Anastomotic Healing After Resection of Left-Colon Stenosis: Effect on Collagen Metabolism and Anastomotic Strength

An Experimental Study in the Rat

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Anastomotic breaking strength and collagen metabolism in the colonic wall were studied after resection of a standardized left-colon stenosis in the rat. An increased complication rate was found in the stenosis group compared with the control group (27 percent *vs.* 2 percent) and the complications arise soon after surgery. The collagen turnover in the anastomotic area, as well as the changes of breaking strength, were equal between the groups in the early healing course, implying that the stenosis group, as an entity, did not show impairment in the studied parameters predisposing for complications. Other factors such as mechanical strain by the increased fecal bulk and increased bacterial load may contribute to occurrence of the anastomotic complications. [Key words: Anastomotic healing; Colonic obstruction; Collagen metabolism; Breaking strength]

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THE METHOD OF CHOICE for surgical handling of acute left-colon obstruction is controversial. Most authors recommend staged operations with either a first step of only a diverting colostomy or a resection with an end colostomy.[1-5] Others, however, advocate resection and direct anastomosis.[6-9] In clinical materials, it is difficult to evaluate the advantages and disadvantages of the different treatment modalities because the results are influenced by many factors including age, nutritional status, type of disease, and duration of the stenosis.

In this study, the healing course of an anastomosis in the left colon after resection of a standardized stenosis[10] was examined. The goal was to evaluate the influence of stenosis on the complication rate and to determine if complications could be explained by changes in anastomotic strength and collagen of the bowel wall. Because anastomotic leakage usually occurs within the first week after surgery, the study was limited to the early course of healing.

Materials and Methods

One hundred eighty-four Wistar male rats, weighing 279 ± 12 gm, were used. Ten of the rats were not operated upon and served as a reference group. The remaining 174 animals were allocated to a stenosis group and a nonstenosis control group. The rats were anesthetized with an intraperitoneal injection of chloral hydrate (25 to 30 mg/100 mg body weight) and the abdomen was opened by a low median incision. In the stenosis group, a 5-mm broad ring of a silicon tube with an inner

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TABLE 1. *Number of Animals in Different Groups*

	Stenosis*	Nonstenosis controls*
Day 0	21	23
Day 2	26 (8)	24
Day 4	31 (8)	28 (1)
Day 7	11 (2)	10
TOTAL	68 (18)	62 (1)

*Numbers within brackets represent animals with anastomotic complications.

diameter of 6.5 mm was placed around the colon between two marginal vessels about 2.5 cm above the peritoneal reflection. In the nonstenosis control group, a similar ring with a diameter of 9.5 mm was implanted in the same way. Four days after application of the ring, the ring segment (1 cm) was resected and an inverted single-layer anastomosis was performed using interrupted 6-0 polypropylene sutures (Surgilen®). Groups of animals were killed with an overdose of ether either immediately after construction of the anastomosis (day 0) or at 2, 4, or 7 days postoperatively (Table 1). The body weight of all animals was recorded and the diameter of the colon measured 1 to 2 cm proximal to the ring/anastomosis on the day of ring implantation, on the day of resection, and at sacrifice. At sacrifice, anastomotic dehiscences and perianastomotic abscesses were registered. Animals with these complications were excluded from further analysis. The animals had free access to standard laboratory diet and water during the entire experimental period.

One hundred seven rats were used for collagen determinations. The collagen content of the bowel wall was analyzed in all animals. In 41 of the animals, collagen synthesis was also determined. These animals were given 5.5 MBq L-proline (2-3-4-5-^{3}H-) (New England Nuclear specific activity 3.7-4.4 × 10^6 MBq/mmol) intravenously in the tail 24 hours before sacrifice.

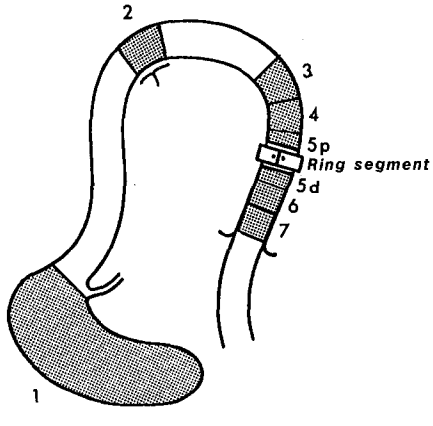

FIG. 1. Sites of stenosis and resected segments.

