The Effect of Butyrate on the Healing of Colonic Anastomoses in Rats

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ABSTRACT

Background: Butyrate, a short-chain fatty acid (SCFA) formed by the fermentation of complex carbohydrates by the bacteria in the colon, is the main source of nutrition for colonocytes. The aim of this experiment was to investigate the effect of butyrate on the healing of colonic anastomosis in a rat model. Materials and Methods: Forty male Wistar rats were fed a fibre-free diet for 2 days. They then underwent laparotomy, transection, and anastomosis of both left and right colon, with a defunctioning caecostomy. The animals were then randomly assigned to receive butyrate or saline enemas from the third postoperative day and underwent another laparotomy on the seventh postoperative day when the bursting pressures of both anastomoses were measured. Results: Out of the 40 rats, 23 were available for the final data analysis. The mechanical strength of the anastomosis was measured by the bursting wall tension (BWT), which was calculated from the bursting pressure and the anastomotic circumference. The anastomoses in the butyrate arm showed a significantly higher BWT for both the right (48.9 ± 64.71 dyne10⁻³/cm, p value .04) and the left (51.44 vs 72.38 dyne10⁻³/cm, p value .01). Conclusion: This experiment suggests that butyrate has a significant role in increasing the mechanical strength of colonic anastomoses in rats.

Keywords: butyrate, healing of colonic anastomoses, short-chain fatty acids

INTRODUCTION

The healing of colonic anastomoses has remained a challenging area for surgeons in spite of many modern advances. Low colonic anastomoses have clinically significant leakage rates of around 16% [1] with radiologically demonstrable ones ranging from 27–49% [2, 3]. The colon has a variety of mainly anaerobic intraluminal bacteria that help in digesting the effluent delivered to the colon, especially the complex sugars contained in dietary fibre leading to the formation of short-chain fatty acids (SCFAs) – butyrate, propionate, and acetate. Butyrate is preferentially metabolized by the colonic mucosa and provides around 80% of colonocyte energy even up to 500 calories per day. The physiological role of butyrate in colonocyte nutrition, epithelial physiology, and disease has encouraged its use in a variety of colonic conditions. In drug-induced Ulcerative Colitis (UC), butyrate has been found to have a cytoprotective effect [4, 5] and has been used experimentally in the treatment of colitis with favorable results [6–9]. Instillation of a solution of SCFAs over 4 to 6 days has demonstrated amelioration of symptoms and healing of colonic epithelium in patients with diversion colitis [10]. It has also been found to be effective in the treatment of radiation proctitis [11]. Intravenous
infusion of butyrate has been found to improve the mechanical strength of colonic anastomoses in rats [12] as has continuous intraluminal infusion of a mixture of SCFAs [13]. Butyrate enemas also strengthened the anastomoses in rats exposed to radiotherapy [14]. The aim of this experiment was to examine the effect of butyrate enemas on the healing of colonic anastomoses in rats. The objective was to measure the bursting wall tension in rats treated or not treated with butyrate enemas.

MATERIALS AND METHODS

Forty male albino rats of the Wistar strain weighing between 200–250 g were fed a specially prepared purified fibre-free diet (AIN-93M) [15] starting 2 days before the initial operation and continuing throughout the experiment. This was to minimize endogenous production of SCFAs in the colon. After 2 days of the diet, the rats were anaesthetized using an ether chamber and intra-peritoneal ketamine (2 mg/kg) and a midline laparotomy was performed under aseptic conditions. The colon was washed out with saline and transected in two places—the right colon 3 cm distal to the ileocaecal junction and the left colon 3 cm proximal to the peritoneal reflection preserving the marginal vessel. An end-to-end anastomosis was then carried out with a single layer of interrupted 6/0 polypropylene sutures. A diversion caecostomy was made to prevent local production of butyrate, and the abdomen was closed in layers. The rats were injected with 20 ml of saline (140 mmol) subcutaneously for the day of the operation and started on oral fibre-free diet from the second day. No analgesics were given after surgery since signs associated with pain were not present. No antibiotics were used.

The rats were then randomly assigned into two groups— butyrate and control. The rats in the butyrate group were given enemas with 10 ml of a solution containing 80 mmol of sodium butyrate and 60 mmol of sodium chloride. The rats in the saline group were given 10 ml of 140 ml sodium chloride enemas. The enemas were started on day 3. On the seventh postoperative day, the rats were anaesthetized again (in a similar manner as indicated above) and the bursting pressures of the two anastomoses were measured in vivo without disturbing the adhesions using an electronic transducer (Hewlett Packard, 78353B). The colon was transected on either side of the anastomosis, and saline was infused at a constant rate into the lumen from one end. At the other end of the anastomosis, another tube led to the transducer, which showed the pressure curve. At the point at which the colon burst, there was a sudden drop in the pressure and the highest pressure was taken as the bursting pressure. The colon was then divided longitudinally and the anastomotic circumference measured. The rats were then sacrificed by transecting the inferior vena cava. While under ether/ketamine anaesthesia. The experiment was cleared by the University Animal Ethics Committee.

