The Effect of Selective Cyclooxygenase-2 Inhibition in Barrett’s Esophagus Epithelium: An In Vitro Study


Background: Individuals with Barrett’s esophagus, in which the normal squamous esophageal epithelium is replaced with a columnar mucosa, are at increased risk for esophageal adenocarcinoma. Mucosal injury may be involved in the progression to neoplasia via the synthesis of prostaglandins and other mediators of inflammation. Cyclooxygenase (COX)-2 is the rate-limiting enzyme involved in prostaglandin synthesis. We examined the effect of inhibiting COX-2 activity in Barrett’s esophageal cells. Methods: Primary esophageal epithelial and fibroblast cell cultures were established from endoscopic biopsy specimens from 20 consecutive patients with Barrett’s esophagus. COX-2 expression and activity were determined on pooled cell cultures by reverse transcription–polymerase chain reaction and prostaglandin E2 (PGE2) enzyme immunoassay, respectively. Proliferation was measured by Ki-67 staining. PGE2 levels were determined in supernatants from epithelial cells treated with the selective COX-2 inhibitor NS-398, proinflammatory cytokines (interleukin 1β and tumor necrosis factor-α), and conditioned medium from fibroblast cultures (both unstimulated and stimulated with proinflammatory cytokines). Results: Esophageal epithelial cells and fibroblasts expressed COX-2 messenger RNA. Compared with control-treated cells, NS-398 decreased proliferation of Barrett’s esophageal epithelial cells by 55% (95% confidence interval = 47.1% to 63.8%; P < .001) and decreased COX-2 activity. The addition of exogenous PGE2 reversed the antiproliferative effect of NS-398 on Barrett’s esophageal epithelial cells. Proinflammatory cytokines did not affect COX-2 activity in esophageal epithelial cells but stimulated COX-2 activity in fibroblasts. However, conditioned medium from unstimulated and stimulated fibroblasts increased COX-2 activity in esophageal epithelial cells. Conclusion: COX-2 is functionally active in Barrett’s esophagus because treatment with the COX-2 inhibitor hinders proliferation of Barrett’s esophageal epithelial cells in culture, but proliferation is restored by treatment with prostaglandin. These results raise the possibility that inhibition of COX-2 may have chemopreventive potential for Barrett’s esophagus. [J Natl Cancer Inst 2002;94: 422–9]
of Barrett’s esophageal epithelial cells by stromal cells remain undefined.

In this study, we examined the expression of COX-2 in Barrett’s esophageal epithelial cells and esophageal fibroblasts and assessed the relative COX-2 activity of these cells in the presence of proinflammatory cytokines. We also evaluated the effect of a selective COX-2 inhibitor, NS-398, on the proliferation of Barrett’s esophageal epithelial cells to determine its chemopreventive potential.

Materials and Methods

Biopsy Specimens

The Institutional Review Board of the Mayo Graduate School of Medicine (Rochester, MN) approved the study, and all patients provided written informed consent to provide tissue. Tissue samples were obtained during routine surveillance endoscopy performed between August 1999 and February 2000 on 20 consecutive patients who were known to have Barrett’s esophagus. Barrett’s esophagus is defined by the presence of specialized columnar metaplasia on biopsy specimens from the esophagus. Four biopsy specimens from each patient were taken for cell culture. Additional biopsy specimens from the Barrett’s segment were obtained for histologic analyses by taking four quadrant biopsies in every centimeter of Barrett’s mucosa. Eighteen of the 20 patients were male, with a mean age of 65 ± 9 years (mean ± SD). Ten patients had Barrett’s esophagus without dysplasia, five had low-grade dysplasia, and five had high-grade dysplasia.

