mechanical strength. Am J Surg 1981;141:222-227.

10. Grant RA. Estimation of hydroxyproline by the AutoAnalyser. J Clin Path 1964;17:685-686.

11. Hawley PR, Faulk WP, Hunt TK, Dunphy JE. Collagenase activity in the gastrointestinal tract. Br J Surg 1970;170:896-900.

12. Hawley PR. Causes and prevention of colonic anastomotic breakdown. Dis Col Rect 1973;16:272-277.

13. Hesp WLEM, Hendriks T, Lubbers EJC, De Boer HHM. A reproducible model for protracted peritonitis in the rabbit. Eur Surg Res 1984;16:317-321.

14. Högström H, Jiborn H, Zederfeldt B, Haglund U. Influence of intraperitoneal Escherichia coli with septicemia on the healing of colonic anastomoses and skin wounds. Eur Surg Res 1985;17: 128-132.

15. Houston KA, Rotstein OD. Fibrin sealant in high-risk anastomoses. Arch Surg 1988;123:230-234.

16. Hulkko OA, Haukipuro KA, Laitinen ST. Fibrin glue protection of primary anastomosis in the obstructed left colon; an experimental study on the rat. Acta Chir Scand 1988;154:49-52.

17. Irvin TT. Collagen metabolism in infected colonic anastomoses. Surg Gynecol Obstet 1976;143:220-224.

18. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomoses: the effect of suture technic on collagen concentration in the colonic wall. Am J Surg 1978;135:333-340.

19. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomoses: III. Collagen metabolism in the colon after left colon resection. Am J Surg 1980;139:398-405.

20. Jönsson K, Jiborn H, Zederfeldt B. Collagen metabolism in small intestinal anastomosis. Am J Surg 1987;154:288-291.

21. Leibovich SJ, Ross R. A macrophage-dependent factor that stimulates the proliferation of fibroblasts in vitro. Am J Path 1976;84:501-513.

22. Nordkild P, Hjortrup A, Kjærgaard J. Tissue adhesives and intestinal anastomosis. Ann Chir Gyn 1986;75:205-208.

23. Peoples JB, Vilk DR, Maguire JP, Elliott DW. Reassessment of primary resection of the perforated segment for severe colonic diverticulitis. Am J Surg 1990;159:291-293.

24. Petrelli NJ, Cohen HC, DeRisi D, et al. The application of tissue adhesives in small-bowel anastomoses. J Surg Oncol 1982;19:59-61.

25. Pohl J, Bruhn HD, Christophers E. Thrombin and fibrin-induced growth of fibroblasts: role in wound repair and thrombus organization. Klin Wochenschrift 1979;57:273-277.

26. Pointner R, Villinger R. Vorbeugung der intraperitonealen Nahtinsuffizienz durch Einsatz von Fibrin kleber. Zbl Chirurgie 1984;109:1146-1148.

27. Scheele J, Herzog J, Mühe E. Fibrin glue protection of digestive anastomoses. Zbl Chirurgie 1978;103:1325-1336.

28. Schrock TR, Deveney CW, Dunphy JE. Factors contributing to leakage of colonic anastomoses. Ann Surg 1973;127:513-518.

29. Stegemann H. Stalder K. Determination of hydroxyproline. Clin Chim Acta 1967;18:267-273.

30. Tagart REB. Colorectal anastomosis: factors influencing success. J Roy Soc Med 1981;74:111-118.

31. Van der Ham AC, Kort WJ, Weijma IM, Van den Ingh HFGM, Jeekel J. The effect of fibrin sealant on the healing colonic anastomosis in the rat. Br J Surg 1991;78:49-53.

32. Waclawiczek HW, Boeckl O. Clinical experience from fibrin sealing in general and thoracic surgery. Zbl Chirurgie 1986;111:16-24.

33. Waclawiczek HW, Rieger R, Hasenöhrl G, Boeckl O. Valuation of additive anastomosis or suture sealing using fibrin adhesive at the gastrointestinal tract. in: Eigler FW, Gross E, Vogt E. (Eds.) Intestinal anastomosis. TM-Verlag, Hameln, 1990:95-102.

34. Wara P, Sφrensen K, Berg V. Proximal faecal diversion: review of ten years' experience. Dis Col Rect 1981;24:114-119.

35. Wise L, McAlister W, Stein T, Schuck P. Studies on the healing of anastomoses of small and large intestines. Surg Gynecol Obstet
1975;141:190-194.

CHAPTER VIII

TRANSIENT PROTECTION OF INCOMPLETE COLONIC ANASTOMOSES WITH FIBRIN SEALANT; an experimental study in the rat

A.C. van der Ham, W.J. Kort, I.M. Weijma, J. Jeekel.

From the Laboratory for Experimental Surgery, Erasmus University Rotterdam, The Netherlands.

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Abstract

Fibrin glue has been used as a protective seal in normal and high-risk anastomoses to prevent leakage. The influence of fibrin adhesive on the healing colonic anastomosis in a control and high-risk model was tested. Resection and anastomosis of the left colon was performed in rats. In group Ia an end-to-end anastomosis was constructed with 12 6-0 polypropylene sutures; in group Ib the anastomosis was sealed with fibrin adhesive. In group II an incomplete anastomosis was constructed with only 4 sutures at 90 therefore potentially leaking. In group IIb additional sealing with fibrin glue was performed. On Days 2,4 and 7 body weight, adhesion formation, anastomotic bursting pressure and collagen concentration were measured. The results showed increased adhesion formation after fibrin sealing. The anastomotic bursting pressure of incomplete anastomoses showed a significant increase after sealing on Day 2 only; on Day 4 and 7 no differences were found. Sealing of control anastomoses caused lower bursting pressures on Day 4. Collagen concentration is significantly reduced after fibrin sealing of normal anastomoses. We conclude that fibrin sealing of control anastomoses inhibits wound healing. Incomplete anastomoses are temporarily protected by fibrin glue sealing. Finally, fibrin sealing of the colon wound does not prevent adhesion formation.

Introduction

Leakage of colonic and rectal anastomosis is a major complication after surgery with a reported incidence of up to 50% associated with a significantly increased mortality and morbidity.[1,2] Many factors are reported to contribute to the healing and integrity of an anastomosis such as adequate blood supply, tension-free anastomosis, inflammation, type of suture or staples, bowel preparation and old age.[2-6] Much attention has been given to the different type of sutures but primarily the integrity of the anastomosis depends on the technique of the individual surgeon.[7] As the leakage rate varies among different surgeons, and technical and anatomical location of the low colorectal anastomoses in the pelvis limit surgery, incomplete anastomoses are a true clinical problem.

It has been shown that by sealing an intestinal anastomosis, with fibrin sealant, a multicomponent tissue adhesive, bacteria can be confined intraluminally, thus reducing peri-anastomotic inflammation.[8] Additional sealing has been advocated in normal and

high-risk colonic anastomosis in human as a method to prevent anastomotic leakage.[9-13] However, experimental studies are conflicting and prospective randomised clinical studies are lacking.[14-17]

In view of these conflicting experimental data a study was designed to evaluate the effect of fibrin sealing on the healing incomplete colonic anastomosis in the rat.

Material and methods

Male Wag/Rij rats (120), weighing 180-230 g, were randomly allocated to four treatment groups and were allowed water (acidified, pH 3.0) and food (AM II, Hope Farms, Woerden, the Netherlands) *ad libitum* pre- and postoperatively. Operations were performed using ether anaesthesia. Through a midline incision 1 cm of the left colon, 3 cm proximal to the peritoneal reflection, was resected. A single-layer end-to-end anastomosis was performed with 12 interrupted, inverting 7-0 polypropylene (Prolene^{R}, Ethicon, Germany) stitches. The resected segment of colon was frozen and kept at -80 C until analysis, to serve as an individual control. The study comprised the following groups: Ia, control sutured anastomosis; Ib sutured anastomosis plus fibrin sealant; IIa, incomplete or leaking anastomosis; IIb, incomplete anastomosis plus fibrin sealant.

Fibrin sealant. The human fibrin sealant (Tissucol^{R}, Immuno Co, Vienna, Austria) was prepared according to the enclosed instructions. Fibrin sealant is a multicomponent adhesive consisting of (1) a freeze-dried protein concentrate of human fibrinogen (120 mg/ml) which is reconstituted in a solution of aprotinin (3000 kIU/ml) and (2) a solution of thrombin (500 IU/ml) and calcium chloride (40 mmol/liter). After preheating to 37 C the components are reconstituted with their solutions and drawn up in separate syringes. These syringes were fitted in a specially designed syringe holder (Duplojet^{R}) enabling mixing and application of the two components simultaneously. Upon mixing and application the fibrinogen was activated to form fibrin and the solutions were transformed into a rubber-like adhesive mass. For one anastomosis 0.2 ml of fibrin sealant was used.

Incomplete anastomosis. Incomplete colonic anastomoses were created by using only four interrupted polypropylene stitches at 90 .[18] On Days 2, 4 and 7, 10 rats from each group underwent relaparotomy. Following ether anaesthesia the laparotomy incision was reopened and the degree of adhesion formation was assessed. We used the following scale: 0,

no adhesions; 1+, minimal adhesions, mainly between anastomosis and omentum; 2+, moderate adhesions, i.e., between omentum and the anastomotic site and between the anastomosis and a loop of small bowel; 3+, severe and extensive adhesions, including abscess formation. Thereafter the rectum was cannulated and the left colon was ligated twice: 1 cm proximal and 1 cm distal to the anastomosis, the latter around the rectal cannula. Care was taken not to disturb the anastomosis and its adhesions. The rectal cannula was connected to an infusionpump (Hospal^{R},Italy), with a side arm to a pressure transducer, in connection with a recorder. The *in vivo* anastomotic bursting pressure was measured by infusing normal saline at a constant rate of 1 ml/min and was marked by abrupt loss of pressure. After measuring the bursting pressure the animal was sacrificed by intrathoracic bleeding. The left colon, including the anastomosis, was excised and dissected free of mesenteric fat tissue. Three rings of tissue measuring 0.5 cm wide were cut: one ring of tissue containing the anastomosis, one ring just proximal to the anastomosis, and one ring distal to the anastomosis. The tissue samples were immediately frozen and kept at -80 C for later hydroxyproline assay.

Hydroxyproline as a measure of collagen was determined using a modified version of the colorimetric assay of Stegemann.[19,20] Collagen concentrations were calculated in micrograms per milligram dry weight. The collagen concentration of the segment of colon resected at the initial operation served as the individual control, and was used to calculate any changes in collagen content.

For statistical analysis of the results, the mean values and the standard error of the mean were calculated. Results were compared using one-way analysis of variance (ANOVA) with the Student-Newman-Keuls test, X^2 test and Fisher's exact test when appropriate. The null hypothesis of no difference was rejected at the P level of 0.05.

Results

Two animals died: one animal of group IIa died immediately after operation, another rat in group IIb died after 1 day. Macroscopically there were no signs of anastomotic leakage nor peritonitis on postmortem examination of the deceased animals. The cause of death of the animals remained unclear. The average body weight loss in group I and II was maximal on Day 2 (fig. 1). Although some reduced body weight loss in group II,

compared to the control group was noted on Day 2, results did not reach level of statistical significance. On Day 4 the loss of bodyweight in group Ib was less than in group Ia (P=0.04). The loss in group IIb was significantly less than that in group Ib (P=0.004). On Day 7 both groups IIa and IIb showed a gain of body weight, compared to loss in group Ia and Ib (P=0.02 and 0.006 respectively).

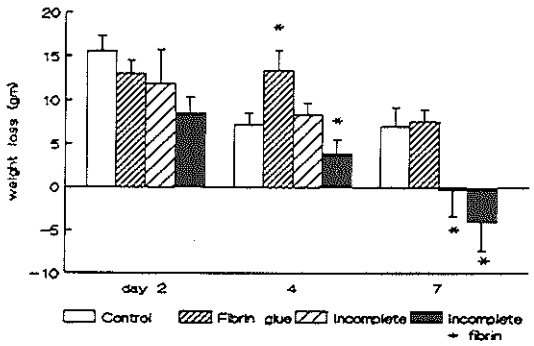

FIG. 1. Loss of body weight following resection and anastomosis of the left colon in rats (n = 10).

