

Effects of Piroxicam on Prostaglandin E₂ Levels in Rectal Mucosa of Adenomatous Polyp Patients: A Randomized Phase IIb Trial¹

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Abstract

Prostaglandin E₂ (PGE₂) has served as a surrogate end point biomarker in colorectal tumor progression. Colonic mucosa PGE₂ levels of patients with colorectal adenomas or carcinomas have been shown to be higher than in control subjects. Our dose-finding study on piroxicam, a nonsteroidal anti-inflammatory drug with chemopreventive effects in preclinical colon carcinoma models, suggested that 7.5 mg/day was well tolerated and associated with significant depression of rectal mucosa PGE₂ concentrations in comparison with baseline values. We therefore conducted a randomized Phase IIb cancer prevention clinical trial to investigate the chemopreventive properties of piroxicam in patients with a history of resected colorectal adenomatous polyps. After a 2-month run-in period, 47 participants were randomized to piroxicam at a dose of 7.5 mg/day, and 49 were randomized to a placebo. Rectal biopsy specimens were taken at the initial visit, at 2 months later during the run-in period, and at 6, 12, and 24 months after the start of the interventions. Mean PGE₂ concentrations in the rectal mucosa of the piroxicam-treated patients differed significantly between visits ($P < 0.001$), and the values at the 6-month visit ($P < 0.001$) and 12-month visit ($P = 0.005$) differed significantly from the average baseline value. Unfortunately, we observed an incidence of adverse gastrointestinal side effects in patients treated with 7.5 mg/day of piroxicam similar to that seen for arthritis patients treated with 20 mg/day. Consequently, the gastrointestinal toxicities appear to override the potential benefit that piroxicam may offer as a long-term colon cancer chemopreventive agent.

Received 4/26/00; revised 9/22/00; accepted 9/29/00.

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¹ Supported in part by Grants CA-41108 and CA-23074 Cancer Center Support Grant from NIH, Bethesda, MD 28092.

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Introduction

Colorectal carcinoma is the second most prevalent malignancy with the second highest cancer mortality rate in the United States (1). Improved screening methods and novel preventive approaches, such as NSAID³ use, are the two most promising strategies for reducing this death rate (2–4). Prostaglandins and the metabolites of arachidonic acid, well known as potent mediators of inflammation and other biological processes, may influence progression of various tumors including colorectal carcinoma (5–7). Increased prostaglandin production, particularly of the E series, has been reported in human colonic tumor cells in both humans and experimental models (5, 8–10). Individuals with colorectal adenomas or carcinomas have been shown to have higher levels of colonic mucosal PGE₂ than control subjects (11–13). Two groups have reported that PGE₂ levels were higher in adenomatous polyps and carcinoma of the colon than in normal mucosa adjacent to or 5–10 cm away from the tumor (14, 15).

The biochemical basis for the anti-inflammatory effects of NSAIDs is attributed to their inhibition of both COX-1 and COX-2, the constitutive (COX-1) and inducible (COX-2) isoforms of the enzyme that catalyze the formation of prostaglandin precursors in the arachidonic acid pathway (16, 17). This inhibition causes a reduction in prostaglandin production. Although their anti-inflammatory effects are well known, several lines of evidence suggest that NSAIDs may also have cancer chemopreventive properties (18–24). Cultured tumor cell line studies, for instance, have demonstrated that various NSAIDs inhibit cell growth, arrest the cell cycle, and induce apoptosis (18–24). Aspirin and indomethacin have been shown to inhibit tumor formation in murine models involving transplanted tumors (25, 26) or chemical- and radiation-induced carcinogenesis (27–36). Studies that involve adenomatous polyposis coli patients have demonstrated that sulindac prevents adenoma recurrence (37). Moreover, in these patients, sulindac and possibly indomethacin caused adenoma regression (38–46). A protective effect of aspirin against colorectal cancer incidence and mortality has been proposed in several epidemiological studies (3, 47–52), although a benefit was not seen in all instances (53, 54). Aspirin use has also been associated with a reduced risk of harboring sporadic large bowel adenomas and of adenoma recurrence (51, 55).