Cell Culture

Barrett’s esophageal epithelial and fibroblast primary cultures. Biopsy specimens for tissue culture were immediately placed on ice in minimal essential medium (Sigma Chemical Co., St. Louis, MO). Within 4 hours from the time of the biopsy, the specimens were minced into approximately 2-mm³ fragments. Primary cell cultures were initiated by maintaining the cells in Barret’s plus media, as previously described. Primary cell cultures were initiated by maintaining the cells in Barret’s plus media, as previously described. Four biopsy specimens from each patient were taken for cell culture. Additional biopsy specimens from the Barrett’s segment were obtained for histologic analyses by taking four quadrant biopsies in every centimeter of Barrett’s mucosa. Eighteen of the 20 patients were male, with a mean age of 65 ± 9 years (mean ± SD). Ten patients had Barrett’s esophagus without dysplasia, five had low-grade dysplasia, and five had high-grade dysplasia.

Control cell lines. The HCA-7 (COX-2-expressing) and HCT-15 (COX-2-deficient) colon cancer cell lines (donated by Dr. S. C. Kirkland, Imperial Cancer Research Fund Histopathology Unit, Royal Postgraduate Medical School, London, U.K.) were used as positive and negative controls, respectively. HCA-7 and HCT-15 cell cultures were maintained in DMEM with 10% FBS and plated at a concentration of 30,000 cells/mL in each well of four-well chamber slides for 72 hours.

Analysis of COX-2 Expression and Activity

COX-2 mRNA expression was assessed by reverse transcription–polymerase chain reaction (PCR). Total cellular RNA was extracted from the Barrett’s esophageal epithelial cell and fibroblast cultures and from the HCA-7 and HCT-15 cell lines with the RNeasy Extraction Kit (Qiagen, Valencia, CA) according to the manufacturer’s protocol. Reverse transcription–PCR was performed using the Qiagen One Step Kit (Qiagen). A master mix of enzymes and buffers was made and, after the addition of the COX-2 primers (5’-TGAAACCCACTTCAAACACAG-3’, and 5’-TCATCAGGCACAGGAGAACAG-3’) (20), 50-μL aliquots of the mix were placed in individual PCR tubes along with 1 μg of the extracted RNA from each sample. The tubes then were placed in a PerkinElmer Gene Amp 9600 system thermocycler and heated to 95°C for 15 minutes to inactivate the reverse transcriptase and activate the HotStarTaq polymerase, which began the PCR cycles (21) of 94°C for 60 seconds, 52°C for 60 seconds, and then 72°C for 60 seconds. Finally, after 40 PCR cycles, the tubes were heated to 72°C for 10 minutes and cooled to 4°C overnight. B-Globin primers were used as internal controls for loading the RNA (22). PCR products were separated by electrophoresis through 1.2% agarose gels and visualized after staining the gels with 0.5 μg/mL ethidium bromide. The size of PCR products was assessed by comparing the PCR products with 2 μg of HaeIII-digested φ174 marker ( Gibco BRL) that was subjected to electrophoresis through the same gel. The predicted sizes of the PCR products were 232 bp for COX-2 and 600 bp for β-globin.

Analysis of COX-2 activity. COX-2 activity was assessed in cell culture supernatants with the use of a competitive PGE2 enzyme immunoassay kit (Cayman Chemical Co., Ann Arbor, MI) according to the manufacturer’s recommendations. Duplicates of known PGE2 concentrations were used to generate a standard curve from which the PGE2 concentrations in the supernatants could be determined. Intra- and interassay coefficients of variance were less than 10%. The specificity of the assay is 100% for PGE2, with a minimal detection limit of 75 pg/mL.

Journal of the National Cancer Institute, Vol. 94, No. 6, March 20, 2002 ARTICLES 423
Final PGE₂ concentrations were converted on a log scale and corrected for the level of protein content of the cell culture; this minimized the effect of any differences in cell number. The total protein concentration of the cell cultures was determined with the use of a Micro BCA protein assay kit (Pierce, Rockford, IL). For some experiments, cell cultures were treated for 24 h with a combination of the cytokines IL-1β (final concentration 10 ng/mL) and TNF-α (final concentration 50 ng/mL) before the PGE₂ concentration was measured in the culture supernatants.

