Antireflux Surgery Normalizes Cyclooxygenase-2 Expression in Squamous Epithelium of the Distal Esophagus

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BACKGROUND: In some patients gastoesophageal reflux disease presents with heartburn and regurgitation symptoms but a relative paucity of endoscopic and clinical findings, while in others symptoms may be minor or absent yet there is significant mucosal damage on endoscopy including the presence of Barrett’s esophagus. The initial injury of gastroesophageal reflux is to the squamous esophageal mucosa, but while substantial research has been devoted to determining which genes are involved in the progression of Barrett’s to dysplasia and cancer, little is known about the gene expression alterations in the squamous mucosa of patients with reflux. We hypothesized that the expression of cyclooxygenase-2 (Cox-2) might be increased in the squamous esophageal mucosa of patients with reflux, and might be a molecular indicator of reflux injury. Further, we hypothesized that Cox-2 expression in the squamous mucosa would be reduced following the elimination of reflux with an antireflux operation.

METHODS: Biopsies of the distal esophageal squamous mucosa were taken 3 cm above the squamocolumnar junction (SCJ) in 28 GERD patients before and after Nissen fundoplication. Following microdissection and RNA isolation, quantitative real-time PCR was used to measure Cox-2 gene expression in paraffin-embedded (n = 16) and fresh frozen (n = 12) tissue. Biopsies from patients (paraffin n = 15, frozen n = 14) with normal acid exposure and no evidence of mucosal injury were analyzed as controls.

RESULTS: Median Cox-2 expression in the squamous epithelium from paraffin embedded biopsies in patients with reflux disease was significantly increased compared to controls (p = 0.037). The presence of esophagitis or Barrett’s esophagus did not significantly alter the expression of Cox-2 compared to patients with nonerosive reflux disease (NERD). After antireflux surgery median Cox-2 expression values were significantly reduced (p = 0.001) and were normalized to levels similar to controls without reflux (p = 0.74). Similar results were observed in the prospectively obtained fresh frozen tissue.

CONCLUSIONS: Cox-2 gene expression is increased in the distal esophageal squamous mucosa of most patients with GERD, and the elevation was similar whether there was mucosal injury in the form of esophagitis or Barrett’s or no visible mucosal injury. This suggests that increased Cox-2 expression may serve as a molecular marker of reflux disease. The increased Cox-2 expression in patients with reflux was usually normalized following antireflux surgery. These findings demonstrate for the first time that gene expression can be altered by surgical correction of reflux. Thus, in addition to symptom control and improvement in the quality of life, perhaps future studies assessing the efficacy of antireflux therapy should also focus on the impact of the therapy on gene expression in the esophageal squamous mucosa.

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INTRODUCTION

Gastroesophageal reflux disease is one of the most common ailments in the Western world (1). Although widely associated with the typical symptoms of heartburn and regurgitation, the disease can manifest clinically in a myriad of fashions. Some patients have severe symptoms yet a paucity of findings on upper endoscopy, while others are asymptomatic but have developed one of the most important consequences of reflux disease—Barrett’s esophagus. It is clear that symptomatic, endoscopic, and clinical findings often are disparate, yet presumably the same process, i.e., gastroesophageal reflux, is occurring in these patients.

Recent biotechnological developments have made it possible to study the molecular changes associated with reflux in a precise fashion. However, most investigative efforts have been directed at Barrett’s and esophageal adenocarcinoma where changes at the genomic level such as p53 and p16 mutations, gene methylation and aneuploidy, as well as abnormal expression of growth factors, cell adhesion molecules, and cell signaling factors have been reported (2). Much less is known about the molecular changes that occur in the distal esophageal squamous mucosa as a consequence of exposure to refluxed gastric juice.

Cyclooxygenase-2 (Cox-2) is one of the rate-limiting enzymes in the conversion of arachidonic acid to prostaglandins. Cox-2 participates in the regulation of a broad range of cellular processes including proliferation, angiogenesis, and resistance to apoptosis. It is also one of the genes that has been implicated in the development of a number of gastrointestinal cancers, including Barrett’s associated esophageal adenocarcinoma (2–6). Recently we reported that among nine genes evaluated in distal esophageal squamous mucosal biopsies only the expression of the Cox-2 gene correlated with the amount of esophageal acid exposure based on 24-h pH monitoring (7). Based on these findings we hypothesized that increased Cox-2 expression in squamous esophageal mucosal biopsies might be a molecular indicator of reflux injury, and may represent a common denominator among patients with various clinical manifestations of reflux disease. Further, we hypothesized that Cox-2 expression in the squamous mucosa would be reduced following the elimination of reflux with an antireflux operation. To test this hypothesis we evaluated Cox-2 gene expression in the squamous mucosa from patients with varying manifestations of reflux disease both before and after Nissen fundoplication, and compared these values to those present in control patients documented to have normal esophageal acid exposure. The study was done retrospectively and verified prospectively.

