Piroxicam Is an Ineffective Inhibitor of N-Nitrosomethylbenzylamine-induced Tumorigenesis in the Rat Esophagus

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ABSTRACT

Epidemiological studies indicate an association between the frequent use of nonsteroidal anti-inflammatory drugs and decreased risk for esophageal cancer. These studies suggest that limiting excess prostaglandin production, via inhibition of cyclooxygenase (COX)-mediated arachidonic acid metabolism, may be an important strategy for the prevention of this type of malignancy. N-Nitrosomethylbenzylamine (N MBA)-induced tumorigenesis in the rat esophagus is a model of human esophageal squamous cell carcinoma used for investigations of chemical carcinogenesis and for the evaluation of putative chemopreventive agents. In this study, we characterized COX-mediated arachidonic acid metabolism in NMBA-induced rat esophageal tumorigenesis by measuring COX-1 and COX-2 expression and prostaglandin E2 production. In addition, we evaluated the ability of piroxicam, a potent COX inhibitor, to prevent postinitiation events of NMBA-induced tumorigenesis in the rat esophagus. After a 2-week acclimatization period, groups of 30 male F344 rats received s.c. injections of NMBA (0.5 mg/kg b.w.) three times/week for 5 weeks. Seventy-two h after the final NMBA treatment and for the remainder of the study, piroxicam was administered in the diet at 200 and 400 ppm. Twenty-five weeks after the initiation of NMBA treatment, we observed an elevation in COX mRNA and protein expression and prostaglandin E2 production in NMBA-treated esophageal tissues compared with normal epithelium. However, these changes were associated with data indicating that a COX inhibitor is not preventive in NMBA-induced rat esophageal tumorigenesis. Administration of piroxicam in the diet produced no significant reductions in esophageal tumor incidence, multiplicity, or size. The reasons for the lack of effect are largely unknown but may be related to the inability of piroxicam to modulate other biochemical pathways involved in NMBA-induced tumorigenesis.

INTRODUCTION

In the United States, it is estimated that 13,100 new cases of esophageal cancer will be diagnosed in 2002, and that 12,600 deaths will be attributed to this malignancy (1). Worldwide measures of cancer incidence estimated >300,000 new cases of esophageal cancer in 1990 for both sexes combined (2). Epidemiological studies indicate that regular users of NSAIDs3 are at reduced risk for developing and dying from esophageal cancer (3–5). In a prospective study of >600,000 adults followed for 6 years, prolonged use of aspirin was associated with reduced esophageal cancer mortality (3). A subsequent study involving >14,000 United States residents followed for 12–16 years indicated a 90% reduced risk of developing esophageal carcinoma with occasional aspirin use (4). In a recent case-control study, current users of aspirin were shown to have a decreased risk for developing both esophageal SCCs and adenocarcinomas (5).

Studies conducted in a mouse model of esophageal cancer demonstrated that the NSAID indomethacin effectively inhibited DEN-induced tumor development (6, 7). When indomethacin was administered in the drinking water during DEN treatment, or 4 months after initiation of DEN treatment, the number of esophageal tumors per centimeter of the esophagus (tumor index) was significantly reduced (6). A more recent study demonstrated the induction of apoptosis by aspirin in cell lines derived from human esophageal SCC (8). During the past 15 years, numerous studies have shown the NSAID piroxicam to effectively inhibit chemically and genetically induced cancer in rodents (9–18). In particular, piroxicam has been shown to inhibit AOM-induced rat colon carcinogenesis (9–13), 4-(methyltriamino)-1-(3-pyridyl)-1-butanone-induced mouse lung carcinogenesis (14), N-butyl-N-(4-hydroxybutyl)nitrosamine-induced rat urinary bladder carcinogenesis (15), 2-acetylaminofluorene-induced rat liver carcinogenesis (16), and in a murine model of familial adenomatous polyposis, spontaneous development of intestinal adenomas (17, 18). In these and in other animal model systems, elevated expression of COX has been associated with neoplastic progression (19–26). Furthermore, recent studies have demonstrated increased expression of COX in human tumors of the colon (27–29), breast (30, 31), lung (32), gastric epithelium (33), pancreas (34), bladder (35), cervix (36), head and neck (37), and in adenocarcinomas and SCCs of the esophagus (38–41).