Analysis of Cell Proliferation and Apoptosis

The 72-h, four-well cultures of Barrett’s esophageal epithelial cells, HCA-7, and HCT-15 were treated with 1, 25, and 50 μmol of the selective COX-2 inhibitor, NS-398 (Cayman Chemical Co.) or treated with an equivalent volume of the diluent dimethyl sulfoxide (DMSO; final concentration 0.1%). The culture media containing the drug or the diluent were replaced every other day for 6 days. Proliferation was assessed by immunostaining the cells with the Ki-67 mouse monoclonal antibody, which detects the nuclei of proliferating cells (Roche Diagnostics, Indianapolis, IN). After the supernatant was removed, the cells on the four-well chamber slides were rinsed three times with phosphate-buffered saline (PBS) at pH 7.4 and air-dried. Slides were stored at 4 °C until stained. Immediately before staining, cells were fixed in ice-cold methanol for 30 minutes. Slides were washed twice with PBT (PBS containing 0.1% bovine serum albumin and 1% Tween 20, made with Sigma reagents). After several washes, slides were incubated with the Ki-67 antibody (10 μg/mL) in PBT at room temperature for 30 minutes. Slides were washed twice with PBT and then incubated with fluorescein-conjugated anti-mouse immunoglobulin G (Roche Diagnostics) (10 μg/mL) in PBS for 30 minutes at room temperature, in the dark. After washing twice with PBS, coverslips were applied to the slides with GEL/Mount (Biomedia Corp., Foster City, CA) and stored at 4 °C in the dark until read. The total number of Ki-67-positive cells, which reflects the number of potentially proliferating cells, was determined in each sample by directly counting the cells on a fluorescence microscope (Axioskop; Carl Zeiss, New York, NY). A minimum of 300 cells was scored for every treatment, and each sample was counted twice by the same technician. A four-well chamber control slide with the immortal keratinocyte HaCats cell line was included with each batch as an internal control for staining. HaCats cells were treated with or without cyclohexamide (100 ng/mL) for 48 hours. For a control, Barrett’s esophageal epithelial cells were treated with DMSO (final concentration 0.1%).

Analysis of Apoptosis

The terminal deoxynucleotidyltransferase-mediated uridine triphosphate nick end-labeling assay (TUNEL assay; Roche Diagnostics) was used to determine whether inhibition of COX-2 activity induced apoptosis. The 72-h, four-well cultures of Barrett’s esophageal epithelial cells were treated with 1, 25, and 50 μmol of the selective COX-2 inhibitor, NS-398 (Cayman Chemical Co.) or treated with an equivalent volume of the diluent DMSO (final concentration 0.1%). The culture media containing the drug or the diluent were replaced every other day for 6 days. Apoptosis was assessed at the end of the experiment. After removal of the supernatant, the Barrett’s esophageal epithelial cells were incubated in a solution of 0.1% Triton X-100, 0.1% sodium citrate for 2 minutes on ice (4 °C). After this permeabilization step, 50 μL of the TUNEL reaction mixture was added to the cells. The cells then were incubated in a humidified chamber for 60 min at 37 °C in the dark. The same technician analyzed all samples under a fluorescence microscope and recorded the percentage of cells undergoing apoptosis. A four-well chamber control slide with HaCats cells was included as an internal control for staining. HaCats cells were treated with 100 μg/mL cyclohexamide to induce apoptosis (positive control) or DMSO (final concentration 0.1%). A well stained without the TUNEL mixture served as a negative control.

Preparation of Esophageal Fibroblast-Conditioned Medium and Testing Its Effect on COX-2 Activity

Fibroblasts were grown to a culture confluence of 75%–90%, rinsed twice with serum-free DMEM, and grown in a serum-free modification of Barrett’s plus media (SFD) containing bovine pituitary extract (140 μg/mL) and bovine serum albumin (0.1%). For the last 24 h of a 72-h incubation, a combination of IL-1β (10 ng/mL) and TNF-α (50 ng/mL) was added to the culture medium. After incubation, the supernatants were removed, centrifuged at 800 g in a bench top centrifuge for 15 minutes at room temperature, passed through a 0.2-μm filter, and then stored at −20°C.