The antineoplastic mechanisms of NSAIDs, however, are not known. The cancer chemopreventive activity of sulindac sulfide and sulfone, for example, has been attributed to an inhibition of cell growth through apoptosis induction (21). Sulindac sulfone inhibited colon tumor formation in the azoxymethane model as effectively as sulindac without reducing prostaglandin levels or significant inhibitory effects on enzymes responsible for regulating prostaglandin levels (56). In contrast, sulindac, piroxicam, and aspirin have been shown to

³ The abbreviations used are: NSAID, nonsteroidal anti-inflammatory drug; PGE₂, prostaglandin E₂; COX, cyclooxygenase; BMI, body mass index.

reduce colonic PGE₂ levels in rodents and humans (56, 57). The inhibition of colon tumors in experimental animals treated with piroxicam has also been shown to correlate with reduced PGE₂ levels (35, 56). Piroxicam has been studied as a potential drug in preventing and treating various tumor types (58–70). Its effects on colon cancer also have been extensively studied (29, 57, 71–81) with one report of complete regression of villous adenomas in a 73-year-old man treated with piroxicam (82).

The present randomized Phase IIb clinical trial was designed to investigate the chemopreventive properties of piroxicam and long-term tolerability in patients with a history of resected colorectal adenomatous polyps.

Materials and Methods

Participants. Male and female participants, 40–80 years of age, residing in the Tucson metropolitan area were considered eligible if they had: one or more adenomatous colorectal polyps removed within the previous 6 months; no history of invasive cancer within 5 years; no severe metabolic disorders or other life-threatening acute or chronic diseases; and no additional therapy planned. Individuals were ineligible if they used aspirin or other NSAIDs or had adverse effects to these agents, had a history of a gastric or duodenal ulcer within 12 months before entry in the trial, or had a colon resection >40 cm or of the ileocecal valve. The study was approved by the University of Arizona Human Subjects Committee. Written informed consent was obtained from all participants before study entry.

Investigational Agents. Piroxicam, 7.5 mg tablets, and matched placebo tablets were kindly supplied by Pfizer (Groton, CT).

Treatment Plan. One-hundred and nine participants were randomized and underwent baseline rectal biopsies at their initial visit (visit 1) and 2 months later (visit 2). Of these original patients, 13 stopped taking the study drug for a variety of reasons and had unsatisfactory biopsies for determining PGE₂ concentration. Of the 96 participants remaining, 47 patients were randomized to piroxicam at a dose of 7.5 mg/day, and 49 were randomized to a placebo. Rectal biopsies were scheduled at 6 months (visit 3), 12 months (visit 4), and 24 months (visit 5) after the initiation of the interventions. In the piroxicam treated group, 44 patients were evaluated at 12 months, and 45 patients underwent a biopsy at 24 months. Of the 49 patients in the placebo group, 44 were evaluated at 12 months, whereas 37 were evaluated at 24 months.

PGE₂ Analysis. PGE₂ analysis for this study was performed as described previously (83). Briefly, during sigmoidoscopy, four biopsies were taken perpendicular to the mucosal surface from the upper half of the rectum. Specimens were placed in cryovials containing aqueous indomethacin and snap-frozen in liquid nitrogen. The tissue was thawed later and homogenized in an indomethacin buffer. Protein determination aliquots were taken, and extraction of a sample was performed. The ¹²⁵I-labeled PGE₂ RIA kit (DuPont NEN, Boston, MA) was used to determine the PGE₂ content in the extracted tissue, made up of one or two biopsies. Two sets of rectal biopsies, taken 8 weeks apart at visits 1 and 2 before randomization, as well as biopsies taken at visits 3, 4, and 5 after the start of the study were used in the analyses.

Statistical Analysis. Demographic and baseline characteristics were summarized using frequency counts and medians as appropriate. Homogeneity of the treatment groups with respect to sex was tested using Fisher's exact test. BMI was computed as the ratio of weight in kg to square of height in m. Wilcoxon's rank-sum test was used to test for differences in any of the demographic or baseline characteristics.

For each participant, the highest grade of severity experi-

Table 1 Patient demographics by assignment to placebo or piroxicam

	Placebo	Piroxicam	All
Total <i>n</i>	55	54	109
% male ^a (<i>n</i>)	55 (30)	70 (38)	62 (68)
Age ^b , yr	67	67	67
BMI ^b , kg/m ²	26.2	26.7	26.3
Energy ^b , kcal/day	1780	1890	1810
Protein ^b , g/day	72	77	75
Fat ^b , g/day	62	65	63
Carbohydrate ^b , g/day	224	233	230
Calcium ^b , mg/day	767	779	768
Dietary fiber ^b , g/day	18	22	19
Alcohol ^b , g/day	1.6	1.5	1.6

^a *P* = 0.11 for differing randomizations by gender (Fisher's exact test).

