Ligands for Peroxisome Proliferator-activated Receptors α and γ Inhibit Chemically Induced Colitis and Formation of Aberrant Crypt Foci in Rats

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Abstract

The biological role of the peroxisome proliferator-activated receptors (PPARs) in various diseases, including inflammation and cancer, has been highlighted recently. Although PPARγ ligands have been found to inhibit mammary carcinogenesis in rodents, the effects on colon tumorigenesis are controversial. In the present study, three different experiments were conducted to investigate the modifying effects of PPARα and PPARγ ligands (PPARα and PPARγ) on colitis and an early phase of colitis-related colon carcinogenesis in male F344 rats. In the first experiment, gastric gavage of troglitazone (PPARγ ligand, 10 or 100 mg/kg body weight) or bezafibrate (PPARα ligand, 10 or 100 mg/kg body weight) inhibited colitis induced by dextran sodium sulfate (DSS) and lowered trefoil factor-2 content in colon mucosa. In the second experiment, dietary administration (0.01 or 0.05% in diet) of troglitazone and bezafibrate for 4 weeks significantly reduced azoxymethane (AOM, two weekly s.c. injections, 20 mg/kg body weight)-induced formation of aberrant crypt foci, which are precursor lesions for colon carcinoma. In the third experiment, dietary administration (0.01% in diet for 6 weeks) of pioglitazone (PPARγ ligand) troglitazone, and bezafibrate effectively suppressed DSS/AOM-induced ACF. Administration of both ligands significantly reduced cell proliferation activity in colon mucosa exposed to DSS and AOM. Our results suggest that synthetic PPARs ligands (PPARα and PPARγ) can inhibit the early stages of colon tumorigenesis with or without colitis.

Introduction

Patients with long-standing ulcerative colitis are at increased risk of developing colorectal cancer compared with the general population (1). Thus, prevention of colonic malignancy by inhibition of ulcerative colitis is an attractive approach in the overall management of colonic cancer (2, 3).

Acute and chronic inflammatory processes may be important among the factors involved in oxidative events leading to DNA damage in the colorectal epithelium (4). DSS can be used to induce both acute and chronic colonic inflammations, depending on the dose and duration of treatment (5). Long-term administration of DSS induces colorectal cancer (6), whereas short-term exposure results in the formation of ACF (7), which are precursor lesions for colon adenocarcinoma (8–10), and enhances the development colorectal cancer. Therefore, the DSS-induced colitis model is useful for studying colitis-associated colorectal neoplasia (3, 11).

Recently, the physiological function and metabolism of PPARs, which are members of the nuclear hormone receptor superfamily, as well as their role in various nutritional states and various diseases including inflammation and cancer have been highlighted (reviewed in Refs. 12–15). PPARs bind to a specific element in the promoter region of target genes. PPAR and other nuclear hormone receptors bind the promoter only as a heterodimer with the receptor for 9-cis-retinoic acid, retinoid X receptor. They activate transcription in response to binding of the hormone (ligand). Eicosanoids and fatty acids can regulate gene transcription through PPARs. At present, several PPARs have been identified including PPARα, PPARβ (PPARδ, NUC-1, or FAAR), and PPARγ. Some ligands, such as polysaturated fatty acids and probably oxidized fatty acids, are shared by the three isotypes. Several compounds including linoleic acid, phytanic acid, conjugated linolenic acid, 8S-hydroxyeicosatetraenoic acid, and leukotriene B4 bind with high affinity to PPARα. PPARs are expressed in the intestine at various levels (16, 17). The ligands for PPARs are suspected to modify carcinogenesis (15). In colon carcinogenesis, conflicting results between in vivo (18, 19) and in vitro (20) studies have been reported on the action of synthetic PPARγ agonists, troglitazone and rosiglitazone, used for the treatment of type II diabetes (21). In vivo studies of other organs showed that GW7845, a new PPARγ ligand, could inhibit rat mammary carcinogenesis (22). DuBois et al. (23) reported aberrant expression of PPARγ in chemically induced colon carcinoma and human colon cancer cell lines. Similar to PPARα (24), PPARγ agonists 15-deoxy-Δ12,14-prostaglandin J2 and troglitazone exhibit anti-inflammatory properties (25, 26). Moreover, PPARγ ligand effectively inhibits colitis in mice (27). Interestingly, NSAIDs including indomethacin, which are candidate chemopreventive agents against colon cancer, activate both PPARα and PPARγ (28).

