SHORT COMMUNICATION

Polyethylene-glycol, a potent suppressor of azoxymethane-induced colonic aberrant crypt foci in rats

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Bulking fibers and high water intake may decrease colon carcinogenesis in rats, and the risk of colorectal cancer in humans. We speculated that a non-fermented polymer, polyethylene-glycol (PEG) 8000, which increases stool moisture, might protect rats against colon carcinogenesis. Thirty female F344 rats were given a single injection of azoxymethane (20 mg/kg), and 7 days later randomized to AIN76 diets containing PEG (to provide 3 g/kg body wt/day), or no PEG (control). Diets were given ad libitum for 105 days, then colon carcinogenesis was assessed by the aberrant crypt foci (ACF) test. ACF were scored blindly by a single observer. Dietary feeding of PEG almost suppressed ACF larger than one crypt, and strikingly decreased the total number of ACF per rat. PEG-fed rats had 100 times less large ACF than controls (0.8 and 83 respectively, P = 0.00001). PEG-fed rats had 20 times less total ACF than control (six and 107 ACF/rat, respectively; P < 0.0001). Two treated rats had no detectable ACF. PEG is 10 times more potent than other chemopreventive agents in this model. Since PEG is generally recognized as safe, its cancer-preventive features could be tested in humans.

Many chemopreventive agents and protective diets have been tested against colorectal cancer in animals. Few of them have been tested also in human clinical trials. However, up to now, no agent was shown to be simultaneously potent enough and safe enough to be recommended on a large scale to people at risk (1–3). Most epidemiological studies suggest that a high intake of vegetables, fruits and cereals, decreases the risk of colorectal cancer, maybe because they contain dietary fibers (4). In rodent studies also, fiber-rich diets often decrease colon carcinogenesis. Wheat bran, a source of fiber that increases the water-holding properties, doubles the fecal weight, but does not inhibit colon carcinogenesis in rats (8). In contrast, rats with high water intake have more moist stools, and smaller preneoplastic lesions in their colons, than rats with normal water intake (9). Recent case-control studies suggest that a high intake of drinking water is associated with a low risk of colon cancer (10,11).

These observations prompted us to speculate that dietary fibers and drinking water might protect people and rodents against colon carcinogenesis, by increasing stool moisture. This hypothesis was tested by feeding rats with a diet supplemented with a non-absorbed and non-fermented polymer that increases the water content of feces, polyethylene-glycol (PEG) 8000. The formula of PEG is H-(O-CH₂-CH₂)ₙ-OH, with n = 8000. The effect of PEG was tested on azoxymethane (AOM)-induced aberrant crypt foci (ACF).

Thirty female F344 rats were obtained from Iffa Credo (Lyon, France) at 4 weeks of age. Rats were housed by pair in stainless steel wire drop-bottom cages. The light cycle consisted of 12 h each of light and dark. The animal room was maintained at 22 ± 2°C. The powdered AIN 76 diet (UAR, Villemoisson, France) and drinking water were provided ad libitum. After 5 days of acclimatization, each rat was given one i.p. AOM injection (20 mg/kg body wt in saline). Seven days later, the rats were randomly allocated to two groups. A control group of 20 rats was given the standard AIN 76 diet. An experimental group of 10 rats was given the AIN 76 diet supplemented with PEG 8000 (ICN, Orsay, France) to provide 3 g/kg body wt/day. We calculated afterwards that the average PEG proportion in diet had been 5%. Body weights, food and water intake, 24 h fecal excretion and humidity, were monitored weekly throughout the study. Since fecal pellets dry after emission, the fecal humidity was also measured twice on pellets obtained directly at the anus (on days 34 and 76 of treatment).