^b Median values reported. Medians did not differ between arms (Wilcoxon's rank-sum test *P*s all exceeded 0.25).

enced for each of 10 general toxicities and 9 blood/urine toxicities was determined, using only toxicity data arising from assessments performed after randomization and, of the general toxicities, those deemed either definitely, probably, or possibly study drug related by the research nurse. The most severe postrandomization toxicities were tabulated for each treatment group, and comparisons between groups were made by Fisher's exact test on counts of grade 0 versus not grade 0. Moreover, collectively looking at all toxicity categories, the Bonferroni adjustment for multiple comparisons was applied, resulting in the need to attain a *P* of 0.003 or less for statistical significance of a given toxicity rate differing between treatment groups.

Treatment regimen adherence was assessed by adherence percentage (the ratio of the difference between the number of pills supplied and the number of pills returned, divided by the number of days elapsed). Using the four adherence assessments made during visits 2–5, the Kruskal-Wallis test was used to gauge whether median adherence percentage differed between visits. The test was performed separately for each treatment group. Because testing for differences in median adherence percentage between treatment groups while controlling for the effect of the visit was difficult, treatment groups were compared only with respect to overall median adherence percentage, computed across all visits, using Wilcoxon's rank-sum test.

The effects of adherence percentage on blood piroxicam concentration and blood piroxicam concentration on rectal mucosal log PGE₂ concentration for the piroxicam arm were examined by computing Spearman correlations between blood piroxicam concentration and adherence percentage or PGE₂ concentration of visits 3, 4 and 5. Blood piroxicam concentration values were set to zero for the placebo arm. The mean and SE for the PGE₂ measurements were computed for each visit in each arm and plotted. The percentage reduction in PGE₂ concentration was computed as the ratio of the difference in geometric mean PGE₂ concentrations between visit 2 and each given intervention visit, divided by the geometric mean at visit 2. The data were missing nonrandomly attributable primarily to the placebo arm lacking more observations at visit 5 than the piroxicam arm. Because the incomplete data rates differed significantly between the arms, each treatment group needed to be modeled separately using a maximum likelihood repeated measures model to determine the effect of a visit on PGE₂ concentration. Although the placebo and piroxicam arms could not fit simultaneously into a single model, comparisons between arms were performed using *t* tests on the PGE₂ concentrations at each visit. Statistical significance was attained with

Table 2 Most severe postrandomization study drug-related^a gastrointestinal toxicities

Toxicity	Group ^b	Grade 0 n (%)	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)
Nausea and vomiting	Placebo	48 (88.9)	2 (3.7)	3 (5.6)	1 (1.9)
	Piroxicam	42 (77.8)	4 (7.4)	8 (14.8)	0 (0.0)
Abdominal pain ^d	Placebo	48 (88.9)	4 (7.4)	2 (3.7)	0 (0.0)
	Piroxicam	38 (70.4)	12 (22.2)	4 (7.4)	0 (0.0)
Heartburn or epigastric discomfort	Placebo	45 (83.3)	7 (13.0)	1 (1.9)	1 (1.9)
	Piroxicam	38 (70.4)	9 (16.7)	4 (7.4)	3 (5.6)
Diarrhea or constipation	Placebo	47 (87.0)	7 (13.0)	0 (0.0)	0 (0.0)
	Piroxicam	41 (75.9)	7 (13.0)	5 (9.3)	1 (1.9)

^a Represents toxicities considered by research nurse to be possibly, probably, or definitely study drug related.

^b Comparisons between groups made by Fisher's exact test on counts of grade 0 versus not grade 0. Unless otherwise noted, toxicity rate did not differ between groups.

