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RESEARCH ARTICLE

EFFECT OF LUMINAL BUTYRATE INSTILLATION ON COLONIC ANASTOMOTIC STRENGTH IN RATS

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ABSTRACT

Background and Aims: Leakage rates from colonic anastomoses continue to be significant. The effect of luminal butyrate on the healing of colonic anastomoses in the ascending and descending colon was investigated in rats.
Methods: Colonic resection and anastomosis was performed in the proximal and distal colon of adult Wistar rats. Daily enemas of butyrate (40 mM) or saline were administered to case and control rats respectively. Animals were sacrificed on the 6th day and colonic segments of 4 cm including the anastomoses were excised from both the ascending and descending colons. Bursting pressure for the anastomotic line was measured for each colonic segment.
Results: The mean bursting pressure was significantly higher (131.96±20.11 mm Hg, mean±SD) in the group receiving butyrate enemas as compared to the control group receiving saline enemas (91.29±15.91) (P<0.001). The effect of butyrate on bursting wall tension was higher in the proximal colon than in the distal colon.
Conclusion: Butyrate facilitates colonic anastomotic healing and results in significantly stronger anastomoses judged by the bursting pressure. There may be a role for local butyrate instillation in healing of colonic anastomoses.

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INTRODUCTION

Breakdown of colonic anastomosis resulting in partial or complete disruption is a consequence of disturbed healing. Low colonic anastomoses are known to have clinically significant leakage rates of 11%, and radiologically demonstrable leakage rates ranging from 27 to 49%. (Tiret *et al*., 2003; Curley *et al*., 1988) The physiology of colon is different from that of small intestine, because it plays host to a large number of bacteria in the lumen. These bacteria produce a variety of metabolites, some of which such as short chain fatty acids (SCFA) – acetate, propionate and butyrate – influence colonic function significantly. The SCFA, are the major source of energy for the colonic epithelial cells, up to 80% of colonocyte energy requirements being derived from butyrate. (Roediger, 1982) They are also of particular value in the maintenance of mucosal integrity by their role in the stimulation of mucosal growth, differentiation of epithelial cells and repair of epithelial defects (mucosal restitution). (Cook and Sellin, 1998) These effects forms the basis of the therapeutic use of butyrate in the treatment of diversion colitis (resulting from a surgically bypassed colon), acute radiation proctitis, and ulcerative colitis. (Harig *et al*., 1989; Scheppach *et al*., 1992; Vernia *et al*., 2000) SCFA given intravenously have been reported to increase the mechanical strength of an anastomosis in the colon. (Rolandelli *et al*., 1997) In another study, intraluminal SCFA instillation prevented the deleterious effects of ischemia on left colonic anastomotic healing. (Topcu *et al*., 2002) The process of cleansing the colon prior to colonic surgery is expected to deplete the luminal SCFA, which in turn could delay healing of colonic anastomoses.

The effects of SCFA on colonic proliferation and metabolism appear to be more striking in the descending colon as compared to ascending colon. (Cook and Sellin, 1998) The present study was undertaken to determine whether butyrate instilled into the lumen of the colon would increase the mechanical strength of surgical anastomoses in the rat colon, and if so whether there would be differences between proximal and distal colon.

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MATERIALS AND METHODS

Animals and experimental protocol

Twenty three male albino rats of the Wistar strain, of approximately one year age and weighing around 250 gm, were used. The rats were kept in cages accommodating four animals each preoperatively, and caged individually after surgery. To keep the level of SCFA in the colon to a minimum, all the animals received a specially prepared fibre free liquid diet (AIN23)[10] for 48 hours preoperatively. Fibre in the colon serves as a source of carbohydrate by bacterial fermentation, resulting in the formation of short chain fatty acids in the lumen of the colon. Rats were anaesthetized using ketamine 90mg/kg administered intraperitoneally as an initial dose, and supplemented as and when required. A midline laparotomy was performed. The descending colon was transected 3 cm from the peritoneal reflection and the ascending colon 4 cm away from the ileo-caecal junction, preserving the marginal vessels. An end to end anastomosis of the transected ends was then performed, using interrupted 6/0 Prolene sutures. A caecostomy was done to relieve the tension on the suture lines and thus protect the anastomosis. Post-operatively, the rats continued to receive the fibre-free diet till day 6, when they were sacrificed. After surgery, they were randomly assigned to receive 7ml of freshly prepared enemas of either 40 mmol/L butyrate (Na 140, Cl 80, and butyrate 40 mmol/L) or normal saline 140 mmol/L (as control). The solution was instilled through a plastic catheter into the rectum, and was observed to come out of the caecostomy.

On the 6th postoperative day, the animals were subjected to a laparotomy under anaesthesia. The colon was dissected out leaving behind the adhesions around the anastomoses and 4 cm segments each of descending and the ascending colons, with the anastomosis at the center, were excised for studies. The excised segments of the colon were washed and placed in a saline buffer. The animals were then sacrificed by incising the inferior vena cava. The Institutional Animal Ethics Committee approved the experimental protocols and animal use for the study.

