Role of the Prostaglandin E Receptor Subtype EP₁ in Colon Carcinogenesis

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Abstract

Although the cyclooxygenase pathway of the arachidonic acid cascade has been suggested to play an important role in colon carcinogenesis, the molecular species of prostanoids and receptors involved have not been fully elucidated yet. We examined the development of aberrant crypt foci (ACFs), putative preneoplastic lesions of the colon, in two lines of knockout mice, each deficient in prostaglandin E receptors, EP₁ and EP₃, by treatment with the colon carcinogen, azoxymethane. Formation of ACFs was decreased only in the EP₁-knockout mice to ~60% of the level in wild-type mice. Administration of 250, 500, or 1000 ppm of a novel selective EP₁ antagonist, ONO-8711, in the diet to azoxymethane-treated wild-type mice also resulted in a dose-dependent reduction of ACFs. Moreover, when Min mice, having a nonsense mutation in the adenomatous polyposis coli gene, were given 500 ppm ONO-8711 in the diet, the number of intestinal polyps was significantly reduced to 57% of that in the basal diet group. These results strongly suggest that prostaglandin E₂ contributes to colon carcinogenesis to some extent through its action at the EP₁ receptor. Thus, EP₁ antagonists may be good candidates as chemopreventive agents for colon cancer.

Introduction

Colon cancer is one of the most common malignancies in humans. Epidemiological studies have revealed a significant decrease in the death rates from colon cancer in individuals who have taken aspirin, a NSAID, for prolonged periods (1). Various NSAIDs also inhibit chemically induced colon carcinogenesis in rodents (2, 3), and the NSAID sulindac reduced the number and size of intestinal polyps in patients with familial adenomatous polyposis (4, 5). The common mechanism of action of NSAIDs, putative preneoplastic lesions of the colon (19), in two lines of mice lacking EP₁ or EP₃ receptors for the colon carcinogen, AOM. To confirm these results, a newly developed selective EP₁ antagonist, ONO-8711, was tested for chemopreventive effects on development of AOM-induced ACFs in mice and of intestinal polyps in Min mice containing a nonsense mutation of the Apc gene.

Materials and Methods

Animals. Male C57BL/6J mice were purchased from CLEA Japan (Tokyo, Japan) and male Min mice were purchased from The Jackson Laboratory (Bar Harbor, ME) at 5 weeks of age. The mouse genes encoding EP₁ or EP₃ receptors were disrupted by gene knockout methods using homologous recombination, as reported previously (14). The generated chimeric mice were back-crossed with C57BL/6J mice, and the resulting wild-type and homozygous mutant male mice of these F₂ progeny were used at 7 (EP₁) and 12 (EP₃) weeks of age. Genotypes of these knockout mice were confirmed by PCR according to the method described previously (15). The animals were housed in plastic cages at 24 ± 2 °C and 55% humidity with a 12-h light-dark cycle. Water and basal diet (AIN-76A; Bio-Serv, Frenchtown, NJ) or experimental diets prepared once every 2 weeks were given ad libitum. Body weights were measured weekly.

AOM-induced ACF Development in Prostanoid Receptor-Knockout Mice. EP₁- and EP₃-deficient homozygous mice (EP₁⁻/⁻ and EP₃⁻/⁻), and wild-type mice received AOM (Sigma Chemical Co., St. Louis, MO) at a dose of 10 mg/kg body weight i.p. once a week for 3 weeks. The numbers of knockout mice treated with AOM were 9 for EP₁ and 10 for EP₃, and those of relative to those apparent in the parental animals (9). These observations suggest that COX-2 and, by inference, the prostanoids formed by the action of this isozyme play an important role in colon carcinogenesis. However, whether prostanoids actually contribute to this process and, if so, the identity of the specific prostanoid responsible remain unknown.

The prostanoids PGE₂, PGA₂, PGF₂α, PGF₃α, and TXA₂ exert their biological actions through binding to specific receptors with seven transmembrane domains. These receptors include DP for PGG₂, FP for PGF₂α, IP for PGA₂, TP for TXA₂, and the four subtypes EP₁ to EP₃ for PGE₂ (10, 11). The recent development of mice lacking the genes encoding these receptors (12–15) facilitated the clarification of the roles of prostanoids and receptors involved in the development of colon cancer.