NSAIDs exert analgesic, antipyretic, and anti-inflammatory effects through the inhibition of COX-catalyzed biosynthesis of prostaglandins (42). Moreover, the ability of these drugs to prevent cancer is thought to be due, in part, to COX inhibition. Currently, there are two known isoforms of COX, both of which catalyze the metabolism of arachidonic acid to prostaglandin H2, a precursor to prostaglandins (43). The COX-1 isofrom is constitutively expressed and produces the prostaglandins important for normal physiological function. COX-2 can be induced by cytokines, growth factors, and tumor promoters and produces prostaglandins at sites of inflammation (44). In carcinogenesis, overexpression of COX is thought to deregulate arachidonic acid metabolism and lead to elevated prostaglandin production (43). Increased prostaglandin levels have been observed in human and animal tissues compared with surrounding normal tissue (45) and are thought to contribute to colon carcinogenesis by influencing cell proliferation, tumor promotion, immune response, and metastasis (45).

In view of the considerable epidemiological and experimental data suggesting the importance of deregulated arachidonic acid metabolism in carcinogenesis, we have measured COX expression and PGE2 production in NMBA-induced tumorigenesis in the rat esophagus, a model of human esophageal SCC. On the basis of these results and the substantial experimental data suggesting the chemopreventive efficacy of piroxicam in animal model systems, we conducted a study to determine the effects of piroxicam on postinitiation events of tumor-

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The abbreviations used are: NSAID, nonsteroidal anti-inflammatory drug; SCC, squamous cell carcinoma; DEN, diethylnitrosamine; AOM, azoxymethane; COX, cyclooxygenase; PGE2, prostaglandin E2; NMBA, N-nitrosomethylbenzylamine; b.w., body weight; RT-PCR, reverse transcription-PCR; HPRT, hypoxanthine-guanine phosphoribosyltransferase; EIA, enzyme immunoassay; TGF-α, transforming growth factor α; LOX, lipooxigenase; PPAR, peroxisome proliferator-activated receptor.
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igogenesis in the rat esophagus. Our results suggest that although there appears to be a correlation between increased COX expression, elevated PGE2 production, and NMBA-induced neoplastic progression, COX inhibition by piroxicam is an ineffective strategy for the prevention of rat esophageal tumorigenesis.

MATERIALS AND METHODS

Experimental Animals. Male Fischer 344 rats, 5–6 weeks-of-age, were purchased from Harlan Sprague Dawley (Indianapolis, IN). The animals were housed 3/cage under standard conditions (20 ± 2°C, 50 ± 10% relative humidity, and 12-h light/dark cycles). Rats were maintained on modified AIN-76A purified diet containing 20% casein, 3% d-l-methionine, 52% cornstarch, 13% dextrose, 5% cellulose, 5% corn oil, 3.5% AIN salt mixture, 1% AIN vitamin mixture, and 0.2% choline bitartrate (Dyets, Bethlehem, PA). Food and water were provided ad libitum. Hygienic conditions were maintained by cage changes (twice/week). Body weight and food consumption measurements were recorded weekly for the duration of the studies.

Chemicals. NMBA, obtained from Ash Stevens, Inc., Detroit, MI, was determined to be >98% pure by high-performance liquid chromatography analysis. DMSO and piroxicam were purchased from Sigma Chemical Company (St. Louis, MO).

Diets. Piroxicam diets were prepared in our laboratory fresh every 1–2 weeks and stored at 4°C. Aliquots of piroxicam were added directly to modified AIN-76A purified diet and mixed for 30 min with a Hobart mixer (Troy, OH). According to toxicity data reported by Rao et al. (11), piroxicam at 200 and 400 ppm in AIN-76A diet appears to be 40 and 80% maximum tolerated dose in male F344 rats. On a weekly basis, portions of the piroxicam diets and control diet were placed in glass feeding jars and fed to the rats.