There were no overt signs of anastomotic leakage or dehiscence in the control or test groups. The adhesion scores are shown in Table 1. Incomplete anastomoses (group IIa) showed more adhesion formation than controls (group Ia) on Day 2 postoperatively. On Day 4 adhesion formation in group Ib was significantly raised following fibrin sealing (P=0.02). This was also the case in groups IIa and IIb (P=0.002). On Day 7, however, significant difference in this respect was only noted between groups IIb and Ib.

The bursting pressures measured in this study are shown in Table 2. On Day 2 the average bursting pressure was highest in group Ib. The average bursting pressure in group IIa (incomplete anastomoses) was 6 mm Hg. After sealing of the incomplete anastomoses

TABLE 1

Adhesions Following Colonic Anastomosis in the Rat

			Day 2				Day 4				Day 7			
Group	n	Died	0	1+	2+	3+	0	1+	2+	3+	0	1+	2+	3+
Ia (control)	30	0	0	6	4	0	0	9	1	0	0	6	4	0
Ib (+ fibrin seal)	30	0	2	4	4	0	0	4	6	0	0	6	4	0
IIa (incomplete)	29	1	0	1	8	0	0	2	7	1	0	3	6	1
IIb (+ fibrin seal)	29	1	0	5	4	0	0	1	9	0	0	1	7	2

* Statistically significant ($P < 0.05$).

Note. Between brackets the relative change compared to the individual control value.

(group IIb) the average bursting pressure was 41 mmHg, which is significantly higher ($P<0.001$; ANOVA). On Day 4, in group Ib the mean bursting pressure (65 mm Hg) was significantly lower than group Ia (123 mm Hg). The sealed incomplete anastomosis in group IIb showed lower mean bursting pressure (68 mm Hg) on Day 4 compared to that in group IIa (97 mm Hg); however, this difference is not significant. On Day 7 the differences of mean bursting pressure are not statistically significant.

TABLE 2

Anastomotic Bursting Pressure of Colonic Anastomosis in the Rat

Group	Day 2		Day 4		Day 7
Ia Control	56 ± 13		123 ± 12	*	193 ± 8
Ib (+ fibrin seal)	72 ± 10	*	65 ± 6	*	195 ± 6
IIa (incomplete)	6 ± 2.4	*	97 ± 9		186 ± 5
IIb (+ fibrin seal)	41 ± 7	*	68 ± 11		169 ± 12

* Statistically significant ($P < 0.05$).

Note. Results are given as mean values ± SEM in mm Hg; between brackets the relative change compared to the individual control value.

On Day 2 the point of disruption during the test of anastomotic bursting pressure was invariably the site of the anastomosis. On Day 4 in two animals in group Ia, the point of rupture was distal, while all other cases ruptured at the anastomosis. On Day 7 in group Ia, in all cases rupture took place outside the anastomosis (8x proximal, 2x distal); in group Ib the anastomosis ruptured once (7x proximal, 2x distal). Both in groups IIa and IIb in four cases the anastomosis was the place of rupture.

Collagen proximal to the anastomosis

On Day 2 collagen concentration was lowest (Table 3). On Day 2 the mean relative loss of collagen in group IIa was 18%, which is significantly less than 48% in group Ia. Group Ib showed a significant higher loss of collagen on Days 4 and 7, compared to groups Ia and IIb. On all three occasions the relative collagen loss was highest in group Ib.

Collagen at the anastomotic site

Maximal relative collagen degradation (55%) was noted at the anastomotic site in group IIb on Day 2. On Day 2 in the incomplete anastomosis collagen reduction is significantly increased after fibrin sealing (group IIb versus IIa). On Day 4 sealing of control anastomoses (group Ib) resulted in significant reduction of collagen concentration ($P=0.002$). This reduction is also significantly higher compared to sealed incomplete anastomoses in group IIb ($P=0.01$). Incomplete anastomoses (group IIa) did not show different collagen concentration changes compared to controls (group Ia) on Days 2 and 4.

On Day 7 the collagen concentration reduction was less in incomplete anastomoses: however, this difference was not statistically different.

TABLE 3

Mean Concentration and Relative Change of Collagen after Construction of Colonic Anastomosis in the Rat

Group	*n*	Day 2	Day 4	Day 7
		Proximal to anastomosis		
Ia	30	91 ± 8 (−48%) *	108 ± 7 (−29%) *	158 ± 7 (−7%) *
Ib	30	82 ± 5 (−50%)	85 ± 9 (−47%)	102 ± 3 (−37%)
IIa	29	116 ± 6 (−18%) *	118 ± 6 (−17%) *	136 ± 8 (−11%)
IIb	29	84 ± 6 (−41%)	109 ± 6 (−24%)	121 ± 7 (−17%)
		Anastomosis		
Ia	30	97 ± 6 (−44%)	113 ± 6 (−26%) *	152 ± 7 (−11%)
Ib	30	77 ± 6 (−52%)	81 ± 9 (−49%) *	139 ± 3 (−15%)
IIa	29	83 ± 6 (−41%) *	93 ± 7 (−35%)	152 ± 5 (−0%)
IIb	29	64 ± 5 (−55%)	98 ± 8 (−31%)	125 ± 8 (−13%)
		Distal to anastomosis		
Ia	30	135 ± 10 (−22%)	146 ± 7 (−6%) *	157 ± 11 (−8%)
Ib	30	131 ± 6 (−19%)	130 ± 5 (−19%)	144 ± 3 (−11%)
IIa	29	120 ± 6 (−14%)	126 ± 8 (−12%)	144 ± 6 (−5%)
IIb	29	108 ± 4 (−25%)	125 ± 8 (−13%)	139 ± 7 (−8%)

* Statistically significant ($P < 0.05$).

Note. Values given are mean values in μg/mg dry wt ± SEM; between brackets the relative change compared to the individual control value.

Collagen distal to the anastomosis

Distal to the anastomoses hydroxyproline decrease was less pronounced than proximal and at the suture line. Although the relative loss of collagen on Day 2 was highest in group IIb, no statistically significant differences were found. On Day 4 relative hydroxyproline loss was significantly higher in group Ib compared with controls. On Day 7 no differences were noted comparing collagen concentration.

Discussion

Reduction of the number of sutures from 12 to 4 led to mechanically weaker anastomoses on Day 2 only; on Day 4 and 7 bursting pressures did not differ. Although no spontaneous dehiscence of the anastomoses was noted, the bursting pressures measured on Day 2 were almost zero. Despite this, no clear leakage of colonic contents took place to cause overt peritonitis. This may be caused by the high consistency of rat feces. This makes it very difficult to create a leaking colonic anastomosis in the rat.

Addition of fibrin glue to the insufficient anastomoses resulted in significant stronger anastomoses on Day 2 only, while in the control group, fibrin sealing caused a significant

decrease of mechanical strength on Day 4. Also, the incomplete anastomosis without sealing is stronger than after addition of fibrin glue, albeit not statistically significant on Day 4 and 7. Thus the early advantage of fibrin sealing of insufficient anastomoses has changed on Day 4 and 7. The inhibitory effect of human fibrinogen on the healing colonic anastomosis cannot be attributed to the use of foreign protein, as in a previous study we have shown that rat fibrinogen has the same effect.[17]

The quantity and quality of collagen in the submucosal layer of the intestinal wall determine the strength of the healing intestine.[21] Following the construction of an anastomosis in the colon there is a significant decrease in collagen.[22-24] The results of this study confirm that the decrease in collagen content is highest just proximal to the anastomosis.[24] The reduced collagen concentration proximal to the anastomosis may account for disruption taking place almost exclusively in this area when the bursting pressure was tested on Day 7.

Local degradation of mature collagen might be the cause of cutting through of sutures, thus causing breakdown of the anastomosis.[3,25] Collagen is very resistant to the action of proteolytic enzymes, but breakdown is initiated by a specific enzyme: collagenase. A significant increase of collagenase was found in the gastrointestinal tract after anastomosis in the colon.[26] The highest collagenase activity is found in the colon, and possibly the high incidence of anastomotic leakage in the colon is due to this enzyme. It was shown that fibrin applied on a colonic anastomosis is infiltrated by inflammatory cells.[17] These inflammatory cells are a known source of collagenase and other proteolytic enzymes. The significant reduction of collagen concentration on Day 4 is likely the result of increased collagen breakdown by these inflammatory cells. Local disturbance of circulation and inflammatory reaction caused by sutures are also known factors influencing local collagen metabolism. Reduction of the number of sutures in our model did not result in a change in collagen metabolism at the anastomotic site. This corresponds with studies on sutureless anastomoses showing no differences in collagen metabolism with control anastomoses.[27,28] However, in the present study, proximal to the anastomosis we found less reduction of collagen concentration after reduction of the number of stitches.

Studies in a model in which adhesions were produced by excision of a section of peritoneum have suggested that fibrin adhesive may decrease intraabdominal adhesion

formation, dependent on the fibrinogen concentration.[29-31] Contrary to this we found increased adhesion formation following fibrin sealing of colonic anastomosis on Day 4. It seems, however, from our results that this difference is only temporary: on Day 7 there are no differences in adhesion formation. Also in the incomplete anastomoses adhesion formation was not reduced by fibrin sealant. A possible cause for the increased adhesion formation, contrary to studies on other models, might be the bacterial contamination of the fibrin clot in our model. On the other hand, we could not confirm increased abscess formation after sealing of colonic anastomosis with fibrin adhesive, as reported by others.[15] Even incomplete anastomoses did not induce abscess formation.

From our results it was concluded that sealing of normal colonic anastomosis in the rat has a negative effect on wound healing. Temporary improvement of mechanical strength of incomplete anastomoses was obtained by fibrin sealing.

Fibrin sealant may be of clinical use to protect technically incomplete colonic anastomoses during the first 48 hr. However, the effect of fibrin sealant on the healing colonic anastomosis, both normal and at risk, during this period needs further study. Finally, fibrin sealant does not prevent adhesion formation in control and incomplete anastomoses.

References:

1. Goligher JC, Graham NG, De Dombal FT. Anastomotic dehiscence after anterior resection of rectum and sigmoid. Br J Surg 1970;57:109-118.

2. Fielding LP, Stewart-Brown S, Blesovsky L, Kearny G. Anastomotic integrity after operations for large-bowel cancer: a multicentre study. Br Med J 1980;281:411-414.

3. Hawley PR. Causes and prevention of colonic anastomotic breakdown. Dis Col Rect 1973;16:272-277.

4. Foster ME, Lancaster J, Leaper DJ. Leakage of low rectal anastomoses - an anatomic explanation. Dis Col Rect 1984;27:157-158.

5. Schrock TR, Devency CW, Dunphy JE. Factors contributing to leakage of colonic anastomoses. Ann Surg 1973;127:513-518.

6. Tagart REB. Colorectal anastomosis: factors influencing success. J Roy Soc Med 1981;74:111-118.

7. Fielding LP, Stewart-Brown S, Dudley HAF. Surgeon-related variables and the clinical trial. Lancet 1978;ii:778-779.

8. Petrelli NJ, Cohen HC, DeRisi D, et al. The application of tissue adhesives in small-bowel anastomoses. J Surg Oncol 1982;19:59-61.

9. Scheele J, Herzog J, Mühe E. Fibrin glue protection of digestive anastomoses. Zbl Chirurgie 1978;103:1325-1336.

10. Pointner R, Villinger R. Vorbeugung der intraperitonealen Nahtinsuffizienz durch Einsatz von Fibrin kleber. Zbl Chirurgie 1984;109:1146-1148.

11. Athanasiadis S, Kuhlgatz Ch, Girona I. Additional fibrin glueing in surgery of colon and rectum. Zbl Chirurgie 1984;109:1107-1111.