Several reports have demonstrated increased levels of PGE₂ in human colon cancer tissue compared with surrounding normal mucosa (16). Moreover, it has been suggested that PGE₂ inhibits programmed cell death and enhances the tumorigenic potential of colonic epithelial cells (17). Among the four subtype receptors, EP₁ to EP₃ for PGE₂, it was only possible to use EP₁- and EP₃-knockout mice for the experiments because the numbers of EP₂- and EP₄-knockout mice available are very limited because of failure of fertilization or death in the neonatal period (15, 18). In the present study, we therefore examined the development of ACFs, putative preneoplastic lesions of the colon, in two lines of mice lacking EP₁, or EP₃, receptors for PGE₂ (14). The results indicated a requirement for the EP₁ receptor in ACF induction by the colon carcinogen, AOM. To confirm these results, a newly developed selective EP₁ antagonist, ONO-8711, was tested for chemopreventive effects on development of AOM-induced ACFs in mice and of intestinal polyps in Min mice containing a nonsense mutation of the Apc gene.

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wild-type mice in each experiment were 10 and 7, respectively. All mice were sacrificed at 5 weeks after the first dosing of AOM. After laparotomy, the entire colon was resected and filled with 10% neutral buffered formalin and then opened longitudinally from the anus to the cecum. Each colon was then fixed flat between sheets of filter paper in 10% neutral buffered formalin, stained with 0.2% methylene blue in saline, and scored under a light microscope for the number of ACFs per colon and mean number of ACs per focus, according to the procedure of Bird (19).

A Selective EP1 Antagonist, ONO-8711. A selective EP1 antagonist, ONO-8711 [6-[(25,35)-3-(4-chloro-2-methylphenylsulfonylaminomethyl)-bicyclo[2.2.2]octan-2-yl]-5-hexenoic acid} was chemically synthesized at ONO-8711 starting the day before the first treatment of AOM until the mean number of ACs per focus between EP1 deficient mice and their wild-type counterparts (data not shown). The mean body weights of the AOM-treated wild-type mice are shown in Table 1. The number of ACFs per colon in EP1 deficient mice was reduced significantly, by ~40%, relative to that for wild-type animals. The mean number of ACs per focus in EP1 deficient mice did not differ from that in the wild-type mice. In contrast, there were no differences in the number of ACFs per colon and the mean number of ACs per focus between EP1 antagonist-treated mice and their wild-type counterparts. The numbers and sizes of polyps as well as their distribution in the intestine were determined with a stereoscopic microscope.

Table 1 Effect of EP1 receptor deficiency on AOM-induced ACF formation in the mouse colon

<table>
<thead>
<tr>
<th>Mice</th>
<th>Mice with ACFs</th>
<th>ACFs/colon</th>
<th>Mean of ACs/focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>10/10</td>
<td>11.8 ± 1.3</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>EP1−/−</td>
<td>9/9</td>
<td>7.6 ± 1.1</td>
<td>1.6 ± 0.1</td>
</tr>
</tbody>
</table>

* Data are means ± SE.

**P < 0.05 versus wild type.

Results

ACF Development in EP1−/− and EP3−/− Knockout Mice. ACFs were detected in the colons of all mice treated with AOM but not in the colons of animals treated with vehicle (saline). The ACFs were located mostly in the distal colon, with fewer present in the middle colon and rectum. The data on ACFs for EP1−/− mice and the wild-type mice are shown in Table 1. The number of ACFs per colon in EP1−/− mice was reduced significantly, by ~40%, relative to that for wild-type animals. The mean number of ACs per focus in EP1−/− mice did not differ from that in the wild-type mice. In contrast, there were no differences in the number of ACFs per colon and the mean number of ACs per focus between EP1−/− mice and their wild-type counterparts. The numbers and sizes of polyps as well as their distribution in the intestine were determined with a stereoscopic microscope.

The results were considered statistically significant at P < 0.05.

Table 2 Inhibition of AOM-induced ACF development in the colons of C57BL/6J mice by ONO-8711

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intake of ONO-8711 (mg/kg/day)</th>
<th>Mice with ACFs</th>
<th>ACFs/colon</th>
<th>Mean of ACs/focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td></td>
<td>10/10</td>
<td>16.3 ± 1.2</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Mice fed ONO-8711 during post-AOM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ONO-8711 (250 ppm)</td>
<td>37</td>
<td>12.7 ± 1.0 (22)c</td>
<td>1.6 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>ONO-8711 (500 ppm)</td>
<td>71</td>
<td>11.3 ± 1.4 (31)d</td>
<td>1.3 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>ONO-8711 (1000 ppm)</td>
<td>141</td>
<td>10.6 ± 1.2 (35)d</td>
<td>1.3 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Mice fed ONO-8711 post-AOM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ONO-8711 (250 ppm)</td>
<td>35</td>
<td>13.4 ± 1.7</td>
<td>1.4 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>ONO-8711 (500 ppm)</td>
<td>72</td>
<td>12.5 ± 1.7</td>
<td>1.3 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>ONO-8711 (1000 ppm)</td>
<td>137</td>
<td>11.3 ± 0.9 (31)d</td>
<td>1.2 ± 0.0</td>
<td></td>
</tr>
</tbody>
</table>

* Data are means ± SE.