To test the effect of esophageal fibroblasts on Barrett’s esophageal epithelial cell COX-2 activity, the culture medium from the 72-h, four-well Barrett’s esophageal epithelial cell cultures was removed and replaced with either 1 mL of SFD medium, SFD medium containing a combination of IL-1β (10 ng/mL) and TNF-α (50 ng/mL), or conditioned medium obtained from unstimulated or IL-1β-stimulated and TNF-α-stimulated fibroblasts. Because of the limited amount of Barrett’s esophageal epithelial cells and fibroblasts available, the combination of the proinflammatory cytokines IL-1β and TNF-α was used to maximally induce COX-2 activity (7–9). Control cells were treated with SFD medium alone or conditioned medium obtained from an unstimulated fibroblast culture. After 24 h, PGE₂ levels were measured in the supernatants of the Barrett’s esophageal epithelial cell cultures to determine COX-2 activity.

Statistical Analysis

Results are expressed as means with 95% confidence intervals (95% CIs). Each experiment was repeated at least four times. Cell proliferation and apoptosis are shown as the percentage of cells proliferating or undergoing apoptosis from a total of at least 300 cells. The COX-2 activity was expressed as a PGE₂ level in nanograms per milligram of protein in the supernatant of cell cultures.

To provide approximate normality for modeling purposes, when the response variable was a proportion, we used the arcsine transformation. The transformed value is equal to the arc-sine of the square root of the proportion. A log transformation was used for experiments in which the variable was the prostaglandin level. Repeated-measures models with a compound symmetric covariance structure, that is, equal correlation be-
between all pairs of treatments, were fit. These models were used because every time the experiment was repeated all the treatments were given. An overall F-test of treatment mean equality was done using each model. When the overall test was statistically significant at the $\alpha = 0.05$ level, we compared the treatment means in a pairwise fashion using the Bonferroni method of multiple comparisons ($t$ tests). Statistical analysis was performed using SAS software (version 6.12; SAS Institute, Cary, NC). All statistical tests were two-sided.

**RESULTS**

**Cell Culture**

In culture, Barrett’s esophageal epithelial cells were polygonal and spread in a monolayer. More than 90% of the cells were positive for periodic acid-Schiff, Alcian blue (pH 2.5), by cytochemical staining; positive for a panel of keratin proteins detected with mouse monoclonal antibodies (KA4, KA5, and RGE53); and positive for a Barrett’s esophagus-specific antigen detected with the monoclonal antibody Mab-Das-1. Less than 10% of the cells were fusiform or polygonal, positive for vimentin, and variably positive for $\alpha$-smooth muscle actin, but negative for the epithelial markers above. Esophageal fibroblast cultures were more than 90% fusiform cells. These cells were negative for periodic acid-Schiff, Alcian blue (pH 2.5), KA4, KA5, RGE53, and Mab-Das-1 staining but were positive for vimentin and variably positive for $\alpha$-smooth muscle actin. The morphologic and cytochemical characteristics of the cultures are similar to the Barrett’s esophageal epithelial and fibroblast cultures previously reported (17–19).

**COX-2 Expression and COX-2 Activity of Barrett’s Esophageal Epithelial Cells and Fibroblasts**

Both Barrett’s esophageal epithelial and esophageal fibroblast primary cell cultures expressed COX-2 mRNA by reverse transcription–PCR (Fig. 1, A). The steady-state COX-2 mRNA levels were comparable after culture in either the presence or absence of the proinflammatory cytokines IL-1$\beta$ (10 ng/mL) and TNF-$\alpha$ (50 ng/mL) for 24 hours (Fig. 1, A). Cells for this experiment were pooled from two patients: one with dysplastic esophageal epithelium and one with nondysplastic esophageal epithelium.

