Contrasting Effects of Subtotal Enteric Bypass, Enterectomy, and Colectomy on Azoxymethane-induced Intestinal Carcinogenesis

Robin C. N. Williamson, Frederick L. R. Bauer, Onno T. Terpstra, Jeffrey S. Ross, and Ronald A. Malt

ABSTRACT

Compensatory hyperplasia after extensive loss of functioning small or large intestine might predispose to the development of neoplasia in the residual adapted bowel. To test this hypothesis, male Fischer rats were randomized to receive 85 to 90% jejunoileal resection or bypass, subtotal colectomy, or no operation (controls). One week later, the first of six weekly s.c. injections of azoxymethane (15 mg/kg/week) was given. At the 36th week postoperatively, mean body weight after enteric bypass was 62% of control values, and after enterectomy or colectomy it was 78 to 79% of control. Adaptation after all three operations was characterized by 22 to 84% increments in villous height and crypt depth in the residual functioning ileum (p = 0.05 to 0.001); the depth of colonic crypts was unchanged. Fewer rats developed intestinal tumors after enteric bypass (36%) than after any of the other treatments (80 to 91%) (p = 0.01 to 0.001). Compared with controls, bypass reduced the number of colonic tumors by 77% (p < 0.001). Although resection did not affect colonic tumor yield, it tripled the incidence of tumors in the duodenum and jejunum (p = 0.025). Colectomy promoted rectal carcinogenesis (p < 0.05). Anastomotic tumors were commoner after intestinal resection. The lower frequency of tumors after jejunoileal bypass contrasts with enhanced carcinogenesis after enterectomy or colectomy. Profound reduction in body weight may prevent the promotional effect of adaptive hyperplasia.

INTRODUCTION

Enhanced susceptibility of proliferating cells to carcinogenic agents probably explains the increased prevalence of experimental colonic tumors after either proximal or distal enterectomy (25, 35) or pancreaticobiliary diversion to mid-small bowel (37), since all 3 operations produce at least transient colonic hyperplasia (23, 24, 32, 36). Following jejunal bypass as opposed to jejunal resection, hyperplasia of the small bowel remaining in circuit is slightly slower in origin but soon develops to the same degree (10, 11, 13, 20, 22, 36); compensatory hyperplasia in the colon, however, is much less easy to detect (6, 36). Subtotal resection of the colon itself elicits adaptive growth of the ileum (1, 39, 40).

Since compensatory hyperplasia of the residual small bowel (and, by implication, the colon) is proportional to the extent of partial enterectomy performed (16, 32, 33), subtotal removal of functioning small bowel might cause maximal enhancement of colorectal carcinogenesis. Patients losing a large proportion of functioning intestine, for example in the surgical treatment of morbid obesity or inflammatory bowel disease, might therefore be at risk of developing bowel cancer in later years. This possibility was tested in rats exposed to the selective intestinal carcinogen, azoxymethane. In fact, jejunoileal and colonic resections increased the frequency of tumors in adjacent sections of bowel, but jejunoileal bypass inhibited carcinogenesis.

MATERIALS AND METHODS

Male Fischer rats (Charles River Breeding Laboratories, Wilmington, Mass.) were received into the animal quarters 7 to 10 days before operation. Quarters were lighted in 12-hr cycles, and animals were housed in cages with open wire-mesh bottoms. Purina rat chow and water were given ad libitum, except that food was withdrawn overnight following operation.

