Differential Effects of Surgical Suture Materials in 1,2-Dimethylhydrazine-induced Rat Intestinal Neoplasia

Ronald N. Calderisi and Hugh J. Freeman

Department of Medicine (Gastroenterology), University of British Columbia Faculty of Medicine and Cancer Research Center, Vancouver, British Columbia V6T 1W5, Canada

ABSTRACT

The incidence, distribution, size, and histopathology of rat small and large bowel tumors induced by sequential administration of 1,2-dimethylhydrazine followed by cecal placement of one of six differing types of suture materials were systematically examined. In addition, measurements of \(\beta\)-glucuronidase activities in large bowel contents followed by fecal trace metal determinations were done. The results indicate that specific slowly absorbed and nonabsorbable suture materials in the absence of a surgical anastomosis promote tumor induction locally in the rat cecum. In addition, cecal suture material composed of multifilament stainless steel wire enhanced tumor development at a "downstream" site in the distal colon, paralleling increased fecal \(\beta\)-glucuronidase activities at this site and implicating a possible luminaly mediated mechanism for colon tumor development in this animal model.

INTRODUCTION

The use of carcinogen-induced animal models to examine factors that may influence the pathogenesis of gastrointestinal neoplasia is well recognized (1). The effects of various dietary factors, such as dietary fiber (4, 8, 9), as well as the influence of various surgical procedures (17, 26) and chronic irritation induced by nonabsorbable suture materials promoting tumor development locally in the gastrointestinal tract have emerged largely from clinical observation. Increased colon cancer rates are reported in long-standing ulcerative colitis (6), foreign bodies (14, 17). However, no systematic study, to our knowledge, has addressed the specific role, if any, of differing suture materials per se in experimental intestinal neoplasia.

In this paper, the effects of 6 different classes of suture materials were examined in rats initially treated with 1,2-dimethylhydrazine, an agent that induces both small and large bowel tumors. The cecum was selected for suture placement, since this is a relatively resistant site to tumor development in this animal model. The results indicate that specific types of suture materials promote tumor induction locally in the rat cecum, while stainless steel significantly increased tumors at a distal colonic site "downstream" from the cecum.

MATERIALS AND METHODS

Animals and Diets. Weaned male Wistar rats (Simonsen Laboratories, Inc., Gilroy, CA) weighing 140 to 160 g were administered water ad libitum at all times and standard laboratory chow pellets (Ralston Purina Co., St. Louis, MO). Rats were randomly assigned to one of 7 groups using suspended wire mesh cages to reduce coprophagia. Relative humidity and environmental air temperature were constant, and a 12-hr light-dark cycle was imposed in a carcinogen containment animal facility.

Carcinogenesis. All rats were weighed weekly and received 1,2-dimethylhydrazine dihydrochloride (Aldrich Chemical Co., Milwaukee, WI) administered as 12 weekly s.c. 25-mg/kg injections. This was prepared as a 0.5% solution in 1 mM EDTA (Mallinckrodt, Inc., St. Louis, MO) adjusted to pH 6.5 with sodium bicarbonate. Previous studies had established this as an effective regimen in our laboratory for small and large bowel tumor induction (8, 9, 18).

Surgical Procedures. All animals were anesthetized with an i.p. injection of pentobarbital (50 mg/kg body weight) and underwent laparotomy 6 weeks after the last carcinogen injection using the methods of Lambert (11). In 6 of the 7 animal groups, a single 3-0 suture was inserted from serosa through mucosa to serosa, and a triplicate tie was securely applied with care to avoid tissue contraction or injury. All sutures were placed within 1 cm of the cecal tip on the antimesenteric aspect approximating the site of the appendix in humans. Suture groups included: surgical gut (absorbable; Ethicon Chromic; Ethicon Sutures, Ltd., Peterborough, Ontario, Canada); polyglycolic acid (absorbable, braided, synthetic; Dexon "S"; American Cyanamid Co., Pearl River, NY); Poliglactin 910 (absorbable, braided, synthetic; Ethicon Vicryl; Ethicon Sutures, Ltd.); surgical silk (nonabsorbable; Ethicon Silk; Ethicon Sutures, Ltd.); polypropylene (nonabsorbable, monofilament; Ethicon Prolene, Ethicon Sutures, Ltd.); and stainless steel (nonabsorbable, multifilament wire; Flexon; American Cyanamid Co.). Table 1 summarizes composition and properties of the different suture types. The abdominal wall was then closed in all groups in 2 layers with 3-0 Dermabond monofilament polyethylene suture (American Cyanamid Co.).

