Chemopreventive Activity of Celecoxib, a Specific Cyclooxygenase-2 Inhibitor, against Colon Carcinogenesis

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Abstract

Epidemiological and laboratory studies suggest that nonsteroidal anti-inflammatory drugs reduce the risk of colon cancer and that the inhibition of colon carcinogenesis is mediated through modulation of prostaglandin production by cyclooxygenase (COX) isozymes (COX-1 and -2). Overexpression of COX-2 has been observed in colon tumors; therefore, specific inhibitors of COX-2 activity could potentially serve as chemopreventive agents. Our recent study indicated that celecoxib (SC-58635), a specific COX-2 inhibitor, suppressed colonic aberrant crypt formation induced by azoxymethane in rats and led us to investigate more specifically the chemopreventive potential of this compound using colon tumors as end points. Five-week-old male F344 rats were fed the control diet (modified AIN-76A) or an experimental diet containing 1500 ppm celecoxib. Two weeks later, all animals except those in the saline-treated groups received s.c. injections of azoxymethane (15 mg/kg of body weight) once weekly for 2 weeks. All groups were kept on their regimen until the experiment was terminated, 50 weeks after carcinogen treatment. Colon tumors were evaluated histopathologically. Remarkably, dietary administration of celecoxib inhibited both incidence and multiplicity of colon tumors by about 93 and 97%, respectively. It also suppressed the overall colon tumor burden by more than 87%. The degree of tumor inhibition was more pronounced with celecoxib than it was with previously evaluated nonsteroidal anti-inflammatory drugs. The results of this study provide evidence, for the first time, that a specific COX-2 inhibitor, celecoxib, possesses strong chemopreventive activity against colon carcinogenesis.

Introduction

Large bowel cancer is one of the leading causes of cancer deaths in Western countries, including North America (1). Several epidemiological studies suggest an inverse association between the risk of colon cancer and intake of NSAIDs, especially aspirin (2-4). Laboratory animal assays have demonstrated colon tumor inhibition by several NSAIDs, including aspirin, piroxicam, sulindac, sulindac sulfone, and ibuprofen, to name a few (5-8). Clinical studies in patients with familial adenomatous polyposis indicate that administration of sulindac causes a regression and prevents recurrence of polyps, the precursor lesion of colon cancer (2). Although the precise mechanism by which NSAIDs inhibit colon carcinogenesis is not clear, it is often attributed to specific or non-specific inhibition of arachidonic acid metabolism via COX enzymes, which, in turn, modulate eicosanoid production and affect cell proliferation, tumor growth, and immune responsiveness (9). The levels of PGs and COX activities are elevated in tumors (10); therefore, suppression of PG production via COX activity by the administration of NSAIDs provided a mechanistic strategy for the chemoprevention of colon cancer. In addition to inhibition of PG production, sulindac, a NSAID, also increases apoptosis in chemically induced colon tumors (11) and in Min mice (12).

Accumulating evidence indicates that conversion of arachidonic acid to PGs is catalyzed by two isoforms, COX-1 and COX-2. Both have been shown to be present in colon tumors of rodents and humans (10, 13-16). COX-1 is believed to be constitutively expressed in most tissues that generate PGs for normal physiological function. Thus, the expression of this isozyme does not fluctuate due to stimuli, whereas COX-2 expression can be induced by various agents, including growth factors and tumor promoters (17-19). Also, prolonged administration of NSAIDs has been associated with side effects, such as gastrointestinal ulceration and renal toxicity. These potential toxicities of NSAIDs are manifested by the inhibition of the constitutive enzyme, COX-1 (20-22). A study by Tsuji and DuBois (23) indicated that intestinal epithelial cells overexpressing the COX-2 gene develop altered adhesion properties and resist undergoing apoptosis; these changes are reversed by treatment with NSAIDs, suggesting that overexpression of COX-2 may alter the development of neoplasms in the intestine. That commonly used NSAIDs inhibit the activity of both isoforms of COX, which accounts for their therapeutic and adverse side effects, and that sensitivity of recombinant COX-2 toward inhibition by NSAIDs is different from that of COX-1 (24, 25) make it likely that specific inhibitors of COX-2 can serve as chemopreventive agents in colorectal cancer without side effects (23). Oshima et al. (16) demonstrated that COX-2 gene knockout and MF Tricyclic, a COX-2 inhibitor, reduced the number and size of the intestinal polyps in Apc(−/−) knockout mice. Celecoxib (SC-58635) is a novel, specific inhibitor of COX-2 with significant anti-inflammatory and analgesic properties (26-28). In a previous study, we reported that celecoxib suppressed preneoplastic lesions in the colon of rats (29); therefore, we designed a preclinical efficacy study that would more fully evaluate this compound for its chemopreventive properties, using colon tumor formation as an end point.

