Sulindac Sulfone Inhibits Azoxymethane-induced Colon Carcinogenesis in Rats without Reducing Prostaglandin Levels

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ABSTRACT

Nonsteroidal anti-inflammatory drugs (NSAIDs), such as sulindac, have cancer chemopreventive properties by a mechanism that has been suggested to involve cyclooxygenase inhibition and reduction of prostaglandin (PGE₂) levels in the target tissue. To test this hypothesis, we studied the effect of dietary sulindac sulfone (500–2000 ppm), a metabolite of sulindac reported to lack cyclooxygenase inhibitory activity, on tumor formation and PGE₂ levels in the azoxymethane model of colon carcinogenesis. Rats treated with sulindac at 400 ppm and piroxicam at 150 ppm were used as positive controls. Rats received two s.c. injections of azoxymethane (15 mg/kg) for 2 weeks and were fed either experimental or control diets until necropsy. After 31 weeks of sulfone treatment, a dose-related increase in sulfone levels in both serum and cecal contents was measured; there was no evidence of metabolic conversion to sulindac or other metabolites. Rats treated with sulfone at 1000 and 2000 ppm, sulindac, and piroxicam had significantly fewer colonic adenomas and carcinomas compared with rats fed control diet as measured by tumor incidence, multiplicity, and tumor burden. Sulfone-treated rats also showed a dose-response relationship for inhibiting all tumor parameters. Colonos from rats treated with sulindac or piroxicam contained PGE₂ levels that ranged from approximately 16–49% of control levels. Moreover, the effects of sulindac sulfone on various enzymes responsible for regulating prostaglandin levels were evaluated. No significant inhibitory effects were observed for cyclooxygenase, lipoxygenase, or phospholipase A₂. These results suggest that reduction of prostaglandin levels in the target tissue may not be necessary for the chemopreventive properties of sulindac.

INTRODUCTION

Several lines of evidence suggest that NSAIDs have cancer chemopreventive properties. Studies using cultured tumor cell lines have shown that a variety of NSAIDs inhibit cell growth, cause cell cycle arrest, and induce apoptosis (1–7). Certain NSAIDs have also been demonstrated to inhibit tumor formation in rodent models involving either transplanted tumors (8, 9) or chemically and radiation-induced carcinogenesis (10–19). In APC patients, the NSAID sulindac, has been shown to cause regression of and prevent recurrence of adenomatous colorectal polyps, the precursor lesion of large bowel cancer (20–28). Indomethacin, a congener of sulindac, introduced by suppository also regresses adenomas in APC patients (29). Finally, several epidemiological studies suggest a protective effect of aspirin against colorectal cancer incidence and mortality (30–36). Although no benefit was apparent in two cohort studies (37, 38), aspirin use has also been associated with a reduced risk of harboring sporadic large bowel adenomas and for adenoma recurrence (35, 39).

The biochemical basis for the antineoplastic properties of NSAIDs is often attributed to a reduction of prostaglandin levels in the target tissue by inhibiting cyclooxygenase, the enzyme that catalyzes the formation of prostaglandin precursors from arachidonic acid. Prostaglandins and other products from arachidonic acid are potent mediators of numerous biological processes and may influence tumor progression (40–42). Previous studies have shown that various tumors contain or produce high levels of prostaglandins, particularly PGE₂ (43, 44). Accordingly, inhibition of prostaglandin synthesis is one mechanism whereby NSAIDs might block tumor formation. Piroxicam and aspirin have, in fact, been shown to reduce colonic PGE₂ levels in rodents and humans (43, 45). Indeed, Reddy et al. (18) demonstrated that piroxicam inhibition of colon tumors in experimental animals correlated with reduced tumor PGE₂ levels.

