The effects of L-748706, a selective cyclooxygenase-2 inhibitor, on N-nitrosomethylbenzylamine-induced rat esophageal tumorigenesis

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Introduction

Esophageal cancer ranks eighth in cancer incidence and fifth in cancer mortality for both men and women worldwide (1). Regions with particularly high occurrences of esophageal squamous cell carcinoma (SCC) include certain parts of China, Central Asia, India, Iran, Puerto Rico, France and the Transkei region of South Africa (2, 3). In the USA, esophageal adenocarcinoma is more prevalent than esophageal SCC, and it is estimated that there will be ~14,520 new cases of esophageal cancer and 13,570 deaths in 2005 (4). Current treatment modalities for the disease have not resulted in improved prognosis, as indicated by an exceptionally low 5-year survival rate (<10%) and the death of ~75% of patients within a year of initial diagnosis (5). Thus, there is a need to develop effective strategies for the prevention of this malignancy and chemoprevention is a potentially viable approach.

Epidemiological studies suggest that the use of non-steroidal anti-inflammatory drugs (NSAIDs) protects against the development of esophageal cancer (6). The ability of NSAIDs to prevent this malignancy is thought to correlate with their inhibition of cyclooxygenase (COX) activities. There are two known isoforms of COX, designated COX-1 and COX-2. Both isoforms catalyze the conversion of arachidonic acid to prostaglandin H₂, an unstable intermediate, which undergoes further metabolism to the parent eicosanoids, PGD₂, PGE₂, PGF₂, PGI₂ and thromboxane-2. Prostaglandins (PGs), the major metabolites of arachidonic acid, appear to be crucial in the process of carcinogenesis due to their ability to modulate mitogenesis, cellular adhesion, immune surveillance, cell proliferation, apoptosis and angiogenesis (7–10). Inhibition of COX enzyme activities by NSAIDs leads to reduced biosynthesis of PGs. Due to the side effects involved with inhibition of COX-1, and the nearly unique expression of COX-2 in premalignant and malignant tissues, much emphasis has been placed on developing NSAIDs that specifically target COX-2.

Overexpression of COX-2 has been observed in a variety of human cancers including cancers of the breast (11), lung (12), colon (13), uterus (14), cervix (15), head and neck (16), skin (17) and esophagus (18, 19). In particular, COX-2 is overexpressed in squamous dysplasia and squamous cell carcinoma of the esophagus in humans (18, 19). In addition, prostaglandin E₂ (PGE₂) levels are elevated in several human cancers including colon, lung and prostate, and esophageal SCC (20–23). Previous studies in our laboratory demonstrated significant increases in COX-2 mRNA expression, as well as PGE₂ production, in N-nitrosomethylbenzylamine (N MBA)-induced preneoplastic and papillomatous tissues of the rat esophagus when compared with untreated controls (24). COX-1 mRNA expression was elevated in esophageal papillomas but not in preneoplastic tissues. Data from genetic studies in animals also provide strong evidence in support of a cause-and-effect relationship between COX-2 and tumorigenesis (25–27). Transgenic mice that overexpress COX-2 in the skin, develop...
epidermal hyperplasia and dysplasia (25). Moreover, the development of skin papillomas and intestinal tumors is markedly suppressed in COX-2 knockout mice (26,27). Pharmacological studies have also shown that the selective inhibition of COX-2 by pharmacologic agents reduces the formation of bladder, intestinal, breast, skin, lung, tongue and esophageal tumors in animals (28–34). This information led to the present study being designed to determine whether L-706, a novel selective COX-2 inhibitor, is capable of inhibiting esophageal tumorigenesis in the F344 rat when administered in the diet following treatment of the rats with NMBA. We also evaluated whether L-706 in combination with piroxicam, an inhibitor principally of COX-1, results in an additive or synergistic inhibition of NMBA-induced esophageal tumorigenesis. In a previous investigation, we found that piroxicam alone was ineffective as an inhibitor of NMBA-induced tumors in the rat esophagus (24).