Determination of Anastomotic strength

The quality of the anastomoses was measured by recording the saline pressure needed to induce leakage at the anastomoses. One end of the excised colonic segment was connected to an automated infusion syringe pump which injected saline at a constant rate of 2 mL/min, while the other end was connected to an electronic transducer (Hewlett Packard, VIRIDIA 24C). Pressure was recorded and the leakage was detected immediately by the loss of pressure. Bursting pressure (BP) was recorded as the maximum pressure (mm Hg) reached before it dropped suddenly. After the measurement of the bursting pressure, the specimen was opened longitudinally and the circumference of the bowel was measured at the level of the anastomosis. The anastomotic strength was measured as the bursting wall tension (BWT) using BP and anastomotic circumference in accordance with Laplace's law, which states that the pressure inside an inflated elastic container with a curved surface, is inversely proportional to the radius and is expressed as, P = 2T/r. (p = pressure, r = radius, T = tension). Accordingly the BWT was calculated using the following formula: (Rolandelli *et al*., 1997)

BWT = BP x 1.36 x anastomotic circumference / 2

Statistical analysis

All data were expressed as mean ± standard deviation (SD). The significance of difference between the means of two groups was assessed using the unpaired Student t test.

RESULTS

Anastomotic dehiscence

A total of 46 anastomoses were performed on 23 rats. 6 anastomoses (4 controls and 2 butyrate) had evidence of perianastomotic abscess formation indicating dehiscence, and hence were excluded from analysis of bursting pressure.

Bursting pressure and bowel wall tension

As shown in Table 1, the bursting pressures were higher in the butyrate instilled colon than in the controls (P<0.001). The calculated bowel wall tension at which the colon burst was also significantly higher in the colons with butyrate instillation than in the saline treated colons (P<0.001) (Table 1).

Table 1. Bursting pressures (BP) and bowel wall tension (BWT) of colonic anastomoses treated with saline (control) or butyrate instillation. Values shown are mean ± SD

Group	n	Mean BP mmHg	BWT Dynes 10^3/cm
Control	14	91.29 ± 15.91	20.24 ± 3.32
Butyrate	26	131.96 ± 20.11	36.05 ± 5.50

Comparison between bursting pressure in proximal and distal colon

As shown in Table 2, butyrate had similar effects on bursting pressure in proximal and distal colon, increasing bursting pressure significantly in both segments of the colon compared to control saline instillation (P= 0.003 and <0.001 respectively).

Table 2. Bursting pressure of day 6 proximal and distal colon anastomoses. Effect of butyrate instillation compared to instillation of normal saline. Values shown are mean ± SD

Proximal colon			Distal colon		
Control N = 7	Butyrate N = 13	P	Control N = 7	Butyrate N= 13	P
88.57 ±14.96	126.84 ±19.95	0.003	94.00 ±17.54	137.07 ±19.69	0.001

DISCUSSION

Colonic anastomoses, especially the ones following distal colonic resections are frequently prone to leakage, with significant morbidity and mortality (Tiret *et al*., 2003; Curley

et al., 1988) Hence there has been a continuing search to improve the strength and healing of colonic anastomoses. Recent advances in our knowledge of the healing process in colonic anastomoses have led to the development of various experimental therapies, designed to augment the strength of colonic anastomoses. The present study reveals that butyrate instillation into the colon, following colonic anastomosis, resulted in significantly greater strength of the anastomosis, as reflected by an increase of the bursting pressure and of bowel wall tension when compared to control rats. Bursting pressures and bowel wall tension in the ascending colon were comparable to values obtained in the descending colon. This observation has not been made previously and indicates that the strength of the anastomosis is the same in both locations and the healing process occurs in the same time frame. This study did not examine mechanistic aspects of the action of butyrate in enhancing anastomotic strength. However, it is possible to speculate on the possible mechanisms that underlie the action of butyrate. Butyrate is the major energy source for colonocyte metabolism. (Roediger, 1982) In addition, it stimulates colonic mucosal proliferation and epithelial differentiation, (Cook and Sellin, 1998) enhances epithelial restitution in response to superficial injury, (Venkatraman *et al*., 1999) and ameliorates colonic inflammation through inhibition of activation of nuclear factor kappa B. (Kvietys and Gronger, 1981) Collagen deposition at the site of anastomosis is necessary to augment the strength of the anastomosis. Adequate delivery of oxygen to the injured site is necessary for hydroxylation of proline and lysine as well as for the stabilization and cross-linking of collagen fibres, which are both components of the healing process. Butyrate stimulates colonic mucosal blood flow, (te Velde *et al*., 2002) which in turn may augment delivery of oxygen to the injured site and facilitate anastomotic healing. Physiological angiogenesis is important in healing of colonic anastomoses; (Venkatraman *et al*., 2003) whether butyrate stimulates physiological angiogenesis is not known. Butyrate is a short chain fatty acid produced normally in the colon by fermentation of unabsorbed carbohydrate. Early postoperative feeding hastens colonic anastomosis healing, and this is accompanied by increased collagen deposition at the site of anastomosis. (Kiyama *et al*., 2000) It is possible that this can be explained by increased SCFA and butyrate production from the fibre component of the diet that was fed. It has been noted that anastomotic healing is more rapid in animals with a normal intestinal bacterial flora as well as ex-germ free animals compared to germ free animals. (Okada *et al*., 1999) Again it is possible that this may be explained by the need for the presence of intestinal bacteria to ferment fibre to SCFA and butyrate. This study suggests that colonic anastomotic healing may be hastened by luminal butyrate, and we suggest that clinical trials of interventions to increase luminal butyrate after colonic surgery will now be appropriate.

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