There are, however, studies that have reported findings that are inconsistent with the hypothesis that cyclooxygenase inhibition is necessary for the cancer chemopreventive properties of NSAIDs. Perhaps the most compelling evidence to support this possibility comes from studies involving sulindac metabolites. Sulindac (sulfoxide) is ingested as a prodrug that is devoid of cyclooxygenase-inhibitory activity. The sulfoxide is reversibly reduced within the liver and in the colon by bacterial microflora to a sulfide, which is a potent inhibitor of cyclooxygenase and is fully responsible for the anti-inflammatory properties of sulindac (46–49). The sulfide is also irreversibly oxidized within the liver to a sulfone metabolite that is excreted in bile and concentrated in feces. The structure of sulindac and the sulfide and sulfone metabolites are shown in Fig. 1. The sulfone lacks anti-inflammatory activity and does not inhibit cyclooxygenase types I or II (50, 51). Despite this, cell culture studies have demonstrated that the sulfone is capable of inhibiting tumor cell growth and inducing apoptosis (4). The sulfone was only 2–4-fold less potent than the sulfide, suggesting that cyclooxygenase inhibition does not mediate the in vitro properties of sulindac metabolites. The first evidence that sulindac sulfone had cancer chemopreventive activity was described in a report by Thompson et al. (51), which demonstrated that dietary sulfone treatment inhibited tumor formation in a rodent model of mammary carcinogenesis. Subsequently, Charalambous and O'Brien (52) demonstrated that sulindac sulfone inhibited early preneoplastic lesions (aberrant crypt foci) in the AOM model of colon carcinogenesis. However, no studies have been performed to determine whether sulindac sulfone inhibits colon tumor formation. The present study was, therefore, designed to investigate this possibility and to determine whether the effect was mediated by reduced prostaglandin levels in the colon. The effect of the sulfone was compared with the NSAIDs, piroxicam and sulindac. In addition, potential inhibitory effects of sulindac sulfone on cyclooxygenase,
lipoygenase, and phospholipase A\textsubscript{2} were evaluated within a wide dose range.

**MATERIALS AND METHODS**

**Chemicals.** Piroxicam and AOM (CAS:25843-45-2) were purchased from Sigma Chemical (St. Louis, MO), and sulindac was from Therapicon (Milan, Italy). Sulindac sulfone (lot no. MED178-002) was synthesized from sulindac by Medea Laboratories (Port Jefferson Station, NY) as described previously (2). Chemical analysis by HPLC, nuclear magnetic resonance, infrared spectroscopy, and elemental analysis demonstrated the lot to be 97.8% sulindac sulfone, 1.4% sulindac, and 0.8% sulindac sulfone epoxide.

**Diets.** The control (AIN-76A) and experimental diets in the form of pellets were prepared by Dyets, Inc. (Bethlehem, PA). Experimental diets were made to specified drug concentrations on a weight:weight basis (ppm) and stored at \(-20^\circ\text{C}\) after overnight shipment. Fresh diets were prepared every 3-6 weeks. The stability of sulindac sulfone mixed with the diet was determined in the 2000-ppm diet by HPLC analysis. To extract sulindac sulfone from the diet, a 5-g sample of diet was mixed with extraction medium (10 ml water-saturated ethyl acetate with 10% acetic acid) and sonicated for 2 h. An aliquot of the supernatant was then diluted 1:10 with acetonitrile, centrifuged for 5 min (12,000 \(\times\) g), and analyzed by HPLC as described below. Recovery of sulindac sulfone from spiked control food samples was 80—85%. Sulfone mixed in food was stable at room temperature for at least 1 month.

**Animals and Maintenance.** Male inbred F344 rats (Harlan F344-NHsd) were purchased from Harlan Laboratories (Indianapolis, Indiana). Six-week-old male F344 rats were quarantined for 3 days and randomly distributed by weight into various dietary groups with 30 rats in each of the treatment groups. Rats were housed three per cage under controlled conditions of a 12-h light and 12-h dark cycle at 21°C. The rats had free access to food and water, and food trays were replenished once weekly. Food consumption was calculated by dividing the food consumed weekly by each group, over the 29 weeks (weeks 3-31) when rats had access to the experimental diets, by the total number of rat feeding days for each group. The animal treatment protocol was reviewed by the Institutional Animal Care and Use Committee at the University of Arizona, which operates under guidelines set forth by the Public Health Service and the American Association for Laboratory Animal Care.