Fifteen weeks after the start of experimental diets, 112 days after the AOM injection, the animals were killed by carbon dioxide asphyxiation. The colons were evaluated for ACF by Bird’s procedure (12). They were excised and flushed with Kreb’s Ringer solution (Sigma, St Quentin, France), then opened longitudinally and fixed flat between coded filter papers in 10% buffered formalin (Sigma). The colons were stained with methylene blue (0.1%) for 6 min, then the mucosal side was observed at 32× magnification. ACF were distinguished from surrounding non-involved crypts by their slit-like opening, increased staining, size and pericryptal zone. The multiplicity (no. of crypts per ACF) was recorded for each ACF in each colon. All colons were scored blindly by a single observer. Moreover, thin sections (4 µm) were obtained from three parts of each colon (proximal, median and distal) from four rats in each group, and stained with hematoxylin–eosin.

Bile acids and cytolytic activity of fecal water were measured as described by Lapre and Vandermeer (13). Fecal water was prepared by adding 0.35 ml of distilled water to 1 g of freeze-dried feces. Samples were incubated for 1 h at 37°C, then centrifuged for 10 min at 40 000 g. The supernatant was removed and stored at –20°C until use. Bile acids in fecal water were determined using a fluorometric enzymatic assay, all reagents coming from Sigma. The reaction mixture consisted of 10 µl of appropriate dilution of fecal water (substrate source), 1.24 ml of 0.1 M Tris buffer (pH 9) and 250 µl of the ‘3-alpha-Flu’ solution, itself containing 67 mM KH₂PO₄, 55 mM NaOH, 4.4 mM sucrose, 1.8 mM NAD, 10 mM

Abbreviations: ACF, aberrant crypt foci; AOM, azoxymethane; PEG, polyethylene-glycol.
PEG 10 3.5 6 6 Na$_4$P$_2$O$_7$·10H$_2$O, 0.13% bovine serum albumin, 714 U/l diaphorase and 107 ± 43 ACF/rat, respectively; $P < 0.0001$ in Welsh’s $t$-test). Two treated rats had no detectable ACF in their colons. PEG-fed rats had, on average, 0.8 ACF larger than 1 crypt, whereas control rats had 83 large ACF per colon (ratio 1:104; $P = 0.00001$ in Mann–Whitney test). The mean crypt multiplicity was halved in PEG-fed rats compared with controls (1.3 ± 0.4 and 2.9 ± 0.4, respectively; $P < 0.0001$ in Student’s $t$-test).

The body weight gains of the rats in the two groups did not differ significantly at any time point. Final body weight was 188 ± 8 g for control rats, and 183 ± 8 g for PEG-fed rats ($P = 0.11$). The daily intake of diets and water was similar in control and treated rats: 10.2 and 10.3 ± 0.7 g/day, respectively, for diet, and 11.8 and 12.2 ± 0.7 ml/day for water. Rats given PEG had watery feces, but clinical signs of toxicity were not observed. The mean left kidney weight was the same in control rats and in PEG-fed rats (0.83 ± 0.03 and 0.82 ± 0.03 g, respectively). Fecal values were strikingly increased in PEG-fed rats ($P < 0.0001$). The enlarged cecum in PEG-fed rats weighed more than three times the control cecum weight ($P < 0.0001$). The large intestinal wall looked thin and regular, almost transparent. Histology showed that PEG did not change the wall and the mucosa thickness. No obvious evidence of inflammation or trauma was seen. The aspect of the crypts was normal in PEG-fed rats, except that goblet-cells with mucus were found all along the entire crypt. This contrasts with crypts in control rats, where the bottom was free of mucus-loaded cells. Stools from PEG-fed rats were moist, and the feces collected during 24 h contained three times more water than control stools ($P < 0.0001$). The humidity of single fecal pellets taken at the anus of rats was 41 ± 2 and 64 ± 2% in control and PEG-fed rats, respectively ($P < 0.0001$). However, fecal pellets were well formed, and we did not observe diarrhoea. Bile acid concentration was halved in feces from PEG-fed rats compared with controls (Table I; $P < 0.0001$), but the daily fecal excretion of bile acid was the same in both groups (4.86 and 4.83 µmol/day). The cytolytic activity of fecal water was three times smaller in PEG-fed rats than in controls ($P < 0.0001$).