Necropsy Studies. Four weeks following surgery, animals were administered an overdose of i.p. pentobarbital, and autopsies were carefully performed. The distribution, size, and incidence of small and large bowel tumors were recorded. Each tumor detected was oriented separately on monofilament mesh, placed in separate vials containing modified Bouin's fixative (750 ml aqueous saturated picric acid-250 ml 38% formaldehyde-10 ml glacial acetic acid), embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Fecal Enzymes. Fecal samples from cecum and distal colon in each group of rats were individually collected at the time of the autopsy and processed as described by Reddy et al. (15). \(\beta\)-Glucuronidase activity was quantitated colorimetrically at 550 nm with a Bausch and Lomb Model 710 spectrophotometer (Bausch and Lomb, Inc., Rochester, NY).
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Table 1
Suture materials and properties

<table>
<thead>
<tr>
<th>Suture</th>
<th>Type</th>
<th>Source</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical gut</td>
<td>Chronic</td>
<td>Collagen from animal intestine</td>
<td>Absorption rapid; minimal tissue reaction</td>
</tr>
<tr>
<td>Polyglycolic acid</td>
<td>Braided</td>
<td>Synthetic homopolymer of glycolic acid</td>
<td>Slowly absorbed; minimal tissue reaction</td>
</tr>
<tr>
<td>Polyglactin 910</td>
<td>Braided</td>
<td>Synthetic copolymer of lactide and glycolide</td>
<td>Polyglycolic acid more rapidly absorbed than Polyglactin 910</td>
</tr>
<tr>
<td>Surgical silk</td>
<td>Braided</td>
<td>Raw silk, a natural protein fiber spun by the silkworm</td>
<td>Very slowly absorbed; moderate tissue reaction</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>Monofilament</td>
<td>Polymer of propylene</td>
<td>Nonabsorbable; minimal tissue reaction</td>
</tr>
<tr>
<td>Stainless steel</td>
<td>Multifilament</td>
<td>Low carbon-iron alloy</td>
<td>Nonabsorbable; minimal tissue reaction</td>
</tr>
</tbody>
</table>

* * * * *

Table 2
Tumor frequency in small and large bowel

<table>
<thead>
<tr>
<th>Group*</th>
<th>No. of animals</th>
<th>No. of tumors/animal</th>
<th>No. of tumors/animal</th>
<th>No. of tumors/animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 (100)$^b$</td>
<td>7.2 ± 1.6$^c$</td>
<td>9 (90)</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>Surgical gut</td>
<td>10 (100)</td>
<td>5.6 ± 0.7</td>
<td>8 (80)</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>Polyglycolic acid</td>
<td>10 (100)</td>
<td>6.8 ± 1.5</td>
<td>8 (80)</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>Polyglactin 910</td>
<td>10 (100)</td>
<td>6.4 ± 0.7</td>
<td>10 (100)</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>Surgical silk</td>
<td>10 (100)</td>
<td>6.4 ± 0.6</td>
<td>8 (80)</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>10 (100)</td>
<td>8.7 ± 1.5</td>
<td>8 (80)</td>
<td>2.0 ± 0.6</td>
</tr>
<tr>
<td>Stainless steel</td>
<td>10 (100)</td>
<td>11.8 ± 1.5$^d$</td>
<td>10 (100)</td>
<td>2.4 ± 0.5</td>
</tr>
</tbody>
</table>

* * * * *

RESULTS

Animal Weights. For the 7 animal groups, mean body weights of each group were virtually identical for the duration of the study. During carcinogen administration, no statistical difference in mean body weight or weekly weight gain among the 7 groups was observed. After completion of the carcinogen protocol until sacrifice, no statistical differences were seen.

Necropsy Studies. At sacrifice, suture materials from the surgical silk, polypropylene, and stainless steel animal groups were still present. In the 70 animal autopsies, 527 tumors were detected macroscopically and confirmed as epithelial neoplasia on histological examination. Of these, 120 (23%) were found in small bowel, and 407 (77%), in large bowel. Tumors were sessile plaques or polypoid lesions and ranged in size from 1 to 22 mm in greatest diameter. These findings are similar to those reported (8, 9, 18) for 1,2-dimethylhydrazine-treated rats. Although relative proportions of small intestinal tumors are greater in this experimental model compared to the proportions reported at small and large intestinal sites in humans, their distribution, relative size, and histopathology are similar to those of human intestinal epithelial neoplasia (1).

Tumor Frequency. Table 2 shows the percentage of animals in each group with small and large bowel tumors. For all groups in this study, all animals had intestinal tumors, and over 80% of animals had tumors detected in either small or large bowel sites alone. Among these 7 groups, no statistical differences in percentages of animals with tumors were observed.