Suppression of tumor growth in vitro (20) is not supported in animal models of familial adenomatous polyposis coli (18, 19). In the present study, we investigated the role of PPARα and PPARγ ligands in preventing colitis induced by DSS and ACF induced by DSS and/or AOM in rats. Because TFF play an important role in the repair and healing of the gastrointestinal tract (29), we measured TFF2 and TFF3 concentrations in the mucosa of rats with experimentally induced colitis. Because control of cell proliferation is important for cancer inhibition (30), we also estimated the cell proliferation activity in...
colonic mucosa exposed to DSS, AOM, and PPAR ligands by counting the number of AgNOR protein per mucosal cell nucleus (AgNORs index). Our results showed that PPARα and PPARγ ligands suppressed DSS-induced colitis and DSS- and/or AOM-induced ACF. This may explain the protective role of ligands for PPARα and PPARγ against colonic ACF and suggests a possible therapeutic effect in colitis-associated colon carcinogenesis.

Materials and Methods

Animals, Chemicals, and Diets. Male F344 rats (Shizuoka Laboratory Animal Center, Shizuoka, Japan), 4 weeks of age, were used in three different experiments. The animals were maintained at Kanazawa Medical University Animal Facility according to the Institutional Animal Care Guidelines. All animals were housed in plastic cages (three or four rats/cage) with free access to drinking water and a basal diet, CE-2 (CLEA Japan Inc., Tokyo, Japan), under controlled conditions of humidity (50 ± 10%), light (12/12 h light/dark cycle), and temperature (23 ± 2°C). They were quarantined for the first 14 days and then randomized by body weight into experimental and control groups. DSS, with a molecular weight of 40,000, was purchased from ICN Pharmaceuticals, Inc. (Costa Mesa, CA). DSS for induction of colitis was dissolved in water at a concentration of 1% (w/v). AOM for induction of ACF was purchased from Sigma Chemical Co. (St. Louis, MO). Powdered CE-2 diet was used as a basal diet throughout the study. PPARα ligand troglitazone (Sankyo Co., Tokyo), PPARγ ligand pioglitazone (Takeda Chemical Industries, Osaka, Japan), and PPARα ligand bezafibrate (Kissei Pharmaceutical Co., Matsumoto, Japan). Experimental diets containing troglitazone, pioglitazone, and bezafibrate were prepared by mixing the respective compound in powdered basal diet CE-2 at a concentration (w/w) of 0.01 or 0.05%.

Experimental Procedure. Three experiments were conducted to examine the effects of PPAR ligands in rats with DSS-induced colitis, AOM-induced ACF, and DSS/AOM-induced ACF.

Experiment 1. A total of 54 male F344 rats were divided into eight experimental and control groups (Fig. 1A). Groups 1–5 were provided with drinking water containing 1% DSS ad libitum for 7 days. Groups 2–5 also received gastric gavage of troglitazone (10 mg/kg body weight for group 2 or 100 mg/kg body weight for group 3) or bezafibrate (10 mg/kg body weight for group 4 or 100 mg/kg body weight for group 5) suspended in 0.75% methyl cellulose containing normal physiological solution every other day for 3 times, starting 24 h after commencement of the study. Groups 6 and 7 were treated with troglitazone and bezafibrate (100 mg/kg body weight), respectively. Group 8 represents untreated control rats. The experiment was terminated at day 8. All animals were sacrificed, and the colons were flushed with saline, excised, cut open longitudinally along the main axis, and then washed with saline. The colon was cut and fixed in 10% buffered formalin for at least 24 h. Histological examination was performed using paraffin-embedded sections after H&E staining. For statistical evaluation, the severity of colonic inflammation was histologically scored in a blind fashion according to the scoring system (31): grade 0, normal; grade 1, focal inflammatory cell infiltration including neutrophils; grade 2, crypt loss with inflammatory cell infiltration or crypt abscess formation; grade 3, mucosal ulceration, or five or more foci of gland loss with inflammatory cell infiltration; and grade 4, two or more areas of mucosal ulceration. At the end of the study, the concentrations of TFF2 and TFF3 in the colonic mucosa of three rats from each group were measured by RIA (32) using region-specific polyclonal antibodies in each case. Total protein concentration was also determined using the protocol of Bradford (33).