Materials and methods

Animals, chemicals and diet

Three hundred and fifty male Fischer 344 (F344) rats, 4–5 weeks of age, were purchased from Harlan Sprague–Dawley (Indianapolis, IN). Rats were housed three per cage under standard conditions (20 ± 2°C; 50 ± 10% relative humidity and 12-h light/dark cycles) and were fed modified AIN-76A purified diet (Dyets, Bethlehem, PA). Food and water were provided ad libitum. Hygienic conditions were maintained by twice weekly cage changes. Body weight and food consumption measurements were recorded weekly for the duration of the bioassay. NMBA, obtained from Ash Stevens (Detroit, MI), was determined to be >98% pure by high-performance liquid chromatography (HPLC). Dimethyl sulfoxide (DMSO) and piroxicam were purchased from Sigma Chemical Company (St Louis, MO). L-706, a proprietary compound, was kindly provided by Merck (West Point, PA). L-706 has a higher binding affinity for COX-2 than does its structurally-related analog, rofecoxib or Vioxx® (R.Dixit, personal communication). A Hobart mixer was used to mix L-706 and piroxicam into modified AIN-76A-purified diet weekly. The diets were stored at 4°C until fed to the animals. L-706 was shown to be well tolerated by a 6-week toxicity study in which weight measurements were evaluated. The dose of piroxicam was chosen based on the toxicity data reported by Rao et al. (35), in which piroxicam at 200 p.p.m. represented 40% of the maximum tolerated dose in male F344 rats.

Post-initiation bioassay

To evaluate the effect of L-706 on NMBA-induced tumorigenesis in the rat esophagus, all animals were randomized into 11 groups and placed on modified AIN-76A-purified diet (Table I). After a 2-week acclimatization period, rats in Groups 4–11 were injected subcutaneously with NMBA at dose levels of either 0.25 mg/kg body weight (body wt) (Groups 4–7) or 0.5 mg/kg body wt (Groups 8–11) for 7 times a week, three times per week for 5 weeks. Vehicle controls (Group 1) received subcutaneous injections of 20% DMSO in water, the solvent for NMBA. Seventy-two hours after the final NMBA treatment, rats were given either 100 p.p.m. L-706 (Groups 5 and 9), or 150 p.p.m. L-706 (Groups 2, 6 and 10), or 150 p.p.m. L-706 + 200 p.p.m. piroxicam in the diet (Groups 3, 7 and 11) for the remainder of the bioassay (Figure 1). At the end of week 25, rats were euthanized by CO2 asphyxiation. In randomly selected animals, portions of the liver, kidney, stomach, colon, small intestine, heart, lung and bladder were collected for microscopic determination of L-706 and piroxicam toxicity. The esophagus of each animal was excised, opened longitudinally and papillomas ≥0.5 mm in a single dimension were counted, mapped and measured. Each esophagus was then cut into halves along its length. One-half of the esophagus was fixed in 10% neutral buffered formalin for 4 h, and then transferred to phosphate buffered saline (PBS) and stored at 4°C before embedding in paraffin. The other half of the esophagus was stripped of the submucosal and muscularis layers. Papillomas were removed from the esophagus. The esophageal tissues and papillomas were stored separately at –80°C.

Histopathology of NMBA-induced epithelial lesions in esophagus

One-half of each formalin-fixed esophagus was cut into thirds and embedded in paraffin with the epithelium uppermost. Serial 4-μm sections were cut and mounted on superfrost plus slides (Histotechniques Laboratories, Powell, OH). An H&E-stained slide was made from each esophagus of animals in all groups and scanned at 100× magnification. Each viewing field was classified into one of five histological categories: normal epithelium, epithelial hyperplasia, low-grade dysplasia, high-grade dysplasia and papilloma. The classification scheme used both gross and microscopic descriptions of hyperplasia and dysplasia as described previously (36).

The effects of L-748706 on esophageal cancer

Table I. Effects of L-706 alone and in combination with piroxicam on NMBA-induced tumorigenesis in the rat esophagus

<table>
<thead>
<tr>
<th>Group</th>
<th>NMBA (mg/kg body wt)</th>
<th>Diet</th>
<th>No. of rats</th>
<th>Tumor incidence (%)</th>
<th>Tumor multiplicity</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td></td>
<td>10</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>150 p.p.m. L-706</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>–</td>
<td>L-706 + Piroxicam</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.25 Control</td>
<td>50</td>
<td>87.8</td>
<td>2.1 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.25 100 p.p.m. L-706</td>
<td>50</td>
<td>81.3</td>
<td>1.4 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.25 150 p.p.m. L-706</td>
<td>50</td>
<td>65.3</td>
<td>1.2 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.25 L-706 + Piroxicam</td>
<td>50</td>
<td>69.4</td>
<td>1.2 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.5 Control</td>
<td>30</td>
<td>100</td>
<td>4.8 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.5 100 p.p.m. L-706</td>
<td>30</td>
<td>100</td>
<td>4.6 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.5 150 p.p.m. L-706</td>
<td>30</td>
<td>100</td>
<td>4.4 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0.5 L-706 + Piroxicam</td>
<td>30</td>
<td>96</td>
<td>4.0 ± 0.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aGroups 3, 7 and 11 were administered diets containing 150 p.p.m. L-706 + 200 p.p.m. piroxicam.
*bSignificantly lower than Group 4 as determined by analysis of variance (P < 0.05).
*cSignificantly lower than Group 4 as determined by χ²-test (P < 0.05).