^c The toxicity monitoring and management program was based on both available data in the literature and discussions with representatives of the drug manufacturer. In general, grade 0 meant no toxicity, and grades 1, 2, and 3 implied mild, moderate, and severe degrees of toxicity, respectively. In the case of nausea and vomiting, grade 2 = intermittent nausea or episodes of vomiting ≤ 1 time/day, and grade 3 = continuous nausea with episodes of vomiting ≥ 2 times/day. Grade 1 abdominal pain involved no medications, whereas grade 2 required evaluation, and grade 3 required a narcotic for pain control. In the case of diarrhea and constipation, grade 2 = daily symptomatic treatment and grade 3 = unexplained bloody diarrhea or new constipation unresponsive to bulking agent. With heartburn or epigastric discomfort, occasional antacid use was acceptable for a mild toxicity (grade 1), whereas grade 2 required daily antacids, and grade 3 was pain unresponsive to antacids or evidence of peptic ulcer, gastrointestinal bleeding, or gastric erosions.

^d $P = 0.03$; borderline significantly different between groups when adjusting for multiple comparisons.

Table 3 Study drug-related^a toxicities assessed^b

Clinical toxicity	Laboratory toxicity
Skin	Liver dysfunction
Fever	Hemoglobin
Headache	Differential WBC
Pulmonary	Platelets
Cardiac	Serum creatinine
General well-being	Other blood chemistries
	Urine protein
	Urine sediment

^a Represents toxicities considered by research nurse to be possibly, probably, or definitely study drug related.

^b Comparisons between groups made by Fisher's exact test on counts of grade 0 versus not grade 0. Toxicity rates did not differ between arms for any of the listed toxicities (all $P > 0.15$).

a P of 0.010 (yielding an overall probability of a type I error of 0.05), because of the need to adjust for multiple comparisons. Unless otherwise stated, statistical significance was defined as obtaining a P of 0.05 or less using two-sided tests.

Results

Demographics. Demographic and baseline characteristics of the treated and untreated subjects are summarized in Table 1. The proportion of males in the piroxicam-treated group (70%) was somewhat higher than that in the placebo group (55%; $P = .11$) but was not statistically different. The median age of the subjects (67 years) was similar to the age of individuals who are screened routinely in this country for colorectal adenomas and cancer. The BMI of the participants corresponded to that of non-participants of the same age. There were no statistically significant differences between the two randomized groups with respect to age, BMI, intakes of total energy, protein, fat, carbohydrates, calcium, dietary fiber, or alcohol with all P s exceeding 0.25. When adjusting for nutrient density, expressed in g/1000 kcal, the results were consistent with the g/day comparison.

Toxicities. Of 19 potential drug-related toxicities examined, gastrointestinal disturbances were the most important and were higher in incidence in the piroxicam group than in the placebo group (see Tables 2 and 3). Episodes of nausea, vomiting, and diarrhea, for example, were more frequent in participants taking piroxicam, as were the rates of abdominal pain and epigastric discomfort, in-

Table 4 Treatment regimen adherence

Visit	Placebo ^a	Piroxicam ^b
	Median adherence percentage (n)	Median adherence percentage (n)
2	98.2 (55)	96.4 (54)
3	98.2 (52)	98.2 (48)
4	97.6 (43)	98.8 (42)
5	98.8 (35)	98.8 (39)
Overall ^c	98.2 (185)	97.8 (183)

^a $P = 0.490$ for differing median adherence percentage between visits for the placebo arm (Kruskal-Wallis test).

^b $P = 0.003$ for differing median adherence percentage between visits for the piroxicam arm (Kruskal-Wallis test).

^c $P = 0.167$ for differing overall median adherence percentage between arms (Wilcoxon's rank-sum test).

cluding heartburn. On the basis of the eligibility requirements, all participants could not have a history of gastric or duodenal ulcers within 12 months before entering the study. In the piroxicam group, 16 participants (30%) suffered from gastritis as defined by a degree of heartburn or epigastric discomfort greater than grade 1 (see Table 2). Moreover, three male participants in this group (5.6%) were diagnosed with duodenal and cecal ulcers, with two of these participants (ages 74 and 75) diagnosed with duodenal ulcers measuring 1 and 1.5 cm by esophagogastroduodenoscopy. The third male patient, age 58, was diagnosed at 1-year colonoscopy to have an asymptomatic cecal ulcer that microscopically showed ischemic changes. In the placebo group, one participant (1.9%), age 47, with grade 3 toxicity was taken out of the study after 30 months. She had a history of adenocarcinoma of the sigmoid colon with lymph node invasion 5 years before the start of the study and was scheduled for an exploratory laparotomy for a new pelvic mass.