COX mRNA Expression Study. The effects of short- and long-term NMBA treatment on COX-1 and COX-2 mRNA expression in the rat esophagus were evaluated in an initial 80-animals bioassay. After a 2-week acclimatization period, animals were randomized and placed into three groups (Table 1). Rats were given s.c. injections with NMBA at 0.5 mg/kg b.w. once/week for 15 weeks or three times/week for 5 weeks. Vehicle controls received s.c. injections of 20% DMSO in water, the solvent for NMBA. Twenty-five weeks after initiation of NMBA treatment, rats were sacrificed by CO2 asphyxiation. The esophagi from each treatment on COX-1 and COX-2 mRNA expression in the rat esophagus were determined to be >98% pure by high-performance liquid chromatography analysis. DMSO and piroxicam were purchased from Sigma Chemical Company (St. Louis, MO).

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<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of rats</th>
<th>Amount administered (ml)</th>
<th>Dose administered (mg/kg b.w.)</th>
<th>Dosing protocol (injection/wk × total wks)</th>
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<td>30</td>
<td>0.2</td>
<td>0.5</td>
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</tbody>
</table>

* DMSO + H2O, vehicle for NMBA.

Fig. 1. Experimental protocol for the Chemoprevention Bioassay. Rats were treated with NMBA at 0.5 mg/kg b.w. three times/week for 5 weeks. Piroxicam diets were administered 72 h after the final NMBA treatment.

The expression of COX-1 and COX-2 mRNA and PGE2 assays, esophagi were stripped of the outer submucosa, snap frozen in liquid nitrogen, and stored at −80°C.

RNA Isolation and Semiquantitative RT-PCR. Total cellular RNA was extracted from the frozen tissues according to a modified protocol supplied with TRIzol Total RNA Isolation Reagent (Life Technologies, Inc., Gaithersburg, MD). RNA samples were evaluated for integrity of 18S and 28S rRNA by ethidium bromide staining and photographed using an AlphaImager 2200 documentation and analysis system (Alpha Innotech Corp., San Leandro, CA). The expression of COX-1 and COX-2 mRNA was normalized against expression of housekeeping genes HPRT (COX Expression Study) or Cyclophilin (Chemoprevention Bioassay) in the same samples. Data represent the mean of two independent RT-PCR reactions.

Immunohistochemistry. Untreated (N = 8) and NMBA-treated tissues (N = 8) from the Chemoprevention Bioassay were fixed in 10% neutral buffered formalin for 4 h. Tissue processing, paraffin embedding, sectioning, and slide preparation was performed by Histotechniques (Powell, OH). Paraffin sections were cut 4-μm thick and mounted on Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA). Slides for COX-1 and COX-2 staining were antigen retrieved in citrate buffer (pH 6) by microwaving at 70% power for 3 min. Tissues were blocked with 3% hydrogen peroxide for 20 min, casein for 20 min, goat serum for 20 min, avidin for 15 min, and biotin for 15 min. Slides were incubated for 1 h at room temperature with a murine polyclonal COX-1 antibody at a dilution of 1:200 in PBS or with a murine polyclonal COX-2 antibody at a dilution of 1:75 in PBS (Cayman Chemical Co., Ann Arbor, MI). Antibody incubation was followed by 20-min incubations with a rat adsorbed link (goat antirabbit immunoglobulin) and streptavidin-horseradish peroxidase label. Visualization of stain-
ing was facilitated by a 5-min incubation with 3.3'-diaminobenzidine. Slides were counterstained with hematoxylin, dehydrated, and cover-slipped. Sections of rat kidney were included on each slide to provide a positive control for COX-1 and COX-2 immunostaining. All reagents for immunohistochemistry were purchased from BioGenex, Inc. (San Ramon, CA). Image capture was with a Nikon brightfield microscope mounted with a high-resolution spot camera and interfaced with a computer loaded with image analysis software (Simple PCI Imaging Systems by Compix, Inc., Cranberry Township, PA).