12. Waclawiczek HW, Boeckl O. Clinical experience from fibrin sealing in general and thoracic surgery. Zbl Chirurgie 1986;111:16-24.

13. Giardino R, Brulatti M, Franchini A. Colonic anastomoses protected with fibrin sealant. In: Schlag G, Redl H (eds). Fibrin sealant in operative medicine. General surgery and abdominal surgery - Vol. 6. Springer-Verlag, Heidelberg, Berlin, 1986:155-158.

14. Nordkild P, Hjortrup A, Kjærgaard J. Tissue adhesives and intestinal anastomosis. Ann Chir Gyn 1986;75:205-208.

15. Houston KA, Rotstein OD. Fibrin sealant in high-risk anastomoses. Arch Surg 1988;123:230-234.

16. Hulkko OA, Haukipuro KA, Laitinen ST. Fibrin glue protection of primary anastomosis in the obstructed left colon; an experimental study on the rat. Acta Chir Scand 1988;154:49-52.

17. Van der Ham AC, Kort WJ, Weijma IM, Van den Ingh HFGM, Jeekel J. The effect of fibrin sealant on the healing colonic anastomosis in the rat. Br J Surg 1991;78:49-53.

18. Henne-Bruns D, Kreischer HP, Schmiegelow P, Kremer B. Reinforcement of colon anastomoses with polyglycolic acid mesh: an experimental study. Eur Surg Res 1990;22:224-230.

19. Stegemann H. Stalder K. Determination of hydroxyproline. Clin Chim Acta 1967;18:267-273.

20. Grant RA. Estimation of hydroxyproline by the AutoAnalyser. J Clin Path 1964;17:685-686.

21. Gottrup F. Healing of incisional wounds in stomach and duodenum: collagen distribution and relation to mechanical strength. Am J Surg 1981;141:222-227.

22. Wise L, McAlister W, Stein T, Schuck P. Studies on the healing of anastomoses of small and large intestines. Surg Gynecol Obstet 1975;141:190-194.

23. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomoses: III. Collagen metabolism in the colon after left colon resection. Am J Surg 1980;139:398-405.

24. Cronin K, Jackson DS, Dunphy JE. Changing bursting strength and collagen content of the healing colon. Surg Gynec Obstet 1968;126:747-753.

25. Jönsson K, Jiborn H, Zederfeldt B. Collagen metabolism in small intestinal anastomosis. Am J Surg 1987;154:288-291.

26. Hawley PR, Faulk WP, Hunt TK, Dunphy JE. Collagenaseactivity in the gastrointestinal tract. Br J Surg 1970;170:896-900.

27. Mastboom WJB, Hendriks Th, de Boer HHM. Intestinal anastomotic healing in the absence of suture material: an experimental study in rats. Int J Colorectal Dis 1991;6:33-37.

28. Haukipuro KA, Hulkko OA, Alavaikko MJ, Laitinen ST. Sutureless colon anastomosis with fibrin glue in the rat. Dis Colon Rectum 1988;31:601-604.

29. Brands W, Jung M, Manegold C, Liedgens P, König M, Stock M. Prophylaxis and therapy of intra-abdominal adhesions with highly concentrated human fibrinogen: experimental and clinical results. in: Waclawiczek HW. (Ed.) Progress in fibrin sealing. Springer-Verlag, Berlin Heidelberg New York, 1989:141-145.

30. Lindenberg S, Steentoft P, Sørensen SS, Olesen HP. Studies on prevention of intra-abdominal adhesion formation by fibrin sealant: an experimental study in rats. Acta Chir Scand. 1985;151:525-527.

31. DeVirgillio C, Dubrow T, Sheppard BB, et al. Fibrin glue inhibits intra-abdominal adhesion formation. Arch Surg 1990;125:1378-1382.

CHAPTER IX

EFFECT OF ANTIBIOTICS IN FIBRIN SEALANT ON HEALING COLONIC ANASTOMOSES IN THE RAT

A.C. van der Ham, W.J. Kort, I.M. Weijma, H.F.G.M. van den Ingh*, J.Jeekel.

From the Laboratory for Experimental Surgery, Erasmus University, and the Department of Pathology*, St. Clara Ziekenhuis, Rotterdam, The Netherlands.

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Summary

In 90 rats, a colonic anastomosis was constructed with 12 interrupted 7/0 polypropylene sutures. Group 1 (n = 30) served as a control group. In group 2 (n = 30) the anastomosis was sealed with fibrin adhesive and in group 3 (n = 30) a mixture of fibrin, clindamycin and cefotaxim. On days 2, 4 and 7, ten animals in each group were killed. Adhesion formation was significantly increased in groups 2 and 3 compared with the control group. On day 2 the anastomosis was significantly stronger after sealing with antibiotic-fibrin mixture. On day 4 the bursting pressure in group 2 was significantly lower than in groups 1 and 3. At the same time the concentration of hydroxyproline was significantly reduced in group 2, but not in group 3. The addition of antibiotics prevents the negative influence of fibrin adhesive on the healing colonic anastomosis and contributes to a stronger anastomosis on day 2 after operation.

Introduction

Leakage from colonic and rectal anastomoses has a reported incidence up to 50 per cent[1] and is associated with significantly increased mortality and morbidity rates[2]. Fibrin sealant is a multicomponent biological adhesive made from concentrated human fibrinogen which can be used to establish haemostasis or as an adhesive in wound repair. Additional sealing with fibrin sealant has been advocated in normal and high-risk colonic anastomosis to prevent anastomotic leakage.[3,4,5,6,7] Experimental studies provide conflicting results and prospective, randomised clinical studies are lacking.[8,9] A negative influence of additional fibrin sealant on the healing colonic anastomosis has recently been demonstrated in the rat.[10] The negative effect might be caused by an increased inflammatory reaction near the anastomosis.[9,10]

The quantity and quality of collagen in the submucosal layer of the intestinal wall determines the strength of the healing intestine.[11] Following colonic anastomosis there is first a period of breakdown of collagen, followed by synthesis.[12,13,14] Collagen is highly resistant to proteolytic agents but is readily degraded by collagenase enzyme. Factors directly influencing the activity of collagenase are infection and inflammation around infected anastomoses.[15,16] Bacteria and inflammatory cells are known to produce collagenase.[17,18] As every colonic anastomosis must be considered to be contaminated

by intestinal bacteria, the fibrin clot will be infected too. It is possible to mix antibiotics with fibrin glue without impairment of its adhesive and clotting properties. High local antibiotic concentrations are slowly released from the fibrin clot.[19]

This study was designed to determine whether the addition of antibiotics abolishes the negative effect of fibrin sealing on the strength and collagen metabolism of the healing colonic anastomosis in the rat.

Materials and methods

Ninety male Wag/Rij rats, weighing 180-230 g, were randomly allocated to three treatment groups and were allowed water (acidified, pH 3.0) and food (AM II; Hope Farms, Woerden, The Netherlands) *ad libitum* before and after operation. Using ether anaesthesia and through a midline incision, 1 cm of the left colon, 3 cm proximal to the peritoneal reflection, was resected. A single-layer end-to-end anastomosis was performed with 12 interrupted, inverting 7/0 polypropylene (Prolene^R, Ethicon, Norderstedt, Germany) stitches in a standard fashion. The resected segment of colon was frozen and kept at -80 C until analysis, to serve as an individual control. The experimental groups were: group 1, control sutured anastomosis; group 2, sutured anastomosis plus human fibrin sealant; group 3, sutured anastomosis plus fibrin-antibiotic complex. The human fibrin sealant (Tissucol^R, Immuno AG, Vienna, Austria) was prepared according to the manufacturer's instructions. The sealant consists of a freeze-dried protein concentrate of human fibrinogen (120 mg/ml) which is reconstituted in a solution of aprotinin (3000 kallidinogenase inactivator units/ml) and a solution of thrombin (500 IU/ml) and calcium chloride (40 mmol/l). In treatment group 3, 1 ml thrombin solution was mixed with 50 mg cefotaxim and 50 mg clindamycin. After preheating to 37 C the components were reconstituted in their solutions and drawn up in separate syringes. On mixing and application the fibrinogen was activated to form fibrin and the solution was transformed into a rubber-like adhesive mass. The fibrin-antibiotic complex contains 25 mg cefotaxim and 25 mg clindamycin per ml of fibrin glue. For one anastomosis 0.2 ml of fibrin sealant was used. On days 2, 4 and 7, ten rats from each group had the laparotomy incision reopened under ether anaesthesia and the degree of adhesion formation was assessed: 0, no adhesions; 1+, minimal adhesions, mainly between anastomosis and omentum; 2+, moderate adhesions,

i.e. between omentum and the anastomotic site and between the anastomosis and a loop of small bowel; 3+, severe and extensive adhesions, including abscess formation.[9] The rectum was then cannulated and the left colon was ligated twice, 1 cm proximal and 1 cm distal to the anastomosis, the latter around the rectal cannula. Care was taken not to disrupt the anastomosis and its adhesions. The rectal cannula was connected to an infusion-pump (Hospal^{R}; Dasco Spa, Medolla, Italy), with a side arm to a pressure transducer connected to a recorder. The *in vivo* anastomotic bursting pressure was measured by infusing normal saline at a constant rate of 1 ml/min, and was marked by an abrupt loss of pressure. After measuring the bursting pressure the animal was killed. The left colon, including the anastomosis was excised and dissected free of mesenteric fat. Three rings of colon tissue 0.5 cm wide were cut: one ring of tissue containing the anastomosis, one ring proximal and one ring distal to the anastomosis. For histological examination a small segment of the anastomotic ring was cut and laced in 4 per cent formaldehyde. The remaining tissue samples were immediately frozen and kept at -80 C for hydroxyproline assay.

Hydroxyproline was determined using a modified version of the colorimetric assay of Stegemann *et al.*[20,21]

Collagen concentrations were calculated in micrograms per milligram dry weight. The collagen concentration of the control colon was used to calculate any change in collagen content.

Sections of 5- m thickness were cut and stained with haematoxylin and eosin. A periodic acid-Schiff, a Gomori and a Weigert-van Gieson stain were used to assess the tissue reaction. The inflammatory infiltrate (neutrophils, eosinophils, macrophages) and tissue organization (fibroblast and capillary proliferation) were scored semiquantitatively as follows: +, numerous; , occasionally present; -, absent.

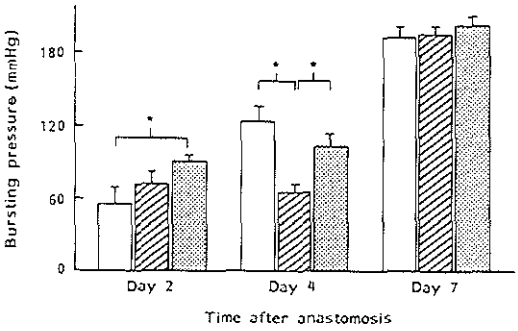

Figure 1 *Colonic bursting pressure on days 2, 4 and 7 after creation of an end-to-end anastomosis. Values are mean(s.e.m.): n = 10. □, Control: ▨, fibrin sealant: ⊠, fibrin sealant plus antibiotics. *P < 0·05 (ANOVA and Newman–Keuls test)*

For statistical analysis the mean values, and the standard error of the mean (s.e.m.) were calculated. Results were compared using one-way analysis of variance (ANOVA) with the Student-Newman-Keuls test, X^2 test and Fisher's exact test when appropriate. The null hypothesis of no difference was rejected at a P value of 0.05.

