Intravenous Butyrate and Healing of Colonic Anastomoses in the Rat

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PURPOSE: Intracolonic infusions of short chain fatty acids promote healing of colonic anastomoses. Because the intravenous route may have wider clinical application, we studied the effect of intravenous n-butyrate on the mechanical strength of colonic anastomoses in the rat. METHODS: After placement of an indwelling intravenous catheter, the descending colon was transected and an anastomosis was performed. Rats were then randomized to receive total parenteral nutrition (TPN group; n = 15) or total parenteral nutrition plus 130 mM/l of n-butyrate (TPN+BUT group; n = 13). On the fifth postoperative day, bursting pressure and bowel wall tension of the anastomoses were measured *in situ*. Anastomotic tissues were analyzed for hydroxyproline. RESULTS: The TPN+BUT group had a significantly higher bursting pressure (107.5 ± 30.3 *vs.* 83 ± 41.0 mmHg; $P = 0.04$) and bowel wall tension (20.7 ± 7.6 *vs.* 14.1 ± 9.9 Newton; $P = 0.03$). Tissue hydroxyproline was not different between the two groups (TPN, 45.8 ± 9.2, and TPN+BUT, 47.9 ± 2.9 μg/mg tissue nitrogen). CONCLUSIONS: We conclude that intravenous butyrate improves mechanical strength of a colonic anastomosis without a detectable change in total collagen content. [Key words: Total parenteral nutrition; n-Butyrate; Short chain fatty acids; Colonic healing]

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Higher incidence of anastomotic disruption in the colon, compared with either gastric or small-bowel anastomoses, is usually attributed to the higher bacterial content and the bulk of its contents.[1] Therefore, patients undergoing surgery receive bowel preparation to reduce bacterial counts and bulk in the colon. However, colonic bacteria are essential for the mucosa because they produce short chain fatty acids: n-butyrate, acetate, and propionate.[2] Deprivation of the colon from short chain fatty acids results in diversion colitis, which can be reversed by intraluminal infusion of short chain fatty acids.[3]

In a rat model of colonic anastomosis, we have demonstrated that the provision of intracolonic short chain fatty acids, including n-butyrate, acetate, and propionate, significantly improved healing of colonic anastomoses.[4] Intracolonic infusions require placement of a catheter into the colon, which adds a potential source for complications. Acetate is normally present in the blood stream and administered in parenteral solutions. Propionate does not appear to exert any direct effect on the colon. n-Butyrate, however, is not present in the bloodstream but is used as the preferred fuel for colonic mucosa.[5] Therefore, we decided to administer intravenous n-butyrate to rats undergoing colonic anastomosis.

METHODS

Twenty-eight male Sprague-Dawley rats (250–275 g) were housed in individual metabolic cages and received a fiber-free liquid diet (VIVONEX® T•E•N, Norwich Eaton, Norwich, NJ) for 48 hours to reduce the effect of residual fiber in the colon. Rats were kept in metabolic cages that have a wired bottom to prevent coprophagy. The surgical procedure was described previously.[4] Briefly, each rat was anesthetized with ketamine (90 mg/kg intraperitoneally) and xylazine (1 mg/kg intraperitoneally) and had a catheter placed in the superior vena cava *via* the right internal jugular vein. The catheter exits in the interscapular area through a steel spring connected to a swivel apparatus. Then a laparotomy is performed, and descending colon is transected, preserving the marginal vessels. An anastomosis is then constructed with interrupted 7-0 silk sutures. Postoperatively, rats were randomly assigned to receive total parenteral nutrition (TPN group; n = 15) or total parenteral nutrition with n-butyrate (TPN+BUT group; n = 13). Calories and sodium were made equivalent for both groups by modifying the concentration of glucose and sodium chloride, as shown in Table 1.

Solutions were prepared on a daily basis. n-Butyrate was added through a 0.22-micron filter as the sodium salt (Sigma, St. Louis, MO). Sixty milliliters of diet was infused daily to provide 1.4 g of nitrogen/kg/day and 230 nonprotein kcal/kg/day. All animals were offered water ad libitum but restricted of any other oral intake. On postoperative day 5, each rat was anesthetized and underwent laparotomy. A hemostatic clip was placed across the bowel 2.5 cm