**PGE, EIA.** Tissues were homogenized in Tris-HCl buffer (pH 7.5) with 0.02 M EDTA and 5 mg/ml indomethacin. PGE2, collection and purification was according to a modified protocol supplied with the Biotrak PGE2 Enzymeimmunoassay System (Amersham Pharmacia Biotech, Piscataway, NJ). Briefly, 0.5 ml of cold 1:4 water/ethanol solution was added to 0.5 ml of tissue homogenate. A 10-μl volume of glacial acetic acid was added to each sample followed by a 5 min incubation at room temperature. Samples were then centrifuged at 2500 × g for 2 min. The supernatant was then applied to a preprimed Amprep C18 minicolumn (Amersham Pharmacia Biotech), and the column was washed with distilled water and hexane. PGE2 was eluted into siliconized glass vials with two 0.75-ml volumes of ethyl acetate. This fraction was evaporated to dryness under a stream of argon and stored at −80°C. According to the protocol provided with the EIA kit, samples were brought up in 1 ml of buffer and assayed in 96-well plates. PGE2 standards ranged from 50 to 6400 pg/ml (standard protocol) or 20 to 64 pg/ml (high sensitivity protocol). Total protein concentration for each tissue homogenate was determined using the DC Protein Assay (Bio-Rad, Hercules, CA). The relative protocol. Total protein concentration for each tissue homogenate was determined using the DC Protein Assay (Bio-Rad, Hercules, CA). The relative concentration of PGE2 was normalized against total protein concentration in the same samples.

**Statistical Analysis.** Differences in relative COX mRNA expression, PGE2 production, body weight, food consumption, and tumor multiplicity and size were analyzed for statistical significance (P < 0.05) using ANOVA, followed by Neuman-Keuls’ multiple comparisons test. Differences in tumor incidence were determined by χ2 test. All statistical procedures were carried out using the NCSS 97 statistical software package (NCSS Statistical Software, Kaysville, UT).

**RESULTS**

**COX mRNA Expression Study.** We evaluated the effects of NMBA treatment on relative COX-1 and COX-2 mRNA expression in the rat esophagus. Moreover, we determined whether these effects varied by NMBA-dosing protocol. Esophagi of vehicle control animals were classified as normal epithelium (N = 4). Tissues classified as preneoplastic epithelium were NMBA-treated esophagi with lesions ≥0.5 mm2 removed (N = 4). Papillomas represent single lesions removed from NMBA-treated esophagi (N = 5). Semiquantitative RT-PCR analysis of RNA isolated from these tissues produced DNA fragments of the expected sizes for the COX-1, COX-2, and Cyclophilin-specific primers (Fig. 2A). NMBA treatment produced a significant increase in COX-1 and COX-2 mRNA expression in papillomas compared with normal and preneoplastic epithelium (P < 0.05; Fig. 2B). Together, the semiquantitative RT-PCR data of both studies indicate a correlation between the up-regulation of COX-1 and COX-2 mRNA expression and neoplastic progression in the rat esophagus.

Immunostaining for COX-1 and COX-2 was conducted to determine whether increases in COX mRNA expression correlated with increases in COX protein expression. Representative COX immunostaining in normal, preneoplastic and papillary tissues is shown in Fig. 3. COX-1 immunostaining was predominantly cytoplasmic and localized throughout the basal and suprabasal layers of the epithelium. Expression was consistently strong in all three tissue types (Fig. 3, A–C). COX-2 immunostaining was also cytoplasmic but showed considerable variation among tissue types with respect to number of positive cells, localization, and staining intensity. In normal esophageal epithelium (Fig. 3D), COX-2 immunostaining was infrequent and weak. COX-2 immunostaining in NMBA-induced papillomas presented with normal and preneoplastic lesions, particularly dysplastic foci (Fig. 3E), was weak in the suprabasal layers but moderate in the thickened basal layer. In NMBA-induced papillomas (Fig. 3F), moderate to strong immunostaining for COX-2 was observed throughout the tissue.