This double-blind study was designed to investigate the chemopreventive potency of celecoxib on AOM-induced colon carcinogenesis in male F344 rats. The ultimate goal of this study was to determine whether this compound is an effective chemopreventive agent against chemically induced colon carcinogenesis in preclinical efficacy studies and, eventually, in human clinical trials.

Materials and Methods

Animals, Diets, Carcinogen, and Chemopreventive Agent. AOM (CAS: 25843-45-2) was purchased from Ash Stevens (Detroit, MI). Celecoxib (SC-58635: 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide; Fig. 1) was kindly supplied by Searle Research and Development (St. Louis, MO). Weanling male F344 rats were purchased from Charles River Breeding Laboratories (Kingston, NY). All ingredients of the semipurified diet were obtained from Dyets Inc. (Bethlehem, PA). The experimental diet was prepared weekly by mixing celecoxib with modified AIN-76A diet, and it was

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2 To whom requests for reprints should be addressed, at American Health Foundation, One Dana Road, Valhalla, NY 10595.

3 The abbreviations used are: NSAID, nonsteroidal anti-inflammatory drug; COX, cyclooxygenase; PG, prostaglandin; AOM, azoxymethane.
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stored in a cold room. Following a 1-week quarantine period, all animals were randomly distributed by weight into experimental and control groups and transferred to an animal holding room that was maintained under controlled conditions, as follows: 12-h light/dark cycle, 21 ± 2°C room temperature, and 50 ± 10% relative humidity.

**Selectivity Assay and Dose Selection.** Prior to evaluation of celecoxib for its potential chemopreventive properties, an in vitro assay was performed to determine the selective inhibition of COX-1 and COX-2 activity by this agent in insect cells expressing either COX-1 or COX-2 by methods described in the literature (25). Upon treatment with celecoxib, the formation of prostaglandin E₂ was decreased in insect cells, as indicated by IC₅₀s for COX-1 and -2 isozymes. These IC₅₀s were 15 ± 3.0 μM for COX-1 and 0.04 ± 0.01 μM for COX-2 (mean ± SD, n = 5), signifying that this agent selectively inhibited COX-2 activity. For dose selection, the adjuvant-induced arthritis-chronic inflammatory model was used to establish the therapeutic blood level of celecoxib. The therapeutic blood level is defined as the lowest dose of the drug that produces maximal anti-inflammatory effect (30). At 0.9 mg/kg of body weight, which corresponds to a plasma level of about 0.3 μg/ml, celecoxib produced the maximal effect. Our finding that daily administration of 1500 ppm celecoxib significantly suppressed the colon aberrant crypt foci formation served as the basis for dose selection in the current study (29).