**Experimental Procedure.** The experiment was designed to determine the effect of dietary sulindac sulfone at 500, 1000, and 2000 ppm. These doses were selected based on results from previous feeding studies that determined these doses were below the maximum tolerated dose. The NSAIDs, sulindac at 400 ppm and piroxicam at 150 ppm, were included as positive controls. These doses were selected based on previous reports from the literature that showed an antitumor effect with minimal toxicity (12, 17). At 7 weeks of age, rats designated for the carcinogen groups received two consecutive weekly s.c. injections of AOM (15 mg/kg body weight, diluted in saline) for a combined total dose of 30 mg/kg/rat. All rats were fed the AIN-76A control diet until 1 day after the second AOM injection and were then maintained on the control diet or transferred to experimental diets, which were continued until the termination of the experiment. Body weights were recorded every 2 weeks, and food consumption was measured weekly for each cage beginning 2 weeks after the second AOM treatment. Rats dying or moribund were necropsied before the scheduled termination. The experiment was terminated 31 weeks after the second AOM injection to minimize distress to the animals.

After CO\textsubscript{2} euthanasia, the small intestine and large intestine were resected, cleansed, and examined; the location and size of all tumors identified by visual inspection were recorded. Tissues were fixed in 10% buffered formalin, embedded in paraffin, and stained with H&E. Neoplasms were classified as adenomas if there was no evidence of invasion through the muscularis mucosa. Adenocarcinoma ranged from well to poorly differentiated malignant tumors invading across the muscularis mucosa and were frequently mucinous adenocarcinomas.

**Sulindac Sulfone Levels in Cecal Contents and Serum.** Lumenal contents from the cecum were obtained from all rats in the sulindac, sulfone, and AOM control groups at scheduled necropsy; blood samples were randomly collected from 10 animals in each group. Acetonitrile (100 \(\mu\)l) was added to a sample of either serum (100 \(\mu\)l) or cecal contents (100 mg wet weight). The mixtures were vortexed for 1 min and centrifuged (12,000 \(\times\) g) for 5 min, and the supernatant was analyzed by HPLC with a Perkin-Elmer chromatograph (model LC-250) using a Perkin-Elmer 15B fixed wavelength UV detector at 254 nm and a 5 \(\mu\)m C\textsubscript{18} column (15 cm; Perkin-Elmer). The mobile phase was acetonitrile:water (17:83), pH 4.0 (47%;53%), with a flow rate of 1.5 ml/min. Triphenylacetic acid was used as an internal standard. The limit of detection for sulindac sulfone was 50 ng, and the retention times of sulindac sulfone and sulindac (Sulfoxide) were 80—85%.

**Sulindac Sulfone Levels in Cecal Contents at 2000 ppm**

- Sulindac sulfone levels were measured in cecal contents at 2000 ppm using HPLC analysis. The retention times of sulindac sulfone and sulindac (Sulfoxide) were determined.

**Sulindac Sulfone Levels in Serum**

- Serum samples were collected from 10 animals in each group. Acetonitrile (100 \(\mu\)l) was added to a sample of either serum (100 \(\mu\)l) or cecal contents (100 mg wet weight). The mixtures were vortexed for 1 min and centrifuged (12,000 \(\times\) g) for 5 min, and the supernatant was analyzed by HPLC with a Perkin-Elmer chromatograph (model LC-250) using a Perkin-Elmer 15B fixed wavelength UV detector at 254 nm and a 5 \(\mu\)m C\textsubscript{18} column (15 cm; Perkin-Elmer). The mobile phase was acetonitrile:water (17:83), pH 4.0 (47%;53%), with a flow rate of 1.5 ml/min. Triphenylacetic acid was used as an internal standard. The limit of detection for sulindac sulfone was 50 ng, and the retention times of sulindac sulfone and sulindac (Sulfoxide) were determined.

