Evaluation of Cyclooxygenase-2 Inhibitor for Potential Chemopreventive Properties in Colon Carcinogenesis

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Abstract

Epidemiological and laboratory studies indicate an inverse relationship between the risk of colon cancer development and intake of nonsteroidal antiinflammatory agents, including aspirin. One of the mechanisms by which nonsteroidal antiinflammatory agents inhibit colon carcinogenesis is through the inhibition of prostaglandin production by cyclooxygenase isozymes (COX-1 and COX-2). Overexpression of COX-2 has been observed in colon tumors. Thus, selective inhibitors of COX-2 could potentially serve as chemopreventive agents. We have assessed the chemopreventive properties of SC-58635, a COX-2 inhibitor, and of sulindac, as a positive control, in a double-blind study, using azoxymethane-induced colonic aberrant crypt foci (ACF) as a measure of efficacy. Five-week-old male F344 rats were fed the control diet (modified AIN-76A) or experimental diets containing 150 or 1500 ppm SC-58635, 320 ppm sulindac, or 1500 ppm placebo. Two weeks later, all animals except those in vehicle (normal saline)-treated groups were s.c. injected with azoxymethane (15 mg/kg of body weight, once weekly for 2 weeks). At 16 weeks of age, all rats were sacrificed and colons were evaluated for ACF. As expected, administration of sulindac suppressed ACF development as much and reduced crypt multiplicity in terms of number of aberrant crypts/focus. Administration of 1500 ppm SC-58635 inhibited total ACF induction and crypt multiplicity by about 40—49%. Our finding that SC-58635 significantly suppressed colonic ACF formation and crypt multiplicity strengthens the hypothesis that a selective COX-2 inhibitor possesses chemopreventive activity against colon carcinogenesis.

Introduction

Large bowel cancer is one of the leading causes of cancer deaths in both men and women in Western countries, including the United States (1). Although several epidemiological and experimental studies suggest a relationship between the risk of development of colon cancer and dietary factors (2), recent epidemiological investigations also indicate an inverse relationship between the intake of NSAIDs, specifically aspirin, and colorectal cancer risk (3, 4). Studies in laboratory animal models have also demonstrated colon tumor inhibition by several NSAIDs, including aspirin, piroxicam, sulindac, sulindac sulphone, and ibuprofen, to cite a few (5—8). Clinical studies in patients with familial adenomatous polyposis indicate that administration of sulindac causes a reduction of polyps (9).

The mechanism(s) by which NSAIDs inhibit colon carcinogenesis is not clearly understood but could possibly involve blockage of COXs, which, in turn, suppresses eicosanoid production, and especially the type 2 series of eicosanoids that affect cell proliferation, tumor growth, and immune responsiveness (10—12). In addition to inhibiting PG production, sulindac significantly blocks the formation of lipoxygenase metabolites such as 8(S)- and 12(S)-hydroxyeicosatetraenoic acid in the colonic mucosa. Sulindac also inhibits tumor developments in F344 rats and increases apoptosis in Min mice (5, 7). Recent studies have clarified that sulindac sulphone, a metabolite of sulindac, does not inhibit COX but induces apoptosis (13). Thus, it is possible that the changes in the activities of the COX and/or lipoxygenase pathways of arachidonic acid metabolism and/or modulation of events other than COX inhibition by NSAIDs may alter tumorigenesis.

At least two COX isozymes, namely COX-1 and COX-2, have been identified in colon tumors of humans and rats (14—16). COX-1 isozyme is believed to be a constitutively expressed gene in most tissues to generate PGs for normal physiological functions; thus, the expression of this isozyme does not fluctuate due to stimuli, whereas the expression of COX-2 can be induced by a variety of agents, including growth factors and tumor promoters (15, 17, 18). Tsujii and DuBois (19) have shown 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, indicating that overexpression of the isozyme may alter the potential for development of neoplasm of intestinal epithelial cells. Also, there is evidence that the sensitivity of recombinant COX-2 toward inhibition by NSAIDs is different from that of COX-1 (20, 21). These observations raise the possibility that selective inhibitors of COX-2 could potentially serve as chemopreventive agents in colorectal carcinogenesis.

ACF, which are recognized as early preneoplastic lesions, have consistently been observed in experimentally induced colon carcinogenesis in laboratory animals (22—25). Pretlow et al. (26) also have shown that these lesions are present in the colonic mucosa of patients with colon cancer and have suggested that aberrant crypts are putative precursor lesions from which adenomas and carcinomas may develop in the colon (23). ACF express mutations in the apc gene and ras oncogene that appear to be biomarkers of colon cancer development (24, 27). There is some evidence that several inhibitors of ACF formation reduce the incidence of colon tumors in laboratory animals (24—25), suggesting that ACF induction can be used to evaluate novel agents for their potential chemopreventive properties against colon cancer.