Dietary PEG strongly suppressed ACF when administered after carcinogen treatment. Results are so significant that they cannot be due to chance alone ($P$-values are much smaller than 0.0001). Four possibilities may, however, reduce the value of our finding: (i) instead of deleting ACF, PEG might modify their aspect, and interfere with scoring; (ii) PEG might suppress AOM-induced tumors; and (iii) PEG might specifically suppress AOM-induced tumors in rats but not in humans. We have started rodent studies to address the three first points, and we discuss here the relevance

### Table I. Effect of feeding PEG 8000 (3 g/kg body wt/day) on fecal values in F344 rats given an AIN 76 diet

<table>
<thead>
<tr>
<th>Group</th>
<th>$n$</th>
<th>Fecal weight (g/day)</th>
<th>Fecal pellets (no./day)</th>
<th>Water in 24 h feces (%)</th>
<th>Cecum weight (g)</th>
<th>Bile acid (µmol/g fresh feces)</th>
<th>Cytolytic activity (% hemolysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>1.5 ± 0.1$^a$</td>
<td>23 ± 3</td>
<td>13 ± 2</td>
<td>2.1 ± 0.4</td>
<td>3.3 ± 0.9</td>
<td>62 ± 29</td>
</tr>
<tr>
<td>PEG</td>
<td>10</td>
<td>3.5 ± 0.4</td>
<td>29 ± 6</td>
<td>39 ± 5</td>
<td>6.6 ± 0.9</td>
<td>1.4 ± 0.5</td>
<td>20 ± 1</td>
</tr>
<tr>
<td></td>
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<td>$P &lt; 0.0001$$^b$</td>
<td>$P = 0.01$$^b$</td>
<td>$P &lt; 0.0001$$^b$</td>
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<td>$P &lt; 0.0001$$^b$</td>
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$^a$Mean ± SD.

$^b$Welsh’s $t$-test.

**Fig. 1. Effect of feeding PEG on AOM-induced ACF in rat colon.** Bars are means (± SEM) of the number of ACF containing a given number of crypts. Data are shown above bars, because PEG was so potent that black bars are too small to be seen. PEG effect was extremely significant (all $P < 0.0001$).

Na$_2$P$_2$O$_5$·10H$_2$O, 0.13% bovine serum albumin, 714 U/l diaphorase and 0.05 mM resazurin. To this reaction mixture was added 10 µl of 2.5 U/ml 3-α-hydroxysteroid-deshydrogenase (enzyme). After 15 min, the fluorescence was measured at 580 nm under a 565 nm excitation: the intensity was proportional to the bile acid concentration. Cytolytic activity of fecal water was assessed by the lysis of red blood cells. Horse blood (Sanofi, France) was centrifuged for 15 min at 1500 l cells (final hematocrit 5%). In each experiment, blanks without erythrocytes, erythrocytes in double-distilled water (100% lysis), and erythrocytes in 154 mM NaCl (0% lysis) were incubated simultaneously. Samples were incubated at 37°C for 120 min in a shaking water bath, then centrifuged for 1 min at 10 000 g. Supernatant was carefully aspirated. Iron was analyzed with an atomic absorption spectrophotometer (Philips PU 9200). The iron concentration in the supernatant is proportional to red blood cell lysis, and data were calculated relative to the 100% lysis samples.

Most results were analyzed for statistical significance with the test of Welch, a modification of Student’s $t$-test that handles unequal variances. Data are given as means ± SD (except SEM in Figure 1). Two-sided $P$-values $<0.05$ were considered significant.

Dietary feeding of PEG virtually suppressed ACF larger than one crypt, and decreased the total number of ACF per rat strikingly (Figure 1). The mean number of ACF per PEG-fed rat was nearly 20 times less than in control rats (6 ± 6 and 107 ± 43 ACF/rat, respectively; $P < 0.0001$ in Welsh’s
of ACF. ACF are present in carcinogen-treated rodent’s colon, and in human colons with a high risk of cancer (12,14). We have shown previously that dietary constituents that increase the growth of ACF, as assessed by the number of large ACF, also increase the incidence of adenocarcinoma in rats (15,16). In the present study, PEG strongly reduced the number of large ACF, which may be better predictors of cancers than total ACF (15–17). Many compounds that inhibit ACF development have been proved as chemopreventive against colon cancer in rodents (17,18), and may be in humans (19). ACF have thus become accepted intermediate markers, although they do not correlate with tumor endpoint in all studies. The results described here suggest that PEG may have chemopreventive effects on colon carcinogenesis, although a long-term experiment, which is under way in our laboratory, is needed to confirm the present findings.