Fig. 1. Experimental protocol for L-706 and piroxicam post-initiation bioassay. Rats were treated with NMBA (0.25 or 0.5 mg/kg body wt) three times per week for 5 weeks. L-706 or L-706 plus piroxicam were administered following NMBA treatment and for the duration of the bioassay.

Immunohistochemical detection of proliferating cell nuclear antigen (PCNA)

The effect of L-706 on esophageal cell proliferation was evaluated by quantitating PCNA immunohistochemical staining. Five esophageal samples were randomly selected from each group and stained with anti-PCNA protein as described previously (36). PCNA-stained slides were then scanned at 200× magnification with a Nikon bright-field microscope equipped with a high-resolution spot camera and computer containing a matrix frame grabber board. Image analysis was conducted using Simple PCI Imaging Systems (Compix, Cranberry Township, PA). The percent labeling index was calculated by dividing the positively stained nuclear area in the esophageal epithelium by the total nuclear area (positively and negatively stained area) as described previously (36).

PGE₂ enzyme immunoassay

This assay was performed according to protocols established in our laboratory (24). Frozen tissues were homogenized in Tris–HCl buffer (pH 7.5) with 0.02 M EDTA and 5 μg/ml indomethacin. PGE₂ recovery and purification was conducted according to protocols provided with the PGE₂ EIA kit (Amersham Pharmacia Biotech, Piscataway, NJ). Purified PGE₂ samples were stored at –80°C. Samples were dissolved in 0.5–1.0 ml of sample buffer and assayed in 96-well plates. The quantities of PGE₂ were determined by both standard and sensitive protocols with PGE₂ standards ranging from 50 to 6400 pg/ml and 20 to 640 pg/ml, respectively. Total protein concentration for each tissue homogenate was determined using the DC Protein Assay (Bio-Rad, Hercules, CA). The concentration of PGE₂ was normalized to micrograms of total protein in the same sample.

Statistical analysis

Data on body weight, food consumption, tumor multiplicity, microscopic esophageal epithelial lesions and PGE₂ concentrations were analyzed using one-way ANOVA, followed by Neuman–Keuls’ multiple comparisons test.
Tumor incidence was analyzed by \( \chi^2 \)-test. All statistical procedures were conducted using the NCSS 97 statistical software package (NCSS Statistical Software, Kaysville, UT). Differences were considered statistically significant at \( P < 0.05 \).

## Results

### General observations

No significant differences were observed in animal body weights or food consumption among all groups during the bioassay (data not shown). Both L-706 and piroxicam were well tolerated and did not result in any gross or histological abnormalities in the esophagus, liver, kidney, stomach, or intestinal tract of the rats.

### Tumor response

The effects of L-706 alone and in combination with piroxicam on NMBA-induced tumor development are shown in Table I. In rats treated with the low-dose of NMBA (0.25 mg/kg body wt), both 150 p.p.m. L-706 alone and 150 p.p.m. L-706 + 200 p.p.m. piroxicam led to a significant reduction in tumor incidence (65.3 and 69.4%, respectively) and multiplicity (1.2 and 1.2 ± 0.2, respectively), relative to NMBA only (87.8%, 2.1 ± 0.2). 100 p.p.m. L-706 also significantly reduced tumor multiplicity (1.4 ± 0.2) but not tumor incidence. In rats treated with the high-dose of NMBA (0.50 mg/kg body wt), however, none of the chemopreventive treatments produced significant reductions in tumor incidence or multiplicity.

### Esophageal epithelial lesions

The effects of L-706, alone and in combination with piroxicam, on the development of preneoplastic lesions were evaluated in animals treated with the low-dose of NMBA as shown in Table II. In groups treated with 150 p.p.m. L-706 (Group 6) or 150 p.p.m. L-706 + 200 p.p.m. piroxicam (Group 7), the percentage of areas exhibiting esophageal hyperplasia are higher and the percentage of areas exhibiting low-grade dysplasia are lower than in NMBA only group (Group 4). All chemopreventive treatments (Groups 5–7) have fewer areas of high-grade dysplasia when compared with preneoplastic lesions in rats treated with NMBA alone (Group 4), but were statistically fewer only in animals that received both L-706 and piroxicam (Group 7). None of the three treatment regimens (Groups 5–7) produced significant reductions in preneoplastic lesions in the esophagus of rats treated with high-dose (0.50 mg/kg body wt) NMBA (data not shown).