The present double-blind study was designed to evaluate the inhibitory activity of SC-58635, a COX-2 inhibitor, on AOM-induced ACF formation in the colon of male F344 rats. The major goal of this study was to determine whether this compound is conceivably an effective chemopreventive agent in preclinical efficacy studies and, eventually, in human clinical trials.

Materials and Methods

Selectivity Assay and Dose Selection. Before evaluating SC-58635 for its potential chemopreventive activity, an in vitro assay was performed to determine the selective inhibition of COX-1 and COX-2 activities by this agent. For
Table 1  Percentage composition of experimental semipurified diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control diet&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Experimental diet&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Corn starch</td>
<td>52.0</td>
<td>52.0</td>
</tr>
<tr>
<td>Dextrose</td>
<td>13.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Alphacel</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Mineral Mix, AIN</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin Mix, AIN revised</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Test compounds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC-58635, 150 or 1500 ppm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulindac, 320 ppm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo, 1500 ppm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Adapted from American Institute of Nutrition Reference Diet (AIN-76A) with the modification of source of carbohydrate.

<sup>b</sup> Test compounds were added to the diets at the expense of cornstarch.

left contralateral footpad volume was measured by the water displacement method 14 days after injection. Animals with paw volumes that were 0.4 ml greater than normal were then randomized and treated with SC-58635, beginning 15 days after adjuvant injection. The drug administration was continued until day 25 postadjuvant injection, and the mean inhibition values on paw volume were determined on the basis of eight Lewis rats. Plasma samples were collected on the final day of dosing and were analyzed for SC-58635; the therapeutic blood level was established as the lowest dose of the drug that produced the maximal antiinflammatory effect (29). SC-58635 produced a maximal effect in rats in this chronic model at a dose of 0.9 mg/kg body weight, which corresponded to a plasma level of approximately 0.3 μg/ml. SC-58635 has a markedly improved safety profile in the rat, presumably as a function of its selectivity for inducing the COX-2 enzyme while sparing the physiologically important COX-1 in the gastrointestinal tract. Therefore, studies were designed to evaluate the effect of SC-58635 on blocking ACF formation and progression at two dose levels, namely 150 and 1500 ppm, when added to the diet. These doses produced plasma levels of SC-58635 of approximately 0.5 and 3.5 μg/ml, respectively.

Animals, Diets, Carcinogen and Chemopreventive Agents. AOM (CAS:25843-45-2) was purchased from Ash Stevens (Detroit, MI). SC-58635 and placebo were coded and supplied by G. D. Searle Research and Development, (St. Louis, MO). Sulindac, a known inhibitor of colon carcinogenesis, was included in the current study as a positive control (7). 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) and were stored at 4°C until the experimental diets were prepared. The rats were held in quarantine for 1 week and had access to modified AIN-76A control diet (Table 1). They were randomly distributed by weight into various dietary groups and were transferred to an animal holding room where they were housed in plastic cages, three rats/cage, under controlled conditions of a 12 h light/12 h dark cycle, 50% relative humidity, and 21°C room temperature. Experimental diets were prepared by mixing SC-58635, sulindac, and placebo with modified AIN-76A control diet.

For dose selection, the adjuvant-induced arthritis-chronic inflammatory model was used to establish the therapeutic blood level of SC-58635. Adjuvant arthritis was induced in male Lewis rats (body weight, 150–175 g, Harlan Sprague-Dawley, Inc.) by injecting 1 mg of Mycobacterium butyricum (Difco) in 50 μl of mineral oil into the right hind footpad. The selectivity assay, insect cells expressing either COX-1 or COX-2 were homogenized and incubated with arachidonic acid (10 μM). The activities of COX-1 and COX-2 were then determined by monitoring prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) production (28). To measure the selective inhibition of COX-1 or COX-2 by SC-58635, 0.001–100 μM levels of this agent were preincubated with crude 1% 3-[(3-cholamidopropyl)dimethylamino]-1-propanesulfonate homogenates (2–10 μg protein) for 10 min prior to the addition of arachidonic acid. The PGE<sub>2</sub> formed was detected by ELISA after a 10-min incubation period (28). The formation of PGE<sub>2</sub> in insect cells expressing COX-1 and COX-2 upon treatment with SC-58635 was 13 ± 3.0 and 0.04 ± 0.01 μM (mean ± SD; n = 5), respectively, indicating that SC-58635 selectively inhibited COX-2 activity.