To determine the effects of up-regulated COX expression on prostaglandin production in the rat esophagus, we measured the relative concentration of PGE2 in normal (N = 15), preneoplastic (N = 15), and papilloma (N = 11) tissues of the Chemoprevention Bioassay. We also measured the relative concentration of PGE2 in preneoplastic

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of rats</th>
<th>Tumor incidence (%)</th>
<th>Tumor multiplicity (mean ± SE)</th>
<th>Tumor size* (mean ± SE)</th>
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<td>30</td>
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<td>2</td>
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<tr>
<td>3</td>
<td>NMBA</td>
<td>30</td>
<td>96.7</td>
<td>2.9 ± 0.3</td>
<td>6.5 ± 1.2</td>
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<td>30</td>
<td>100</td>
<td>2.7 ± 0.3</td>
<td>6.7 ± 1.6</td>
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<tr>
<td>5</td>
<td>NMBA + 400 ppm piroxicam</td>
<td>30</td>
<td>90.0</td>
<td>2.8 ± 0.3</td>
<td>5.9 ± 1.0</td>
</tr>
</tbody>
</table>

* Tumor size, volume calculated as length × width × depth × 1/6 assuming a prolate spheroid shape (46).
Confidence intervals are shown. Means and 95% confidence intervals are shown.

Cyclophilin expression correlates with neoplastic progression in the rat esophagus. Means and 95% confidence intervals are shown.

DISCUSSION

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(N = 14) and papilloma tissues (N = 8) treated with piroxicam. EIA analysis indicated a >15-fold increase in PGE2 levels in papillomas compared with normal epithelium (P < 0.05) and a >10-fold increase compared with neoplastic epithelium (P < 0.05; Fig. 4A). A 1.6-fold increase in PGE2 levels was observed in neoplastic epithelium compared with normal, but this difference was not significant.

Piroxicam in the diet at 200 and 400 ppm reduced PGE2 levels in preneoplastic tissues to levels below those measured in normal epithelium (P < 0.05; Fig. 4B). In papillomas, piroxicam in the diet at 400 ppm reduced PGE2 levels 3.8-fold, but this difference was not significant (P = 0.07; data not shown).

Thus, we have shown that elevated COX mRNA and protein expression and PGE2 production are correlated with neoplastic progression in the rat esophagus. Despite these findings, the COX inhibitor piroxicam was shown to be ineffective as an inhibitor of NMBA-induced rat esophageal tumorigenesis. Furthermore, our results suggest that PGE2 inhibition may not be a definitive indicator of the mechanism by which COX inhibitors are chemopreventive in the rat esophagus, and possibly, in other model systems.

In this study, we have characterized COX-mediated arachidonic acid metabolism in NMBA-induced tumorigenesis in the rat esophagus by measuring COX-1 and COX-2 expression and PGE2 production. Using semiquantitative RT-PCR, we demonstrated that COX-1 and COX-2 mRNA levels were elevated in esophageal epithelium treated with NMBA. In the first of two independent bioassays, our data indicated that COX-1 and COX-2 mRNA expression was increased 2- and 5-fold, respectively, in papillomas compared with normal epithelium. The reproducibility of this data was confirmed in the second bioassay where we demonstrated respective 1.5- and 4-fold increases in COX-1 and COX-2 mRNA expression. With respect to preneoplastic epithelium, measures of COX mRNA expression may have been underestimated because of the heterogeneous nature of these samples. To obtain sufficient quantities of RNA for RT-PCR, the entire NMBA-treated esophageal epithelium, with papillomas removed, was analyzed and classified as preneoplastic. Given that these samples consisted of lesions ranging from mild hyperplasia to dysplasia, COX mRNA expression in preneoplastic epithelium may have been ‘diluted’ because of the occurrence of less advanced lesions.