### PCNA labeling indices

PCNA labeling indices (LIs) for each experimental group are shown in Table III. In brief, PCNA LIs in non-NMBA-treated animals ranged from 18.2 to 19.9% (Groups 1–3).

### PGE\(_2\) production

A previous study in our laboratory showed increased expression of COX-2 mRNA and protein in NMBA-treated rat esophagus (24). The present study determined the effects of inhibition of COX-2 activity on the production of PGE\(_2\), one of the major metabolites of COX-2 activity. The relative concentrations of PGE\(_2\) were measured in preneoplastic and papillomatous esophageal tissues in both low- and high-dose NMBA-treated animals as shown in Table IV. For reference purposes, the levels of PGE\(_2\) in the esophagus of rats that were not treated with NMBA (i.e. normal esophagus) was 0.023 ± 0.006 ng/mg total protein (Table IV).

In low-dose NMBA-treated animals, both 100 and 150 p.p.m. dietary L-706 significantly reduced PGE\(_2\) levels in preneoplastic esophageal epithelium by 77.7 and 79.4%, respectively.
when compared with preneoplastic epithelium in rats treated with NMBA only (P < 0.05). In papillomas, 100 and 150 p.p.m. L-706 reduced PGE2 levels by 33.8 and 53.8%, respectively (not significant). The combination treatment however, produced 89.3 and 81.8% reductions in PGE2 levels in preneoplastic esophageal epithelium and in papillomas, respectively (P < 0.05).

In high-dose NMBA treated rats, both 100 and 150 p.p.m. dietary L-706 reduced PGE2 levels in preneoplastic esophageal epithelium and in papillomas when compared with the corresponding tissues in rats treated with NMBA only (P < 0.05). The combination treatment further reduced PGE2 production as evidenced by 95.6 and 90.7% reductions in preneoplastic epithelium and in papillomas, respectively (P < 0.05).

**Discussion**

Cyclooxygenases are the rate limiting enzymes required for the conversion of arachidonic acid to a series of biologically active PGs and thromboxanes. PGs are considered to be crucial in the development of cancer due to their multiple effects in carcinogenesis (7–10). Thus, inhibition of COX activity, subsequently blocking the formation of downstream products of PGs, appears to be a feasible approach for the prevention of cancer. Selective inhibition of COX-2, which avoids thwarting the housekeeping function of COX-1, appears to be particularly promising.

In the present study, the potential inhibitory effects of L-706, a selective COX-2 inhibitor, on NMBA-induced esophageal tumorigenesis in F-344 rats, were investigated. In low-dose (0.25 mg/kg body wt) NMBA-treated animals, we found that dietary L-706 reduced tumor incidence and multiplicity in a dose-dependent manner when compared with NMBA controls. Inhibition of tumor development correlated with reductions in esophageal cell proliferation and in PGE2 levels in preneoplastic epithelium. Our data are in agreement with other studies evaluating the effects of selective COX-2 inhibitors on tumorigenesis in the rat esophagus and in other animal model systems. Li et al. (34) reported that JTE-522, a selective COX-2 inhibitor, produced a significant dose-responsive reduction in tumor multiplicity in NMBA-induced esophageal SCC in the rat. Furukawa et al. (37) found that nimesulide, another COX-2 inhibitor, prevented N-nitrosobis(2-oxopropyl) amine-induced pancreatic carcinogenesis in hamsters when administered post-initiation. Celecoxib, a selective COX-2 inhibitor, was approved for the prevention of colon polyps in patients with familial adenomatous polyposis (38).