Experiment 2. A total of 56 male F344 rats were divided into eight experimental and control groups (Fig. 1B). Groups 1–5 were treated with AOM by two weekly s.c. injections (20 mg/kg body weight). Rats in groups 2 and 3 were fed a diet containing 0.01 and 0.05% troglitazone, respectively, for 4 weeks, commencing 1 week before the first dose of AOM. Groups 4 and 5 were fed a diet mixed with 0.01 and 0.05% bezafibrate, respectively, for 4 weeks, commencing 1 week before the first injection of AOM. Groups 6 and 7 were given a diet containing 0.05% troglitazone and bezafibrate alone. Group 8 served as the untreated control. Rats were sacrificed at week 4 by ether overdose to assess the incidence of colonic ACF. The frequency of ACF was determined according to the method described in our previous report (10). At necropsy, the colons were flushed with saline, excised, cut open longitudinally along the main axis, and then washed with saline. They were cut and fixed in

Fig. 1. Experimental protocols. A, experiment 1; B, experiment 2; C, experiment 3. bw, body weight; i.g., intragastrically.

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10% buffered formalin for at least 24 h. Fixed colons were dipped in 0.5% solution of methylene blue in distilled water for 30 s, briefly washed with distilled water, and placed on a microscope slide with the mucosal surface up. Using a light microscope at a magnification of ×40, ACF were distinguished from the surrounding “normal-appearing” crypts by their large size (10).

**Experiment 3.** A total of 55 male F344 rats were divided into nine experimental and control groups (Fig. 1C). Group 1 was administered two weekly s.c. injections of AOM on weeks 3 and 4 at a dose of 20 mg/kg body weight. Groups 2–5 were administered 1% DSS in drinking water for 7 days and two weekly s.c. injections of AOM (20 mg/kg body weight), commencing 7 days after DSS administration. Groups 3, 4, and 5 were fed diets containing 0.01% pioglitazone, troglitazone, and bezafibrate for 6 weeks, respectively. Groups 6–8 were administered diets mixed with 0.01% pioglitazone, troglitazone, and bezafibrate, respectively. Group 9 consisted of untreated control rats. The severity of colitis and development of ACF were evaluated at week 6, as described in Experiments 1 and 2. In addition, the AgNORs index was counted on one-step silver-stained sections (34) in four rats in each of groups 1–5 and in three rats in each of groups 6–9. Measurements were performed on 25 crypts/rat.

**Statistical Analysis.** All data were expressed as mean ± SD. Data were compared by one-way ANOVA, followed by a Bonferroni/Dunn post-hoc test. P < 0.05 denoted the presence of a statistically significant difference.

**Results**

**Experiment 1.** The mean body weights of rats of groups 2–5, which were treated with 1% DSS and troglitazone or bezafibrate, were slightly lower than that of group 1 treated with 1% DSS alone at the end of the study (Table 1). The mean liver and relative liver weights did not differ among the groups. Scoring of severity of colitis showed significant reduction in groups 2–5, compared with group 1 (P < 0.005, P < 0.001, P < 0.02, and P < 0.002, respectively). The concentrations of TFF2 and TFF3 in colonic mucosa are shown in Table 2. Although the concentrations of TFF3 were comparable among the groups, those of TFF2 in groups 2–5 were lower than that of group 1, and the value of group 3 was significantly smaller than group 1 (P < 0.05).

**Experiment 2.** Body, liver, and relative liver weights at the end of the experiment are shown in Table 3. The mean body weight of group 1 (AOM alone) was significantly lower (P < 0.001) than group 8 (untreated). Mean liver and relative liver weights of rats among groups 1–6 did not significantly differ. Dietary administration of 0.05% bezafibrate significantly elevated the mean liver and relative liver weights compared with the untreated control (P < 0.05 and P < 0.01, respectively). ACF analysis showed that administration of 0.05% troglitazone or bezafibrate caused a significant reduction in the numbers of ACF/colon (P < 0.01 or P < 0.001), as shown in Table 3. Administration of 0.05% troglitazone significantly reduced aberrant crypts/focus (P < 0.005).