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proximal to the anastomosis, and no attempt was made to dissect the anastomosis. A pursestring suture was placed around the anus with 2-0 silk to hold a pressure catheter introduced through the anal canal. The catheter was then connected simultaneously to an infusion pump and a pressure transducer. The transduced pressure signal was digitalized and entered into a personal computer using analytic software (Notebook Labtech, Wilmington, MA). The Notebook program includes a graphic interface that allows post-hoc analysis of the pressure curve. Rate of rise of intraluminal pressure and area under the pressure curve were calculated (Fig. 1). The anastomosis was then excised, and the circumference was measured. By applying Laplace's law bowel wall tension was calculated as described previously.[4] The colon was then transected 5 mm proximal and 5 mm distal to the suture line, resulting in a 1-cm-long strip of anastomotic tissue. This strip was divided in three equal parts, and all three were weighed separately (wet weight). One sample was used to determine percent water content by drying it in an oven to constant weight. A second sample was hydrolyzed in concentrated hydrogen chloride and assayed for hydroxyproline,[6] whereas the third sample was frozen and stored. Hydroxyproline was measured by the Prockop method, which involves oxidation of hydroxyproline to pyrrole and then conversion to a relatively specific chromophore with *p*-dimethylaminobenzaldehyde. Absorption of this chromophore is read at 560 λ with a spectrophotometer. The third sample was used to measure nitrogen content of the tissue by Kjeldahl analysis. Hydroxyproline is expressed in micrograms per milligram of tissue nitrogen. Data were analyzed using unpaired Student's *t*-test; a *P* value of less than 0.05 was deemed significant.

Table 1.
Composition of Diet Formulas

	TPN	TPN + BUT
BUT (% calories)	0	6
Sodium butyrate (mM/l)	—	130
Glucose (g/L)	250	233.8
Nitrogen (g/l)*	6.8	6.8
NaCl (mEq/l)	130	0
KCl (mEq/l)	20	20
KPO_4 (mEq/l)	15	15
$CaCl_2$ (mEq/l)	8	8
$MgCl_2$ (mEq/l)	3	3
Vitamins (ml/l)†	0.1	0.1

TPN = total parenteral nutrition; BUT = n-butyrate.
* Aminosyn 8.5% (Abbott Laboratories, North Chicago, IL).
† M.V.I. 9 + 3 (Lymphomed, Melrose Park, IL).

RESULTS

All animals survived the experiment. Both groups had a similar percentage of weight loss during the

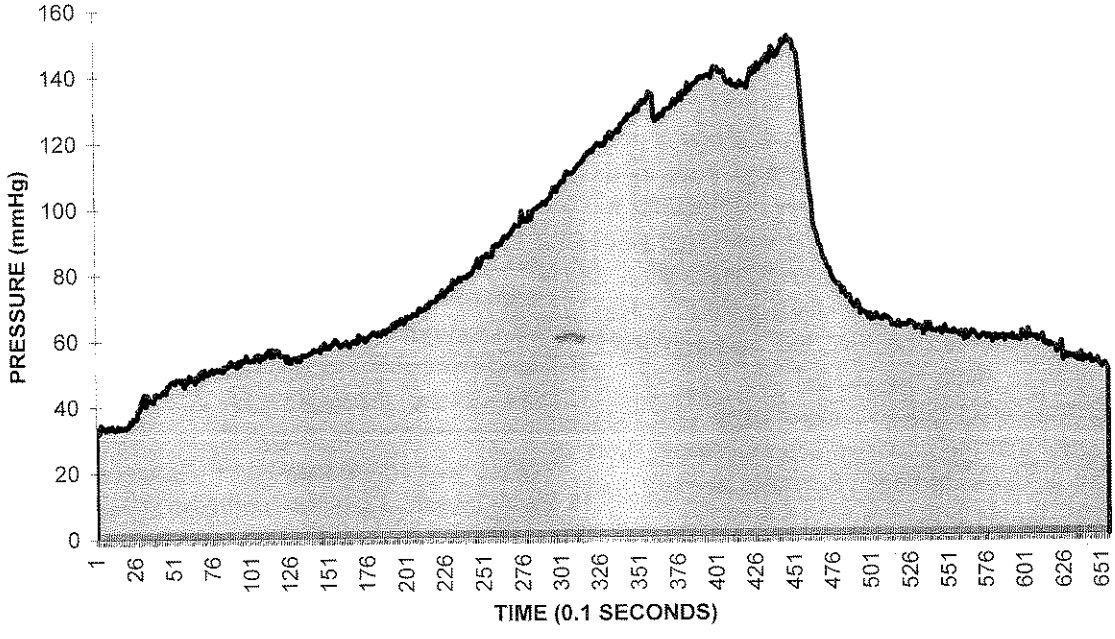

Figure 1. An example of a bursting pressure graph generated by transduced pressure necessary to burst an anastomosis. Rate of rise of intraluminal pressure and area under pressure curve were calculated (data shown in Table 2).