**Experimental Procedure.** At 5 weeks of age, the rats were fed either control diet (modified AIN-76A) or experimental diet containing 1500 ppm celecoxib. At 7 weeks of age, all animals except those intended for saline treatment received s.c. injections of AOM (15 mg/kg body weight) once weekly for 2 weeks. The rats were then maintained on control or experimental diets until termination of the experiment. Body weights were recorded weekly for the first 8 weeks and then every 4 weeks. Animals were monitored daily for their general health. The experiment was terminated 50 weeks after the second AOM treatment, at which time all animals were killed by CO₂ euthanasia. After laparotomy, the entire stomach and intestines were resected and opened longitudinally, and the contents were flushed with normal saline. Using a dissection microscope, small and large intestinal tumors were noted grossly for their location, number, and size. The length, width, and depth of each tumor were measured with calipers. Tumor volume was calculated using the formula $V = L \cdot W \cdot D / 6$ (31), where $V$ is volume, $L$ is length, $W$ is width, and $D$ is depth. All other organs, including kidneys and liver, were also grossly examined under the dissection microscope for any abnormalities. Tumors were fixed in 10% buffered formalin, embedded in paraffin blocks, and processed for histological evaluation by routine procedures with H&E staining. The stained sections were examined for tumor types according to the classification of Pozharisski (32), with minor modifications.

**Statistical Analysis.** Body weights, tumor incidence, tumor multiplicity, and tumor volume were compared between the animals fed the control and the celecoxib diet. Tumor incidence, expressed as percentage of tumor-bearing animals, was analyzed by Fisher’s exact probability test, whereas tumor multiplicity, expressed as the mean number of tumors per animal, was analyzed by the unpaired Welch’s t test, accounting for unequal variance. Differences in body weights and tumor volume between the groups were analyzed by Welch’s t test and ANOVA. Differences were considered statistically significant at $P < 0.05$.

**Results**

**General Observations.** The body weights of animals treated with vehicle or AOM and fed the control diet or celecoxib, respectively, were comparable throughout the study (Table 1). In saline-treated animals, chronic administration of celecoxib did not produce any

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**Table 1** Effect of celecoxib on body weights in male F344 rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No. of rats</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>24</th>
<th>32</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOM-treated</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet (AIN-76A)</td>
<td>36</td>
<td>115 ± 10</td>
<td>224 ± 14</td>
<td>284 ± 19</td>
<td>351 ± 23</td>
<td>395 ± 25</td>
<td>442 ± 27</td>
<td>437 ± 37</td>
<td>430 ± 40</td>
</tr>
<tr>
<td>Celecoxib (1500 ppm)</td>
<td>36</td>
<td>116 ± 9</td>
<td>219 ± 14</td>
<td>283 ± 18</td>
<td>352 ± 22</td>
<td>398 ± 22</td>
<td>429 ± 25</td>
<td>450 ± 34</td>
<td>458 ± 28</td>
</tr>
<tr>
<td>Saline-treated</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control diet (AIN-76A)</td>
<td>9</td>
<td>113 ± 9</td>
<td>231 ± 8</td>
<td>293 ± 13</td>
<td>371 ± 15</td>
<td>419 ± 15</td>
<td>454 ± 15</td>
<td>476 ± 14</td>
<td>484 ± 12</td>
</tr>
<tr>
<td>Celecoxib (1500 ppm)</td>
<td>9</td>
<td>115 ± 8</td>
<td>286 ± 16</td>
<td>286 ± 22</td>
<td>351 ± 26</td>
<td>401 ± 24</td>
<td>439 ± 20</td>
<td>466 ± 24</td>
<td>470 ± 20</td>
</tr>
</tbody>
</table>

* Values represent mean ± SD.
A Weeks after the last AOM injection.