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**Table 1** Effects of PPARs ligands on DSS-induced colitis (experiment 1)

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>n</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>Relative liver weight</th>
<th>Score of colitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1% DSS</td>
<td>10</td>
<td>154 ± 6*</td>
<td>8.4 ± 1.1</td>
<td>5.45 ± 0.58</td>
<td>3.60 ± 0.66</td>
</tr>
<tr>
<td>2</td>
<td>1% DSS/troglitazone (10 mg/kg bw i.g.)</td>
<td>8</td>
<td>148 ± 10</td>
<td>8.5 ± 1.5</td>
<td>5.73 ± 0.90</td>
<td>1.88 ± 0.78</td>
</tr>
<tr>
<td>3</td>
<td>1% DSS/troglitazone (100 mg/kg bw i.g.)</td>
<td>8</td>
<td>149 ± 9</td>
<td>8.5 ± 1.2</td>
<td>5.75 ± 0.98</td>
<td>1.00 ± 0.87</td>
</tr>
<tr>
<td>4</td>
<td>1% DSS/bezafibrate (10 mg/kg bw i.g.)</td>
<td>8</td>
<td>148 ± 11</td>
<td>8.3 ± 0.8g1</td>
<td>5.67 ± 0.67</td>
<td>1.88 ± 1.05</td>
</tr>
<tr>
<td>5</td>
<td>1% DSS/bezafibrate (100 mg/kg bw i.g.)</td>
<td>8</td>
<td>147 ± 3</td>
<td>9.1 ± 0.8</td>
<td>6.22 ± 0.54</td>
<td>1.50 ± 1.00</td>
</tr>
<tr>
<td>6</td>
<td>Troglitazone (100 mg/kg bw i.g.)</td>
<td>4</td>
<td>158 ± 3</td>
<td>8.2 ± 0.9</td>
<td>5.23 ± 0.67</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Bezafibrate (100 mg/kg bw i.g.)</td>
<td>4</td>
<td>153 ± 7</td>
<td>9.6 ± 1.8</td>
<td>6.29 ± 0.93</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>None</td>
<td>4</td>
<td>159 ± 5</td>
<td>8.3 ± 1.1</td>
<td>5.22 ± 0.50</td>
<td>0</td>
</tr>
</tbody>
</table>

* Liver weight/100 g body weight. bw, body weight; i.g., intragastrically.

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**Table 2** Colonic tissue concentrations of endogenous TFF2 and TFF3 (experiment 1)

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>n</th>
<th>TFF2 (pmol/mg protein)</th>
<th>TFF3 (pmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1% DSS</td>
<td>3</td>
<td>2.13 ± 0.24b</td>
<td>105.0 ± 33.3</td>
</tr>
<tr>
<td>2</td>
<td>1% DSS/troglitazone (10 mg/kg bw i.g.)</td>
<td>3</td>
<td>1.60 ± 0.14</td>
<td>102.7 ± 35.6</td>
</tr>
<tr>
<td>3</td>
<td>1% DSS/troglitazone (100 mg/kg bw i.g.)</td>
<td>3</td>
<td>0.92 ± 0.15</td>
<td>94.0 ± 31.1</td>
</tr>
<tr>
<td>4</td>
<td>1% DSS/bezafibrate (10 mg/kg bw i.g.)</td>
<td>3</td>
<td>1.66 ± 0.16</td>
<td>100.3 ± 31.3</td>
</tr>
<tr>
<td>5</td>
<td>1% DSS/bezafibrate (100 mg/kg bw i.g.)</td>
<td>3</td>
<td>1.01 ± 0.18</td>
<td>97.9 ± 23.7</td>
</tr>
<tr>
<td>6</td>
<td>Troglitazone (100 mg/kg bw i.g.)</td>
<td>3</td>
<td>0.68 ± 0.08</td>
<td>59.3 ± 13.3</td>
</tr>
<tr>
<td>7</td>
<td>Bezafibrate (100 mg/kg bw i.g.)</td>
<td>3</td>
<td>0.71 ± 0.07</td>
<td>58.4 ± 6.7</td>
</tr>
<tr>
<td>8</td>
<td>None</td>
<td>3</td>
<td>0.67 ± 0.07</td>
<td>56.0 ± 8.9</td>
</tr>
</tbody>
</table>

* Mean ± SD. bw, body weight; i.g., intragastrically.