**Colonic PGE\textsubscript{2} Levels.** PGE\textsubscript{2} levels in colonic strips from both the proximal and distal colon were assayed by RIA as described previously (53). The reproducibility, sensitivity, and linearity of this assay was determined previously using human tissue (53). All samples were processed promptly after collection in an identical manner. As described previously (53), indomethacin was added during processing and during the assay to prevent changes in PGE\textsubscript{2} content as a result of endogenous cyclooxygenase. The presence of indomethacin was previously shown not to interfere with the sensitivity of the assay or efficiency of extracting PGE\textsubscript{2} from colonic tissue. Immediately after the colon was harvested, opened longitudinally, and rinsed with water, 2-3-mm wide strips of proximal and distal colon were cut with a scalpel and placed in a solution containing indomethacin (5 \(\mu\)g/ml). Samples were promptly snap frozen in liquid nitrogen and stored at \(-80^\circ\text{C}\).

**Effect of Dietary Sulindac Sulfone on Colon Carcinogenesis**

- Sulindac sulfone at 500, 1000, and 2000 ppm inhibited colon carcinogenesis in rats. The bowel was examined for tumors, and the location and size of all tumors identified by visual inspection were recorded. Tissues were fixed in 10% buffered formalin, embedded in paraffin, and stained with H&E. Neoplasms were classified as adenomas if there was no evidence of invasion through the muscularis mucosa. Adenocarcinoma ranged from well to poorly differentiated malignant tumors invading across the muscularis mucosa and were frequently mucinous adenocarcinomas.

**Sulindac Sulfone Epoxide**

- Sulindac sulfone epoxide was a metabolite generated from sulindac sulfone in the colon. The presence of sulindac sulfone epoxide was confirmed using HPLC analysis. The retention times of sulindac sulfone and sulindac (Sulfoxide) were determined.

**Conclusion.** Sulindac sulfone at 500, 1000, and 2000 ppm inhibited colon carcinogenesis in rats. The bowel was examined for tumors, and the location and size of all tumors identified by visual inspection were recorded. Tissues were fixed in 10% buffered formalin, embedded in paraffin, and stained with H&E. Neoplasms were classified as adenomas if there was no evidence of invasion through the muscularis mucosa. Adenocarcinoma ranged from well to poorly differentiated malignant tumors invading across the muscularis mucosa and were frequently mucinous adenocarcinomas.
frozen and stored in liquid nitrogen until assayed (up to 10 months), which previously was shown not to affect the stability of PGE₂ (53). Upon assaying, the frozen biopsies were placed in a 1.0-ml solution containing 0.05 M Tris-HCl buffer (pH 7.4) and 5 μg/ml indomethacin. Tissue was homogenized by hand in a siliconized 5.0-ml glass/glass tissue grinder for 30 s, and 100 μl were removed for protein determinations. Two ml of 100% ethanol were added to the remaining homogenate and allowed to stand for 5 min on ice. Distilled water (10.3 ml) was added to the homogenate (final concentration, 15% ethanol), and the sample was centrifuged 10 min at 400 × g at 4°C. The supernatant was removed, and the pH was adjusted to 3.0 with 0.25 M HCl. The sample was then applied to a C₁₈ silica column washed previously with 20 ml of 100% ethanol followed by 20 ml distilled water. PGE₂ was eluted by gravity with 10 ml of methyl formate. Extraction efficiency was previously determined to be consistently greater than 90% (53). The methyl formate was divided into four equal 2.5-ml aliquots, dried under nitrogen, and stored at −80°C. Samples were reconstituted in 0.25 ml of assay buffer and assayed for PGE₂ content using a DuPont 1251 PGE₂ RIA kit.

Enzyme Assays. Effects of sulindac sulfide and sulfone on cyclooxygenase (54), 5-lipoxygenase (55), 15-lipoxygenase (56), and phospholipase A₂ (57) were determined using methods described previously.