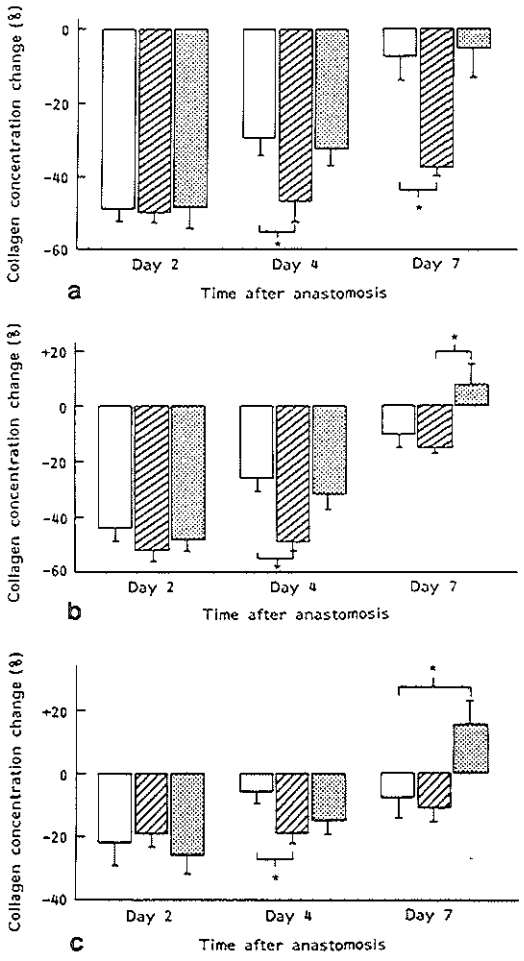

Figure 2 *Relative changes in collagen concentrations* a *proximal to colonic anastomosis;* b *in colonic anastomotic area;* c *distal to colonic anastomosis. Values are mean(s.e.m.) percentage changes compared with preoperative values:* n = *10.* □, *Control;* ▨, *fibrin sealant;* ⊡, *fibrin sealant plus antibiotics.* *P < *0·05 (ANOVA and Newman–Keuls test)*

Results

All animals survived and no animal showed macroscopic evidence of leakage from the anastomosis. There were no differences in change of body-weight.

The results of adhesion formation are presented in *Table 1.* Adhesion formation was more extensive in groups 2 and 3 compared with group 1 by day 4. No animal showed abscess formation.

The results of anastomotic bursting pressure measurements are presented in *Figure 1.* On day 2 bursting invariably occurred at the anastomosis. On day 4, 80 per cent of bursting in the control group occurred at the anastomosis; in groups 2 and 3 rupture without exception took place at the suture line. On day 7 the rupture invariably occurred outside the anastomosis in groups 1 and 3; in one animal in group 2 the anastomosis was the place of rupture. Bursting was most

likely to take place proximal to the anastomosis in 80, 70 and 90 per cent of animals in groups 1, 2 and 3 respectively. On day 7 the colon wall was significantly weaker proximal to the anastomosis compared with the distal colon ($P < 0.01$).

The values of collagen concentration around the anastomosis and relative change as compared with individual normal values are presented in *Table 2*. Hydroxyproline concentration was lowest on day 2 in all groups. The relative loss of collagen was prominent in all groups on most occasions. Overall there were slight differences in relative collagen loss between groups 1 and 3; these changes became statistically significant only on day 7. *Figure 2* illustrates the relative change of collagen around the anastomosis.

Histological examination showed remnants of fibrin glue outside the anastomosis on days 2 and 4 (*Figure 3*); on day 7, however, this fibrin was not demonstrated. On days 2 and 4 the fibrin seal in groups 2 and 3 was heavily infiltrated and surrounded by neutrophilic granulocytes. On day 4 this neutrophilic infiltrate was more pronounced in group 2 than in group 3 (*Figure 4*). The intestinal wall showed infiltration of neutrophilic and eosinophilic granulocytes and macrophages. Apart from the number of neutrophils, no clear differences could be found between the treatment groups.

On day 2 fibroblasts were present only occasionally. On days 4 and 7 macrophages and fibroblasts were abundant in all groups, while granulocytes had reduced in number again. No differences were noted concerning the number of fibroblasts and capillary proliferation.

Discussion

Fibrin sealant contributes to wound healing by producing local haemostasis and by stimulating the influx of macrophages which produce factors that cause angiogenesis, fibroblast proliferation and collagen production.[22,23,24,25] Additional sealing has been advocated for normal and high-risk colonic anastomoses in patients to prevent anastomotic leakage.[3-7] In the rat model, however, application of human and rat fibrinogen has a negative effect on the healing colonic anastomosis.[10] Since anastomoses are contaminated and fibrin is a culture medium for pathogens, antibiotics in the adhesive may prevent infection. Clindamycin and cefotaxim can be added to fibrin adhesive without impairing its clotting behaviour.[9]

Table 1 *Adhesions of colonic anastomoses in the rat*

Group	*n*	Day 2				Day 4				Day 7			
		0	1+	2+	3+	0	1+	2+	3+	0	1+	2+	3+
1 (Control)	30	0	6	4	0	0	9	1	0	0	6	4	0
2 (Fibrin sealant)	30	2	3	5	0	1	3	6	0*	0	6	4	0
3 (Fibrin sealant and antibiotics)	30	0	4	4	2	0	0	3	7*	0	2	5	3

*$P < 0{\cdot}05$ (group 1 *versus* groups 2 and 3, χ^2 test)

Table 2 *Concentration and relative change of collagen in colonic anastomoses in the rat*

Site	Group	Day 2	Day 4	Day 7
Proximal to anastomosis	1	91(8) (−49)	109(7) (−29)*	158(11) (−7)*
	2	82(5) (−50)	85(9) (−47)	102(4) (−38)
	3	68(6) (−48)	81(4) (−32)	92(5) (−5)
Anastomosis	1	97(6) (−44)	113(6) (−26)*	152(7) (−11)
	2	77(6) (−52)	81(9) (−49)	139(3) (−15)*
	3	67(3) (−48)	80(4) (−32)	106(6) (+8)
Distal to anastomosis	1	135(10) (−22)	146(7) (−6)*	157(11) (−8)*
	2	131(6) (−19)	130(5) (−19)	144(3) (−11)
	3	98(6) (−26)	102(4) (−15)	113(7) (+15)

Values are mean(s.e.m.) expressed in micrograms per milligram dry weight; values in parentheses are the relative percentage changes compared with the individual control value. *$P < 0{\cdot}05$ (day 4: group 1 *versus* group 2; day 7: proximal, group 1 *versus* group 2; anastomosis, group 2 *versus* group 3; distal, group 1 *versus* group 3; ANOVA and Newman–Keuls test)

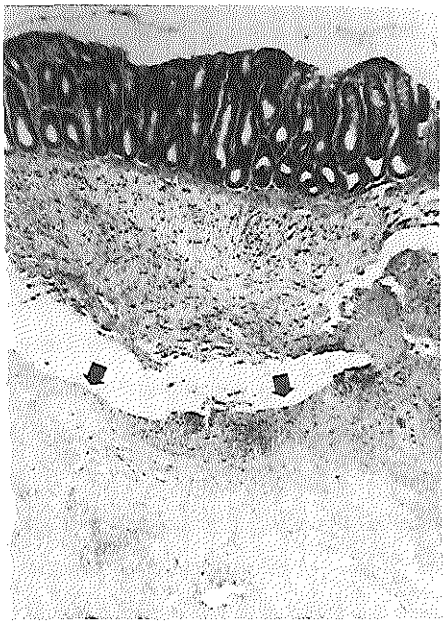

Figure 3 *Histological section on day 4 after operation of a fibrinogen-sealed colonic anastomosis (arrows). The submucosa and fibrin sealant show cellular infiltrate. (Haematoxylin and eosin stain, original magnification ×80)*

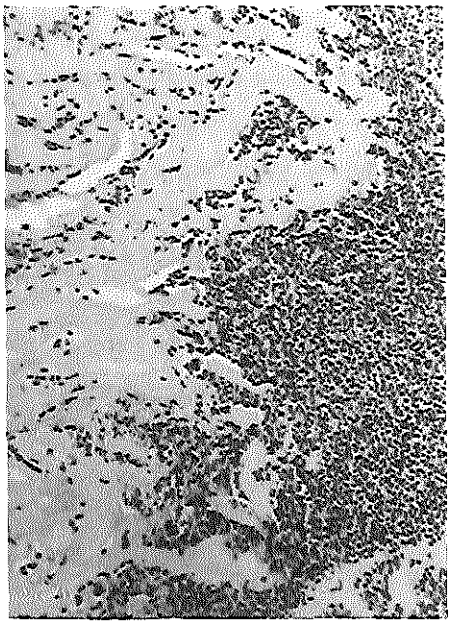

Figure 4 *Detail of fibrin sealant showing massive cellular infiltrate of mainly neutrophilic granulocytes. (Haematoxylin and eosin stain, original magnification ×160)*

The application of fibrin sealant resulted in increased adhesion formation around the anastomosis on day 4. This was more prominent after addition of antibiotics. On day 7 this difference was not present. Others have found that fibrin glue prevents adhesion formation to peritoneal-muscular defects.[26] A possible explanation for the difference may be the presence of colonic bacteria in this model.

On day 2 the bursting pressure was significantly higher after sealing with antibiotic-fibrin complex. This did not correlate with diminished collagen degradation, and may be caused by mechanical bonding properties of the adhesive. Addition of antibiotics might inhibit degradation of the fibrin clot during the first few days and improve the adhesive property.

Application of fibrinogen produced a lower bursting pressure on day 4, but this was prevented by addition of antibiotics to the fibrin sealant. In group 2 the lower bursting pressure on day 4 correlated with reduced collagen concentration. This decreased collagen concentration may account for the lower bursting pressure because bursting pressure is mainly dependent on submucosal collagen.[14] Addition of antibiotics to the sealant gave almost the same hydroxyproline values on day 4 as in control animals. The reduced degradation of collagen after addition of antibiotics may be explained by reduced bacterial infection and inflammatory reaction. On day 4 the neutrophilic infiltration of the anastomoses with antibiotic-fibrinogen was significantly reduced.

On day 7 the net hydroxyproline change in the anastomotic segment as well as distal to it was positive, and significantly higher in group 3. This did not result in a higher bursting pressure. At this time the intestine is weakest proximal to the anastomosis, as shown by the point of disruption. On day 7, therefore, bursting pressure is not a good measure of anastomotic collagen concentration. The finding of asymmetrical collagen changes around the anastomosis has been reported in other studies.[14,27] This study demonstrated that collagen changes were more marked proximally and explains why the rupture occurred almost exclusively proximal to the anastomosis on testing the bursting pressure on day 7. No explanation for this pattern of collagen distribution can be found.

Histological examination showed excessive accumulation of neutrophilic granulocytes in the fibrin sealant covering the anastomosis on days 2 and 4. Fibrinogen gives rise to infiltration of granulocytes and local fibrin deposition may predispose local infection and inflammation. Under normal conditions, deposition of fibrin occurs within 3 h and the

wound is infiltrated by granulocytes, followed by macrophages during the first 24 hours.[28] Foreign material, e.g. fibrin, prolongs the inflammatory phase and provides a favourable site for bacterial proliferation. A significant reduction in the lowering of hydroxyproline concentration around the anastomosis was observed by Mastboom *et al.* using germ-free rats.[29] The results of the present study with antibiotics suggest that local bacterial infection contributes to collagen degradation following the construction of a colonic anastomosis.

Neutrophilic granulocytes and bacteria are sources of proteolytic enzymes including collagenase and may cause increased collagen breakdown of the intestinal wall. The postoperative decrease of breaking strength of colonic anastomoses is not apparent in rats with neutropenia.[30] In this model the increased number of neutrophils at the site of the anastomosis is responsible for the increased loss of hydroxyproline in the colonic wall following the addition of fibrin sealant. The results do not show enhanced healing of colonic anastomosis following sealing with fibrin glue, contrary to some other studies.[3-7] Addition of antibiotics to the fibrin sealant improved the mechanical properties of the anastomosis on day 2 and abolished the decrease of bursting pressure on day 4. The addition of antibiotics may be useful in the clinical application of fibrin sealant.

References

1. Goligher JC, Graham NG, De Dombal FT. Anastomotic dehiscence after anterior resection of rectum and sigmoid. Br J Surg 1970;57:109-118.