Treatment Adherence. Table 4 summarizes treatment regimen adherence which was excellent for both placebo- and piroxicam-treated participants with median adherence percentages across all visits of 98.2 and 97.8%, respectively ($P = 0.167$). The median adherence percentage did not differ significantly across visits for the placebo group ($P = 0.490$). The adherence percentage did differ significantly across visits for the piroxicam treated groups ($P = 0.003$), although the only substantial divergence from the overall median adherence per-

Table 5 Log concentrations of PGE₂ (pg/mg protein) in rectal mucosa of participants^a

	Clinic visit	Baseline 1	Baseline 2	Intervention 3 ^b (6 mo.)	Intervention 4 ^c (12 mo.)	Intervention 5 ^d (24 mo.)
Placebo ^e	<i>n</i>	41	40	42	44	37
	Mean	5.18	5.33	5.17	5.17	5.36
	SE	0.13	0.19	0.17	0.12	0.16
Piroxicam ^f	<i>n</i>	44	43	43	44	45
	Mean	5.19	5.19	4.48 ^g	4.78 ^g	4.85
	SE	0.14	0.14	0.12	0.14	0.12
% reduction in PGE ₂ ^h				50.8	33.6	28.8

^a Comparisons between means conducted within context of maximum likelihood repeated measures modeling (model estimated means not shown), unless otherwise stated.

^b $P = 0.001$ for means differing between arms (two sample *t* test).

^c $P = 0.033$ for means differing between arms (two sample *t* test).

^d $P = 0.012$ for means differing between arms (two sample *t* test).

^e $P = 0.632$ for differing means between visits for the placebo arm.

^f $P < 0.001$ for differing means between visits for the piroxicam arm.

^g $P \leq 0.005$ for mean differing from average of baseline visits 1 and 2.

^h $(\mu_2 - \mu_1)/\mu_2 \times 100\%$, where μ_1 is geometric mean (the value derived from exponentiation of the mean log PGE₂ concentration) of piroxicam arm PGE₂ concentration at visit 1.

centage was at placebo run-in visit 2. Spearman correlations between blood piroxicam concentration and treatment adherence percentage for visits at 6, 12, and 24 months were non-significant ($P > 0.30$). Similarly, when comparing PGE₂ concentration in rectal mucosal biopsies and blood piroxicam concentration, Spearman correlations at 6, 12, and 24 months of treatment were non-significant ($P > 0.35$).

Summary statistics for rectal mucosal biopsy PGE₂ concentrations are provided in Table 5 and the means (± 1 SE) are plotted in Fig. 1. When repeated measures analysis was applied to the placebo arm, no visit effect was detected ($P = 0.632$), which implies stability of rectal mucosal PGE₂ levels. In contrast, the mean concentration of rectal mucosal PGE₂ levels in the piroxicam-treated participants differed significantly from baseline values ($P < 0.001$). Moreover, at the 6-month visit ($P < 0.001$) and the 12-month visit ($P = 0.005$) of treatment, PGE₂ rectal mucosal concentrations differed significantly from their average baseline values. Although no statistical difference was seen at 24 months after starting treatment ($P = 0.019$), the mean PGE₂ rectal mucosal concentration in the piroxicam study participants remained notably lower than the baseline value. Comparisons of mean rectal mucosal PGE₂ concentrations between the placebo treatment group and piroxicam treatment group at each visit demonstrated that at 6 months (visit 3) of piroxicam treatment, there were significantly different means ($P = 0.001$). Lastly, as shown in Table 5 and Fig. 1, the observed percentage reduction in PGE₂ concentrations, relative to baseline, were greatest at 6 months (visit 3) and 12 months (visit 4) of piroxicam treatment with decreases of 50.8 and 33.6%, respectively.

Discussion

Piroxicam has been approved for use on a dosing schedule of 20 mg/day for the management of the complications of arthritis. At this dose level, piroxicam causes gastrointestinal symptoms in 20% of arthritis patients. Because it demonstrated colon cancer chemopreventive effects in preclinical models and could be taken once a day, we performed a Phase IIa dose-finding study of piroxicam in participants with a history of resected colorectal adenomas (75). This early study suggested that a piroxicam dose of 7.5 mg/day was well tolerated and associated with significant reductions in rectal mucosal PGE₂ concentrations in comparison with baseline values. The results of the present study, however, reveal that even the relatively low 7.5 mg/day piroxicam dose was associated with a 5.6% intestinal ulcer rate and common symptoms of gastritis in ~30% of our participants. As has been shown

in a recent meta-analysis, piroxicam was associated with the third highest relative risk of developing serious upper gastrointestinal complications in comparison with 11 other NSAIDs studied (84).

