Polyethylene Glycol 8000 and Colon Carcinogenesis: Inhibition in the F344 Rat, Promotion in the Min Mouse

Dinaz Naigamwalla, Marie C. Chia, Thien T. Tran, Alan Medline, Kazy Hay, Steven Gallinger, and W. Robert Bruce

Abstract

It has recently been reported that 5% polyethylene glycol 8000 (PEG 8000; M₈₀₀₀) in the diet markedly inhibits the development of colonic tumors in carcinogen-treated rats. To assess the possible use of this agent as a preventive or treatment agent for patients with familial adenomatous polyposis, we determined the effect of PEG 8000 on spontaneous carcinogenesis in the Min mouse. PEG at a 5% concentration in the diet of Min mice did not affect the number of small intestinal or cecal tumors but did increase the number of colon tumors and the number of animals with colonic tumors (2 of 18 versus 12 of 22 animals; P < 0.001). Although the chemopreventive effect of PEG 8000 in rats is remarkable, we suggest a cautious approach in long-term testing of PEG as a chemopreventive agent for subjects at risk for colonic neoplasia.

Introduction

Corpet and Parnaud recently reported that 5% (w/w) PEG₈₀₀₀ (M₈₀₀₀) in the diet markedly inhibits the number and size of ACF, tumor incidence, and tumor multiplicity in F344 rats given the colon carcinogen AOM (1–3). They suggested that because of its low toxicity, PEG might be considered a chemopreventive agent for human colon cancer. The clinical effectiveness of PEG 8000 in colon cancer prevention could be readily assessed in patients with FAP. Before initiating such studies, however, we chose to evaluate the effect of PEG on colon carcinoma in the Min mouse, a strain bearing a mutation in the Apc gene similar to that observed in patients with FAP (4).

Materials and Methods

The 40 three-week-old Min mice used for this study were progeny of crosses of male Apca(+/−)/Msh2(+/−) and female Apca(+/+)Msh2(+/+) mice maintained at the Research Annex at the Samuel Lunenfeld Research Institute, Mount Sinai Hospital (Toronto, Canada) for studies of the combined effect of the Apc gene with a defect in DNA mismatch repair [Msh2(−/−)]. Those with genotype Apca(+/−)/Msh2(+/−) and Apca(+/+)Msh2(+/+) were identified (4) and transferred to microisolator cages and, after removing some of the males for further crosses, randomized by genotype and sex to a control diet (AIN76A; Dyets, Bethlehem, PA) or to the same diet with 5% (w/w) PEG 8000 (Sigma, St. Louis, MO). In our colony, Apca(+/−)/Msh2(+/−) and Apca(+/+)Msh2(+/+) mice are similar with respect to small intestine and colon polyps and rarely develop colonic ACF (4). These two genotypes are also very similar phenotypically to standard Min mice (4). The mice were examined daily for evidence of toxicity until they were 100 days of age, at which time, they were sacrificed by cervical dislocation. Their small intestines, cecums, and colons were removed, cut open longitudinally, stretched flat, fixed in formalin, and coded. The small intestines and cecums were stained with 0.2% methylene blue and examined under a dissecting microscope at ×40 magnification to determine the number of adenomas. The colons were examined in the same way, but because many of the apparent tumors were large lymphoid aggregates, all colonic tumors larger than 0.5 mm in diameter were excised and examined histologically to determine whether they were adenomatous polyps. Because the data for tumor number (and for ACF) were skewed and not normally distributed, they were analyzed after a square-root transformation, and results were given as mean and 95% CI. When differences were detected by ANOVA, Student’s t tests were used and considered significant when two-sided P ≤0.05.