Fig. 1. Unsectioned methylene blue-stained rat colon. A, topographic view of colon mucosa of saline-treated control animal. Note that no ACF are identified. ×40. B, several AOM-induced ACF with two or more aberrant crypt/focus are identified (arrows). ×100. C, two aberrant crypt/focus. ×100. D, multicrypt/focus. ×100.
Crypts. In the present study, sulindac, which has been shown to be a strong inhibitor of colon carcinogenesis in animal assays and has reduced polyplasticity in patients with familial polyposis, was also found to be an effective inhibitor of total occurrences of ACF/colon (36%) and of multicrypt clusters containing two, three, or four or more crypts/focus (31–40%). Administration of 1500 ppm SC-58635 significantly suppressed the total number of ACF/colon (40% inhibition) as compared to control diet. aberrant crypt multiplicities per focus were significantly decreased (41–49%). The lower dose of SC-58635 had only minimal impact on ACF formation. As expected, administration of placebo had no effect on ACF inhibition.

Discussion

The prolonged administration of NSAIDs has been associated with side effects such as gastrointestinal ulceration and bleeding, as well as renal toxicity. Because aspirin, sulindac, piroxicam and indomethacin, commonly used NSAIDs, have little or no selectivity for inhibition of COX-1 or COX-2 activity (30, 31), the development of more specific and potent, yet minimally toxic, inhibitors of COX-2 may provide useful cancer chemopreventive agents. The present study was undertaken to evaluate the selective COX-2 inhibitor SC-58635 for its potential chemopreventive activity against ACF formation in the colon. ACF are putative preneoplastic lesions. Because multiplicity of four or more aberrant crypts/focus has been a consistent predictor of colon tumor outcome (32, 33), the present study used this criterion to evaluate SC-58635 for its potential chemopreventive properties. The results of this study support our earlier efficacy study with sulindac (7) and provide additional evidence that crypt multiplicity and ACF are predictive of colon tumor incidence. Sulindac was found to be a strong inhibitor of chemically induced colon carcinogenesis in animal models and is currently under evaluation in human clinical chemoprevention trials (7, 9). COX-2 inhibitors reduce inflammatory PG synthesis without affecting PG levels in normal tissues that result from COX-1 activity (20). The fact that a selective COX-2 inhibitor like SC-58635 can inhibit colonic ACF formation and crypt multiplicity further suggests a role for similar compounds as potential chemopreventive agents against colon cancer. This finding is significant because SC-58635, through its ability to inhibit COX-2 expression, has the potential to block the development and/or progression of colon carcinogenesis, and at the same time, this agent possesses minimal or no gastric toxicity. The lack of an inhibitory effect of the low dose of SC-58635 that was effective in modulating adjuvant-induced arthritis in rats may indicate that higher blood levels of this agent are needed to achieve adequate colonic exposure; however, it is noteworthy that the higher dose of SC-58635 did not induce any symptoms attributable to toxicity. It is possible that, in addition to COX-2 inhibition, SC-58635 may also increase apoptosis, thereby inhibiting ACF formation. Further experiments, including preclinical efficacy studies, are warranted to fully evaluate this compound for its chemopreventive properties.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Total no. of ACF/rat</th>
<th>1 crypt</th>
<th>2 crypts</th>
<th>3 crypts</th>
<th>4 or more crypts</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC-58635, 1500 ppm</td>
<td>71 ± 15'</td>
<td>16 ± 6.5</td>
<td>35 ± 7.7</td>
<td>34 ± 4.6</td>
<td>35 ± 7.9</td>
</tr>
<tr>
<td>SC-58635, 150 ppm</td>
<td>127 ± 13</td>
<td>10 ± 4.3'</td>
<td>22 ± 6.8'</td>
<td>20 ± 6.8'</td>
<td>18 ± 5.8'</td>
</tr>
<tr>
<td>Placebo, 1500 ppm</td>
<td>111 ± 35</td>
<td>15 ± 7.7</td>
<td>34 ± 11.8</td>
<td>31 ± 10.1</td>
<td>31 ± 10.2</td>
</tr>
<tr>
<td>SC-58635, 1500 ppm</td>
<td>77 ± 14'</td>
<td>11 ± 6.3'</td>
<td>24 ± 8.5'</td>
<td>21 ± 6.6'</td>
<td>21 ± 5.8'</td>
</tr>
<tr>
<td>SC-58635, 150 ppm</td>
<td>127 ± 13</td>
<td>16 ± 4.6</td>
<td>44 ± 7.0</td>
<td>35 ± 6.8</td>
<td>33 ± 6.6</td>
</tr>
<tr>
<td>Placebo, 1500 ppm</td>
<td>111 ± 35</td>
<td>15 ± 7.7</td>
<td>34 ± 11.8</td>
<td>31 ± 10.1</td>
<td>31 ± 10.2</td>
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<td>31 ± 10.1</td>
<td>31 ± 10.2</td>
</tr>
</tbody>
</table>

\(a\) Values are means ± SD (n = 12).

\(b\) Animals were fed either control diets or experimental diets containing SC-58635, sulindac, or placebo and treated with AOM or saline (vehicle).

\(c\) Significantly different from control diet group treated with AOM; \(P < 0.001\).

\(d\) Significantly different from control diet group treated with AOM; \(P < 0.05\).
Acknowledgments

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References

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