The statistically significant decrease in rectal mucosal PGE₂ concentrations seen in the piroxicam-treated participant group occurred during the first 12 months of treatment. The most pronounced reduction occurred at 6 months from the start of piroxicam, with statistically significant differences in mean rectal mucosal PGE₂ levels within the piroxicam-treated participants when compared with baseline concentrations and with rectal mucosal concentrations in the placebo group participants. Despite evidence of a relatively high rate of upper gastrointestinal toxicity in our participants, we did not see large reductions of rectal mucosal PGE₂ concentrations associated with the low dose piroxicam intervention. In fact, this relatively small piroxicam-induced PGE₂ concentration-lowering effect diminished over time, so that at 24 months of treatment the effect became quite small. Nevertheless, the safety data and the relatively small effect of piroxicam on rectal mucosal PGE₂ concentrations at the low dose of 7.5 mg/day argue against further exploration of piroxicam as a colon cancer preventive agent. On the basis of the safety data and the less than expected effect of piroxicam on rectal mucosal PGE₂ concentrations at the relatively low dose of 7.5 mg/day, we do not feel that piroxicam should be developed further as a colon cancer preventive agent.

Although median adherence percentage values did not differ significantly across visits for the placebo arm, median adherence percentages did differ significantly across visits in the piroxicam arm. No trend was seen, however, when blood piroxicam concentration and adherence percentages or blood piroxicam concentration and rectal mucosal PGE₂ levels were compared. The lack of a correlation is likely related to the variability of absorption of p.o. administered piroxicam and the variability of piroxicam inhibition of COX-1. Statistical limitations of the study were the small number of participants enrolled and the incomplete data for some patients at designated clinic visits. Statistical significance in rectal mucosal PGE₂ concentrations, however, was documented between the two study arms despite these limitations. In conclusion, our results show a decrease in the surrogate biomarker PGE₂ in the rectal mucosa of piroxicam-treated patients in comparison with the randomized placebo group. Unfortunately, we observed an incidence of adverse gastrointestinal side effects with 7.5 mg/day of piroxicam similar to that seen for arthritis patients treated with 20 mg/day. Consequently, the occurrence of these toxic effects appear to override any benefit that piroxicam may offer as a long-term

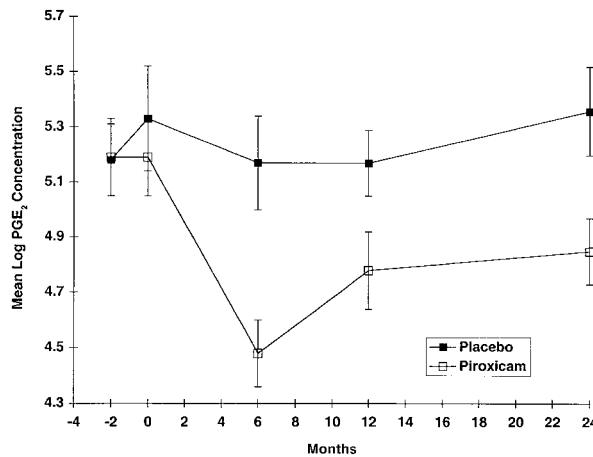


Fig. 1. Rectal mucosal biopsy mean log PGE₂ concentrations \pm 1 SE for placebo and piroxicam intervention groups as measured at -2, 0, 6, 12, and 24 months.

colon cancer chemopreventive agent. Indeed, toxic side effects have limited COX-1 inhibitors in general as potential chemopreventive agents. Less toxic substitutes for piroxicam could prove useful as colon cancer chemopreventive agents such as the development of drugs like celecoxib that selectively inhibit COX-2, sulindac sulfone, which inhibits neither COX-1 nor COX-2, or antagonists to the EP1 receptor of PGE₂ (19, 21, 73, 84, 85). Indeed, the results of this study underscore the potential advantage of selective COX-2 inhibitors, inasmuch as these agents have much reduced gastrointestinal toxicity (85).

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