Table 2 also shows mean number of small and large bowel tumors detected per rat in each group. Compared with controls, both mean total intestinal tumors and mean total large bowel tumors were significantly increased in the stainless steel group compared to the control group (both, $p < 0.05$). Moreover, the stainless steel group had significantly more intestinal tumors than each of the other suture groups (surgical gut, $p < 0.005$; polyglycolic acid, $p < 0.025$; polyglactin, $p < 0.005$; silk, $p < 0.005$) except for the polypropylene group which failed to achieve statistical significance. In the small bowel, there were no statistical differences among the 7 animal groups. In the large bowel,
the stainless steel group had significantly more tumors than the other suture groups (surgical gut, p < 0.005; polyglycolic acid, p < 0.05; polyglactin, p < 0.005; silk, p < 0.01) except for polypropylene which failed to achieve statistical significance. Polypropylene, although not statistically different from the control group, was significantly greater than the surgical gut (p < 0.05) and polyglactin (p < 0.05) suture groups.

**Subsite Distribution.** Table 3 shows the distribution and frequency of tumors in the large bowel. Except for the surgical gut group where no cecal tumors were observed, at least 50% of the animals in each of the other 5 suture groups had cecal tumors. This compared to a 20% frequency in the control group. The mean number of cecal tumors was greater in each of these suture groups compared to controls, and statistical significance was achieved with polyglactin, silk, and stainless steel wire (all p < 0.05). In the proximal colon, the percentage of animals in each group with tumors at this site was comparable with no difference among the 3 groups were observed. Other trace metals that showed no differences included: aluminum, barium, beryllium, bismuth, cadmium, calcium, cobalt, copper, lead, magnesium, manganese, molybdenum, nickel, phosphorus, strontium, titanium, tungsten, vanadium, and zinc.

<table>
<thead>
<tr>
<th>Subsite</th>
<th>Control</th>
<th>Surgical gut</th>
<th>Polyglycolic acid</th>
<th>Polyglactin 910</th>
<th>Surgical silk</th>
<th>Polypropylene</th>
<th>Stainless steel</th>
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</thead>
<tbody>
<tr>
<td>Cecum No.</td>
<td>48(48)</td>
<td>41(41)</td>
<td>48(48)</td>
<td>46(46)</td>
<td>48(48)</td>
<td>48(48)</td>
<td>94(94)</td>
</tr>
<tr>
<td>No. of tumors</td>
<td>6(6)</td>
<td>6(6)</td>
<td>6(6)</td>
<td>6(6)</td>
<td>6(6)</td>
<td>6(6)</td>
<td>6(6)</td>
</tr>
<tr>
<td>No. of tumors/animal</td>
<td>1.0 ± 0.5</td>
<td>1.0 ± 0.5</td>
<td>1.0 ± 0.5</td>
<td>1.0 ± 0.5</td>
<td>1.0 ± 0.5</td>
<td>1.0 ± 0.5</td>
<td>1.0 ± 0.5</td>
</tr>
</tbody>
</table>

**Proximal colon**

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Surgical gut</th>
<th>Polyglycolic acid</th>
<th>Polyglactin 910</th>
<th>Surgical silk</th>
<th>Polypropylene</th>
<th>Stainless steel</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>6(6)</td>
<td>6(6)</td>
<td>6(6)</td>
<td>6(6)</td>
<td>6(6)</td>
<td>6(6)</td>
<td>6(6)</td>
</tr>
<tr>
<td>No. of tumors</td>
<td>17(17)</td>
<td>16(16)</td>
<td>12(12)</td>
<td>10(10)</td>
<td>14(14)</td>
<td>10(10)</td>
<td>20(20)</td>
</tr>
<tr>
<td>No. of tumors/animal</td>
<td>1.7 ± 0.5</td>
<td>1.0 ± 0.5</td>
<td>1.0 ± 0.5</td>
<td>1.0 ± 0.5</td>
<td>1.4 ± 0.5</td>
<td>1.0 ± 0.5</td>
<td>2.0 ± 0.5</td>
</tr>
</tbody>
</table>