L-706, alone, and in combination with piroxicam, also reduced PGE2 levels in the esophagus of animals treated with the high-dose of NMBA (0.5 mg/kg body wt) however, none of the chemopreventive treatments reduced tumor development in these animals. One possible explanation for this result is that the chemopreventive treatments failed to reduce the levels of esophageal PGE2 far enough to prevent cancer. As shown in Table V, in rats treated with the lower dose of NMBA (Groups 4–7), L-706 alone (Groups 5 and 6) and L-706 + piroxicam (Group 7) reduced PGE2 levels in esophageal preneoplastic tissues to 0.5–1.1 of the amounts in normal (vehicle control) tissues. In contrast, in rats treated with the higher dose of NMBA (Groups 8–11), PGE2 levels in preneoplastic esophageal tissues of rats administered the chemopreventives ranged from 2.1–15.5-fold higher than those in normal tissues. Thus, from a mechanistic point of view, it would appear that in the rat esophagus, PGE2 levels must be reduced by non-steroidal anti-inflammatory drugs to levels that approximate those found in normal tissues in order to reduce tumor development.

PGE2 stimulates cellular proliferation in a variety of cell types including mouse mammary epithelial cells (39), human

**Table IV. Effects of L-706 alone and in combination with piroxicam on PGE2 production in rats treated with NMBA**

<table>
<thead>
<tr>
<th>Group</th>
<th>NMBA (mg/kg body wt)</th>
<th>Diet</th>
<th>PGE2 (Mean ± SE)(^a)</th>
<th>Inhibition(^b)</th>
<th>Papilloma</th>
<th>PGE2 (Mean ± SE)</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.25</td>
<td>Control</td>
<td>0.112 ± 0.018</td>
<td>0</td>
<td>0.936 ± 0.233</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td>100 p.p.m. L-706</td>
<td>0.025 ± 0.005</td>
<td>77.7%</td>
<td>0.620 ± 0.081</td>
<td>33.8%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.25</td>
<td>150 p.p.m. L-706</td>
<td>0.023 ± 0.006</td>
<td>79.4%</td>
<td>0.432 ± 0.157</td>
<td>53.8%</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.25</td>
<td>L-706 + Piroxicam(^c)</td>
<td>0.012 ± 0.004</td>
<td>89.3%</td>
<td>0.170 ± 0.042</td>
<td>81.8%</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.5</td>
<td>Control</td>
<td>1.122 ± 0.288</td>
<td>0</td>
<td>4.000 ± 1.356</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.5</td>
<td>100 p.p.m. L-706</td>
<td>0.357 ± 0.061</td>
<td>68.2%</td>
<td>0.748 ± 0.123</td>
<td>81.3%</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.5</td>
<td>150 p.p.m. L-706</td>
<td>0.307 ± 0.089</td>
<td>72.6%</td>
<td>0.620 ± 0.074</td>
<td>84.5%</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0.5</td>
<td>L-706 + Piroxicam(^c)</td>
<td>0.049 ± 0.012</td>
<td>95.6%</td>
<td>0.374 ± 0.055</td>
<td>90.7%</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Fold changes in PGE2 levels relative to levels in normal esophagus (NE).
\(^b\)Groups 7 and 11 were administered diets containing 150 p.p.m. L-706 + 200 p.p.m. piroxicam.
\(^c\)Significantly lower than Group 8 as determined by analysis of variance (P < 0.05).

**Table V. Fold changes in PGE2 levels in preneoplastic esophageal tissues and in papillomas of rats treated with NMBA only or NMBA plus L-706 and piroxicam relative to normal esophagus**

<table>
<thead>
<tr>
<th>Group</th>
<th>NMBA (mg/kg body wt)</th>
<th>Diet</th>
<th>Pneoplasia</th>
<th>Papilloma</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.25</td>
<td>Control</td>
<td>4.9(^e)</td>
<td>40.7(^e)</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td>100 p.p.m. L-706</td>
<td>1.1</td>
<td>27.0</td>
</tr>
<tr>
<td>6</td>
<td>0.25</td>
<td>150 p.p.m. L-706</td>
<td>1.0</td>
<td>18.8</td>
</tr>
<tr>
<td>7</td>
<td>0.25</td>
<td>L-706 + Piroxicam(^b)</td>
<td>0.5</td>
<td>7.4</td>
</tr>
<tr>
<td>8</td>
<td>0.5</td>
<td>Control</td>
<td>48.8</td>
<td>173.9</td>
</tr>
<tr>
<td>9</td>
<td>0.5</td>
<td>100 p.p.m. L-706</td>
<td>15.5</td>
<td>32.5</td>
</tr>
<tr>
<td>10</td>
<td>0.5</td>
<td>150 p.p.m. L-706</td>
<td>13.3</td>
<td>27.0</td>
</tr>
<tr>
<td>11</td>
<td>0.5</td>
<td>L-706 + Piroxicam(^b)</td>
<td>2.1</td>
<td>16.3</td>
</tr>
</tbody>
</table>