The F344 rats (26 male, 5-week-old rats obtained from Harlan Sprague-Dawley, Inc., Indianapolis, IN) were housed individually in wire-bottomed cages at the Division of Comparative Medicine, University of Toronto (Toronto, Canada). After acclimatization on rodent Chow (Ralston Purina International, Stratthroy, Canada) for 7 days, they were initiated with a single dose of AOM (20 mg/kg body weight, i.p.; Sigma). Seven days later, they were randomized by weight to the AIN-76A diet or to the 5% PEG diet. They were sacrificed approximately 100 days after initiation, and their colons were removed, coded, and assessed for ACF as described previously (5, 6). The colons were flushed clean of feces and other debris with Kreb’s solution, cut longitudinally, fixed flat (mucosal side up) in 10% formalin, and coded for binding. After fixation, they were stained lightly with 0.2% methylene blue and examined at ×40 magnification. ACF were distinguished on the basis of their enlarged crypts, increased pericryptal space, and dark stain and scored for the number of ACF/colon and ACF multiplicity (aberrant crypts/ACF).

The SWR mice (24 male, 6-week-old mice; Harlan Sprague-Dawley, Inc.) were housed in microisolator cages at the Department of Comparative Medicine, University of Toronto. After acclimatization, they were initiated with three doses of AOM (10 mg/kg) given at weekly intervals. One week after the last dose, they were randomized with regard to diets; they were sacrificed at 100 days, and their colons were scored for ACF. Some animals had macroscopic polyps larger than 0.5 mm in diameter that were confirmed to be adenomas by histological examination.

Results and Discussion

PEG 8000 produced no visible evidence of toxicity and did not affect weight gain (data not shown). The average numbers of tumors in the small intestine and cecum were not affected by dietary PEG or by Msh2 genotype or sex (Table 1, columns 4 and 5). By contrast, an initial examination of the data for colon tumors (Table 1, column 7) showed that more animals receiving PEG had adenomatous tumors than control animals (12 of 22 animals versus 2 of 18 animals). Logistic regression using sex, genotype, and diet as predictors supported this impression. It showed that genotype was not significant (P = 0.27), whereas diet (PEG) was very significant (P < 0.001), as was sex (P = 0.002). Because the data for male animals were limited in number and significantly different, a separate analysis for the females was relevant. Again, logistic analysis indicated no interaction between diet and genotype (P = 0.18), a significant effect of diet...
(P < 0.001), and a nonsignificant effect of genotype (P = 0.32). Thus, there was no effect of PEG on the tumorigenesis process in the small bowel or cecum, but there was an increase in the fraction of animals with colon tumors, as well as in the total number of colon tumors/animal (Table 1, column 6) in the PEG-treated female Min mice.

This unexpected result regarding colon tumors in Min mice led us to reexamine the effect of PEG 8000 on AOM-induced ACF in the rat. The results (Fig. 1, top) confirm the striking protective effect of dietary PEG 8000 on ACF observed by Corpet and Parnaud (1, 2). The average number of ACF per colon was reduced from 62.0 to 14.8 (CI = 56.3–68.1 to 10.8–19.4; P < 0.001) per colon. The number of large ACF (greater than 4 aberrant crypts/ACF in size) was reduced from 22.5 to 0.62 (20.8–24.2 to 0.11–1.55; P = 0.001) per colon. The number of ACF/colon did not reach statistical significance (0.42 to 0.21).

The marked difference in response of carcinogen-treated rats and the Min mice to PEG 8000 prompted us to evaluate the effect of PEG 8000 on a mouse strain susceptible to AOM colon carcinogenesis (7).

The results (Fig. 1, bottom) showed no difference between the average number of ACF/colon in the animals receiving PEG 8000 and those on the control diet [2.89 (1.74–4.32) versus 2.46 (1.28–4.04); P = 0.54]. Adenomatous polyps were observed in animals receiving PEG 8000 but not in control diet-fed animals, although their average number per colon did not reach statistical significance (0.42 ± 0.29 to 0; P = 0.21).

PEG 8000 thus inhibits ACF growth in the initiated F344 rat, does not inhibit ACF growth or carcinogenesis in the initiated SWR mouse, and promotes colon carcinogenesis in the Min mouse.