After sacrifice, the colon was dissected along the mesenteric border. In the animals used for studies of collagen synthesis, nine defined segments from the colonic wall were taken for analysis (Fig. 1). In the remaining animals, only two anastomotic segments (5 mm on each side of the anastomotic line) were taken for analysis of collagen content. Collagen was determined as hydroxyproline (Hypro) according to Stegeman and Stalder[11] and Pikkarainen.[12] The incorporation of ^{3}H-proline into collagen was determined according to Juva and Prockop[13] as applied to colonic healing by Jiborn *et al.*[14] Radioactivity was measured in a liquid scintillation counter (LKB-Wallac 81000). Collagen content was calculated as μg hypro/specimen and collagen synthesis was determined as the specific activity of ^{3}H-hypro (dpm ^{3}H-hypro/μmol hypro).

Eighty-eight animals were used for determination of anastomotic breaking strength (21 of these animals were also used for collagen determination). The animals were killed in groups, immediately after construction of the anastomosis and 2, 4, and 7 days postoperatively. The breaking strength of the anastomosis was tested with sutures in place using a specially constructed tensiometer that provided constantly increasing force. The force at rupture was recorded.

In the *reference group* of ten animals, no ring was implanted, but a resection and a standardized one-layer anastomosis was performed and immediately tested for breaking strength.

Statistical Methods: The chi-square test was used for comparison of anastomotic complications between the two groups. For the remaining statistical analysis, Student's *t* test for unpaired observations was used. Probability levels are represented by * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$.

Results

Weight Development: All animals lost weight after the first operation (ring-application) (Fig. 2). The weight loss was more pronounced in animals with stenosis. The second operation (resection) caused further weight loss initially in both groups. One week after resection both groups had regained pre-experimental weight.

Intestinal Diameter: Four days after ring implantation, the wider ring used in the nonstenosis control group had not caused any significant dilation of the intestine proximal to the ring. Application of the narrow ring (stenosis group) resulted in a significant increase in the intestinal diameter proximally (Fig. 3). After resection of the ring segment, the intestinal diameter successively decreased. Four days after resection the diameter in the stenosis group did not differ from that of the controls.

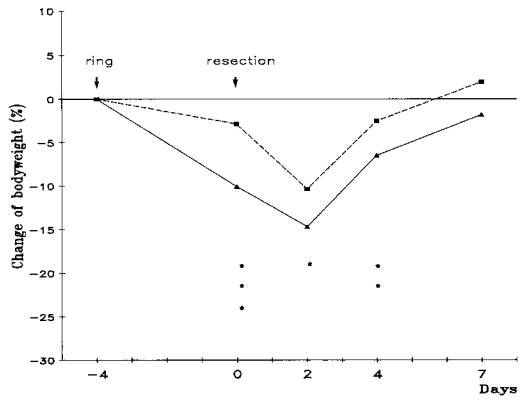

FIG. 2. Change of bodyweight (%) after ring implantation and resection. ▲——▲ represents the stenosis group and ■---■ represents the nonstenosis control group.

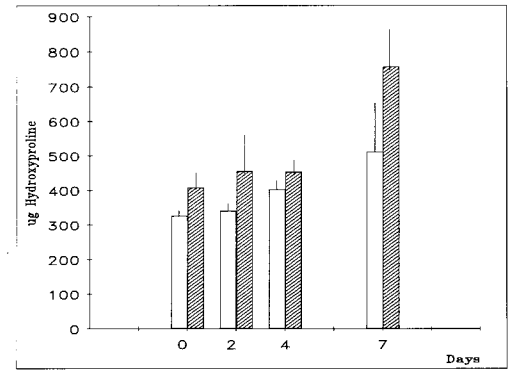

FIG. 4. Collagen content expressed as μg Hypro in the anastomotic segment from day of resection to day seven postoperatively (M±SEM). Hatched bars represent the stenosis group and open bars represent the nonstenosis control group.

Anastomotic Complications: Eighteen of 68 resected animals in the stenosis group had anastomotic complications (27 percent). Five of these animals died. Complications were found on all studied postoperative days. In the nonstenosis control group anastomotic complications occurred in only 1 of 62 resected animals (2 percent). The difference between the groups was highly significant ($P < 0.001$).

Collagen Content: The collagen content in the two anastomotic segments did not differ between the stenosis and nonstenosis control groups on any of the studied days. Because the collagen content was equal in the proximal and distal anastomotic segments, the sum of the values of the two segments has been used (Fig. 4).