Because PGE2 levels are directly dependent on COX-2 activity, quantitative measurements of PGE2 can be used to assess relative COX-2 activity. The PGE2 level in epithelial cell culture supernatants was 0.2 ng/mg protein (95% CI = 0 to 1.2), which was statistically significantly different from the 2 ng/mg protein (95% CI = 1.0 to 3.1) from fibroblasts ($P = .002$). Treatment of Barrett’s esophageal epithelial cells with IL-1$\beta$ and TNF-$\alpha$ increased the PGE2 level in culture supernatants from 0.2 ng/mg protein (95% CI = 0 to 1.2) to 0.9 ng/mg protein (95% CI = 0 to 2.3), a statistically nonsignificant difference ($P = .044$, Bonferroni adjusted $\alpha = 0.005$). However, IL-1$\beta$ and TNF-$\alpha$ treatment of esophageal fibroblasts statistically significantly increased the PGE2 level from 2 ng/mg protein (95% CI = 1 to 3.1) to 2.9 ng/mg protein (95% CI = 1.9 to 3.9) ($P<.001$, Bonferroni adjusted $\alpha = 0.005$) (Fig. 1, B). COX-2 activity (i.e., PGE2 levels) was also statistically significantly higher in the culture supernatants of IL-1$\beta$-treated and TNF-$\alpha$-treated fibroblasts than in those of IL-1$\beta$-treated and TNF-$\alpha$-treated Barrett’s esophageal epithelial cells ($P<.001$, Bonferroni adjusted $\alpha = 0.005$). The COX-2-expressing HCA-7 colon cancer cell line, which served as a positive control, expressed COX-2 mRNA and produced high PGE2 levels of 3.4 ng/mg protein (95% CI = 2.4 to 4.4), whereas the COX-2-deficient HCT-15 colon cancer cell line, which served as a negative control, did not express COX-2 mRNA and had undetectable levels of PGE2.

**Proliferation, Apoptosis, and COX-2 Inhibition**

To determine a functional role for COX-2 activity in Barrett’s esophageal epithelial cells, we assessed cell proliferation in the presence of a selective COX-2 inhibitor (NS-398). A 6-day treat-
ment with NS-398 statistically significantly decreased the percentage of proliferating Ki-67-positive Barrett’s esophageal epithelial cells ($P<.001$). There was a dose-dependent relationship between NS-398 concentration and inhibition of proliferation (Fig. 2, A). Compared with the proliferation of Barrett’s esophageal epithelial cells treated with medium containing DMSO (11.6% proliferating cells; 95% CI = 6.4% to 16.8%), the proliferation of Barrett’s esophageal epithelial cells was inhibited when the cells were treated with 1 μmol (8.5% proliferating cells; 95% CI = 0.6% to 16.3%; $P = .038$), 25 μmol (8% proliferating cells; 95% CI = 0.6% to 15.3%; $P = .018$), or 50 μmol NS-398 (5.5% proliferating cells; 95% CI = 2.6% to 8.3%; $P<.001$; Bonferroni adjusted $\alpha = 0.0083$).

To determine whether the inhibition in proliferation was associated with an increase in the percentage of apoptotic cells, we next assessed the percentage of TUNEL-positive cells in Barrett’s esophageal epithelial cells 6 days after treatment with NS-398. Compared with Barrett’s esophageal epithelial cells treated with DMSO (7.8%; 95% CI = 5.6% to 9.8%), there was no statistically significant difference in the percentage of cells undergoing apoptosis among each of the treatment groups (7% [95% CI = 5.3% to 8.6%], 8% [95% CI = 4.9% to 11.0%], and 7.2% [95% CI = 4.9% to 9.4%], for 1, 25, and 50 μmol, respectively, $P = .874$) (Fig. 2, B).

We confirmed that treatment with NS-398 inhibited COX-2 activity by measuring PGE$_2$ levels. PGE$_2$ levels in culture supernatants of cells treated with 50 μmol of NS-398 for 6 days declined from 0.9 ng/mg protein (95% CI = 0 to 1.2 ng/mg) to below the detection level of the assay (0.075 ng/mL).