The mechanism by which PEG 8000 affects colon carcinogenesis is not known. PEG is, according to Wattenberg’s usage, a suppressing agent, because the inhibition takes place during the post-initiation phase of carcinogenesis (20). Therefore, PEG does not inhibit carcinogenesis by modulating the metabolism of the carcinogen. Three speculative mechanisms by which PEG can inhibit ACF could be considered. (i) PEG increased the fecal bulk, partly because it is not absorbed, but mostly because it has water-holding properties. PEG increases the osmotic pressure and stool moisture. Consequently, fecal components were diluted by PEG and by fecal water. The fecal concentration of bile acids, considered promoting agents, and the fecal water toxicity, were reduced by PEG. We do not think, however, that fecal dilution is enough to explain the high protection afforded by PEG. Indeed, some dietary fibers like wheat bran or psyllium can double the fecal bulk or triple the cecum weight. However, their preventive effect on AOM-induced ACF is small (21–23). (ii) PEG is considered as a demulcent that protects the epithelia from irritation and mechanical abrasion. We have proposed that the promotion of colon cancer by thermolyzed casein (16) might be due to the abrasive effect of casein particles (24). The hard fecal pellets of rats may erode the colonic mucosa and promote colonic tumors, maybe by increasing cell proliferation (5,6). In addition, in the development of colon cancer, there is a deficiency of Goblet cells that make mucin. PEG may coat the surface, lubricate the colon and protect the mucosa against mechanical injuries; i.e. PEG could replace the function of the lost mucin. (iii) PEG may act directly on colonic cells by an unknown mechanism.

At least 80 dietary chemopreventive agents have been tested against AOM-induced ACF in rats. The five most active were n-3 fatty acids in perilla oil (25), inulin with *Bifidobacterium longum* (26), wheat bran (21) and piroxicam or aspirin (27,28). Compared with their respective controls, these agents decrease the number of ACF per colon by factors of 3.9, 3.7, 3 and 2.8, respectively. The median potency of the 35 best agents was 1.7. In the present study, PEG decreased the number of ACF by a factor of 18. Auraptene and ursodeoxycholic acid are the most potent agents to reduce the ACF multiplicity. They reduce the multiplicity by a factor of 1.33 (29,30), while PEG reduced it by 2.28. The number of large ACF could be a better predictor than the total number of ACF for colon cancer in rats. The most potent agents to reduce this endpoint are piroxicam and ursodeoxycholic acid that reduced the number of large ACF 4- to 8-fold (27,30), while in the present study PEG reduced this number 83- to 120-fold.

In conclusion, dietary PEG strongly suppressed AOM-induced ACF in rats when included in an AIN-76 diet. PEG might overcome some unknown ‘defect’ in this semi-purified diet. A similar ‘defect’ might exist in the Western diet, which PEG might correct as well. PEG does not appear to belong to any previously recognized class of chemopreventive agents, and is roughly 10 times more potent than other agents in the ACF model. PEG toxicity is very low, and LD50 in rats is >50 g/kg (31). PEG is generally recognized as safe (a GRAS substance), and is allowed in foods and drugs. For instance, 20 g/day of PEG 4000 is used as a common laxative in France. Based on proportion in food or on body area, the protective dose in rats would translate to 20 or 40 g/day in humans. Such treatment may be associated with unpleasant side-effects (e.g. diarrhoea) which may limit the usefulness to this approach in humans. We nevertheless think that, if PEG was shown to lead to a reduction of macroscopic tumors in animals, its preventive features might eventually be tested in humans at high risk for colorectal cancer.

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**References**


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