Rats weighing 126 ± 9.3 g (S.D.) (N = 190) were randomly assigned to 1 of 4 groups. Controls had no operation (Chart 1A). In the other 3 groups, laparotomy was carried out through a midline incision under light ether anesthesia. The small bowel was delivered and measured between the ligament of Treitz and the iliacolum valve by gently stretching 5-cm segments against a ruler. The combined length of jejunum and ileum varied from 85 to 90 cm. In subtotal (85 to 90%) enteric bypass (Chart 1B), the small bowel was divided 5 cm distal to the ligament of Treitz and the distal cut end was closed and invaginated; the proximal transected end of the jejunum was anastomosed end-to-side to the terminal ileum, 5 cm proximal to the ileocalval valve. The operation provided a long (75- to 80-cm) self-emptying blind loop, similar to an end-to-side jejunoileal bypass procedure for obesity in humans (26). Subtotal (85 to 90%) enterectomy (Chart 1C) was carried out by resection of a similar length (75 to 80 cm) of mid-small bowel, leaving 5-cm stumps of jejunum and ileum, which were joined by end-to-end anastomosis. Subtotal colectomy (Chart 1D) required division of the small bowel immediately proximal to the ileocalval valve and of the large bowel at the level of the pelvic brim (rectosigmoid junction); intestinal continuity was then restored by end-to-end ileorectal anastomosis. All anastomoses were performed with 6-0 silk sutures.

Rats in each group received either an aqueous solution of azoxymethane (15 mg/kg/week) or sterile water by 5 weekly s.c. injections, beginning 1 week after operation or control observation. Dilute solutions of azoxymethane (Ash Stevens, Inc., Detroit, Mich.) were stored at -20° until needed and then thawed and further diluted to a concentration of 1 to 2 mg/ml...
Mortality rates in animals with carcinogen or vehicle

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats at outset</th>
<th>Early post-operative deaths</th>
<th>Subsequent deaths (before 27 wk)</th>
<th>Rats surviving (after 30 wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoxymethane Control</td>
<td>26</td>
<td></td>
<td>4</td>
<td>22</td>
</tr>
<tr>
<td>Enteric bypass</td>
<td>32</td>
<td>5</td>
<td>5</td>
<td>22</td>
</tr>
<tr>
<td>Enteric resection</td>
<td>32</td>
<td>8</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Colectomy</td>
<td>30</td>
<td>6</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>Vehicle Control</td>
<td>14</td>
<td></td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Enteric bypass</td>
<td>19</td>
<td>3</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Enteric resection</td>
<td>19</td>
<td>4</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Colectomy</td>
<td>18</td>
<td>4</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

Weight Loss. In control animals, administration of azoxymethane did not prevent a weight gain of about 20 g/week during the first few weeks. Enteric bypass produced a mean 12% loss of total body weight within 48 hr of operation (Chart 2). Thereafter, weight remained approximately static for 7 to 10 days before gradually returning to preoperative levels after 2 to 3 weeks. Body weight after bypass remained substantially lower than in any of the other 3 groups for the remainder of the experiment, though differences from animals with intestinal resection did not reach significance during the course of azoxymethane injections. When sacrificed at 36 weeks, rats with bypass weighed 62% of the amount of control rats and 78 to 80% that of rats with enterectomy or colectomy.

Enterectomy and colectomy were associated with a slightly greater postoperative weight loss (20%) than was enteric bypass, but surviving rats gained weight more rapidly and appeared healthier thereafter. By the end of the experiment, rats with subtotal resection of the small or large intestine weighed 78 to 79% of the amount of unoperated controls. Throughout the experiment, body weights of animals receiving water were...
similar to those receiving azoxymethane, regardless of the experimental procedure.

All 3 operations markedly increased the intake of food and water during the early weeks. Colectomy caused a particularly profuse diarrhea initially, but after a few weeks movements returned to a semisolid consistency.

**Intestinal Adaptation.** The jejunal and ileal segments remaining in circuit after midbowel resection or bypass showed macroscopic hypertrophy, elongation, and dilation. By contrast, the caliber of the long blind loop after bypass showed progressive tapering away from the site of anastomosis. No gross changes in colonic appearance were detected.