---

**Table 3** Effects of PPAR ligands on AOM-induced ACF formation in male F344 rats (experiment 2)

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment (no. of rats examined)</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>Relative liver weight (g/100 g body weight)</th>
<th>ACF/colon</th>
<th>ACFs/focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AOM alone (12)</td>
<td>166 ± 36b</td>
<td>10.3 ± 1.4</td>
<td>4.73 ± 0.49</td>
<td>83 ± 6</td>
<td>2.01 ± 0.24</td>
</tr>
<tr>
<td>2</td>
<td>AOM + 0.01% troglitazone (8)</td>
<td>172 ± 11</td>
<td>10.8 ± 1.0</td>
<td>4.89 ± 0.44</td>
<td>68 ± 16</td>
<td>1.66 ± 0.21</td>
</tr>
<tr>
<td>3</td>
<td>AOM + 0.05% troglitazone (8)</td>
<td>176 ± 5f</td>
<td>11.1 ± 1.0</td>
<td>4.99 ± 0.28</td>
<td>55 ± 13f</td>
<td>1.54 ± 0.13f</td>
</tr>
<tr>
<td>4</td>
<td>AOM + 0.01% bezafibrate (8)</td>
<td>181 ± 5f</td>
<td>12.3 ± 1.1</td>
<td>5.38 ± 0.23</td>
<td>75 ± 8</td>
<td>1.97 ± 0.20</td>
</tr>
<tr>
<td>5</td>
<td>AOM + 0.05% bezafibrate (8)</td>
<td>178 ± 9</td>
<td>11.8 ± 0.5</td>
<td>4.88 ± 0.19</td>
<td>53 ± 9f</td>
<td>1.85 ± 0.10f</td>
</tr>
<tr>
<td>6</td>
<td>0.05% troglitazone (4)</td>
<td>194 ± 3</td>
<td>9.2 ± 0.8</td>
<td>4.72 ± 0.36</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0.05% bezafibrate (4)</td>
<td>193 ± 9</td>
<td>12.3 ± 0.5f</td>
<td>6.34 ± 0.14f</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>None (4)</td>
<td>182 ± 6</td>
<td>9.0 ± 1.0</td>
<td>4.89 ± 0.33</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Mean ± SD. f Significantly different from group 8 (P < 0.05).

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INHIBITION OF COLITIS AND ACF BY PPAR LIGANDS

Table 4 Effects of PPAR ligands on AOM and/or DSS-induced ACF formation in male F344 rats (experiment 3)

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment (n)</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>Relative liver weight (g/100 g body weight)</th>
<th>ACF/colon</th>
<th>ACs/focus</th>
<th>AgNORs index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AOM alone (8)</td>
<td>234 ± 7a</td>
<td>10.5 ± 1.4</td>
<td>4.5 ± 0.49</td>
<td>84 ± 13</td>
<td>2.0 ± 0.21</td>
<td>1.6 ± 0.21a</td>
</tr>
<tr>
<td>2</td>
<td>1% DSS + AOM (10)</td>
<td>226 ± 7</td>
<td>11.0 ± 1.5</td>
<td>4.9 ± 0.65</td>
<td>115 ± 22</td>
<td>2.4 ± 0.29</td>
<td>1.9 ± 0.13</td>
</tr>
<tr>
<td>3</td>
<td>1% DSS + AOM + 0.01% pioglitazone (7)</td>
<td>231 ± 7</td>
<td>11.0 ± 0.6</td>
<td>4.8 ± 0.34</td>
<td>71 ± 24′</td>
<td>1.8 ± 0.17b</td>
<td>1.2 ± 0.22</td>
</tr>
<tr>
<td>4</td>
<td>1% DSS + AOM + 0.01% troglitazone (7)</td>
<td>219 ± 8</td>
<td>9.7 ± 0.6</td>
<td>4.4 ± 0.33</td>
<td>57 ± 14′</td>
<td>1.6 ± 0.14</td>
<td>0.9 ± 0.15</td>
</tr>
<tr>
<td>5</td>
<td>1% DSS + AOM + 0.01% bezafibrate (7)</td>
<td>216 ± 9</td>
<td>14.2 ± 1.8</td>
<td>6.5 ± 0.63ab</td>
<td>39 ± 18’</td>
<td>1.7 ± 0.16’</td>
<td>0.9 ± 0.16’</td>
</tr>
<tr>
<td>6</td>
<td>0.01% pioglitazone (4)</td>
<td>250 ± 7</td>
<td>9.7 ± 1.4</td>
<td>3.9 ± 0.57</td>
<td>0</td>
<td>0</td>
<td>0.8 ± 0.12</td>
</tr>
<tr>
<td>7</td>
<td>0.01% troglitazone (4)</td>
<td>222 ± 8</td>
<td>11.0 ± 1.0</td>
<td>4.9 ± 0.36</td>
<td>0</td>
<td>0</td>
<td>0.7 ± 0.19</td>
</tr>
<tr>
<td>8</td>
<td>0.01% bezafibrate (4)</td>
<td>226 ± 6</td>
<td>11.7 ± 0.6</td>
<td>5.2 ± 0.26</td>
<td>0</td>
<td>0</td>
<td>0.7 ± 0.11</td>
</tr>
<tr>
<td>9</td>
<td>None (4)</td>
<td>221 ± 8</td>
<td>10.2 ± 1.0</td>
<td>4.5 ± 0.34</td>
<td>0</td>
<td>0</td>
<td>0.7 ± 0.09</td>
</tr>
</tbody>
</table>