2. Fielding LP, Stewart-Brown S, Blesovsky L, Kearny G. Anastomotic integrity after operations for large-bowel cancer: a multicentre study. Br Med J 1980;281:411-414.

3. Scheele J, Herzog J, Mühe E. Fibrin glue protection of digestive anastomoses. Zbl Chirurgie 1978;103:1325-1336.

4. Pointner R, Villinger R. Vorbeugung der intraperitonealen Nahtinsuffizienz durch Einsatz von Fibrin kleber. Zbl Chirurgie 1984;109:1146-1148.

5. Athanasiadis S, Kuhlgatz Ch, Girona I. Additional fibrin gluing in surgery of colon and rectum. Zbl Chirurgie 1984;109:1107-1111.

6. Waclawiczek HW, Boeckl O. Clinical experience from fibrin sealing in general and thoracic surgery. Zbl Chirurgie 1986;111:16-24.

7. Giardino R, Brulatti M, Franchini A. Colonic anastomoses protected with fibrin sealant. In: Schlag G, Redl H (eds). Fibrin sealant in operative medicine. General surgery and abdominal surgery - Vol. 6. Springer-Verlag, Heidelberg, Berlin, 1986:155-158.

8. Nordkild P, Hjortrup A, Kjærgaard J. Tissue adhesives and intestinal anastomosis. Ann Chir Gyn 1986;75:205-208.

9. Houston KA, Rotstein OD. Fibrin sealant in high-risk anastomoses. Arch Surg 1988;123:230-234.

10. Van der Ham AC, Kort WJ, Weijma IM, Van den Ingh HFGM, Jeekel J. The effect of fibrin sealant on the healing colonic anastomosis in the rat. Br J Surg 1991;78:49-53.

11. Gottrup F. Healing of incisional wounds in stomach and duodenum: collagen distribution and relation to mechanical strength. Am J Surg 1981;141:222-227.

12. Wise L, McAlister W, Stein T, Schuck P. Studies on the healing of anastomoses of small and large intestines. Surg Gynecol Obstet 1975;141:190-194.

13. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomoses: III. Collagen metabolism in the colon after left colon resection. Am J Surg 1980;139:398-405.

14. Cronin K, Jackson DS, Dunphy JE. Changing bursting strength and collagen content of the healing colon. Surg Gynec Obstet 1968;126:747-753.

15. Hawley PR, Hunt TK, Dunphy JE. Etiology of colonic anastomotic leaks. Proc Roy Soc Med 1970;63:28-30.

16. Yamakawa T, Patin CS, Sobel S, et al. Healing of colonic anastomoses following resection for experimental "diverticulitis" Arch Surg 1971;103:17-20.

17. Ohlsson K. Polymorphonuclear leukocyte collagenase. In Woolley D, Evanson J, editors. Collagenase in normal and pathologic connective tissue. Chicester, England, 1980, Wiley, pp 209-222.

18. Peterkovsky B. Bacterial collagenase. In: Methods in enzymology, LW Cunningham, DW Frederiksen (eds). Academic Press, New York 1982;82:453-471.

19. Pointner R, Kofler, Offer Ch, Schwab G. The kinetics of antibiotic release from a fibrin-clotting system: an animal; experiment. In: Schlag G, Redl H (eds). Fibrin sealant in operative medicine. General surgery and abdominal surgery - Vol. 6. Springer-Verlag, Heidelberg, Berlin, 1986:194-198.

20. Stegemann H. Stalder K. Determination of hydroxyproline. Clin Chim Acta 1967;18:267-273.

21. Grant RA. Estimation of hydroxyproline by the AutoAnalyser. J Clin Path 1964;17:685-686.

22. Banerjee SK, Glynn LE. Reactions to homologous and heterologous fibrin implants in experimental animals. Ann NY Acad Sci 1960;86:1054-1057.

23. Leibovich SJ, Ross R. A macrophage-dependent factor that stimulates the proliferation of fibroblasts in vitro. Am J Path 1976;84:501-513.

24. Pohl J, Bruhn HD, Christophers E. Thrombin and fibrin-induced growth of fibroblasts: role in wound repair and thrombus organization. Klin Wochenschrift 1979;57:273-277.

25. Petrelli NJ, Cohen HC, DeRisi D, et al. The application of tissue adhesives in small-bowel anastomoses. J Surg Oncol 1982;19:59-61.

26. deVirgillio C, Dubrow T, Sheppard BB. Fibrin glue inhibits intra-abdominal adhesion formation. Arch Surg 1990;125:1378-1382.

27. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomosis; the effect of suture technic on collagen concentration in the colonic wall. Am J Surg 1978;135:333-340.

28. Hesp WLEM, Hendriks Th, Schillings PHM, Lubbers EJC, de Boer HHM. Histologic features of wound repair: a comparison between experimental ileal and colonic anastomoses. Br J Exp Path 1985;66:511-518.

29. Mastboom W.J.B, Hendriks T, de Boer H.H.M. Collagen changes around intestinal anastomoses in germ-free rats. Br J Surg 1989;76:797-801.

30. Högström H, Haglund U. Neutropenia prevents decrease in strength of rat intestinal anastomosis: partial effect of oxygen free radical scavengers and allopurinol. Surgery 1986;99:716-720.

CHAPTER X

GENERAL DISCUSSION AND CONCLUSIONS

10.1 Discussion

The studies on the influence of fibrin sealant on the healing colonic anastomoses, demonstrate that fibrin sealant does not improve the healing of colonic anastomosis in the rat; even more so, fibrin seems to weaken the new-made anastomosis(Chapter V). Some authors have claimed a beneficial effect of fibrin sealant, and advised its use as a routine in normal and high-risk anastomosis in man. However, this advise does not have a strong scientific basis. In fact results of animal studies are conflicting and so far clinical studies are retrospective and lack a good control group. More experimental data are needed before a well considered conclusion can be drawn. Certainly the present results in which the healing of rat colonic anastomoses seems to be impeded, call for more restraint in the use of fibrin sealant in clinical pratice.

Many of our conclusions are based on the parameter of bursting pressure as a measure of anastomotic strength. For example it was a common finding, in normal and high risk situations, that the bursting pressure on day 4 after division and anastomosis of the colon was lower in the animals that had received fibrin glue application. Although we have reason to believe that the bursting pressure is the best method to assess mechanical strength of a fresh anastomosis, this parameter has been a topic of much debate. In the literature on healing of experimental intestinal anastomoses several procedures of measurement of mechanical strength are presented. Generally, two methods can be identified: **breaking strength** and **bursting strength**, expressed as bursting pressure or bursting wall tension. The breaking strength is measured by applying an increasing force in a longitudinal direction to anastomotic segments until disruption takes place. The anastomotic bowel segment cannot be left in situ, as breaking strength measurements are done in a tensiometer. Removing a colonic anastomosis from the abdomen during the first few days after construction, detaching adhesions, may severe the anastomosis and thus influence the measurement. In order to avoid damage to the anastomosis during the early phase of healing, the sutures should be left in place. Tension strength measurements then reflect the "suture holding capacity" of the tissue: the capability of the bowel wall to retain sutures. The anastomotic strength is thus dependent on the number of sutures and the amount and structure of the connective tissue encircled by the sutures.[1]

The measurement of the "bursting pressure" is accomplished by distension of a bowel segment with gas or liquid until a leak develops, and records the pressure at the time of rupture. The rate of inflation must be standardized as this is known to influence the outcome. Because according to LaPlace's law the wall tension is related to the radius of the intestine, and different anastomotic techniques will affect the distension of the intestine, some studies prefer to use bursting wall tension instead of bursting pressure.[2] However, in those studies where both parameters were used, differences found were reflected by both bursting pressure and bursting wall tension, and as such conclusions drawn were the same.[3,4] Because generally the diameter of an anastomosis is smaller than the adjacent colon, and lacks elasticity, LaPlace's law explains why rupture will take place outside the anastomosis, as soon as the anastomosis has gained some strength. When rupture takes place outside the anastomosis, bursting pressure measurements no longer reflect the strength of the anastomoses.[5] In our study this was already the case after 7 days. In view of this information it is essential to indicate the point where rupture takes place, in studies on anastomotic strength, when the bursting pressure is used. In those studies that have measured both breaking strength and bursting strength, corresponding results were found for the two parameters.[6,7] A comprehensive analysis comparing the different mechanical parameters of intestinal anastomotic healing was recently published.[8] The conclusion drawn was that the bursting pressure is a good parameter to monitor anastomotic repair, as long as rupture takes place within the anastomosis, which is generally the case within the first week postoperatively. In our studies we measured the *in situ* bursting pressure, taking care not to disturb any adhesions on the anastomosis, that might have helped to seal small leaks. Especially in the model of "incomplete anastomoses" (Chapter VII) removal of the anastomosis from the abdomen before measuring the mechanical strength would detache "sealing" adhesions making the results unreliable.

Corresponding with the results of the study on normal sutured anastomoses, also in ischaemic anastomoses (Chapter VI) and in the presence of faecal peritonitis (Chapter VII), no improvement of anastomotic strength was achieved by fibrin sealant. Only during the first 2 days of an incomplete anastomosis (Chapter VII), a significant increased bursting pressure could be reached by fibrin sealing. This difference must have been attributed to the direct mechanical sealing effect of fibrin, because collagen degradation in

this group was statistically elevated compared to controls. On day 4 postoperatively again no improvement of bursting pressure was present any more. It was thus found that fibrin sealant does only give transient mechanical support to a technically insufficient anastomosis, during the first 2 days of healing.

From our bursting pressure studies we conclude that the fibrin glue loses its adhesive properties on the anastomoses between day 2 and 4. This conclusion is supported by the finding that fibrin sealant was still present on the anastomosis on day 2 and 4 after operation, but on day 7 no fibrin sealant could be detected any more. Fibrin deposits are degraded in 3 ways: by proteolytic enzymes produced by inflammatory cells, by phagocytosis by macrophages and finally by bacterial lysis. *In vitro* studies have shown that bacteria, e.g. *Streptococcus faecalis*, can completely lyse a clot of fibrin sealant within 1 to 5 days.[9] The same study shows that several bacteria, including other common colonic strains, readily grow and multiply in fibrin sealant. Bacterial infection of fibrin sealant on colonic anastomosis may be likely to contribute to its rapid breakdown. In our studies using a combination of fibrin sealant and antibiotics however, no alterations in the degradation of the fibrin sealant could be demonstrated.(Chapter IX) Nonetheless this does not exclude that the adhesive properties of the fibrin clot might be prolonged due to antibiotics. It would be interesting to investigate *in vitro* whether antibiotics added to fibrin sealant can prolong the lifetime after bacterial contamination.

The strength of a new constructed anastomosis depends mainly on the integrity of the connective tissue of the wound margins holding the sutures. During the early phase of anastomotic healing, there is a considerable decrease of anastomotic bursting pressure, corresponding with collagen concentration loss. The claimed positive influence of fibrin sealant on collagen metabolism of the healing wound (see Chapter II) could not be confirmed in our models of colonic anastomoses. The decrease of bursting pressure changes correlated with an increased loss of collagen in the wound margins, with lowest levels measured on day 2. Also proximal and distal to the anastomosis a decrease of collagen could be noted. Collagen content is the net result of collagen breakdown and collagen synthesis, and the strength of newly synthesized collagen is inferior to that of mature collagen.[10] In the early phase of anastomotic wound healing, collagen concentrations are least on day 2. In our experimental ischemic and peritonitis models, the recovery of

collagen concentration is inhibited, in comparison to controls, with low values on day 4. This pattern of slow collagen concentration regain was seen in all groups that were treated with fibrin sealant. This indicates that either increased collagen degradation continues longer after fibrin sealing, or that formation of new collagen is inhibited. To elucidate this problem and differentiate between collagen breakdown and synthesis, studies using radioactive labelled proline are required. As soon as the fibrin seal was cleared on day 7, collagen concentration levels were the same as in controls. The possible increased breakdown of collagen and lower bursting pressure cannot be attributed to a reaction to foreign (human) protein because the application of rat fibrin sealant, gave identical results as human fibrinogen (Chapter IV). Pilot studies with a (rat) fibrin clot applied on the anastomosis gave also identical results. Only the addition of antibiotics to the fibrin sealant could prevent the adverse effect on the bursting pressure and collagen metabolism (Chapter IX). Moreover, bursting pressure was significantly higher on day 2, compared to non-sealed controls, while fibrin sealant alone did not give significant increase in bursting pressure on day 2. Histologic examination has shown increased inflammatory reaction, especially inside the applied fibrin sealant neutrophil granulocytes were numerous. This neutrophilic infiltration score was less after addition of antibiotics. However, results of inflammatory cell counts, exclusively on the basis of histology, are semi-quantitative and as such must be considered with care.