Experimental anastomoses can be tested in two ways. One method is to identify leakage or breakdown by radiological methods [16]. The other method is to test the mechanical strength of the anastomosis. This can be done by longitudinal stretch force or increasing the intraluminal pressure, which has been found to be a better reflection of physiological strain [17, 18]. It has been shown that the Bursting Wall Tension (BWT) best represents the force responsible for the loss of anastomotic continuity. This was calculated from Laplace’s law using the Bursting Pressure (BP) and the anastomotic circumference (AC). The derived formula is

$$\text{BWT} = \frac{\text{BP} \times 1.36 \times \text{anastomotic circumference}}{2\pi}$$

The data were analyzed using the SPSS software.

RESULTS

Of the 40 rats used in the experiment, six died immediately following anaesthesia or in the immediate postoperative period. Three animals died during the course of the experiment. There were no demonstrated anastomotic leaks in any of these animals. Thirty-one animals survived till the seventh postoperative day of which eight animals were used to standardize the bursting pressure measurement. Data from 23 animals was available for analysis—12 in the saline arm and 11 in the butyrate arm.

The bursting pressures were higher in the butyrate than the saline group in the right colon (168.54 vs 150.5; p value .03) and the left colon (188.62 vs 168.54; p value .06). The anastomotic circumference was also different in the butyrate and saline arms (right – 1.64 vs 1.37; p value .03 and left –1.68 vs 1.47; p value .27). The bursting wall tension was different in both the groups (64.71 vs 51.44 dyne 10^{-3}/cm, p value .01 for the right and 72.38 vs 51.44 dyne 10^{-3}/cm, p value .01 for the left).

DISCUSSION

Butyrate plays an important role in normal colonic epithelial integrity and nutrition as well as inflammatory and healing processes in the colon. There is a growing body of evidence of its therapeutic effect in the healing of inflammatory bowel disease. There are a number of factors that play a role in the healing of colonic anastomoses. Intraluminal contents have a significant part to
play as suggested by the pathophysiology of diversion colitis.

The rat model has been widely used in the study of colon carcinoma and has been suggested for utilization in studies as it applies to the human situation [19]. Research has shown that anastomoses have lower leak rates when not diverted [20]. Previous studies had looked at the role of butyrate in only left colon anastomoses. This study indicates that intraluminal butyrate increases the mechanical strength of anastomoses in both the right and left colon. Similar to our findings, other authors have also reported that anastomoses rupture adjacent to the suture line in a majority of cases [21]. This is possibly due to the inflammatory phase of healing extending in a small zone on either side of the anastomosis. In this zone, there is no mechanical support by the suture material, possibly causing a relative weakness. Another site of weakness may be the entry point of the suture material on either side of the anastomosis.

The main limitation of this study was the low numbers. Due to initial difficulties in standardizing the experimental and measurement protocols, a number of the animals died or were sacrificed for standardization of measurement and could not be used for data analysis. However, on analyzing the 23 remaining animals, there was a significant difference in the two arms consistent to both left and right colonic anastomoses. We feel this is clear evidence that butyrate does provide mechanical strength to colonic anastomoses in rats. The follow-up period of 7 days was settled upon based on clinical experience in humans that it is around this time that most anastomotic leaks occur following operation.

The next challenge is to reproduce these findings in humans. There is much scope for studies in this regard with butyrate being cheap and easily available. As anastomotic leak is a dreaded complication in colonic surgery, it will be of great interest to colonic surgeons to see if these findings can be duplicated in human anastomoses. With the healing of colonic anastomoses still proving to be an unfinished chapter, “... the ideal method of uniting intestinal wounds,” to put it in the words of Nicholas Senn (1844–1908), president of the Association of Military Surgeons of America, “has yet to be devised.”[22]

CONCLUSIONS

The main conclusion of this study is that intraluminal butyrate increases the mechanical strength of anastomoses in both the right and left colon in rats as measured by the bursting wall tension. It also increases the bursting pressure and the anastomotic circumference. There is a difference in the mechanical strength of anastomoses in the right and left colon. This opens up exciting possibilities for the use of butyrate in lowering the morbidity of human colonic anastomoses, which are still a challenge to the colorectal surgeon.

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REFERENCES