study (8 ± 4 percent). In two animals from the TPN group, the anastomosis was dehiscent, and therefore, no pressure tracing could be obtained. Bursting pressure was significantly higher in the TPN+BUT group than in the TPN group ($P = 0.04$; Table 2). Circumference of the anastomosis was slightly larger in the TPN+BUT group (18 ± 2.4 mm) than in the TPN group (16.8 ± 2.6), but this difference did not reach statistical significance ($P = 0.3$). Bowel wall tension, however, was significantly higher in the TPN+BUT group compared with the TPN group (20.7 ± 7.6 *vs.* 14.1 ± 9.9 Newton; $P = 0.03$). Area under the curve, between the time infusion started and the time of bursting, was calculated using the Labtech notebook software. This area (integral) was higher in the TPN+BUT group but, because of the large variability in the groups, the difference did not reach statistical significance. Rate of pressure rise (slope/integral) was lower in the TPN+BUT group than in the TPN group, but again this difference was not statistically significant.

Concentration of nitrogen was not different between groups, 112 ± 6 and 114 ± 14 mg/g dry weight for the TPN and TPN+BUT groups, respectively. Concentration of hydroxyproline per gram of anastomotic tissue was not different between the two groups: TPN, 45.8 ± 9.2, and TPN+BUT, 47.9 ± 2.9 μg/mg tissue nitrogen.

DISCUSSION

Healing of a wound is reflected by its resistance to mechanical strain. In this animal model, intravenous n-butyrate increases the mechanical strength of a colonic anastomosis as shown by an increased bursting pressure and bowel wall tension. In previous studies, we demonstrated a significant increase in mechanical strength of colonic anastomoses with addition of pectin to an elemental diet[7] and with intraluminal infusion of short chain fatty acids.[4] Luminal stimuli provide a strong trophic stimuli to the intestine.[8] Even luminal infusion of electrolytes enhanced healing of a colonic anastomosis compared with complete diversion,[4] indicating that a mechanical, nonnutritive stimulus promotes healing. Delivery of nutrients into the colonic lumen early in the postoperative period is impossible in patients. Therefore, we decided to investigate the effect of intravenous infusion of short chain fatty acids and, specifically, n-butyrate on colonic healing. By maintaining animals on complete bowel rest while receiving total parenteral nutrition, we observed colonic atrophy and impaired healing, similar to that associated with diverting colostomies.[4, 9] Two animals in this study had subclinical dehiscence of the anastomoses, which we had seen before with diverting colostomies.[4] To avoid an accidental disruption of the anastomosis by surgical dissection, we developed an *in situ* method for measurement of bursting pressure. This method was sufficiently sensitive to detect a difference in mechanical strength in the colons of rats with total parenteral nutrition-induced atrophy. However, by leaving the anastomosis attached to surrounding viscera, the slope and integral of the pressure curve became highly variable, depending on intensity of adhesions.

The concentration of hydroxyproline in a wound has been used as an index of collagen content. In this study and in previous reports, we found no significant difference in hydroxyproline concentration between study groups.[4, 10] Collagen is first degraded in the submucosa around the site of transection and later resynthesized.[11, 12] It has been hypothesized that the impaired healing of colonic anastomoses may relate to an increased collagenase activity rather than a decrease in collagen synthesis.[13, 14] This is substantiated by the fact that luminal bacteria produce collagenases, and that endotoxins derived from bacteria stimulate collagenases. Because n-butyrate is the preferred fuel for epithelial cells of the colon,[15] we postulate that one of its possible mechanisms on colonic healing is a faster epithelization reducing collagenolytic stimuli from the colonic lumen.

Table 2.
Parameters of Anastomotic Healing

	TPN	TPN + BUT
Dehiscence	2	0
Bursting pressure (mmHg)	83.0 ± 41.0	107.5 ± 30.3*
Bowel wall tension (Newton)	14.1 ± 9.9	20.7 ± 7.6*
Integral	11,306 ± 13,319	17,266 ± 18,581
Slope	5.29 ± 2.02	4.6 ± 2.13

TPN = total parenteral nutrition; BUT = n-butyrate.
* $P < 0.05$.

CONCLUSION

We have demonstrated that intravenous n-butyrate, administered in conjunction with total parenteral nutrition during the postoperative period, enhances healing of colonic anastomoses in the rat. Because n-butyrate is trophic to the colonic mucosa, we pos-

tulate that healing may be enhanced by a faster epithelization of the anastomosis with decreased collagenolysis.

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