\(^e\)Fold changes in PGE2 levels relative to levels in normal esophagus (NE).
\(^b\)Groups 7 and 11 were administered diets containing 150 p.p.m. L-706 + 200 p.p.m. piroxicam.
endometrial epithelial cells (40), human oral SCC (41), and human colon carcinoma cells (42). NSAIDs/COX-2 inhibitors have been reported to suppress cellular proliferation by blocking PGE\(_2\) production (41,42). In agreement with these findings, our study demonstrates that the PCNA LI is highest in the esophagi of rats treated with NMBA only, and these esophagi contained the highest levels of PGE\(_2\) (Tables III and IV). Cellular proliferation in low-dose NMBA-treated esophagi was inhibited in a dose-dependent manner by L-706 and this correlated with reduced levels of PGE\(_2\). In high-dose NMBA-treated animals however, L-706 did not produce a significant reduction in the PCNA LI suggesting that the reduction in PGE\(_2\) levels was not adequate to inhibit cell growth or tumorigenesis.

Another possible explanation for the lack of tumor inhibition in rats treated with the higher dose of NMBA may be that this dose produces more pronounced changes in gene expression in the esophagus than the lower dose of NMBA. We have identified multiple molecular alterations in the esophagus of rats treated with NMBA (43). Preneoplastic lesions and papillomas from NMBA-treated esophagus were found to contain Ha-ras and p53 transition mutations, and elevated levels of inducible nitric oxide synthase (iNOS), transforming growth factor-\(\alpha\) (TGF-\(\alpha\)), epidermal growth factor receptor (EGFR), and cyclins \(\mathcal{D}_1\) and \(\mathcal{E}\) (44–48). It is possible that these genes, or other genes, are altered to greater extents in the esophagus of high-dose (0.5 mg/kg) NMBA-treated rats thus making them more difficult to modulate with chemopreventive agents. Combinations of chemopreventive agents that modulate multiple molecular targets may be required to prevent esophageal cancer in rats treated with the higher dose of NMBA. In this regard, we reported that \(S,S'\)-1,4-phenylene-bis(1,2-ethanediyl)bis-isothiourea (dihydrobromide) (PBIT), a selective iNOS inhibitor, significantly inhibited tumor progression in the esophagus of rats that were pre-initiated with NMBA (49). Studies are underway to examine the effects of PBIT in conjunction with COX-2 inhibitors.

Piroxicam exhibits chemopreventive activity in several animal model systems however, it was ineffective in the NMBA-induced rat esophagus model when added to the diet at 200 p.p.m. (24). Nevertheless, in the present study, we decided to use piroxicam in conjunction with L-706 since both COX-1 and COX-2 are overexpressed in the esophagus of NMBA-treated rats, and piroxicam exhibits inhibitory activity principally for COX-1 (50). Piroxicam was used at 200 p.p.m. in the diet, which is 40% of the maximum tolerated dose for this compound in male F344 rats (35). Our results indicated that piroxicam did not enhance the inhibitory effects of L-706 on tumor burden, but the combination treatment did produce a significant reduction in the incidence of high-grade dysplasia, and it reduced the production of PGE\(_2\) and cellular proliferation to a greater extent than 150 p.p.m. L-706 alone. To produce even greater inhibitory effects on esophageal tumorigenesis in this model may require targeting other genes in addition to the cyclooxygenases.

In summary, L-706, a novel selective COX-2 inhibitor, reduced esophageal tumor incidence and multiplicity in a dose-responsive fashion in rats pretreated with the low-dose of NMBA. Its ability to inhibit tumor development in this model system when added to the diet following treatment of animals with NMBA is significant in that a large number of chemopreventives have been ineffective in rat esophagus when administered in the diet post-initiation (43). Inhibition of tumor development by L-706 was correlated with reductions in esophageal cell proliferation rates and PGE\(_2\) levels. Its ineffectiveness in preventing tumor development in high-dose NMBA-treated animals could be due to an insufficient reduction in PGE\(_2\) levels in the esophagus. L-706 in combination with piroxicam produced somewhat greater inhibitory effects on tumor incidence, tumor multiplicity and cell proliferation rates in rats treated with both doses of NMBA than L-706 alone. Mechanistically, it would appear that non-steroidal anti-inflammatory drugs must reduce PGE\(_2\) levels to nearly those levels seen in normal esophagus to elicit tumor preventative effects in the esophagus of NMBA-treated rats.

Conflict of Interest Statement: None declared.

References


