The Cyclooxygenase-2 Inhibitor Celecoxib Is a Potent Preventive and Therapeutic Agent in the Min Mouse Model of Adenomatous Polyposis

Russell F. Jacoby, Karen Seibert, Carolyn E. Cole, Gary Kelloff, and Ronald A. Lubet

Abstract

Epidemiological and animal studies suggest that nonsteroidal anti-inflammatory drugs (NSAIDs) may reduce colon cancer risk. NSAIDs nonselectively inhibit both the constitutive cyclooxygenase (COX) 1 associated with side effects and the desired therapeutic target COX-2, which is induced in inflammation and neoplasia. We used the adenomatous polyposis coli (Apc) mutant Min mouse model to determine whether the selective COX-2 inhibitor celecoxib is effective for adenoma prevention and/or regression, and whether it might be safer than the nonselective NSAID previously shown to be most effective in this model, piroxicam. Min mice (n = 120) were randomized to treatment with celecoxib (0, 150, 500, or 1500 ppm celecoxib mixed in the diet) or piroxicam. To distinguish prevention from regression effects, groups were treated either “early” (before adenomas develop) or “late” (after most adenomas are established). Celecoxib caused dramatic reductions in both the multiplicity and size of tumors in a dose-dependent manner (P < 0.01). Early treatment with 1500 ppm of celecoxib was effective for prevention, decreasing tumor multiplicity to 29% and tumor size to only 17% of controls (P < 0.01). Late treatment demonstrated regression effects, reducing tumor multiplicity and size by about half. In contrast to the significant toxicity of piroxicam, which caused ulcers complicated by perforation and bleeding, celecoxib caused no gastrointestinal side effects and did not inhibit platelet thromboxane B2 at plasma drug levels similar to those obtained in early clinical trials in humans. These results provide the first evidence that selective inhibitors of COX-2 are safe and effective for the prevention and regression of adenomas in a mouse model of adenomatous polyposis and strongly support ongoing clinical trials in humans with the same syndrome. The broader population of patients with common sporadic adenomas that have somatic mutations of the same gene (APC) may also benefit from this treatment approach.

Introduction

Colorectal cancer is one of the most common malignancies in industrialized countries and is the second leading cause of cancer death among men and women in the United States (1). Prevention initiatives using pharmacological approaches are now under investigation. The type of patient selected for these trials as well as the choice of drug influences the balance between benefits and side effects. Potential cumulative toxicity is an important concern because individuals who have not yet overtly manifested disease are exposed to drug treatment for long durations. More favorable risk:benefit ratios might be expected in populations with a higher likelihood of developing neoplasia, such as individuals with a personal or family history of colon cancer or multiple adenomatous polyps (2, 3). Molecular diagnostic methods are now identifying such individuals with specific genetic susceptibilities who may demand therapeutic interventions including chemoprevention (4). The development of chemopreventive agents with minimal side effects could expand the potential treatment cohort to include not only these high-risk patients but also the more general population. In this regard, NSAIDs have previously been considered the most promising agents for colon tumor prevention, based on both epidemiological and animal model data. However, the new class of specific COX-2 inhibitors may be preferable because they appear to be much safer than the traditional nonspecific NSAIDs.

A relatively small percentage of human colon cancers arise in families with strong predisposition alleles such as FAP. However, the APC gene that is mutated in the germ line in FAP kindreds is also mutated somatically early during the development of most colon cancers in sporadic cases and in hereditary nonpolyposis colon cancer.

Because these forms of colon cancer share mutations in APC, an animal model with an alteration in this gene would be most appropriate for testing chemopreventive agents targeting the benign precursor stage adenoma. The Min mutant mouse has an autosomal dominant heterozygous nonsense mutation of the mouse Apc gene (5), homologous to human germ-line and somatic APC mutations. The Min mouse model is particularly advantageous for testing chemopreventive agents targeted against early-stage lesions because scores of adenomas grow to a grossly detectable size within a few months on a defined genetic background [the inbred mouse strain C57BL/6J (Min+/-; Ref. 6). Because Min mice develop adenomas as a result of inactivation of the same tumor suppressor gene known to be involved in the pathogenesis of most colon cancer in humans, experiments with this model are likely to be relevant to the design of human chemoprevention clinical trials (3, 6).

We have performed a series of experiments using the Apc mutant Min mouse model to investigate a variety of chemopreventive agents. Data from our previous studies demonstrated that the NSAID piroxicam is very effective for suppressing adenomas, with a dose-response curve for tumors parallel to that observed for prostaglandin inhibition (7). Accumulating evidence suggests that the entire class of NSAIDs shares the property of suppressing colon cancer and/or adenomatous polyps (7–15). Additional studies should clarify whether these drugs act primarily through cyclooxygenase pathway effects or other mechanisms and the relative importance of COX-1 or COX-2 inhibition (16–23). Toxicity (e.g., ulcers, bleeding, and renal impairment) attributed to COX-1 inhibition may limit the usefulness of NSAIDs because side effects may occur at dosages necessary for effective COX-2 inhibition. Celecoxib is a specific COX-2 inhibitor approved by the Food and Drug Administration that does not inhibit COX-1 at therapeutic doses in humans. This study examines whether celecoxib is safer than the traditional NSAID piroxicam, and whether celecoxib...
is effective for the prevention and/or regression of adenomas in the Min mouse model.