Enteric bypass caused slightly greater morphometric changes in the ileum than did either enterectomy or colectomy. Villi were 39 to 84% taller after bypass than those in any of the other 3 groups, and crypts were 22% deeper than those in either controls or rats with colectomy. Although enterectomy and colectomy increased villous heights in the terminal ileum by 24 to 32% of control values, neither operation altered crypt depth at this site (Chart 3). Midileal villous height was not affected by small bowel bypass or large bowel resection, but both procedures caused a slight (16%) increase in crypt depth (Chart 3).

Neither resection nor bypass of the small bowel significantly altered the depth of colonic crypts at 36 weeks as compared with control values.

**Prevalence of Small Bowel Tumors.** Approximately 1 in every 3 control animals had an enteric tumor at autopsy (Table 2). These tumors were scattered through the small bowel, 3 each in the duodenum and terminal ileum and 2 in the upper jejunum (Chart 4A). Neither enteric bypass nor colectomy significantly altered the total number of rats with small bowel tumors; however, enteric bypass did reduce the number of tumors in the duodenojejunal segment ($p < 0.05$). After colectomy, only 2 enteric tumors were found, both in the duodenum (Chart 4D). After bypass, 3 tumors were encountered at the anastomosis and a fourth arose nearby in the distal ileum (Chart 4B).

Following subtotal enterectomy, the number of rats with small bowel tumors (70%) was greater than in any other group (Table 2). Although much of this increase was due to a high yield of anastomatic tumors (Chart 4C), enterectomy also produced many more duodenojejunal tumors per rat (0.85) than either enteric bypass (0), colectomy (0.11), or controls (0.23) (Chart 4). In the remaining small bowel, the increased tumor yield after enteric resection (Chart 4C) made a particularly striking contrast to the decreased tumor yield after enteric bypass (Chart 4B).

**Prevalence of Large Bowel Tumors.** Eighty-six % of control animals had one large bowel tumor or more, as opposed to 27% of animals with enteric bypass (Table 2; $p < 0.001$). All these tumors were distributed throughout the colon and upper rectum, but spared the cecum (Chart 4). Twenty-two control rats produced a total of 30 colorectal tumors (Chart 4A); the same number of rats with small bowel bypass produced only 7 tumors (Chart 4B; $p < 0.001$). Following enteric bypass, the overall number of rats with tumors situated either in the large bowel alone or throughout the intestinal tract was less than one-half that occurring in any other group (Table 2).

Compared with controls, enterectomy altered neither the number nor the distribution of colorectal tumors (Chart 4C). After subtotal colectomy, many tumors arose at the site of ileoileal anastomosis, but in addition the number of rectal tumors was increased 3-fold (Chart 4D). Only after colectomy were tumors encountered in the middle and lower rectum.

**Tumor Histology.** The 3 different histological types of cancer did not correlate with the different geographical regions of the intestinal tract, except that colloid cancer did not affect the rectum. Papillary tumors predominated in controls and in rats with enteric bypass, but after enterectomy and colectomy tubular carcinomas were slightly more common, occurring especially at anastomoses and in the rectum. In control animals, only 13% of tumors were noninvasive; operation increased the proportion of carcinoma in situ to 33 to 45%. There was no correlation between the size of tumor and depth of invasion. All colloid cancers were deeply invasive, but many
Tumors and metastases (N = 22) (N = 22) (N = 20) (N = 18) Intestinal tumors
Small bowel tumors
Large bowel tumors
Anastomotic tumors
Metastases

Table 2
Percentage of rats with intestinal tumors and metastases

<table>
<thead>
<tr>
<th>Tumors and metastases</th>
<th>Control (N = 22)</th>
<th>Enteric Bypass (N = 22)</th>
<th>Enterectomy (N = 20)</th>
<th>Colectomy (N = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal tumors</td>
<td>91</td>
<td>36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80</td>
<td>83</td>
</tr>
<tr>
<td>Small bowel tumors</td>
<td>32</td>
<td>14</td>
<td>70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11</td>
</tr>
<tr>
<td>Large bowel tumors</td>
<td>86</td>
<td>27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>70</td>
<td>83</td>
</tr>
<tr>
<td>Anastomotic tumors</td>
<td>14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>50</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Metastases</td>
<td>23</td>
<td>9</td>
<td>25</td>
<td>17</td>
</tr>
</tbody>
</table>

<sup>a</sup> p < 0.05 versus enterectomy, colectomy; <sup>b</sup> p < 0.01 versus enterectomy, colectomy (χ² test).
<sup>c</sup> p < 0.01 versus enterectomy; <sup>d</sup> p < 0.01 versus enterectomy, colectomy (χ² test).

