Collagenase inhibition in the healing colon[1]

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Summary: A randomized controlled trial was performed to assess the effect of intravenous aprotinin (Trasylol) on the healing of experimental colonic anastomoses in the rabbit following a standard left colonic resection anastomosis. Assessment of tensile strength was by means of both bursting pressure and breaking strength. Those animals subjected to bursting pressure assessment received intravenous aprotinin 80 000 KIU (kallikrein inhibitory units) at the time of anaesthesia, and postoperatively 160 000 KIU per day given in divided doses for three days. Control animals received saline placebo. A further group of animals received a lower loading and maintenance aprotinin dose (40 000 KIU and 60 000 KIU per day respectively) with control animals receiving saline. Breaking strength was employed as the means of assessment.

The mean bursting pressures were 47.7 ± 2.9 mmHg and 37.5 ± 3.4 mmHg for aprotinin and controls respectively ($P < 0.05$). The mean difference in collagen content of the anastomosis compared to the resected specimen was $+1.25 \pm 0.50$ μg/mg and -1.02 ± 0.47 μg/mg for aprotinin and placebo groups ($P < 0.005$).

The mean breaking strength in the aprotinin group was 169.6 ± 74.5 g and 110.0 ± 65.9 g for the saline group ($P < 0.02$). The mean difference in collagen content of the anastomosis compared to the resected specimen was $+0.95 \pm 0.69$ μg/mg and -1.5 ± 0.78 μg/mg for the aprotinin and saline groups respectively ($P < 0.05$).

The significant elevation of both bursting pressure and breaking strength assessments, with a significant improvement in the collagen content of the anastomoses, may be the result of collagenase inhibition following the use of intravenous aprotinin in the experimental model.

Introduction

The reported incidence of colonic anastomotic leakage ranges from 0 to 30% in recent literature (Matheson & Irving 1975, Eykyn *et al.* 1979) with individual surgeon variation being between 0.5 and 30% (Fielding *et al.* 1980).

Many factors have been implicated in the aetiology of anastomotic leakage. Sound surgical technique with a tension-free anastomosis and a good blood supply is of prime importance. Other factors can be conveniently divided into those of local and general importance. The presence of a locally active enzyme, collagenase, was first demonstrated by Hawley *et al.* (1970) in rabbit colon. A significant increase in the level of this enzyme occurred in experimental colonic resection and anastomosis in rabbits, and was associated with a decrease in bursting wall tension and colonic collagen.

If this enzyme could be inhibited, a reduction of the intense collagenolysis which takes place in the early phase of healing of an anastomosis may result. A number of proteolytic enzyme inhibitors of latent collagenase are known; these include soya bean trypsin inhibitor, lima bean trypsin inhibitor, nitrophenyl guanidinobenzoate, and diisopropyl phosphofluoridate. Complete inhibition of collagenase with the commercially available enzyme inhibitor aprotinin (Trasylol) has been demonstrated (Latner *et al.* 1973).

The present study was designed to investigate the effect of intravenous aprotinin on the

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healing of experimental colonic anastomoses in the rabbit. Anastomotic strength was evaluated by the measurement of bursting pressure and breaking strength. In addition, the collagen content of each anastomosis was measured.

Materials and method

Fifty-eight male white New Zealand rabbits (weight 2–2.5 kg) were employed. After anaesthesia with 0.5 ml alphadolone-alphaxalone (Althesin, Glaxo Ltd), the animals were maintained on a gas-air mixture (1 : 1) with fluothane delivered via a cat facial mask. Animals received randomly either 80 000 KIU aprotinin (Trasylol) intravenously or a similar volume of normal saline. Additional increments of Althesin were administered during the course of the operation to maintain anaesthesia. Each animal was subjected to laparotomy, and a standard left colonic resection performed 8 cm from the peritoneal reflection, resecting a 2 cm portion of colon. This resected specimen was retained for collagen content estimation. An end-to-end anastomosis was constructed using a 6/0 Mersilk single-layer inverting interrupted suture technique employing a standard number of sutures.

Postoperatively the animals received intravenously either a total of 160 000 KIU aprotinin per day in divided doses or similar volumes of normal saline; treatment continued for three days. Animals were sacrificed on the fourth postoperative day. At this time a 5 cm length of colon was removed with the anastomosis in the mid-portion. In the first 40 animals, after careful removal of the retained faecal material, the diameter of the colon at the anastomosis was measured. The colon was subjected to bursting pressure measurement using a standard infusion of 10 ml per minute of water. Measurements were made at a standard time following sacrifice of the animals.

The collagen content of both the resected specimen and the anastomosis was determined by modification of the Sirius Red dye staining technique described by Junqueira *et al.* (1979). The resected specimen and the anastomosis were separately homogenized and 100 µl aliquots of homogenate were placed on cover slips and allowed to dry overnight. Each cover slip was then washed in Xylene, removing lipid which may have been present which would otherwise interfere with the staining technique. Using a picrosirius solution, staining was achieved and excess dye was removed with hydrochloric acid. The dye taken up by the collagen was then eluted using sodium hydroxide and the colour obtained read in a spectrophotometer at 540 nm, against collagen standards.