A special aspect of the colon, compared to other parts of the gastrointestinal tract, is its high bacterial load. Therefore every colonic anastomosis must be considered to be contaminated, where fibrin may act as a culture medium for bacteria. In fact this will lead to the condition of an infected anastomosis, when a fibrin seal has been applied. This is confirmed by the findings in our study on anastomotic healing in case of faecal peritonitis (Chapter VII). Mechanical and biochemical parameters of sealed control anastomoses, and anastomoses with peritonitis are comparable. As both bacteria and neutrophilic granulocytes are known to produce collagenase, there are 2 possible explanations for the antibiotic effect seen in our study. Either antibiotics inhibit the growth of collagenase producing bacteria or it is a combination of reduced bacterial colonization and due to this reduced collagenase producing neutrophils. Further studies using bacterial cultures and

anti-neutrophil serum could possibly answer the question whether bacteria or neutrophils invading the fibrin sealant, or both are responsible for the collagen changes.

Inflammatory reaction during the early wound healing phase is a known cause of collagenolysis. Inhibition of the inflammatory reaction by means of anti-neutrophil serum, prevents the decrease of anastomotic collagen content.[11] Eicosanoids are known to play an important role in inflammatory reactions, however, their role in wound healing is rather unknown. We found that the eicosanoid profile of colonic anastomotic tissue in the rat, shows low activity of cyclooxygenase, but high production of lipoxygenase products, especially 12-HETE (Chapter IV). At the same time peritoneal macrophages showed increased 12-HETE production. Due to similarities in the profiles of colonic anastomoses and peritoneal macrophages it is very suggestive that the increase in 12-HETE found in the anastomosis is of macrophage origin, and that macrophages play an important role in the colonic anastomotic healing process from as early as day 1 after surgery. From our results it is to be expected that NSAIDs can hardly inhibit the increased inflammatory response in the healing colonic anastomosis. It is suggestive that a more potent effect will be reached by means of drugs that selectively block the lipoxygenase pathway. NSAIDs are potent inhibitors of cyclooxygenase, but do not affect the lipoxygenase pathway. Although the effect of NSAIDs on the healing of gastrointestinal anastomoses was hardly studied, it was found that NSAIDs influence collagen metabolism and cause a reduction of the decrease of hydroxyproline concentrations in colonic anastomosis in the rat.[12] On the other hand these NSAIDs increased susceptibility to surgical infections, causing increased morbidity and mortality in the same study.

While peritoneal adhesions in general are harmful and can cause intestinal obstruction even after a long period, they may also be beneficial in case of intestinal anastomoses. During the early phase peritoneal adhesions are known to seal potential leaks of the anastomosis. In this respect peritoneal adhesions may even be beneficial. Since the deposition of fibrin is an integral part in the process of adhesion formation, it was to be expected that increased adhesion formation was found after fibrin sealing. However, some studies carried out by others have found a significant decrease in adhesion formation with fibrin glue, in a model of peritoneal defects.[13,14] The inhibitory action of fibrin sealant on adhesion formation was found to be dependent on the fibrinogen concentration and the

thickness of the applied layer of the fibrin glue, where only a high concentration and a thick layer were effective. In our model this inhibitory action of fibrin sealant was not confirmed, on the contrary, increased adhesion formation was found. Besides, whether this adhesion formation is permanent cannot be said, because we did not examen later than 7 days postoperatively.

In our studies increased adhesion formation was found especially in animals with faecal peritonitis and ischemia of the anastomosis. This agrees with the fact that ischemia and inflammation are factors known to produce permanent adhesions in the peritoneum in humans and animals.[15] The formation of adhesions is thought to be dependent on reduction in plasminogen activity of the peritoneum after a local peritoneal lesion, which allows local fibrin to form fibrinous adhesions between traumatized areas.[16] Topical administration of recombinant tissue plasminogen activator (rt-PA) proved to be highly effective in prevention of primary and recurrent peritoneal adhesions.[17] However in the same study there was no effect of rt-PA on the occurrence of adhesions on colonic anastomosis. By analogy with rt-PA also fibrin glue prevents adhesions of peritoneal defects, as reported in the literature, but does not prevent adhesions on colonic anastomoses, as found in this study. Interestingly it was recently found that t-PA reverses the deleterious effect of infection on colonic wound healing.[18] In the same study an infected clot was placed on a colonic anastomosis in the rat, causing a lower bursting pressure and a lower collagen content, which could be prevented by administration of t-PA. This finding supports our findings and the conclusion that fibrin sealing of colonic anastomosis is harmful.

In conclusion:

- Prostaglandins and other cyclo-oxygenase products are not produced in excess in a healing colonic anastomosis in the rat, and probably do not play a major role in uncomplicated healing of the large intestine.
- Lipoxygenase metabolism forming leukotrienes and especiallly 12-HETE is increased significantly and may be of macrophage origin. 12-HETE may play a role in (colonic) wound healing.

- Fibrin sealing of normal and high-risk anastomoses of the rat colon, does not improve wound repair; on the contrary there seems to be a negative effect.
- This negative influence cannot be attributed to a specific immunological reaction to foreign protein, but may be caused by an increased inflammatory reaction on the fibrin mass.
- Addition of antibiotics to fibrin sealant improves the mechanical strength of colonic anastomoses on day 2, and prevents the negative influence of fibrin sealant on day 4.
- Fibrin sealant does not prevent adhesion formation to colonic anastomoses, but generates increased adhesion formation.
- Excess collagen concentration decrease of colonic anastomoses, caused by fibrin sealant, can be prevented by adding antibiotics.
- The routine use of fibrin sealant in colonic anastomotic surgery in human does not seem to be justified.

References

1. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomoses. Breaking strength of the colon after left colon resection and anastomosis. Am J Surg 1978;136:595-599.
2. Nelson TS, Anders CJ. Dynamic aspects of small intestinal rupture with special consideration of anastomotic strength. Arch Surg 1966;93:309-314.
3. Irvin TT, Hunt TK. Reappraisal of the healing process of anastomosis of the colon. Surg Gynecol Obstet 1974;138:741-746.
4. Asencio-Arana F, Martinez-Soriano F. Stimulation of the healing of experimental colon anastomoses by low-power lasers. Br J Surg 1988;75:125-127.
5. Ballantyne GH. Intestinal suturing. Review for experimental foundations for traditional doctrines. Dis Colon Rectum 1983;26:836-843.
6. Rosin RD, Exarchakos G, Gilmore OJA, Ellis H. Topical noxythiolin in colonic healing. Br J Surg 1978;65:603-606.
7. Young HL, Wheeler MH. Collagenase inhibition in the healing colon. J Roy Soc Med 1983;76:32-36.
8. Hendriks Th, Mastboom WJB. Healing of experimental intestinal anastomoses: parameters of repair. Dis Colon Rectum 1990;33:891-901.
9. Hoffmann S, Moesgaard F. Bacterial lysis of fibrin seal in vitro. Eur Surg Res 1988;20:75-81.
10. Peacock EE J. In: Wound repair. Structure, synthesis and interaction of fibrous protein and matrix. Philadelphia. WB Saunders Co, 1976:145-202.

11. Högström H, Haglund U. Neutropenia prevents decrease in strength of rat intestinal anastomosis: partial effect of oxygen free radical scavengers and allopurinol. Surgery 1986;99:716-720.

12. Mastboom WJB, Hendriks T, van Elteren P, de Boer HHM. The influence of NSAIDs on experimental intestinal anastomosis. Dis Col Rect 1991;34:236-243.

13. Lindenberg S, Steentoft P, Sørensen SS, Olesen HP. Studies on prevention of intra-abdominal adhesion formation by fibrin sealant: an experimental study in rats. Acta Chir Scand. 1985;151:525-527.

14. DeVirgillio C, Dubrow T, Sheppard BB, et al. Fibrin glue inhibits intra-abdominal adhesion formation. Arch Surg 1990;125:1378-1382.

15. Ellis H. The aetiology of post-operative abdominal adhesions; an experimental study. Br J Surg 1962;50:10-16.

16. Buckman RF, Woods M, Sargent L, Gervin A.S. A unifying pathogenetic mechanism in the aetiology of intraperitoneal adhesions. J Surg Res 1976;20:1-5.

17. Menzies D, Ellis H. The role of plasminogen activator in adhesion prevention. Surg Gynecol Obstet 1991;172:362-366.

18. Houston KA, McRitchie DI, Rotstein OD. Tissue plasminogen activator reverses the deleterious effect of infection on colonic wound healing. Ann Surg 1990;211:130-135.

SUMMARY

Leakage of colonic anastomoses remains a serious complication in abdominal surgery with high mortality and morbidity. In 1990 over 9000 persons underwent resection of the colon or rectum in The Netherlands. Although the exact incidence of leakage is unknown, based on the literature this figure must be over 10 % for clinical leakage. Every general surgeon knows the sequelae of this feared complication, causing peritonitis and a mortality rate of more than 30%.

In **Chapter I** an introduction on the problem of colonic anastomotic healing is given, and a short historical overview is given. Intestinal surgery as we know it today started in the 19th century, however the discussion and research on improvement of anastomotic wound healing still continues. The first period of wound healing is called the lag phase and is dominated by an inflammatory reaction. This inflammatory reaction is primarily meant to clear the wound of debris and injured tissue. During this period there is a significant decrease of collagen content in the wound margins of the colonic anastomosis. Inflammatory cells, e.g. neutrophils and macrophages are potent sources of the enzyme collagenase. The collagen content of the anastomosis is lowest around the third day, followed by rapid recovery due to increased collagen synthesis, giving normal content by day 7. The integrity of an early anastomosis depends on the submucosal collagen layer holding the sutures. The importance of this layer for the mechanical strength of the anastomosis, has been proven repeatedly, and collagen content of an anastomosis is an important parameter in studies on colonic anastomotic healing. The amount of hydroxyproline in tissue, being a specific aminoacid in collagen, serves as parameter of collagen content. Eicosanoids, leukotrienes and prostaglandins, the biologically active derivatives of arachidonic acid are mediators of acute inflammation and consequently also of the wound healing process. The exact role of these substances and their inhibitors, corticosteroids and NSAIDs in the wound healing process in general, and colonic wound healing specifically, is not very clear. It is known however that i.p. administration of prostaglandin E_2 (PGE_2) influences the healing of colonic anastomosis in a negative way. **Chapter IV** reports on a study on the production of eicosanoid products in tissue samples of the healing rat colon following resection and anastomosis. Non-operated colon tissue

metabolizes small amounts of arachidonic acid into cyclooxygenase and lipoxygenase products. However, following construction of an anastomosis there is increased production of lipoxygenase products, while cyclooxygenase activity remains low. PGE_2 and other cyclooxygenase products are not produced in excess following anastomosis of the colon, and probably do not play a major role in the uncomplicated healing of the large intestine the rat. During the first 8 days of repair in the anastomosed colon tissue a statistically significant increase of 12-hydroxyeicosatetraenoic acid (12-HETE) production was found compared to control colon tissue (P=0.001). At the same time peritoneal macrophages from these rats show increased 12-HETE production as well. The HPLC-profile of peritoneal macrophages strongly resembles that of colonic tissue samples, indicating the presence of infiltrating macrophages. The possible role of macrophages in anastomotic wound healing is still under discussion. Lipoxygenase metabolism and specific drugs that modify this pathway of eicosanoid metabolism, in order to modify macrophage function need more attention in research on (colonic) wound healing, and may possibly give useful tools to modulate the process of wound healing.