We next determined whether PGE$_2$ directly affected proliferation (Fig. 3). A 48-hour treatment with 1 ng of exogenous PGE$_2$ increased the proliferation of Barrett’s esophageal epithelial cells by 39.5% (95% CI = 18.8% to 60%; $P = .001$). PGE$_2$ increased the proliferation of Barrett’s esophageal epithelial cells by 18.8% (95% CI = 0.6% to 16.3%; $P = .001$), unchanged in those treated with NS-398 and PGE$_2$ (95% CI = 0.6% to 16.3%; $P = .821$), and statistically significantly increased in those treated with PGE$_2$ (95% CI = 2.6% to 8.3%; $P<.001$; Bonferroni adjusted $\alpha = 0.0083$) (Fig. 3).
Bonferroni adjusted $\alpha = 0.0083$) relative to the control DMSO-treated cells. A 48-hour treatment with NS-398 (50 $\mu$mol) inhibited the proliferation of Barrett’s esophageal epithelial cells by 33% (95% CI = 28% to 37%; $P = .001$, Bonferroni adjusted $\alpha = 0.0083$) relative to the control DMSO-treated cells. However, when Barrett’s esophageal epithelial cells were treated concomitantly with PGE$_2$ and NS-398, there was no change in proliferation ($P = .821$).

To confirm that the effects of NS-398 were specific for COX-2, we compared the effects of NS-398 on COX-2-expressing and COX-2-deficient colon cancer cell lines. Compared with DMSO-treated controls, treatment with NS-398 (50 $\mu$mol for 6 days) decreased the proliferation of Barrett’s esophageal epithelial cells by 55% (95% CI = 47% to 64%; $P < .001$, Bonferroni adjusted $\alpha = 0.0083$). Similarly, treatment with NS-398 (50 $\mu$mol) inhibited the proliferation of HCA-7 cells by 52% (95% CI = 19% to 85%; $P < .001$, Bonferroni adjusted $\alpha = 0.0083$). However, treatment with NS-398 (50 $\mu$mol) inhibited the proliferation of HCT-15 cells by only 6% (95% CI = 0% to 20%; $P = .067$, Bonferroni adjusted $\alpha = 0.0083$).

**Esophageal Fibroblasts Modulate COX-2 Activity of Barrett’s Esophageal Epithelial Cells**

Although the proinflammatory cytokines IL-1$\beta$ and TNF-$\alpha$ had minimal direct effect on the COX-2 activity (PGE$_2$ levels) of Barrett’s esophageal epithelial cells, we postulated that there could be a paracrine loop in Barrett’s esophagus whereby these cytokines could activate stromal cells that in turn could stimulate the proliferation of the epithelial cells. We next examined whether esophageal fibroblasts could modulate the COX-2 activity of the Barrett’s esophageal epithelial cells by a possible stromal–epithelial interaction. When Barrett’s esophageal epithelial cells were treated with the conditioned medium from unstimulated fibroblasts, the PGE$_2$ level statistically significantly increased to 1.4 ng/mg protein (95% CI = 0 to 2.8 ng/mg; $P = .004$, Bonferroni adjusted $\alpha = 0.0083$). When the Barrett’s esophageal epithelial cells were treated with conditioned medium from 10 ng/mL IL-1$\beta$-stimulated and 50 ng/mL TNF-$\alpha$-stimulated fibroblasts, the PGE$_2$ level underwent an even greater (and statistically significant) increase to 2.3 ng/mg protein (95% CI = 0.8 to 3.7 ng/mg; $P < .001$, Bonferroni adjusted $\alpha = 0.0083$) (Fig. 4). This level may be an overestimate, however, because there was PGE$_2$ in the conditioned medium from the IL-1$\beta$-stimulated and TNF-$\alpha$-stimulated fibroblasts (2.9 mg/ng protein; 95% CI = 1.9 to 3.9 ng/mg protein). However, PGE$_2$ is very unstable in the conditioned medium, and the maximum PGE$_2$ concentration that could have persisted and transferred in the conditioned medium to the epithelial cells was 1.3 ng/mg protein. This assumption is based on our measurements of PGE$_2$ in conditioned media at room temperature; after 24 h, in the absence of any cells, the concentration of PGE$_2$ decreased by 55.2% (95% CI = 46.5% to 64.3%). After correcting for this transferred amount, the IL-1$\beta$-stimulated and TNF-$\alpha$-stimulated fibroblast-conditioned media statistically significantly increased the PGE$_2$ level secreted from the Barrett’s esophageal epithelial cells to 1 ng/mL protein (95% CI = 0.3 to 1.6 ng/mL; $P = .002$, Bonferroni adjusted $\alpha = 0.0083$).