**Table 2** Effect of celecoxib on the incidence and multiplicity of AOM-induced colonic tumors in male F344 rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Total*</th>
<th>Adenomas</th>
<th>Noninvasive</th>
<th>Invasive</th>
<th>Total</th>
<th>Adenomas</th>
<th>Noninvasive</th>
<th>Invasive</th>
<th>Multiplicity* (no. of tumors/rat)</th>
<th>Adenocarcinomas</th>
<th>Noninvasive</th>
<th>Invasive</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOM-treated</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control diet (AIN-76A)</td>
<td>85</td>
<td>9</td>
<td>41</td>
<td>76</td>
<td>1.91</td>
<td>0.13</td>
<td>1.06</td>
<td>0.23</td>
<td>0.09 ± 0.28</td>
<td>0.59 ± 0.77</td>
<td>0.03 ± 0.16</td>
<td>0.12 ± 0.16</td>
</tr>
<tr>
<td>Celecoxib (1500 ppm)</td>
<td>6² (93)²</td>
<td>0 (100)</td>
<td>3³ (93)</td>
<td>3³ (96)</td>
<td>1.26</td>
<td>1.01</td>
<td>1.26 ± 1.01</td>
<td>0.03 ± 0.16</td>
<td>0.03 ± 0.16 (98)</td>
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<tr>
<td>Saline-treated</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control diet (AIN-76A)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Celecoxib (1500 ppm)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
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</tbody>
</table>

* Includes adenomas and noninvasive and invasive adenocarcinomas.

* Values represent mean ± SD.

* Significantly different from control group by Fisher’s exact probability test ($P < 0.000001$).

* Values in parentheses represent percentage inhibition compared to control group.

* Significantly different from control group by Fisher’s exact probability test ($P < 0.0001$).

* Significantly different from control group by Welch’s t test ($P < 0.000001$).

* Significantly different from control group by Welch’s t test ($P < 0.001$).
coxib at 1500 ppm did not induce any toxic side effects, such as body weight loss, gastrointestinal ulceration, or bleeding. It is also noteworthy that a pilot endoscopic study showed no difference in gastroduodenal mucosa damages between celecoxib group and placebo group (28). Thus, specific inhibitors of COX-2 that induce very few toxic side effects but have increased chemopreventive potency may provide a new and effective approach to the chemoprevention of colon cancer.

Although the precise mechanism by which celecoxib inhibits colon carcinogenesis is not certain, the available data support the hypothesis that arachidonic acid metabolism is altered through COX activity, thereby reducing eicosanoid production (33). Various findings suggest that PGs have a role in the pathogenesis of colon cancer because they modulate several signal transduction pathways (10, 17, 23). Several studies have also demonstrated the role of COX-2 metabolites, particularly prostaglandin E$_2$, in colon tumor promotion (33, 34). Our recent study indicated that increased levels of immunoreactive COX-2 are present not only in colon tumors but also in as yet tumor-free colon mucosa, as early as 1 week after carcinogen administration (35). Oshima et al. (16) indicated that induction of COX-2 is a very early event in the sequence of polypl formation to colon carcinogenesis. This study demonstrated that administration of celecoxib produced colon tumor-inhibitory effects, both at the initiation phase and during promotion and progression phases of carcinogenesis. The increased level of COX-2 protein may result in elevated PG levels in these tumors. Several studies have shown that COX-2 but not COX-1 gene expression and protein expression are markedly elevated in most human colorectal cancers, as compared with accompanying normal mucosa (10, 13, 14). This is also true for AOM-induced colon cancer in the rat model (15) and in Min mice (12). DuBoise et al. (17, 23) have demonstrated that the COX-2 gene is induced following growth factor or tumor promoter stimulation of rat intestinal epithelial cells and that COX-2 overexpression is linked to changes in cellular adhesion and inhibition of apoptosis. Thus, the metabolic products derived from its catalytic formation appear to play a role in tumor promotion and progression.

In conclusion, administration of celecoxib, a specific COX-2 inhibitor, suppressed the incidence and multiplicity of colon tumors and the total tumor burden induced by AOM in male F344 rats. The degree of inhibition was more pronounced with celecoxib than it was with NSAIDs, which were evaluated using similar protocols. These results suggest that celecoxib could serve as an effective chemopreventive agent with low toxicity against colon cancer development in humans.

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References


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