Although the direct cause of anastomotic failure may often not be clear there are a number of recognised systemic and local risk factors. Systemic risk factors are: age, malnutrition, malignancy and cancer chemotherapy. A number of local factors that influence anastomotic healing are: level of the anastomosis, intestinal obstruction, intra-abdominal infection, faecal loading, peritoneal spill, severe trauma, ischaemia of the intestine and radiotherapy of the bowel. In experimental and clinical studies, much attention has been paid to suture techniques, suture materials, and the use of staplers in order to improve the safety of intestinal anastomoses. Bowel preparation and use of antibiotics have found a definite place in colonic surgery, although they cannot prevent leakage as such, but do prevent complications. The construction of a protective colostomy reduces the incidence of clinical leakage, but needs an extra operation to close the stoma, again creating a colonic anastomosis.

Many experimental attempts were done to protect the anastomosis by means of an external cover of different synthetic materials, but all these attempts resulted in increased leakage. Only the intraluminal coverage by means of a latex tube (Coloshield®) has been found useful and is applied clinically, since a few years. Several authors have claimed a

beneficial effect of (external) sealing of the intestinal anastomoses with fibrin sealant. Fibrin sealant is a biologic glue, based on highly concentrated human fibrinogen, that can be converted to solid fibrin by addition of thrombin. Detailed information on the composition and characteristics of fibrin glue is given in **Chapter II.** Fibrin sealant can be used as an haemostatic agent, as a seal to prevent leakage of air or fluid, or as an adhesive instead of sutures. Experimental work has shown that fibrin sealant may contribute favourably to wound healing by producing local haemostasis, stimulating the influx of macrophages, angiogenesis, fibroblast proliferation and collagen production. Many of the characteristics of fibrin sealant depend on the concentrations of the components of the fibrin sealant. Most studies on the aspects of fibrin sealant and wound healing were done in skin wounds. There are only a few good experimental studies on the effect of fibrin sealant on the healing intestinal anastomosis and the results are confusing: while most studies claim improved anastomotic healing, others report the opposite.

Fibrin glue has found its way in very many fields of general surgery and many other specialities. In abdominal surgery it is now frequently used clinically as an haemostatic after traumatic rupture or partial resections of parenchymous organs e.g. liver, spleen and kidney. Furthermore many surgeons use fibrin sealant to seal all kind of intestinal anastomoses, although the experimental basis is still lacking.

We have studied the effect of fibrin sealant on colonic anastomoses in rats during the first week. The study on the effect of human- and rat fibrinogen on bursting pressure, collagen changes around the anastomoses, adhesion formation and inflammatory reaction is described in **Chapter V.** Both sealants had the same effect. Adhesion formation was significantly increased after sealing compared with controls. The anastomotic bursting pressure on day 4 postoperatively was significantly lower following fibrin sealing. This finding corresponds with significantly reduced collagen concentrations around the anastomoses on day 4. Histologic examination showed massive infiltration of the fibrin seal with inflammatory cells on day 2 and 4, while on day 7 the sealant had been cleared. From the results of this study it was concluded that fibrin sealant does not improve the healing of sutured colonic anastomoses in rats. On the contrary there seems to be a negative influence, as measured by bursting pressure and hydroxyproline concentration. This negative effect cannot be attributed to a reaction to foreign protein as rat-fibrin sealant had

the same effect. Addition of fibrin sealant caused an increased inflammatory response around the anastomosis.

The negative effects of fibrin sealant found on colonic anastomoses under non-complicated conditions does not necessarily mean that this is the same in compromised anastomoses. Above all fibrin sealant has been specially advocated in high-risk intestinal anastomoses.

Ischemia of the wound margins is a feared complication of intestinal surgery causing increased incidence of leakage. The study on the effect of fibrin sealant on ischemic anastomoses in rats is described in **Chapter VI**. Colonic ischemia was induced by division of the mesenteric blood vessels for 2 cm on either side of the anastomosis. Ischemia gave rise to increased formation of perianastomotic adhesions and abscesses. The anastomotic bursting pressure was significantly reduced although collagen concentration was not significantly lower. Sealing of ischaemic anastomoses however did not improve mechanical strength, and lowered collagen content of perianastomotic tissue. Also adhesion formation was not prevented by fibrin sealing. It was concluded from this study that fibrin sealing does not improve the healing ischemic colonic anastomosis.

In the same way the effect of fibrin sealant on colonic anastomotic healing in case of peritonitis, was studied as described in **Chapter VII**. Peritonitis was induced by means of leaving 200 mg of powdered autoclaved rat faeces in the abdominal cavity. Faecal peritonitis caused increased adhesion and abscess formation, with or without fibrin sealant. The anastomotic bursting pressure was significantly reduced in case of peritonitis compared with controls on days 4 and 7; this could not be prevented by fibrin sealant. Collagen concentration is significantly reduced in case of peritonitis with or without fibrin sealant on day 4 and 7. The collagen concentrations of sealed control anastomoses are comparable to findings in the peritonitis model. We conclude that faecal peritonitis causes a reduced mechanical strength of colonic anastomosis and reduction of anastomotic collagen concentration. This cannot be prevented by means of fibrin sealing of the anastomosis.

Much attention has been given to the different type of sutures but primarily the integrity of the anastomosis depends on the technique of the individual surgeon. As the leakage rate varies among different surgeons, and technical and anatomical location of the low

colorectal anastomoses in the pelvis limit surgery, incomplete anastomoses are a true clinical problem. A model of a technically insufficient anastomosis was created by using only 4 interrupted sutures at 90, as described in **Chapter VIII**. The results of this study showed increased adhesion formation after fibrin sealing. The anastomotic bursting pressure of incomplete anastomoses showed a significant increase after sealing on day 2 only; on day 4 and 7 no differences were found. We conclude that by application of fibrin sealing, incomplete anastomoses are temporarily protected by fibrin glue sealing.

Infection of fibrin sealant with bacteria from the colonic lumen is likely to take place after sealing of colonic anastomoses. Antibiotics can be added to the fibrin sealant without affecting its clotting behaviour. In the study as described in **Chapter IX** the anastomosis was sealed with fibrin adhesive to which clindamycin and cefotaxim was added. Adhesion formation was significantly increased after administration of fibrin sealant and fibrin antibiotic combination. On day 2 the anastomosis was significantly stronger after sealing with antibiotic-fibrin mixture. Addition of antibiotics prevented the decrease of bursting pressure provoked by fibrin sealant on day 4. Also the corresponding lowering of collagen content induced by fibrin sealant was countered. From these results it was concluded that addition of antibiotics prevents the negative influence of fibrin adhesive on the healing colonic anastomosis and contributes to a stronger anastomosis on day 2 after operation.

Finally it was concluded that fibrin sealant does not improve healing of normal and high-risk anastomoses in rats, due to an increased inflammatory reaction. Addition of antibiotics prevents the negative effect of fibrin sealant, and further studies using this combination are mandatory.

SAMENVATTING

In de abdominale chirurgie is lekkage van een colon anastomose nog steeds een ernstige complicatie, die een hoge mortaliteit en morbiditeit kent. In 1990 werd in Nederland bij meer dan 9000 patiënten resectie van het colon of rectum uitgevoerd. Hoewel de exacte incidentie van lekkage onbekend is, wordt deze in de literatuur op meer dan 10% geschat. Elke algemeen chirurg kent de ernstige gevolgen van deze gevreesde complicatie, die peritonitis veroorzaakt en een mortaliteit heeft van meer dan 30%. In **Hoofdstuk I** wordt een inleiding gegeven op de problematiek van de genezende colon naad, evenals een kort historisch overzicht. Intestinale chirurgie zoals we die tegenwoordig kennen ontstond in de 19e eeuw, maar onderzoek naar en discussie over de beginselen en methoden ter verbetering van anastomose genezing is nog steeds gaande. De eerste periode van het wondgenezings proces wordt gedomineerd door een ontstekingsreactie. Deze ontstekingsreactie is primair gericht op het opruimen van lichaamsvreemd materiaal en beschadigd en necrotisch weefsel. Gedurende deze periode daalt het collageen gehalte in de wondranden van de colon anastomose significant. Ontstekingscellen, zoals neutrofiele granulocyten en macrophagen zijn bekende producenten van collagenase. Het collageen gehalte van de anastomose is minimaal rond de 3e dag na operatie, waarna door verhoogde collageen synthese snel herstel intreedt, waardoor op de 7e dag het gehalte weer normaal is. Gedurende de 1e dagen hangt de stabiliteit van een anastomose af van de submucosale collageenlaag, waarin de hechtingen zijn gevat. Het belang van deze laag voor de mechanische sterkte van een anastomose, is bij herhaling bewezen, en derhalve is het collageen gehalte van een anastomose een belangrijke parameter bij onderzoek naar genezing van anastomosen. De hoeveelheid hydroxyproline in weefsel, een aminozuur dat vrijwel uitsluitend in collageen voorkomt, dient als een maat voor collageen.

Eicosanoiden, zowel leukotrienen als prostaglandinen, de biologisch actieve derivaten van arachidon zuur, zijn mediatoren van acute ontstekingsreacties, en spelen in die hoedanigheid ook een rol bij wond genezing. De kennis over de rol van deze stoffen, en van de stoffen die hun produktie remmen, zoals corticosteroiden en NSAIDs, in het wondgenezings proces en in het bijzonder voor de genezing van de colon, is summier.

Wel is bekend dat i.p. toediening van prostaglandine E_2 de genezing van colon anastomosen negatief beïnvloedt. **Hoofdstuk IV** geeft een beschrijving van het onderzoek naar de produktie van eicosanoiden, met behulp van 'high performance liquid chromatography' in de genezende colon anastomose na resectie en anastomose bij de rat. In controle colon weefsel worden slechts kleine hoeveelheden arachidon zuur omgezet door cyclooxygenase en lipoxygenase. Na het aanleggen van een anastomose, is er echter een sterk verhoogde produktie van lipoxygenase afhankelijke produkten, terwijl de activiteit van cyclooxygenase zeer laag is. Prostaglandine E_2 and andere cyclooxygenase produkten worden niet in verhoogde mate gevormd, en spelen dan ook waarschijnlijk geen rol van betekenis bij ongecompliceerde genezing van de colon naad bij de rat. Gedurende de eerste 8 dagen werd een statistisch significante toename van de produktie van 12-hydroxyeicosatetraenoic zuur (12-HETE) gevonden in vergelijking met controle colon weefsel. Gedurende deze zelfde periode tonen ook peritoneale macrophagen verhoogde 12-HETE vorming. Het patroon van de eicosanoiden synthese van de peritoneale macrophagen toonde sterke gelijkenis met het eicosanoiden patroon geproduceerd door het weefsel in de colon anastomose. Dit wijst erop dat de gevormde 12-HETE zeer waarschijnlijk door macrophagen geproduceerd wordt, en dat deze cellen de anastomose vroeg infiltreren. De mogelijke betekenis van 12-HETE voor darmnaad genezing en de rol van macrophagen in deze wordt besproken. Er is meer onderzoek nodig naar de rol van het lipoxygenase metabolisme en medicamenten die dit metabolisme beïnvloeden, met betrekking tot wond genezing. Dit onderzoek kan mogelijk leiden naar middelen die het wond genezingsproces kunnen beïnvloeden.