Materials and Methods

Min Mouse Breeding and Genotyping. C57BL/6J(Min+/+) male mice from the original line derived at the University of Wisconsin (6) were bred with C57BL6J(+/+) females. Progeny were genotyped by an allele-specific PCR assay for the known Min nonsense mutation in the Apc gene as described previously (24).

Animal Care and Drug Treatment. Experimental protocols were approved by the Institutional Animal Care committee. Min+/− mice were treated with celecoxib or piroxicam mixed in the diet according to our usual method (24). Treatments were begun either at age 30 days (soon after weaning) or, in other groups, after a delay to age 55 days, when adenomas were already well established. Mice were sacrificed by CO2 inhalation at age 80 days, before the tumor burden caused any obvious morbidity.

Thromboxane B2 and Piroxicam Assays. At the time of sacrifice, blood was collected in heparinized tubes, and plasma was separated immediately and frozen at −70°C for later assay. Piroxicam levels were measured in plasma by high-performance liquid chromatography using a method based on that of Mazek and Vacha (25). Thromboxane B2 was measured in plasma as described previously (24), using a RIA kit (New England Nuclear Research Products, Boston MA).

Tumor Scoring. Intestinal segments were examined by an individual unaware of the animal’s drug treatment status. Tumor number, tumor location, and tumor diameters were recorded to a precision of less than 0.1 mm using an Olympus SZH10 stereo dissecting microscope as described previously (24).

Statistical Design and Analysis. This protocol used a one-way layout design to compare tumor growth in Min mice under various chemopreventive or treatment strategies (26). The randomization method we developed and used encouraged balanced recruitment over time to all treatment arms within the protocol and allows calculation of exact Ps (7, 26). Statistical significance was accepted only when P < 0.05. All measurements are reported as the mean ± SE.

Results

Effect of Treatment with Celecoxib or Piroxicam on Plasma Levels of These Drugs and the COX-1 Product Thromboxane B2.

Drug levels of celecoxib and piroxicam were measured at the time of sacrifice in blood plasma obtained from animals exposed continuously to the indicated concentrations of these agents mixed in the diet (Table 1). As expected, the results demonstrate a nearly linear dose-dependent increase in celecoxib levels. Celecoxib at these doses did not cause any apparent toxicity or decrease in body weight (data not shown). Piroxicam at 50 ppm caused numerous ulcerations throughout the GI tract, and in some mice, there was significant bleeding, but there was no mortality. In previous dose-range experiments we performed, perforated ulcers and mortality occurred in less than 5% of mice treated with two to four times this amount of piroxicam.

The biological effects of piroxicam and celecoxib were further assessed by measuring thromboxane B2 levels in plasma, which reflect the COX-1-mediated synthesis of this prostanoid by platelets. As expected, thromboxane B2 levels were lower after treatment with the nonselective NSAID piroxicam [decreased by 43% and 80% compared with control (Table 1)]. In contrast, even the highest dose of celecoxib failed to significantly decrease thromboxane B2. These results demonstrated the lack of any significant inhibition of COX-1 at the doses of celecoxib used, as expected for a COX-2-selective drug.

Celecoxib Prevents and Regresses Adenomas in Apc Mutant Min Mice. The effect of celecoxib on total tumor multiplicity in Min mice is shown in Table 2. Tumor data are also reported separately for each intestinal segment (proximal, middle, or distal small intestine and colon). For the standard protocol, exposure to drug in the diet begins at age 30 days, soon after the mice are weaned, and continues until untreated animals begin to have morbidity from increased tumor burden at age 80 days. Celecoxib caused a significant (P < 0.01) dose-dependent decrease in total tumor multiplicity (Table 2A). The response to the NSAID piroxicam is in good agreement with our previous data in this model system (7). In addition to decreasing tumor multiplicity by up to 70%, the highest doses of celecoxib decreased the volume of those tumors that did grow out by approximately 50% (P < 0.01). Thus, the total tumor load, which reflects both tumor multiplicity and volume, was decreased by >85% (P < 0.01).

When treatment did not begin until age 55 days, after adenomas are already established, celecoxib reduced total tumor multiplicity to 48% of that of the control group (Table 2B). Celecoxib significantly decreased both tumor multiplicity and size when administered late, after most adenomas had already developed, but the magnitude of these effects was somewhat less than that seen when drug treatment was started earlier (compare Figs. 1 and 2).