DISCUSSION

Jejunoileal bypass not only failed to promote carcinogenesis in the residual adapted bowel but actually inhibited the development of intestinal tumors. By contrast, jejunoileal resection trebled the yield of tumors in the duodenum and remaining jejunum, and subtotal colectomy caused a similar increase in the number of rectal tumors. The findings after intestinal resection are consistent with our recent reports that colorectal carcinogenesis can be enhanced by partial enterectomy or pancreaticobiliary diversion (25, 35, 37). Indeed, the present observation that small bowel resection promotes cancer in bowel proximal to the site of resection supports the previous hypothesis (37) that postoperative increments in the number of distal tumors depend on adaptive mucosal hyperplasia rather than on greater excretion of bile acids. Partial enterectomy produces some compensatory growth of the duodenum and jejunum as well as of the ileum (8, 16, 32–34).

The contrasting effects of enteric excision and exclusion on small bowel carcinogenesis can scarcely be attributed to any transient differences in adaptive response (36). Bypass caused slightly greater morphometric increments in the ileum at 36 weeks than did equivalent resection. Despite reflecting gross alterations in mucosal cellularity (36), however, villous height and crypt depth are not very sensitive indices of adaptation (4). The absence of any demonstrable atrophy in the lower part of the blind loop might therefore be due either to the inadequacy of these simple histological measurements or to the fact that ileum is less dependent than jejunum on the presence of luminal nutrients for preservation of mucosal integrity (34, 36). Differential adaptation might perhaps contribute to the respective tumor yields in the colon, since structural adaptation of the large bowel follows partial enterectomy but probably does not follow enteric bypass (23, 31, 36). In the present study the depth of colonic crypts was unchanged 36 weeks after either procedure.

Subtotal small bowel bypass probably protects against cancer by sharply reducing body weight. Since resection and bypass leave the same length of functioning small bowel in papillary and tubular cancers grew copiously into the lumen without invading the muscularis propria.

The number of metastases was not affected by operation (Table 2); secondary deposits were almost always located in the regional lymph nodes, with occasional serosal and pulmonary metastases. No tumors were found outside the intestinal tract. No neoplasms occurred in any rats receiving water, although one granuloma and one inflammatory ulcer were discovered on suture lines.
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Chart 4. Number and distribution of intestinal tumors after operation. Each dot represents a single tumor. Significance (Mann-Whitney U test) as follows. Duodenal tumors: enterectomy \( p = 0.025 \) versus control, \( p < 0.005 \) versus colectomy, \( p < 0.001 \) versus enteric bypass; enteric bypass \( p < 0.05 \) versus control. Total small bowel tumors: enterectomy \( p < 0.001 \) versus control, enteric bypass, colectomy. Large bowel tumors: enteric bypass \( p < 0.001 \) versus control, \( p < 0.002 \) versus enterectomy. Rectal tumors: colectomy \( p < 0.05 \) versus control, enterectomy, \( p = 0.001 \) versus enteric bypass.

...cinoma of the ileum in humans, except in Crohn's disease (7, 19, 21). The tumors at the site of ileal anastomoses probably arose from the adjacent jejunum or rectum, since ileal mucosa appears to possess local protective mechanisms against cancer. No tumors at all were encountered in the excluded intestine after small bowel bypass, although about one-third of Crohn's cancers affect short-circuited loops of intestine (14).

ACKNOWLEDGMENTS

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