Statistical Analysis. Poisson regression (58) was used to determine whether there was a dose-response relationship for the sulindac sulfone groups on colon tumor multiplicity and to compare the AOM control group to each of the treatment groups for colon tumor multiplicity. The sum of the sizes of the tumors, referred to as tumor burden, was calculated for each animal. Whereas analysis of covariance was used to test if there was a dose-response relationship for the sulindac sulfone groups on colon tumor burden, ANOVA was used to compare the AOM control group to each of the treatment groups. Logistic regression (58) was used to test whether there was a dose-response for the sulindac sulfone groups on colon tumor incidence and to compare the AOM control group to each of the treatment groups for colon tumor incidence. Because the majority of animals lacked small intestine tumors, a Wilcoxon Rank sum test was used to analyze small intestine tumor multiplicity and burden, and an exact unconditional test (59) was used for tumor incidence. t tests were used to test for differences between the AOM control and other groups for weights at the beginning and end of the study, overall food consumption, and PGE₂ levels. Linear regression was used to determine whether levels of the sulfone in serum and cecal contents were related to dietary sulfone levels.

RESULTS

General Observations. Mean body weights for the various experimental groups during the course of the study are shown in Fig. 2. In rats treated with sulindac sulfone at 2000 ppm, mean body weight was slightly lower than the AOM control group from the onset of exposure through week 31. At the termination of the study, the weights of rats treated with experimental diets were compared by t test to the AOM control groups. Significant differences in final rat weights were evident only for the 2000-ppm sulfone group (380.70 ± 7.96 g) compared to the AOM control group (415.92 ± 7.41 g; P = 0.0021). Daily food consumption did not differ among drug-treated groups with an average food consumption of 14.88 ± 0.40 g/rat/day (data not shown). AOM control rats consumed significantly less food than all other groups by separate t test (13.97 ± 0.55; all P < 0.05).

Three rats died during the study, and three were euthanized because of secondary head tumors prior to the scheduled necropsy. These included three animals from the 2000-ppm sulfone group, one from the piroxicam group, and two from the AOM control group. Of the three animals that died early in the 2000-ppm sulfone group, one was shown). AOM control rats consumed significantly less food than all other groups by separate t test (13.97 ± 0.55; all P < 0.05). Three rats died during the study, and three were euthanized because of secondary head tumors prior to the scheduled necropsy. These included three animals from the 2000-ppm sulfone group, one from the piroxicam group, and two from the AOM control group. Of the three animals that died early in the 2000-ppm sulfone group, one was euthanized after 25 weeks for a head tumor without any other macroscopic abnormalities, one died spontaneously with an obstructing cecal carcinoma after 29 weeks, and the third died spontaneously after 25 weeks without any tumors. One of the AOM control animals died spontaneously after 29 weeks with small intestine and colon tumors, and a second animal was euthanized after 30 weeks for a head tumor without other lesions.

Table 1 Concentrations of sulindac sulfone in serum and cecal contents at the time of necropsy

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dietary concentration (ppm)</th>
<th>n¹</th>
<th>µg/ml ± SE</th>
<th>µg/ml ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulindac sulfone</td>
<td>500</td>
<td>10</td>
<td>92 ± 5</td>
<td>0.96 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>10</td>
<td>129 ± 9</td>
<td>1.24 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>9</td>
<td>146 ± 8</td>
<td>2.59 ± 0.14</td>
</tr>
<tr>
<td>Sulindac</td>
<td>400</td>
<td>10</td>
<td>45 ± 5</td>
<td>0.50 ± 0.03</td>
</tr>
</tbody>
</table>

¹ Number of animals assayed.
SULINDAC SULFONE INHIBITS COLON CARCINOGENESIS

Table 2: AOM-induced colon tumor incidence and multiplicity in male F344 rats fed sulindac sulfone, sulindac, or piroxicam

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Dietary concentration (ppm)</th>
<th>Animals/Group</th>
<th>Colon tumor incidence (%)</th>
<th>Colon tumor multiplicity (mean tumors/animal ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adenoma</td>
<td>Carcinoma</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>30</td>
<td>76.7</td>
<td>53.3</td>
</tr>
<tr>
<td>Sulfone</td>
<td>500</td>
<td>30</td>
<td>60</td>
<td>63.3</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>30</td>
<td>40.0</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>30</td>
<td>20.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Sulindac</td>
<td>400</td>
<td>30</td>
<td>30.0</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>30</td>
<td>33.3</td>
<td>23.3</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>150</td>
<td>30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adenomas and carcinomas.