Collagen Synthesis: Stenosis for four days resulted in an increase of collagen synthesis proximal to the stenosis (Fig. 5). The increase was two to three times that of the controls in the proximal direction at least up to the major flexure except for the closest proximal segment where the difference was not significant. No difference was found in the cecal segment. In the proximal anastomotic segment the collagen synthesis in the stenosis group was significantly higher than that in the

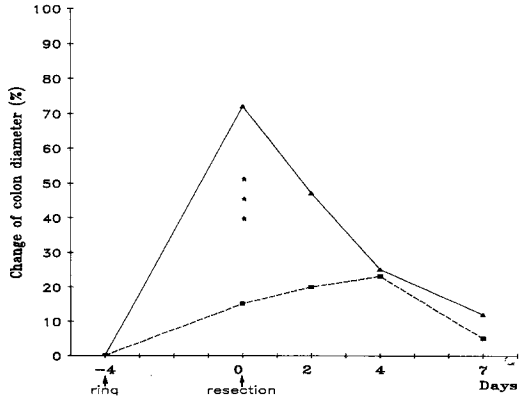

FIG. 3. Change of colon diameter (%) after ring implantation and resection. ▲——▲ represents the stenosis group and ■---■ represents the nonstenosis control group.

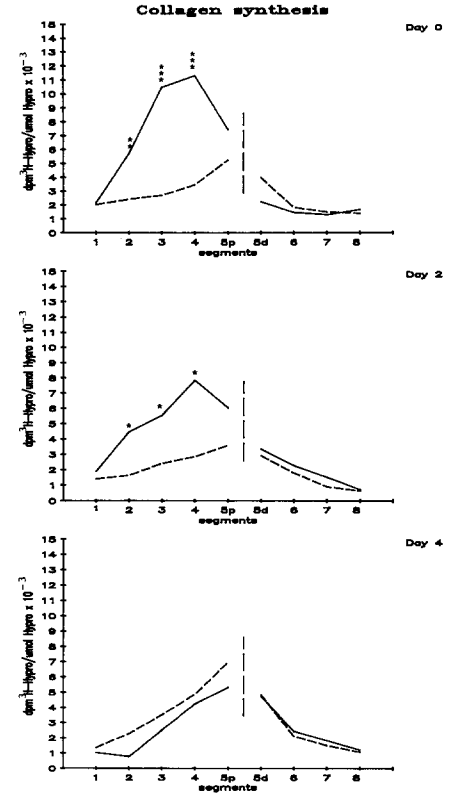

FIG. 5. Collagen synthesis (dpm ^{3}H-hypro/μmol hypro) in different colonic segments (Fig. 1) from day of resection (day 0) to day 4 postoperatively. Vertical broken line denotes ring location and anastomotic line respecatively. ——— represents the stenosis group and ------ represents the nonstenosis control group.

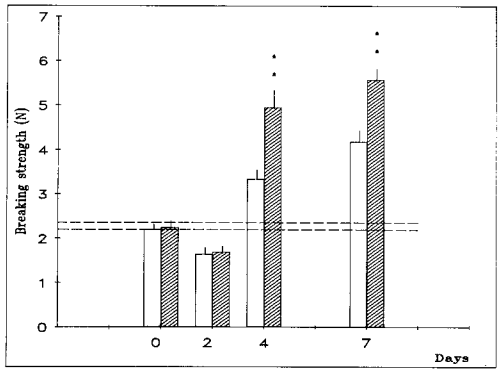

FIG. 6. Anastomotic breaking strength (N) from day of resection to day 7 postoperatively (M ± SEM). The area between the broken horizontal lines represents the strength of the reference group. Hatched bars represent the stenosis group and open bars represent the nonstenosis control group.

distal segment ($P < 0.05$). The distal segments did not differ between the two groups.

Two days after resection of the stenosis the collagen synthesis decreased proximal to the anastomosis but was still significantly higher than in the nonstenosis control group. Four days after resection the stenosis group and the nonstenosis control group had similar patterns of collagen synthesis in the entire colon with an increased activity toward the anastomotic line on both sides of the anastomosis.

Anastomotic Breaking Strength: Four days after ring implantation (day 0) there was no difference in anastomotic breaking strength between the stenosis group and the nonstenosis control group (Fig. 6). Furthermore, the anastomotic strength was the same as in the reference group. A significant and equal decrease of anastomotic strength (25 percent) was found in both groups two days postoperatively. Four days after resection both groups showed increased anastomotic strength compared with that on the day of resection (day 0). In the stenosis group the strength was about 30 percent higher than that of the control group ($P < 0.01$). The difference between the groups remained on the seventh postoperative day.