a Mean ± SD.

Table 4 Effects of PPAR ligands on AOM and/or DSS-induced ACF formation in male F344 rats (experiment 3)

Experiment 3. As shown in Table 4, the final mean body weight of group 1 (AOM alone) was slightly higher than that of group 9 (untreated). The mean body weights of all groups did not significantly differ. Administration of 0.01% bezafibrate (group 5) significantly increased liver (P < 0.05) and relative liver weights (P < 0.01).

ACF analysis (Table 4) showed that treatment with 1% DSS and AOM (group 2) elevated the number of ACF/colon and aberrant crypts/focus. In groups 3–5, which were treated with 1% DSS, AOM, and 0.01% pioglitazone (group 3), 0.01% troglitazone (group 4), or 0.01% bezafibrate (group 5), both biomarkers were significantly smaller than those of group 2 (P < 0.05; P < 0.001; P < 0.005; P < 0.01, or P < 0.002). As summarized in Table 4, the mean AgNORs index of group 1 (AOM alone) was significantly higher than group 9 (untreated; P < 0.001). DSS administration increased this value. Treatment with PPAR ligands (groups 3–5) significantly decreased AgNORs index compared with group 2 (P < 0.001).

Discussion

The results of the present study clearly indicated that PPAR ligands (either PPARγ or PPARα) inhibited DSS-induced colitis and AOM-induced ACF. In addition, both ligands inhibited DSS/AOM-induced ACF formation, which are precursor lesions for colon carcinoma in the presence of colitis (7). Our data on inhibition of chemically induced colitis are in agreement with those reported by Su et al. (27). The results of experiments on ACF formation support those of Sarraf et al. (20), in which troglitazone reduced the growth rate and induced differentiation of human colon tumor cell lines, both in culture and in nude mice. To our knowledge, this is the first report demonstrating that PPARγ ligands (PPARγ or PPARα) could inhibit colitis and ACF formation.

In Experiment 1, both troglitazone (a PPARγ ligand) and bezafibrate (a PPARα ligand) effectively inhibited DSS-induced colitis. TFF2 and TFF3 are known to protect gastrointestinal injury (29). In Experiment 2 and 3, administration of bezafibrate significantly inhibited AOM-induced and DSS/AOM-induced colonic ACF. The treatment caused hepatomegaly, but no preneoplastic or neoplastic lesions were identified in the liver. In Experiment 3, the frequency of ACF in rats treated with DSS and AOM was greater than that of AOM alone, suggesting that colitis caused by DSS enhanced ACF formation initiated by AOM. Such enhancing effects of inflammation on colon carcinogenesis has also been reported by other researchers (31).