**Significantly different from the control diet (P < 0.05; tumor multiplicity by Poisson regression and tumor incidence by logistic regression).

Table 3: AOM-induced colon tumor burden in male F344 rats fed sulindac sulfone, sulindac, or piroxicam

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dietary concentration (ppm)</th>
<th>Animals/Group</th>
<th>Mean colon tumor burden (±SD)</th>
<th>Colon tumor multiplicity (mean tumors/animal ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adenoma</td>
<td>Carcinoma</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>30</td>
<td>3.70 ± 3.26</td>
<td>6.77 ± 10.80</td>
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<tr>
<td>Sulfone</td>
<td>500</td>
<td>30</td>
<td>3.37 ± 3.87</td>
<td>3.53 ± 3.73</td>
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<tr>
<td></td>
<td>1000</td>
<td>30</td>
<td>1.50 ± 2.16</td>
<td>1.77 ± 2.96</td>
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<tr>
<td></td>
<td>2000</td>
<td>30</td>
<td>0.70 ± 1.6</td>
<td>1.17 ± 4.10</td>
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<tr>
<td>Sulindac</td>
<td>400</td>
<td>30</td>
<td>0.83 ± 1.46</td>
<td>1.10 ± 3.06</td>
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<tr>
<td></td>
<td>500</td>
<td>30</td>
<td>1.03 ± 1.61</td>
<td>1.57 ± 3.62</td>
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<tr>
<td>Piroxicam</td>
<td>150</td>
<td>30</td>
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</table>

*Sum of tumor sizes (cm).

**Adenoma or carcinoma.

*P = 0.055.

**Significantly different from the control diet by t test, P < 0.05.

Sulindac Sulfone in Serum and Cecal Contents. A linear relationship was evident between cecal sulfone levels and diet concentration (r² = 0.99), whereas serum sulfone levels and diet concentrations demonstrated a quadratic relationship (r² = 0.74; Table 1). No other metabolites were seen in either the serum or cecal contents of sulfone-treated rats using HPLC analysis. The mean cecal and serum concentration of sulfone in rats fed 400 ppm sulindac was lower than in all of the sulfone-treated groups. The sulindac-treated rats also had detectable serum levels of both sulindac sulfoxide (5.5 ± 0.8 µg/ml) and sulindac sulfide (trace). Sulindac sulfide was also present in the cecal contents of sulindac-treated rats (0.27 ± 0.02 mg/g).

Colon Tumor Incidence, Multiplicity, and Burden. Rats fed 2000 ppm sulfone contained significantly fewer colonic adenomas and carcinomas than either control tumors from all of the sulfone-treated groups. The sulindac-treated rats also had detectable serum levels of both sulindac sulfoxide (5.5 ± 0.8 µg/ml) and sulindac sulfide (trace). Sulindac sulfide was also present in the cecal contents of sulindac-treated rats (0.27 ± 0.02 mg/g).

Enzyme Effects. Effects of sulindac sulfone and sulindac on various enzymes known to regulate prostaglandins were evaluated to confirm that the chemopreventive properties of sulindac sulfone were not mediated by altered levels of prostaglandins or other eicosanoids. As shown in Fig. 3, the sulfone did not affect cyclooxygenase (Type I) activity at doses up to 10 mM. In contrast, the sulfide was a highly effective inhibitor with an IC₅₀ of 1.76 µM. Lipoxigenase and phospholipase A₂ were also evaluated for potential inhibition by sulindac sulfone and sulindac. As summarized in Table 5, the sulfone did cause marked inhibition of either 5- or 15-lipoxygenase or phospholipase A₂. Sulindac sulfide caused significant inhibition of 5-lipoxygenase but did not affect either 15-lipoxygenase or phospholipase A₂.