Our data are in agreement with measures of COX mRNA expression described in a recent study of chemoprevention in the rat esophagus (51) and in other animal model systems (20, 21, 23). Li et al. (51) reported that NMBA treatment at 1.0 mg/kg b.w. administered five times/week for 5 weeks, followed by once/week for 10 weeks, produced a 5-fold elevation in COX-2 mRNA expression in papillomas compared with normal epithelium. In a study reported by Gustafson-Svärds et al. (21), COX-1 and COX-2 mRNA expression was shown to be elevated 3- and 28-fold, respectively, in AOM-induced colon tumors compared with saline-treated normal mucosa. Moreover, normal-appearing mucosa within the AOM-treated colon was shown to have elevated COX-1 and COX-2 mRNA expression compared with normal mucosa. In a similar study reported by DuBois et al. (20), AOM treatment was shown to produce 2.2- to 30-fold increases in COX-2 mRNA expression in colonic adenocarcinomas compared with normal mucosa. However, they reported no change in COX-1 mRNA expression according to AOM treatment. In the Min mouse, intestinal adenomas were shown to have ~3-fold elevation in COX-2 mRNA expression compared with normal intestinal mucosa (23).

The increase in COX-1 mRNA expression reported by Gustafson-Svärds et al. (21) and demonstrated in this study is a novel observation in animal model systems of carcinogenesis. Induction of COX-1 mRNA and protein expression has been reported in vitro (52) and, similar to our findings, was typically less than that observed with COX-2. We suggest that the discrepancy between our COX-1 mRNA and protein data may be because of the inability of immunohistochemical techniques to clearly distinguish expression differences <2-fold. With respect to COX-2, the increases in mRNA expression observed in this study were likely because of the induction of gene expression. Immunostaining for COX-2 indicated that NMBA treatment produced increases, not only in the number of esophageal epithelial cells expressing COX-2, but in the levels of COX-2 protein expressed. COX-2 gene expression has been shown to be induced by various cytokines, growth factors, and tumor promoters (44). Previous studies in our laboratory have demonstrated up-regulated expression of TGF-α and epidermal growth factor receptor in NMBA-induced tumorigenesis in the rat esophagus (53). Our laboratory has also shown high frequency of G to A transition mutations in codon 12 of the Ha-ras oncogene in NMBA-induced papillomas (54, 55). In vitro studies have shown that Ha-ras transfection can produce overexpression of TGF-α and epidermal growth factor receptor (56, 57). Furthermore, studies of rat intestinal epithelial cells indicate that TGF-α treatment and Ha-ras transfection both produce increased expression of COX-2 mRNA and protein and elevated levels of prostaglandins (58, 59). We suggest that increased expression of TGF-α and its receptor, potentially the result of mutational activation of Ha-ras, produce enhanced signaling through a pathway that results in the induction of COX-2 gene expression and, thus, may explain the increased mRNA levels observed in this study.

Increased prostaglandin levels have been observed in human and animal tumors compared with surrounding normal tissue (45) and are thought to contribute to colon carcinogenesis by influencing cell proliferation, tumor promotion, immune response, and metastasis (45). In this study, our data are in agreement with measures of PGE2...
reported by Li et al. (51) and demonstrate significantly elevated levels of PGE$_2$ in NMBA-induced papillomas compared with normal and preneoplastic esophageal epithelium. As indicated for the RT-PCR analysis, measurement of PGE$_2$ in preneoplastic tissues may be underestimated because of our broad classification of preneoplastic epithelium. We suggest that induced COX gene expression deregulates arachidonic acid metabolism in NMBA-induced tumorigenesis and results in elevated PGE$_2$ production.