**Distal colon**

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Surgical gut</th>
<th>Polyglycolic acid</th>
<th>Polyglactin 910</th>
<th>Surgical silk</th>
<th>Polypropylene</th>
<th>Stainless steel</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>9(9)</td>
<td>9(9)</td>
<td>9(9)</td>
<td>9(9)</td>
<td>9(9)</td>
<td>9(9)</td>
<td>9(9)</td>
</tr>
<tr>
<td>No. of tumors</td>
<td>36(36)</td>
<td>37(37)</td>
<td>36(36)</td>
<td>30(30)</td>
<td>26(26)</td>
<td>28(28)</td>
<td>66(66)</td>
</tr>
<tr>
<td>No. of tumors/animal</td>
<td>3.6 ± 0.9</td>
<td>3.7 ± 0.9</td>
<td>3.6 ± 0.9</td>
<td>3.1 ± 0.7</td>
<td>2.6 ± 0.4</td>
<td>4.2 ± 0.6</td>
<td>6.6 ± 1.9</td>
</tr>
</tbody>
</table>

**DISCUSSION**

This study shows that suture materials can promote the development of large bowel tumors in a chemically induced animal model of intestinal neoplasia. Tumor enhancement was observed locally at the site of suture placement in rat cecum with both slowly absorbed and nonabsorbable suture materials as well as further “downstream” in the distal colon with stainless steel multifilament wire. In the cecum, nonabsorbable suture materials caused increased numbers of tumors locally, and that was statistically significant for both surgical silk and stainless steel wire. In addition, similar results were observed with a slowly absorbed suture material, polyglactin 910, a synthetic copolymer of glycolic acid and lactic acid.
and lactic acids. These results are consistent with an earlier study (14), showing increased numbers of cecal tumors in dimethylnitrosamine-treated male albino rats following creation of a cecal diverticulum with a purse-string synthetic thread suture. However, in that paper (14), severe necrosis of the cecal wall in the region of the diverticulum was seen in association with a marked peridiverticular inflammatory response, a situation avoided in the present study. In a paper by Williamson et al. (26), small and large bowel anastomoses were created in azoxymethane-treated rats with silk sutures. Increased numbers of suture line tumors were reported. In the present study using a different carcinogen, increased numbers of tumors were seen with silk sutures demonstrating an effect independently of intestinal transection and subsequent anastomoses. Thus, the results in this paper are consistent with previous studies, but they indicate (on the basis of a systematic examination of several specific classes of suture materials) that the presence of a foreign body per se in the form of a surgical ligature enhances tumor development locally independently of surgically created bowel anastomoses.

In the distal colon, significantly increased numbers of tumors were also observed in the group with stainless steel cecal sutures compared to both the control and other suture groups. A systemic effect seems unlikely since increased numbers of tumors were not seen elsewhere in either the small or large bowel (i.e., proximal colon). Rather, some luminal factor may be critical in mediating this foreign body effect in the distal large intestine. Initially, we wondered if this might be due to a metallic component of the suture material, such as iron, being elaborated directly into the lumen. However, subsequent detailed trace metal determinations, including iron, demonstrated no differences. Alternatively, the presence of multifilament wire may produce indirect effects, possibly permitting bacteria to grow quantitatively within the interstices of the wire filaments or leading to qualitative changes in their metabolic activities. Because β-glucuronidase is thought to reflect metabolic activities of bowel microflora and is diet dependent (4, 10, 15, 16), it may play a role in human and experimental colon carcinogenesis (1, 4, 10, 15, 16, 18) and is believed to be an enzyme involved in the final activation of carcinogens in the intestinal lumen (4, 10, 18, 21, 25). In some previous studies (4, 10), increased activity of this enzyme was associated with a higher tumor incidence rate in this animal model. Finally, direct administration of a β-glucuronidase inhibitor led to decreased colon tumor formation in rodents treated with carcinogen (21). In our study, β-glucuronidase activity was increased only in the lumen of distal colon of the stainless steel group. If tumor enhancement bears any direct relationship to luminal microbial metabolism as reflected in this enzyme, the data in this study could imply differential mechanisms for tumor enhancement between cecum and distal colon, particularly for the stainless steel group. It must be emphasized, however, that enzyme studies were done only at the time of autopsy and not prior to or during carcinogen administration. Further studies would be required to determine if increased enzyme activity per se was responsible for increased carcinogen metabolism and resultant enhanced tumor frequency.

While this study indicates that some suture foreign body materials enhance carcinogenesis in this experimental animal model, it would be premature to extrapolate directly to conditions present in human intestine. The surgical procedure in this study bears no direct resemblance to operative methods ordinarily used in gastrointestinal surgery. On the other hand, the presence of luminal metallic foreign bodies resulting from increasingly widespread use of mechanical stapling devices for surgical anastomoses perhaps deserves further evaluation with respect to their potential long-term effects in the pathogenesis of colon carcinogenesis.

REFERENCES


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