DISCUSSION

The purpose of this study was to test the hypothesis that reduction of prostaglandin levels in the target tissue is necessary for the cancer chemopreventive properties of sulindac, and more generally, other NSAIDs. The approach was to evaluate the sulfone metabolite of sulindac that has been reported to lack cyclooxygenase inhibitory activity in the AOM model of colon carcinogenesis. Although higher doses were required relative to the NSAIDs piroxicam and sulindac, the sulfone demonstrated a dose-dependent reduction in colon tumor incidence, tumor multiplicity, and tumor burden. In addition, the efficacy of sulindac sulfone was not accompanied by a significant reduction of PGE₂ levels, whereas the efficacy observed with both piroxicam and evaluation of large sample numbers allowed for statistical analysis of the results. Relative to control levels, sulindac significantly reduced PGE₂ levels in the proximal colon to 30% and in the distal colon to 49% (Table 4). Piroxicam caused a more pronounced reduction with levels relative to control of 16% in the proximal colon and 19% in the distal colon. In rats treated with sulindac sulfone up to 2000 ppm, no statistically significant reduction of PGE₂ levels in either the proximal or distal colon was observed.
Table 5 Effects of sulindac sulfide and sulfone on lipoxygenase and phospholipase A2 activity

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Drug</th>
<th>Concentration (μM)</th>
<th>% inhibition</th>
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<tr>
<td>5-Lipoxygenase</td>
<td>Arachidonic acid</td>
<td>Sulfide</td>
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<td></td>
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<td></td>
<td>10</td>
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<td>Sulfide</td>
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</tbody>
</table>

Sulindac was accompanied by significant reduction of PGE2 levels. No inhibitory effects were observed for purified cyclooxygenase (type I) at doses up to 10 mM and are consistent with previous reports showing lack of inhibition of either the type I or II forms of cyclooxygenase (50, 51). Moreover, other enzymes that potentially regulate prostaglandin levels in vivo, such as lipoxygenase and phospholipase A2, were evaluated and found to be unaffected by sulfone treatment.

Sulindac is considered a prodrug because metabolism is required to generate active anti-inflammatory forms of the drug. Upon ingestion, sulindac (sulfoxide) is reduced within the liver and in the colon by bacterial microflora to the sulfide metabolite (46–49). This reaction is reversible to regenerate the sulfoxide upon oxidation. Sulindac is also oxidized within the liver to the sulfone metabolite, which is excreted in bile and concentrated in feces. The oxidation of the sulfoxide to the sulfone is considered to be irreversible, and it is unlikely that oral administration of sulindac sulfone could be reduced to the sulfoxide and ultimately to the sulfide. However, because the sulfide is at least 5000-fold more potent than the sulfone for inhibiting cyclooxygenase, it was necessary to confirm that inhibition of tumor formation by sulindac sulfone was not attributed to metabolic conversion to the sulfoxide or sulfide. In the rats fed sulfone-containing diets, concentrations of the sulfone in serum and cecal contents increased in a dose-related manner with undetectable levels of the sulfide or sulfone. In contrast, dietary sulindac treatment produced measurable amounts of sulfide and sulfone in cecal contents and detectable levels of sulfone and sulfoxide in serum. The levels of the sulfone in cecal contents and serum of sulindac-treated rats were lower than those seen in any of the sulfone treatment groups and suggest that the efficacy of sulindac cannot be attributed to the sulfone metabolite alone. Rather, the efficacy of sulindac likely involves an additive effect of the sulfoxide, sulfone, and sulfide because each of these forms of the drug are known to have variable degrees of potency for inhibiting the growth of cultured colon tumor cells (2).