Discussion

The current study confirms findings in earlier experimental and clinical studies[7,9,15–17] of a significantly higher rate of anastomotic complications after resection of left-colon stenosis compared with resection of nonstenotic controls (27 percent *vs.* 2 percent; $P < 0.001$). Anastomotic complications were observed on all days studied and as many were observed on postoperative days 2 and 4 as on day 7. This implies that the complications arise early, *i.e.*, during the inflammatory phase of healing, at which time the anastomotic strength depends mainly on the sutures and the suture-holding capacity of the bowel wall and only to a minor extent on the newly synthesized collagen. Thus, the mechanical properties of the bowel wall might have been influenced by the stenosis. In previous experimental studies, no attempts[15–17] were made to clarify the cause of anastomotic complications. We have studied the anastomotic area biochemically, by measuring collagen turnover, and mechanically, by determination of anastomotic breaking strength, to evaluate if the stenosis group, as an entity, shows changes predisposing to anastomotic complications. Because suitable methods are not available to study collagen breakdown in the bowel wall, we have measured collagen synthesis and the net amount of collagen to obtain an estimate of the collagen turnover. Anastomotic breaking strength was determined with sutures in place which, in the early healing course, gives a measure of the suture-holding capacity of the bowel wall

On the day of resection, *i.e.*, four days after induction of stenosis, there was no difference in suture-holding capacity between the stenosis and the nonstenosis control groups. Neither were there any significant differences in collagen amount or collagen synthesis in the anastomotic area. Thus, on the day of resection the stenosis group showed no impairment predisposing for development of anastomotic complications. One difference was noted, however, between the two groups. In the stenosis group the collagen synthesis in the proximal anastomotic segment was significantly higher than that of the distal one. As collagen content was equal in both segments, this implies an increased lysis of collagen in the proximal segment after stenosis.

In the early postoperative period, anastomotic breaking strength normally decreases.[18–20] This initial strength reduction was confirmed in the current study. No difference was found between the two groups. The decrease in breaking strength was not accompanied by any changes in collagen amount in either of the groups, which is in accordance with earlier reports.[18,19] As a response to the surgical trauma of resection, collagen synthesis normally increases in the anastomotic area.[21,22] In this study an increase was found in the distal anastomotic segment but no further increase of collagen synthesis was found proximal to the anastomotic line in the stenosis group. On the contrary, the collagen synthesis proximal to the anastomotic segment had decreased from its high level at operation. Four days postoperatively both groups showed an equal pattern of collagen synthesis. Thus, the increased activity of collagen synthesis induced by the stenosis did not add to the response of the surgical trauma of resection, but was compensated instead by relief of the stenosis.

From the fourth postoperative day on, there was a marked increase in breaking strength in both groups, but

the increase was significantly greater in the stenosis group. The increasing breaking strength was not correlated to any significant increase in collagen amount in the anastomotic area. This might be explained by a crude sample technique. Because breaking strength represents the mechanical properties only of tissue hold-by sutures, changes in this narrow zone might be hidden in the wider biopsy taken (5 mm). We have, however, not been able to standardize closer biopsies near the wound gap. The more rapid strength gain in the stenosis group during the phase of fibroplasia (days 4 to 7) probably is related to a greater mechanical strain in the anastomosis provoked by the passage of a greater amount of feces in this group. An indirect support for this statement are the findings in the opposite situation with fecal decompression by a proximal colostomy, which results in a reduced strength gain in the anastomosis.[23] Udén *et al.*[24] have also shown that re-establishment of fecal flow by colostomy closure results in a rapid response with strength gain in left-colon anastomosis. Thus, healing during the phase of fibroplasia seems to be speeded up in the stenosis group compared with controls.

The reason for the increased risk of anastomotic complications must be sought in the early healing course (inflammatory phase). We have not found any deficiencies in suture-holding capacity or collagen amount in the anastomotic area, neither at the time of resection nor during the early healing course, which could explain the greater complication rate. One possible explanation is that the fecal impaction in the stenosis group will cause a higher mechanical load exceeding the critical level of anastomotic strength in some animals. Another explanation for the higher complication rate could be an enhanced local inflammatory reaction elicited by an increased load of bacteria in feces, increased risk of contamination during resection, or increased risk of postoperative leakage due to the difference in diameter of the two bowel ends.

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