In this study, we have shown piroxicam in the diet at 200 and 400 ppm to have no effect on NMBA-induced tumorigenesis in the rat esophagus. Similar to these findings, our laboratory previously reported that dietary administration of the NSAID sulindac did not influence the development of NMBA-induced esophageal preneoplastic lesions or papillomas (60). Rats were treated with NMBA at 1.0 mg/kg b.w. three times/week for 5 weeks, and sulindac was administered in the diet at 125 ppm before, during, and after NMBA treatment or only after NMBA treatment. We suggest that the inability of sulindac to inhibit tumorigenesis was due, in part, to an NMBA treatment protocol that may have overwhelmed any inhibitory effects (60). On the basis of a report by Rao et al. (61) suggesting that the maximum tolerated dose of sulindac in male F344 rats is 400 ppm, it is also conceivable that our dietary concentration of sulindac (125 ppm) was below an effective dose. In this study, despite using a modified NMBA treatment protocol (0.5 mg/kg b.w. three times/week for 5 weeks) and dietary concentrations of piroxicam at 40 and 80% maximum tolerated dose in male F344 rats (11), we observed no effect of a COX inhibitor on NMBA-induced tumorigenesis in the rat esophagus.

Results of PGE$_2$ EIA conducted in our laboratory suggest that piroxicam in the diet at 200 and 400 ppm significantly reduced the 1.6-fold elevation in PGE$_2$ levels in NMBA-induced preneoplastic tissues to levels below those in normal epithelium. In addition, 400 ppm piroxicam in the diet produced a 74% reduction in PGE$_2$ levels in papillomas. Thus, our data suggest that the administration of piroxicam in the diet delivers pharmacologically effective drug concentrations to esophageal tissues. However, the ability of piroxicam to inhibit PGE$_2$ production was not correlated with reductions in tumor incidence or multiplicity in the rat esophagus and, thus, confounds the role of deregulated COX-mediated arachidonic acid metabolism in NMBA-induced tumorigenesis. Studies of chemoprevention in 7,12-dimethylbenz[a]anthracene-induced rat mammary tumorigenesis also indicate uncertainty in the importance of inhibiting excess prostaglandin production (62, 63). Carter et al. (62) reported that although indomethacin was an effective inhibitor of tumorigenesis and carprofen produced no inhibitory effects, both NSAIDs significantly inhibited PGE$_2$ production in mammary glands. Therefore, similar to our
present findings with piroxicam, the ability of carprofen to inhibit PGE$_2$ production was not correlated with inhibition of tumorigenesis. In addition, Kitagawa et al. (63) reported that although piroxicam was ineffective, the LOX inhibitor esculetin produced significant inhibition of mammary tumorigenesis. These results suggest that COX-mediated prostaglandin production may not play a critical role in the development of certain cancers. More importantly, they emphasize the importance of considering alternative mechanisms by which NSAIDs are chemopreventive.

We suggest that the lack of effect observed with piroxicam in this study is due, in part, to the inability of this particular NSAID to modulate other mediators of eicosanoid biosynthesis potentially involved in esophageal tumorigenesis. Recent studies suggest that the induction of apoptotic cell death is an important mechanism by which NSAIDs prevent the development of colon cancer (64, 65). The ability of NSAIDs to induce apoptosis in colon cancer cells has been shown to be independent of COX inhibition (66) and possibly related to LOX-mediated eicosanoid biosynthesis. In human tumors of the colon and esophagus, Shureiqi (67) reported that although piroxicam was ineffective, the LOX activator of PPAR$_\gamma$ (70). Indomethacin, an effective inhibitor of tumorigenesis in the mouse esophagus (6, 7), has been shown to significantly induce 15-LOX expression (71) and, compared with piroxicam, is a potent activator of PPAR$_\gamma$ (70). We suggest that the lack of effect observed with piroxicam in the rat esophagus is related to a limited ability to affect these pathways.

In this study, we have shown a COX inhibitor, piroxicam, to be an ineffective inhibitor of tumorigenesis in the rat esophagus. The reasons for the lack of effect are largely unknown but are likely related to the limited ability of piroxicam to modulate additional biochemical pathways that may be involved in NMBA-induced tumorigenesis, including LOX-mediated eicosanoid biosynthesis and PPAR signaling. Before the design of future studies, we will address the role of these pathways in NMBA-induced rat esophageal tumorigenesis and, when selecting agents to evaluate, consider the ability of certain NSAIDs to modulate additional biochemical pathways involved in cancer.

**REFERENCES**

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