In a further group of 38 male New Zealand white rabbits a lower dose of aprotinin was utilized and the breaking strength of each anastomosis measured. Collagen content was determined according to the method already described. Animals were allocated randomly and received a lower loading dose of 40 000 KIU aprotinin intravenously or a similar volume of normal saline. The animals were then anaesthetized and subjected to colonic resection and anastomosis as in the first group.

Postoperatively each animal received intravenously either 60 000 KIU aprotinin per day in divided doses or similar volume of normal saline. Treatment continued for three days with sacrifice of the animals on the fourth postoperative day. At sacrifice a 5 cm portion of colon was excised and after carefully removing retained faecal matter the mesentery was dissected free from the colon and the anastomosis subjected to breaking strength estimations using a specially constructed tensiometer. Collagen content of the resected specimen and the anastomosis was estimated using the picrosirius staining technique already described.

Results

The anastomoses subjected to bursting pressure measurements had mean diameters of 10.61 ± 0.39 mm for the aprotinin group animals ($n = 20$) and 10.56 ± 0.41 mm for the saline group ($n = 20$). The mean bursting pressure was 47.7 ± 2.9 mmHg ($n = 20$) compared to 37.0 ± 3.4 mm Hg ($n = 20$) for the aprotinin and saline groups respectively ($P < 0.05$, log transformed t test).

The change in collagen content for each of the treatment groups is shown in Figure 1. With each animal acting as its own control, the mean difference in collagen content of the

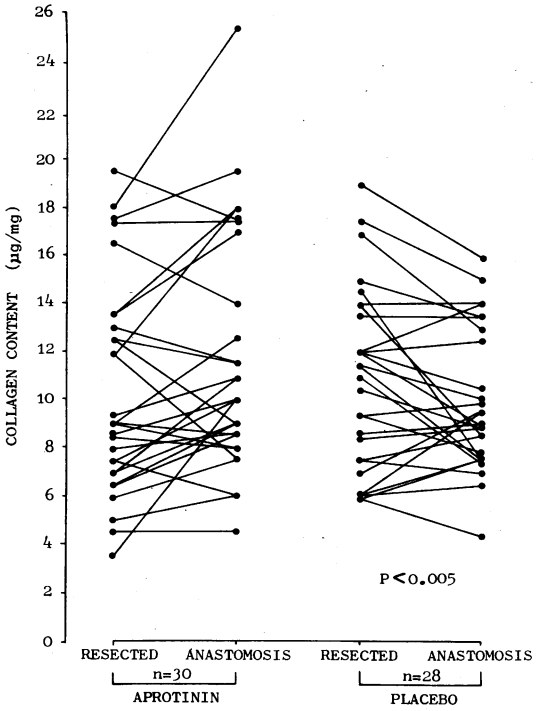

Figure 1. Change in collagen content of anastomosis compared with resected specimen for aprotinin and saline treatment group (aprotinin *v.* saline, $P < 0.005$)

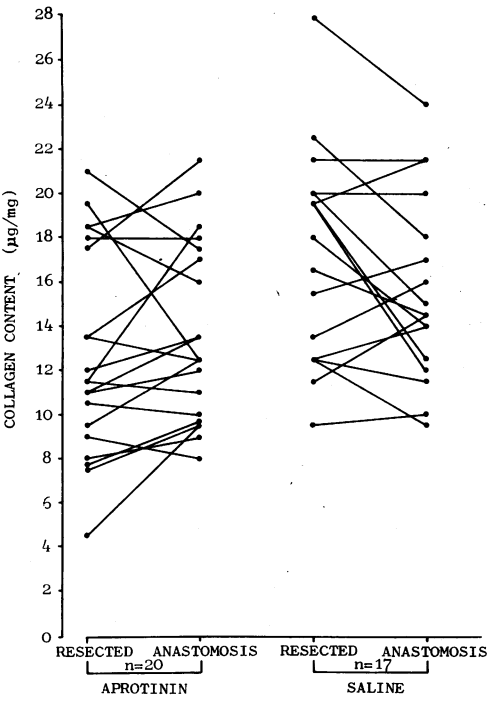

Figure 2. Change in collagen content of anastomosis compared with resected specimen for lower-dose aprotinin and saline treatment groups (aprotinin *v.* saline, $P < 0.05$)

anastomosis compared to the resected specimen was $+1.25 \pm 0.50$ µg/mg for aprotinin treatment animals ($n = 30$) and -1.02 ± 0.47 µg/mg for those receiving saline ($n = 28$) ($P < 0.005$, unpaired t test on the paired differences).

In the second series of experiments, one animal died in the postoperative period from a chest infection, leaving a total of 37 animals in whom results could be obtained. Anastomotic collagen was estimated in 37 animals and colonic anastomotic breaking strength in 33. At the time of removal of the colon and dissection of the mesentery free from the area of the anastomosis, four anastomoses were judged to be damaged by this procedure and, therefore, these were not subjected to tensile strength testing. Sutures were not removed prior to the breaking strength measurement as it was felt that in this early postoperative period such a manoeuvre was likely to damage the anastomosis.