Although total tumor multiplicity decreased significantly as a result of celecoxib treatment, the magnitude of treatment effect varied depending on the intestinal region, with a notable proximal to distal gradient. There was a somewhat less effective degree of inhibition in the proximal small intestine (duodenum). More striking decreases in tumor multiplicity were observed in the middle portion of the small intestine, and the greatest decreases were in the distal portion of the small intestine (Table 2). The colon response was always difficult to assess in the Min mouse model because there were relatively few

<table>
<thead>
<tr>
<th>Drug dose in ppm</th>
<th>Drug in blood (μg/ml)</th>
<th>Thromboxane B2 (ng/ml)</th>
<th>COX-1 (% control)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Early treatment (age 30–80 days)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0.338 ± 0.060</td>
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<tr>
<td>Celecoxib</td>
<td>150</td>
<td>0.09 ± 0.01</td>
<td>0.371 ± 0.121</td>
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<td></td>
<td>500</td>
<td>0.26 ± 0.10</td>
<td>0.124 ± 0.030</td>
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<td></td>
<td>1500</td>
<td>1.80 ± 0.73</td>
<td>0.504 ± 0.202</td>
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<td>Piroxicam</td>
<td>50</td>
<td>NDa</td>
<td>0.331 ± mean</td>
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<td>0.199 ± 0.134</td>
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<tr>
<td><strong>B. Late treatment (age 55–80 days)</strong></td>
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<tr>
<td>Control</td>
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<td>0</td>
<td>1.049 ± 0.329</td>
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<tr>
<td>Celecoxib</td>
<td>150</td>
<td>0.10 ± 0.01</td>
<td>1.083 ± 0.357</td>
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<tr>
<td></td>
<td>500</td>
<td>0.25 ± 0.01</td>
<td>0.229 ± 0.074</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>0.70 ± 0.26</td>
<td>1.031 ± 0.119</td>
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<tr>
<td>Piroxicam</td>
<td>50</td>
<td>0.05 ± 0.02</td>
<td>0.781 ± mean</td>
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<td>74</td>
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<td>0.224 ± 0.138</td>
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* ND, not determined.
tumors in that segment even in untreated animals, but a decrease was observed in the number of colon tumors after celecoxib treatment.

Discussion

The objective of this study was to examine the chemopreventive efficacy of celecoxib in a mouse model of colon tumorigenesis. Celecoxib (Celebrex) is one of a new class of inhibitors that specifically target COX-2 but not COX-1. These drugs were designed to exploit recently discovered subtle structural differences between the active sites of the COX isoenzymes (27). The rationale for examining the potential efficacy of celecoxib as a colon tumor chemopreventive agent was at least 3-fold. First, there are extensive data in animal models and substantial epidemiological data in humans that NSAIDs, which inhibit both forms of COX, are effective inhibitors of colon carcinogenesis. Second, the specific COX-2 inhibitor used in this study does not appear to have certain of the side effects associated with NSAIDs that presumably result from the latter also inhibiting COX-1 activity nonspecifically (21). Third, the finding in animal models and humans that COX-2 is overexpressed in colon adenomas and cancers predicts that compounds targeted against this enzyme specifically are likely to be effective against colon tumors (28–30).

Celecoxib has been shown to prevent azoxymethane-induced colon tumors in the rat. Unfortunately, the applicability of that study to FAP is uncertain because it is not known whether that carcinogen-induced tumors in the rat. Unfortunately, the applicability of that study to FAP is uncertain because it is not known whether that carcinogen-induced tumorigenesis. Second, the specific COX-2 inhibitor used in this study does not appear to have certain of the side effects associated with NSAIDs that presumably result from the latter also inhibiting COX-1 activity nonspecifically (21). Third, the finding in animal models and humans that COX-2 is overexpressed in colon adenomas and cancers predicts that compounds targeted against this enzyme specifically are likely to be effective against colon tumors (28–30).

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Piroxicam and other NSAIDs inhibit thromboxane B2 and cause GI
celecoxib actually gained slightly more weight than their untreated
weight loss or impairment of weight gain was observed at any of the
complete absence of any GI ulcerations or bleeding. Furthermore, no
expected, is further supported by our physiological data indicating a
COX-1 activity in platelets. Our biochemical evidence for lack of
piroxicam at a dose of 50 ppm reduced thromboxane B2 levels to
their nonspecific inhibition of COX-1. We have shown previously that
plasma thromboxane B2. This prostanoid is generated solely by
effects we observed were in the absence of any significant effects on
in a range that is well tolerated in humans. The tumor suppression
in a mouse model of APC and strongly support ongoing
cs were initially presented in abstract form (31), a short-term
indicates that piroxicam nonspecifically inhibits both isoenzymes, but
previous piroxicam thromboxane and tumor response data are in good
Barboms in a mouse model of FAP at a dose of 400 mg twice daily. Because the
broader population of patients with common sporadic adenomas have
somatic mutations of the same gene (APC), they might also benefit from a similar treatment approach. The combination of clinical data in
FAP patients, combined with the efficacy in Min mice reported in the
present study and in the AOM rat model, strongly supports the clinical
tests presently being initiated to examine the efficacy of celecoxib in
preventing the recurrence of adenomas in individuals after polypec-
tomy of one or more sporadic adenomas.

Acknowledgments

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