The protective effect in the rat has been attributed to the increased water content of the feces in animals consuming PEG (1, 2). Under the conditions of our study, however, the moisture of freshly collected feces was significantly increased by the consumption of PEG 8000 in each of the models of colon carcinogenesis, ranging from 13.1 ± 2.4% to 21.6 ± 2.2% for the Min mouse, from 25.3 ± 2.9% to 52.2 ± 2.0% for the rat, and from 13.5 ± 2.3% to 32.4 ± 2.3% for the SWR mouse, indicating that a failure to increase fecal water was not responsible for the differences in the effect of PEG. Fiber can reduce glycemic response, inhibit insulin resistance, and affect carcinogenesis (8). We found that dietary PEG, however, had no effect on glucose tolerance as assessed by oral glucose tolerance and no effect on postprandial levels of energy substrates (glucose, triglycerides, and free fatty acids) in F344 rats (data not shown). The protective effect of PEG could be associated with the development of ACF, which are not frequently observed in the Min mouse. However, carcinogenesis in these mice is inhibited by dietary changes that also affect ACF, by reductions in dietary fat (9), by the addition of some fibers (10), and by nonsteroidal anti-inflammatory drugs (11), although the inhibition is not always observed with changes in fat or fiber (12).

Finally, it is possible that the protective effect of PEG is a consequence of its demulcent or soothing effect (13). Compounds such as PEG are known to improve cell growth in tissue culture (14) and to facilitate membrane rescaling after injury (15, 16). High molecular weight PEGs are thought to be confined to the lumen (17), where they might reduce “spontaneous” injury to the surface epithelial barrier in the F344 rat that had received a single treatment with AOM. The colonic mucosal barrier function in the Min mouse or repeatedly carcinogen-treated SWR mouse may be compromised (18–20). In this situation, PEG may not be confined to the surface but may interact with epithelial cells deeper in the crypt or with cells in the lamina propria to promote the carcinogenesis process.

FAP patients harbor small precursor lesions with Apc mutations. It is our opinion that such individuals should not receive PEG over long periods in clinical chemoprevention trials until the effect of this remarkable agent is more completely understood.

Table 1 The effects of 5% dietary PEG (M, 8000) on intestinal tumors in Min mice

<table>
<thead>
<tr>
<th>Diet</th>
<th>Sex</th>
<th>Genotype</th>
<th>Small intestinal tumors</th>
<th>Cecal tumors</th>
<th>Colon tumors</th>
<th>Animals with colon tumors/total animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(95% CI)</td>
<td>(95% CI)</td>
<td>(95% CI)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Male</td>
<td>Msh2 (+/–)</td>
<td>21.3 (1.2–65.9)</td>
<td>0.25 (0.2–2.2)</td>
<td>0.0</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Msh2 (+/–)</td>
<td>7.8 (3.4–14.0)</td>
<td>0.1 (0.006–0.6)</td>
<td>0.09 (0.0–0.9)</td>
<td>0/6</td>
</tr>
<tr>
<td>PEG</td>
<td>Male</td>
<td>Msh2 (+/–)</td>
<td>14.5 (6.8–25.3)</td>
<td>0.02 (0.02–0.2)</td>
<td>0.08 (0.006–0.4)</td>
<td>2/7</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Msh2 (+/–)</td>
<td>8.6 (3.0–17.1)</td>
<td>0.1 (0.006–0.6)</td>
<td>0.2 (0.2–1.9)</td>
<td>1/6</td>
</tr>
</tbody>
</table>

A. Values are the average number of tumors per animal with 95% CI. Within a column, numbers sharing a common letter are not significantly different from each other as determined by analysis of variance (P ≥ 0.05). Numbers not sharing a common letter are significantly different from one another.

B. Logistic analysis shows that the effect of genotype is not significant (P = 0.27), diet is significant (P < 0.001), and so is sex (P < 0.001).
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References

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