Given the correlation between increased COX-2 expression and colonic carcinoma and/or inflammation, the chemopreventive effects of NSAIDs seems to be mediated, at least in part, by COX inhibition (36). We demonstrated previously that indomethacin, a NSAID, inhibited colon tumorigenesis as well as colitis induced by a naturally occurring carcinogen, 1-hydroxynaphthoquinone (37). Interestingly, some NSAIDs act as peroxisome proliferators (28), suggesting that they might also regulate gene expression as part of their chemopreventive mechanism. Inhibition of colonic inflammation and decrease in cell proliferation by PPARs ligands might be responsible for their chemopreventive effects on colitis-associated colon carcinogenesis, as seen in Experiment 3. Damage to DNA by reactive oxygen and nitrogen species contributes to inflammatory diseases, including colitis-related colon tumorigenesis (4). PPARγ (24) and PPARβ/γ (25) are involved in inflammation control, and both inhibit inducible nitric oxide synthase (26, 38). Several NSAIDs bind to PPARα and PPARγ (28). Their anti-inflammatory activities might be mediated through inhibition of COX-1 and/or COX-2. PPARγ could depress COX-2 induction (15). Therefore, activation of this PPAR may contribute to the anti-inflammatory activity of these drugs. Furthermore, immunomodulation by the ligands (12) might contribute to inhibition of colitis and colon carcinogenesis.

PPARγ is aberrantly expressed in chemically induced rodent colon cancer and in several human colon cancer cell lines (23). It can be up-regulated by treatment with butyrate, which induces differentiation of colon cancer cell line, Caco-2 cells (39). Development of human colon cancer is often associated with mutations in PPARγ gene (40). In addition, Lehmann et al. (28) reported that NSAIDs could bind and activate PPARγ, providing the molecular basis for the preventive effects of these drugs in colon carcinogenesis. In their study, NSAIDs also activated PPARα. The results of the present study (experiments 2 and 3), in which ligands for PPARα and PPARγ effectively prevented the development of ACF, lend support to the above findings. Although PPARγ is the predominant type of PPARs in the colon (16, 17) and its expression increases upon differentiation in human colonic adenocarcinoma cells (41), PPARα is also expressed in the colon (16) and could differentiate malignant tumor cells (42).

Recently, Suh et al. (22) reported that a new ligand for PPARγ (GW7845) inhibits nitrosourea-induced mammary carcinogenesis in female rats. Subsequently, Mehta et al. (43) reported that troglitazone effectively inhibited preneoplastic mammary lesions in female BALB/c mice induced by 7,12-dimethylbenz[a]anthracene. Importantly, the inhibitory effects noted in their study were enhanced by treatment with a retinoid X receptor-selective retinoid, LG10068 (43). Although the effects of PPARγ ligands on intestinal tumorigenesis are conflicting (18–20), the results of the present study (experiments 2 and 3) suggest possible chemopreventive effects by PPARα and PPARγ ligands in colon tumorigenesis with or without colitis.

Because troglitazone can cause hepatic dysfunction (44), other PPARα ligands, such as pioglitazone and GW7845, deserve further investigation with respective to their colon cancer chemopreventive effects. Interesting reports have been described recently. Human colon cancer cells express low levels of 13-S-hydroxyoctadecadienoic acid, a potent natural ligand of PPARγ, and 15-lipoxygenase-1, suggesting that 13-S-hydroxyoctadecadienoic acid production via 15-lipoxygen-
ase-1 down-regulation plays a role in colon tumorigenesis (45). As for PPARα ligands, some (fibrates, phthalate ester plasticisers, pesticides, and hypolipidemic drugs) are considered to be nongenotoxic hepatic carcinogens in rodents (46), although these data are not relevant to humans (47). Thus, PPARα ligands are also candidate chemopreventive agents against colon carcinogenesis.

In conclusion, we demonstrated in the present study that synthetic PPARα ligands (PPARα and PPARγ) could inhibit DSS-induced colitis, AOM-induced ACF, and DSS/AOM-induced ACF. PPARγ ligands could induce growth arrest and differentiation in human colon cancer cell lines (41). In addition, these ligands may inhibit tumor angiogenesis (48). Combined together, the present results and those of previous studies suggest that PPAR activation may be beneficial through inhibition of the early and/or late stages of colon tumorigenesis.

Acknowledgments

We thank Ayumi Nagaoka for secretarial assistance. We also express our thanks to the staff of the Research Animal Facility.

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10. Cooper, H. S., Murthy, S., Kido, K., Yoshihata, K., and Flanigan, A. Dysplasia and hypolipidemic drugs) are considered to be nongenotoxic hepatic carcinogens in rodents (46), although these data are not relevant to humans (47). Thus, PPARα ligands are also candidate chemopreventive agents against colon carcinogenesis.

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Ligands for Peroxisome Proliferator-activated Receptors α and γ Inhibit Chemically Induced Colitis and Formation of Aberrant Crypt Foci in Rats

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Cancer Res 2001;61:2424-2428.

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