Hoewel de directe oorzaak van naaddehiscentie vaak niet duidelijk is, zijn er een aantal systemische en lokale risicofactoren bekend. Systemische risicofactoren zijn: hoge leeftijd, maligniteit en chemotherapie. Lokale risicofactoren zijn: het niveau van de anastomose, darmobstructie, intra-abdominale infectie en ontsteking, slechte darm voorbereiding, faecale verontreiniging van het peritoneum, ernstig trauma, ischaemie van de darm en bestraling van de darm. Met het oog op verbetering van anastomose genezing is er veel experimenteel en klinisch onderzoek gedaan naar hechttechniek, hechtmaterialen en nietapparaten. Darmvoorbereiding en antibiotica hebben hun nut inmiddels bewezen, hoewel ook hiermee lekkage niet kan worden voorkomen. En hoewel het aanleggen van

een beschermend stoma proximaal van de anastomose, de kans op lekkage vermindert, moet bij deze methode rekening gehouden worden met een extra operatie voor het opheffen van het stoma, met weer een anastomose.

Er is veel experimenteel onderzoek geweest naar de mogelijkheid om een anastomose te beschermen d.m.v. een uitwendige afdekking met synthetisch materiaal, doch steeds gaf dit aanleiding tot toename van lekkage. Slechts de intraluminale bedekking met een latex buis (Coloshield^{R}), is nuttig gebleken en wordt sinds enkele jaren met succes toegepast. Meerdere auteurs hebben beweerd dat het afdichten van intestinale anastomosen met fibrine lijm een gunstige werking heeft. Fibrine kleefstof is een biologische lijm, waarvan de werking berust op de laatste stap van het stollingssysteem. Fibrine kleefstof bevat hoog geconcentreerd humaan fibrinogeen dat onder invloed van thrombine kan worden omgezet in fibrine. Meer gedetailleerde informatie over de samenstelling, werking en toepassing van dit produkt wordt gegeven in **Hoofdstuk II**. Fibrine kleefstof wordt enerzijds als haemostaticum gebruikt, b.v. voor het afdichten van resectievlakken van parenchymateuze organen, anderzijds als hechtlijm i.p.v. hechtingen. Experimenten hebben aangetoond dat fibrine kleefstof de wondgenezing kan bevorderen door het creëren van lokale bloedstolling, door stimulatie van de chemotaxis van macrophagen en fibroblasten, de bevordering van angiogenesis, fibroblasten proliferatie en collageen produktie. De verschillende eigenschappen zijn afhankelijk van de concentraties van de samenstellende delen van fibrine kleefstof. Het meeste onderzoek naar de eigenschappen van fibrine kleefstof met betrekking tot wondgenezing is uitgevoerd met huidwonden. Er zijn weinig publikaties van goed opgezette gerandomiseerde experimentele studies naar het effect van fibrine kleefstof op wondgenezing van de darm, en de bevindingen zijn wisselend. De meeste studies toonden goede resultaten, enkele daarentegen beweren het tegenovergestelde. Fibrine kleefstof wordt klinisch toegepast door vele chirurgische specialismen. In de abdominale chirurgie wordt het tegenwoordig frequent gebruikt als haemostaticum na rupturen of partiële resectie van parenchymateuze organen, zoals lever, milt of long. Ook wordt fibrine kleefstof klinisch toegepast voor het afdichten van darmnaden, hoewel het experimentele bewijs van het nut hiervan ontbreekt. Dit proefschrift beschrijft een experimenteel onderzoek naar het effect van fibrine kleefstof op dikke darmnaden bij ratten in de 1e week.

De studie naar het effect van menselijk- en ratte fibrinogeen op 'bursting pressure', collageen metabolisme in de anastomose, adhesie vorming en ontstekingsreactie wordt beschreven in **Hoofdstuk V**. Beide soorten kleefstof hadden dezelfde uitwerking. De ernst van de verklevingen was na het gebruik van kleefstof significant groter dan in de controlegroep. De 'bursting pressure' van de anastomose op de 4e dag na operatie was significant lager na het gebruik van fibrine kleefstof. Deze bevinding past bij de gevonden significant lagere collageen concentraties rondom de anastomose op dag 4. Histologisch onderzoek toonde sterke infiltratie van de fibrine laag door ontstekingscellen op dag 2 en 4, doch op dag 7 was de kleefstof volledig opgeruimd. De conclusie van dit onderzoek was dat fibrine kleefstof de genezing van de colon anastomose bij de rat niet verbetert. Wat betreft sterkte van de naad en de hydroxyproline concentratie is er juist een negatieve invloed. Dit negatieve effect kan niet worden verklaard door een reactie op vreemd eiwit, daar het ratte fibrinogeen tot dezelfde resultaten leidde. Aanbrengen van fibrine kleefstof veroorzaakte een toegenomen ontstekingsreactie rondom de anastomose.

De gevonden negatieve uitwerking van fibrine lijm op normale, optimale anastomosen, betekent niet vanzelfsprekend dat dit ook het geval is voor gecompromitteerde anastomosen. Daar komt bij dat fibrine kleefstof met name voor *high-risk* darmnaden wordt aanbevolen.

Ischaemie van de wondranden is een gevreesde complicatie van darm chirurgie en geeft in verhoogde mate aanleiding tot naadlekkage. Het onderzoek naar de invloed van fibrine kleefstof op ischaemische anastomosen bij ratten wordt beschreven in **Hoofdstuk VI**. Ischaemie van het colon werd gecreëerd door aan beide kanten van de anastomose over een afstand van 2 cm de mesenteriale bloedvaten te coaguleren en door te nemen. De aldus ontstane ischaemie veroorzaakte in verhoogde mate verklevingen met de anastomose, evenals abcessen. De 'bursting pressure' van de darmnaad was significant lager alhoewel de collageen concentratie niet significant was afgenomen. Het gebruik van fibrine kleefstof verbeterde de sterkte van de ischaemische anastomose niet, en veroorzaakte een daling van het collageen gehalte van het darmweefsel rondom de naad. Ook de adhesie vorming kon door fibrine lijm niet worden voorkomen. De conclusie van dit onderzoek was dat fibrine kleefstof de genezing van ischaemische anastomosen niet corrigeert. Op dezelfde wijze werd het effect van fibrine lijm op de naadgenezing bij peritonitis onderzocht; dit wordt

beschreven in **Hoofdstuk VII**. Peritonitis werd teweeggebracht door 200 mg verpulverde en geautoclaveerde ratte faeces in de buik achter te laten. Faecale peritonitis, zowel met als zonder fibrine kleefstof, veroorzaakte toename van de adhesie- en abces vorming. De bursting pressure van de anastomose was significant lager i.c.m. peritonitis, vergeleken met controles op dag 4 en 7, en veranderde niet met fibrine kleefstof. De collageen concentratie was significant gedaald bij peritonitis, onafhankelijk van gebruik van fibrine lijm, op dag 4 en 7. De collageen concentraties van de controle anastomosen met fibrine kleefstof zijn vergelijkbaar met de uitkomsten van het peritonitis model. De conclusie van dit onderzoek is dat faecale peritonitis een afname van de mechanische sterkte van colon naden veroorzaakt, evenals een verlaging van de collageen concentratie. Dit effect kan niet worden voorkomen met behulp van fibrine kleefstof.

In de literatuur is veel aandacht besteed aan vele verschillende hechttechnieken, doch primair is de integriteit van een anastomose afhankelijk van de technische uitvoering van de individuele chirurg. Door het feit dat het percentage naadlekkages, tussen verschillende operateurs significant kan verschillen, en dat m.n. de anatomische lokalisatie van de lage rectumnaad, in het kleine bekken, het opereren ernstig kan beperken, is het verklaarbaar dat incomplete c.q. technisch insufficiënte anastomosen een reëel klinisch probleem vormen. Een experimenteel model van een insufficiënte anastomose werd gecreëerd door met slechts 4 geknoopte hechtingen op 90 een anastomose te maken, zoals beschreven in **Hoofdstuk VIII**. De resultaten van dit onderzoek toonden wederom een toegenomen adhesie vorming na fibrine lijm gebruik. De 'bursting pressure' van insufficiënte anastomosen toonden alleen op dag 2 een significante verbetering, na gebruik van fibrine lijm; op de 4e en 7e dag werden geen verschillen gevonden. De conclusie van dit onderzoek was dat fibrine kleefstof een tijdelijke bescherming geeft van technisch insufficiënte anastomosen.

Het is onvermijdelijk dat fibrine kleefstof bij gebruik op een colon anastomose, geïnfecteerd raakt met bacteriën vanuit het lumen van het colon. Het is mogelijk gebleken antibiotica aan de fibrine lijm toe te voegen zonder aantasting van de stollings eigenschappen. In het onderzoek zoals beschreven in **Hoofdstuk IX** werd de anastomose afgesmeerd met fibrine kleefstof waaraan clindamycine en cefotaxim waren toegevoegd. De toegenomen vorming van adhaesies door fibrine kleefstof was door het toevoegen van

antibiotica niet verminderd. Op dag 2 was de sterkte van de naad significant beter na gebruik van fibrine kleefstof met antibiotica. Tevens voorkwam de toevoeging van antibiotica de daling van de 'bursting pressure' op dag 4 zoals gezien bij gebruik van fibrine lijm zonder antibiotica. Ook de bijpassende daling van het collageen gehalte door fibrine kleefstof verdween na toevoeging van antibiotica. Hieruit werd de conclusie getrokken dat toevoegen van antibiotica de negatieve werking van fibrine kleefstof op de genezing van de colonnaad neutraliseert, en bijdraagt aan een sterkere anastomose op dag 2 na de operatie. Tot slot worden in **Hoofdstuk X** na een algemene discussie de voornaamste conclusies gepresenteerd. De belangrijkste conclusie was dat fibrine kleefstof niet bijdraagt aan de verbetering van de genezing van normale en 'high-risk' colonnaden bij de rat, als gevolg van een toename van de ontstekingsreactie bij de naad. Toevoegen van antibiotica verhindert dit negatieve effect van fibrine lijm, maar uitgebreider onderzoek naar de effecten van deze combinatie zijn vereist.

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CURRICULUM VITAE

Arie Cornelis van der Ham werd op 11 april 1952 geboren te Nieuwerkerk aan den IJssel. Na het doorlopen van de MULO werd de Rijks Scholen Gemeenschap te Gouda bezocht, alwaar in 1971 het HBS-B diploma werd behaald. Hierna studeerde hij geneeskunde aan de Erasmus Universiteit te Rotterdam, hetgeen werd afgerond door afleggen van het artsexamen in 1978. Tijdens de studie werkte hij enige tijd als student-assistent op de afdeling Celbiologie en Genetica onder leiding van Prof.Dr. R. Benner. Na het behalen van het artsdiploma werden voorbereidende stages gelopen in het kader van uitzending naar de tropen. Na 6 mnd arts-assistentschap gynaecologie in het St.Clara Ziekenhuis te Rotterdam (Dr. J.M. Versteeg) en 12 mnd chirurgie in het Oude en Nieuwe Gasthuis te Delft (Dr. J.W. Eckhardt) werd de 'Tropencursus' voor artsen gevolgd in 1979. Vervolgens werkte hij 3 jaar als tropenarts in het St. Elisabeth Hospital 'Mukumu' in Western Province, Kenya. Na terugkeer in Nederland in 1983 werd hij opgeleid tot huisarts aan Erasmus Universiteit te Rotterdam. Tijdens deze éénjarige opleiding werd gedurende 6 mnd gewerkt in de huisartsen praktijk van M.W. Kraaijenhagen, huisarts-opleider te Reeuwijk. Van 1984 tot 1990 werd hij opgeleid tot algemeen chirurg in het Sint Clara Ziekenhuis te Rotterdam door de opleiders A.A. van Puyvelde (tot 1985) en Dr. T.I. Yo. Tijdens de opleiding werd gedurende 6 maanden experimenteel chirurgisch werk gedaan in het Laboratorium voor Chirurgie van de Erasmus Universiteit te Rotterdam onder leiding van Dr. W.J. Kort. Na zijn inschrijving in het specialisten register in 1990 is hij werkzaam als algemeen chirurg in het Ziekenhuis St. Jansdal te Harderwijk.