**DISCUSSION**

Barrett’s esophagus is characterized by the replacement of normal squamous esophageal epithelium with a specialized columnar mucosa in response to chronic gastroesophageal reflux (1). It is considered a premalignant condition that is associated with a 30- to 125-fold increased risk of esophageal adenocarcinoma relative to occurrence in the general population (23). The incidence of esophageal adenocarcinoma is rapidly increasing among Caucasian males (2,3). The prognosis of esophageal adenocarcinoma is poor, with a 5-year survival rate of 13%–15% (24,25). Unfortunately, no medical therapy has been proven to attenuate the progression of Barrett’s esophagus to esophageal cancer. Although mucosal ablative therapies have been proposed, their application is difficult and costly (26). Therefore, developing a chemopreventive strategy for the management of Barrett’s esophagus would be of great benefit.

Our findings indicate that COX-2 activity may be a potential target for the prevention of progression from premalignant Barrett’s esophagus. Because Barrett’s esophagus is associated with chronic inflammation and injury caused by the gastric contents during reflux, we focused on potential mediators of inflammation. COX-2 is an inducible isoform of cyclooxygenase, which is expressed in epithelial and stromal cells in response to injury, growth factors, tumor promoters, and cytokines (27–30). Animal studies (31), *in vitro* cell culture experiments (32,33), and recent human studies (34,35) all have shown that COX-2 is involved in colon carcinogenesis and that selective inhibition of COX-2 has antineoplastic effects. By contrast,
there is limited information on the potential role of COX-2 in the development of esophageal adenocarcinoma from Barrett’s esophagus. Wilson et al. (14) showed that COX-2 mRNA and protein expression are higher in Barrett’s esophagus than in normal esophagus. Recently, Shirvini et al. (5) studied the expression of COX-2 in esophageal biopsy specimens by immunostaining and immunoblot analysis and demonstrated that expression of COX-2 is higher in Barrett’s esophagus and esophageal adenocarcinoma than in normal esophagus and duodenum. Expression of COX-2 was substantially higher in dysplastic Barrett’s esophagus and cancer than in nondysplastic Barrett’s esophagus, whereas COX-1 expression was unchanged (5). COX-2 activity was not addressed in these studies (5,6), however, and it was not clear if the increased COX-2 expression had a functional relevance.

Our data suggest that COX-2 is not only expressed but is also functionally active in Barrett’s esophagus because the inhibitor NS-398 statistically significantly inhibited the proliferation of Barrett’s esophageal epithelial cells. At a concentration of 50 μmol, NS-398 decreased the proliferation of Barrett’s esophageal epithelial cells by more than 50% and completely inhibited COX-2 activity. NS-398 had comparable effects on the proliferation of esophageal epithelial cells by more than 50% and completely inhibited COX-2 activity. NS-398 statistically significantly inhibited the proliferation of Barrett’s esophagus and adenocarcinoma: ex vivo induction by bile salts and acid exposure. Gastroenterology 2000; 118:487–96.


Palanca-Wessels MC, Barrett MT, Galipeau PC, Rohrer KL, Reid BI, Rabinovich PS. Genetic analysis of long-term Barrett’s esophagus epithe-


NOTES

Supported in part by Public Health Service grants CA85992–01 and CA78870–01 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by the Mayo Foundation. Manuscript received July 17, 2001; revised December 18, 2001; accepted January 23, 2002.