** Numbers in parentheses indicate percentage of inhibition compared with the basal diet group.

***Versus basal diet: **P < 0.05; ***P < 0.01.
8711 did not affect body or organ weight in either the AOM- or vehicle-injected groups. Thus, our pharmacological approach confirmed a role for the EP1 receptor in ACDF development.

**Effect of an EP1 Antagonist on Intestinal Polypl Development in Min Mice.** Most polyps were located in the small intestine, with only a few apparent in the colons of the basal diet and ONO-8711 groups. Administration of ONO-8711 at a dose of 500 ppm in the diet for 7 weeks reduced the total number of polyps to 56% of the value for the basal diet group; the number of polyps in the middle and distal parts of small intestine were reduced to 61 and 54%, respectively, of the basal diet values (Table 3). Furthermore, ONO-8711 reduced the number of polyps in each size group (Fig. 2). Histological analysis revealed no differences in polyps between mice fed the basal diet and those exposed to ONO-8711. Administration of ONO-8711 did not affect body weight, food intake, or behavior of Min mice.

**Discussion**

In the present study, examination of the effects of EP1 and EP3 receptors on AOM-induced ACF formation in mice provided evidence of an involvement of the PGE receptor subtype EP1, but not EP3, in colon carcinogenesis. The involvement of the EP1 receptor in colon carcinogenesis was confirmed using a novel EP1 antagonist, ONO-8711, in terms of ACF development. This antagonist was also effective in decreasing ACF formation, even when administered after AOM treatment. This indicates that the reduction of ACF formation by the antagonist was mediated not at the level of AOM metabolism but rather through postinitiation processes. Moreover, ONO-8711 clearly suppressed intestinal polypl formation in Min mice.

It has been reported that the number of AOM-induced ACFs per colon is reduced by 34–53%, relative to control values, by administration of traditional NSAIDs (sulindac and piroxicam) and COX-2-selective inhibitors (nimesulide and celecoxib) in rodents (21–23). The observed suppression potential is comparable to that of the EP1 selective inhibitors (nimesulide and celecoxib) in rodents (21–23).

In conclusion, the data obtained in the present study strongly suggest that PGE2 mediates carcinogenic changes by acting at the EP1 receptor in the colon. To confirm this involvement of EP1 receptor in colon carcinogenesis experiments with EP1-knockout mice and the EP1 antagonist are being conducted in our laboratory. Moreover, to extend our understanding, cross-breeding of EP1 gene knockout and Apc gene knockout mice, and the expression of EP1 receptor in the colon need to be examined.

**References**

9. Oshima, M., Dinchuk, J. E., Kargman, S. L., Oshima, H., Hancock, B., Kwong, E., Trzaskos, J. M., Evans, J. F., and Taketo, M. M. Suppression of intestinal polyposis by the antagonist was mediated not at the level of AOM metabolism but rather through postinitiation processes. Moreover, ONO-8711 clearly suppressed intestinal polypl formation in Min mice.

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Inhibition of COX-1 by traditional NSAIDs such as indomethacin, sulindac, and piroxicam is accompanied by gastrointestinal side effects that limit the long-term application of these drugs as chemopreventive agents. Such adverse effects may be avoided by drugs that selectively target COX-2. Several types of prostanoids are produced as a result of COX activity in a variety of cells in response to various physiological or pathological stimuli (26). In light of the present results, selective EP1-receptor antagonists may prove particularly beneficial as chemopreventive agents for colon cancer with toxicities even lower than those of COX-2-selective inhibitors.

In conclusion, the data obtained in the present study strongly suggest that PGE2 mediates carcinogenic changes by acting at the EP1 receptor in the colon. To confirm this involvement of EP1 receptor in colon carcinogenesis experiments with EP1-knockout mice and the EP1 antagonist are being conducted in our laboratory. Moreover, to extend our understanding, cross-breeding of EP1 gene knockout and Apc gene knockout mice, and the expression of EP1 receptor in the colon need to be examined.

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