Of the 33 animals subjected to tensile strength measurement the mean (± 1 s.d) breaking strengths expressed in grams were 169.6 ± 74.5 for the aprotinin group ($n = 16$) and 110.0 ± 65.9 for the saline group ($n = 17$) ($P < 0.02$, log transformed t test). Of the 37 animals in whom collagen content was estimated, the change in content between the anastomosis and the resected specimen was calculated (Figure 2). The mean (± 1 s.e. (mean)) change in collagen content was $+0.95 \pm 0.69$ µg/mg for the aprotinin group ($n = 20$), -1.5 ± 0.78 µg/mg for the saline group ($n = 17$) ($P < 0.05$, unpaired t test on the paired differences).

Discussion

Adherence to the principles of good surgical technique helps to ensure that the technical failure of an anastomosis does not occur. However, various factors are known to influence the healing of a colonic anastomosis and these include general factors such as malnutrition (Irvin

1976), protein depletion (Daly *et al.* 1972), trauma (Irvin & Hunt 1974), and blood loss (Whitaker *et al.* 1970). More locally-acting factors which may influence such a disruption include sepsis (Clarke *et al.* 1977), faecal loading (Goligher *et al.* 1970), foreign bodies, especially the use of drains (Manz *et al.* 1970), and possibly the proteolytic enzyme collagenase (Hawley *et al.* 1970). A number of collagenolytic enzymes have been previously identified in human bone (Vaes 1971), skin (Woolley *et al.* 1978), leukocytes (Kruze & Wojtecka 1972) and granulocytes (Lazarus *et al.* 1968). In the colon collagenase was first demonstrated in rabbits by Hawley *et al.* (1970), but has subsequently been identified in both human gastric mucosa and human cadaveric colonic tissue (Woolley *et al.* 1976, Kortman & Von Bary 1977).

A gross simplification of anastomotic healing can be represented by the concept of collagen synthesis occurring simultaneously with collagenolysis, with the final strength of the anastomosis being determined by the balance of these two opposing processes. Experimental work has shown both in the rat and the rabbit that a period of collagenolysis occurs in the early phase of healing and that this predominates until the fourth postoperative day. Collagen synthesis then takes over as the dominant factor, and there is a rapid increase in the tensile strength of an anastomosis. The present study was designed to investigate the possibility that the early collagenolytic phase of healing of a colonic anastomosis might be influenced by the intravenous administration of the proteinase inhibitor, aprotinin (Trasylol). Assessment of the strength of the anastomosis was coupled with assay of the collagen content of the experimental colonic anastomosis.

The tensile strength of anastomoses can be measured by means of bursting pressure and breaking strength. In a comprehensive study on the healing of colonic anastomoses in the rat, Jiborn *et al.* (1978*a, b,* 1980), have been able to show experimentally that breaking strength may more accurately reflect the strength of an anastomosis. They have investigated both bursting pressure and breaking strength and have shown that there was little correlation between bursting pressure and collagen synthesis in a colonic anastomosis, while correlation did exist between breaking strength and collagen synthesis. In the first part of the present study bursting pressure was employed as a means of tensile strength estimations. A significant elevation in bursting pressure occurred after animals had received intravenous aprotinin and this was associated with a mean increase in the collagen content of the anastomosis compared to the resected specimen. However, the dosage of aprotinin used in this first study was high. Therefore a second series of experiments using a lower dose of aprotinin was performed and, in view of the studies of Jiborn *et al.* (1978*a, b*), breaking strength was employed as the means of tensile strength measurement. A significant improvement in the strength of a colonic anastomosis in animals treated with aprotinin occurred with elevation of the breaking strength, associated with an increase in the collagen content of the anastomosis compared to the resected specimen. The improvement in collagen content was less marked than when the higher dose of aprotinin was used.

Aprotinin (Trasylol), produced from bovine lung sources, is a proteinase inhibitor which has been shown *in vitro* to inhibit collagenase. Whilst it has been suggested that it has a relative short half-life *in vivo*, and relatively large amounts of the drug are required in the serum for it to be effective (Cuschieri 1977, Worthington & Cuschieri 1976), more recent pharmacokinetic data suggest that it has two half-lives and can move rapidly from the serum into the extracellular compartment (Kaller *et al.* 1978). Associated with this is its low molecular weight which may enable it to gain access to sites that are inaccessible to the larger circulating inhibitors.

This study has shown that when aprotinin is given intravenously to rabbits in which a left colonic anastomosis has been constructed, there is a significant elevation of the bursting pressure associated with a significant change in the collagen content of the anastomosis compared to the resected specimen. Likewise, improvement in the breaking strength of the anastomosis is also associated with an improvement in the collagen content of the anastomosis compared to the resected specimen when a lower total dosage of aprotinin is employed.

These improvements may be the result of inhibition of the proteolytic enzyme collagenase by aprotinin, which is associated with a consequent reduction of collagenolysis at the anastomosis during the early phase of healing.

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