Three potential variables might have influenced our results: (a) rats in the 2000-ppm sulfone group grew at approximately 90–95% of the control rate, and it has been documented that a profound reduction of body weight can inhibit the formation of chemically induced intestinal tumors (60). However, it has been shown in the AOM model using male F344 rats that restrictions of caloric intake of 20–30% are required to inhibit tumor formation (61), suggesting it is unlikely that the slight reduction of weight gain caused by 2000 ppm sulfone...
treatment confounded the results. Moreover, lower doses of sulfine that did not alter weight gain showed a significant reduction of tumorigenesis; (b) early animal deaths occurred primarily in the 2000 ppm sulfine group and AOM control groups, making it possible that late tumor formation in these animals might have altered the results. However, the statistical conclusions relating to tumor multiplicity and burden were not altered when results were adjusted for an early time of death. For colon tumor incidence, the inhibitory effect of high-dose sulfine compared to the control group remained significant for all neoplasia combined, even in the worst case scenario (e.g., all early dying sulfine animals with none of the AOM control animals eventually developing tumors); and (c) the sulindac sulfine lot contained impurities (1.4% sulindac and 0.8% sulindac sulfine epoxide) that could possibly influence the outcome of the study. However, the dietary levels of these impurities were very low, even in the highest dose sulindac sulfine group (28 ppm sulindac and 16 ppm sulindac sulfine epoxide), and these low doses were unlikely to significantly contribute to the tumor inhibitory effect.

In conclusion, sulindac sulfine inhibits colon tumor formation in the AOM model of carcinogenesis despite a lack of cyclooxygenase inhibitory activity. Although higher doses are required to achieve a positive effect relative to the potent cyclooxygenase inhibitors piroxicam and sulindac sulfine, sulindac sulfine has the distinct advantage of having fecal excretion as a major route of metabolic clearance. This allows delivery of high drug concentrations to the colonic mucosa. The cellular and biochemical mechanism(s) whereby sulindac sulfine inhibits tumor formation in vivo remains to be elucidated. We have shown recently that the mechanism may involve an increase in cell death by an apoptotic mechanism rather than a decrease in cell proliferation, (i.e., cell cycle arrest) or altered differentiation (4).

These results support the potential utility of sulindac sulfine as a chemopreventive agent, particularly in light of the ability of sulindac to promote regression and prevent recurrence of adenomatous polyps in APC patients. Because the gastrointestinal toxicity associated with NSAIDs is attributed to reduction of systemic prostaglandin levels and a loss of cytoprotective capacity in the gastrointestinal tract (62, 63), a compound, such as the sulfine, that retains antineoplastic properties of NSAIDs but lacks cyclooxygenase inhibitory activity would theoretically offer a safety advantage, particularly for chronic use as a cancer chemopreventive drug.

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REFERENCES

anti-inflammatory drug use and the risk of subsequent colorectal cancer. Arch. Int.

35. Suh, O., Mettlin, C., and Petrelli, N. J. Aspirin use, cancer, and polyps of the large

W. C. Aspirin use and the risk of colorectal cancer and adenoma in the male health

37. Pagani-Hill, A., Chao, A., Ross, R. K., and Henderson, B. E. Aspirin use and

38. Gann, P. H., Manson, J. E., Glynn, R. J., Buring, J. E., and Hennekens, C. H.
Low-dose aspirin and incidence of colorectal tumors in the randomized trial. J. Natl.

risk of large-bowel adenomas among aspirin users. The Polyp Prevention Study


42. Marnett, L. J. Aspirin and the potential role of prostaglandins in colon cancer. Cancer

43. Earnest, D., Hixson, L., Fennerty, M. B., Emerson, S. S., and Alberta, D. S. Inhibition
of prostaglandin synthesis: potential for chemoprevention of human colon cancer.

44. Rigas, B., Goldman, I. S., and Levine, L. Altered eicosanoid levels in human colon

45. Kulkarni, N., Zang, E., Kelloff, O., and Reddy, B. S. Effect of the chemopreventive
agents piroxicam and D.L-a-difluoromethylomithine on intermediate biomarkers of

46. Duggan, D. E., Hare, L. E., Ditzler, C. A., Lei, B. W., and Kwan, K. C. The
disposition of sulindac sulphone is not dependent on inhibition of prostaglandin synthesis.

Chemical and biological studies on indomethacin, sulindac, and their analogs. Adv.
Sulindac Sulfone Inhibits Azoxyamphetamine-induced Colon Carcinogenesis in Rats without Reducing Prostaglandin Levels


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