MATERIALS AND METHODS

Study Populations, Tissue Samples, and Definitions

REFLUX GROUP, RETROSPECTIVE STUDY. This study population was strictly defined to allow precise correlation of Cox-2 gene expression levels from biopsies obtained before and after Nissen fundoplication. All patients were seen by a member of the thoracic/foregut division at the University of Southern California between 1995 and 2004, and those that met the following criteria were included:

1. Gastroesophageal reflux disease proven by increased esophageal acid exposure on 24-h pH monitoring.
2. Primary Nissen fundoplication done in a standardized fashion at the University of Southern California.
3. An upper endoscopy with biopsies before and at least 6 months after surgery, with an intact fundoplication by endoscopic assessment.
4. Availability of paraffin-embedded tissue blocks from biopsies taken at a similar location within squamous epithelium before and after Nissen fundoplication with adequate RNA for gene expression analysis.

Sixteen patients met the criteria for inclusion and comprised the retrospective reflux group.

REFLUX GROUP, PROSPECTIVE STUDY. To confirm the findings of the retrospective group we performed a prospective study in 12 patients from whom fresh frozen biopsies were analyzed. This study population was comprised patients evaluated by a member of the thoracic/foregut division at the University of Southern California between 2002 and 2004 who were found to have increased esophageal acid exposure on 24-h pH monitoring and subsequently had a primary Nissen fundoplication. Endoscopic biopsies were obtained from these patients prospectively before and after Nissen fundoplication and kept frozen in liquid nitrogen until analysis.

CONTROL, NONREFLUX GROUP. A separate control was used for each study group based on whether the tissue samples were fresh frozen or embedded in paraffin. There were 15 paraffin specimen controls and 14 frozen specimen controls. All control patients had documented normal esophageal acid exposure on 24-h pH monitoring and a normal endoscopy. Biopsies were taken from the distal esophagus 3 cm proximal to a normal appearing SCJ, and all biopsies demonstrated squamous epithelium without any evidence of histologic esophagitis.

UPPER ENDOSCOPY WITH BIOPSIES. Upper endoscopy was performed in a standardized fashion in all patients. The locations of the diaphragmatic crural impression, gastroesophageal junction (GEJ), and squamocolumnar junction (SCJ) were determined in each patient. The presence and extent of erosive esophagitis or columnar lined segments was documented. Routine preoperative biopsies in all patients included retroflexed biopsies at the GEJ, and antegrade biopsies of the antrum, body of the stomach, and every 2 cm from the GEJ to the SCJ if a columnar lined esophagus was present. Lastly, biopsies were taken from squamous mucosa 3 cm proximal to the SCJ. Following Nissen fundoplication, biopsies were again taken according to the same protocol. Furthermore, the postoperative endoscopy included a careful
evaluation of the appearance of the fundoplication to confirm the integrity of the repair.

DEFINITIONS. Patients were considered to have gastroesophageal reflux based on the presence of reflux symptoms with documentation of increased esophageal acid exposure on 24-h pH monitoring. Barrett’s esophagus was diagnosed when there was an endoscopically visible segment of columnar mucosa in the distal esophagus, which on biopsy demonstrated intestinal metaplasia. Patients with a biopsy showing intestinal metaplasia within cardiac mucosa from a normal appearing GEJ were considered to have cardia intestinal metaplasia within cardiac mucosa from a normal mucosa in the distal esophagus, which on biopsy demonstrated intestinal metaplasia, and were not included in the Barrett’s group. The presence or absence of Helicobacter pylori was determined by Giemsa staining of antral biopsies in all patients.

ANTIREFLUX SURGERY. Patients underwent Nissen fundoplication in a standardized fashion by a member of the thoracic/foregut division at USC as previously described (8, 9). The procedure in all patients included esophageal mobilization, reduction of any associated hiatal hernia, crural closure. The operations were performed laparoscopically or as an open abdominal or transthoracic approach based on the severity of reflux and associated anatomic abnormalities including the size of the hiatal hernia.

This study was approved by the Institutional Review Board of the University of Southern California, Keck School of Medicine. Written informed consent was obtained from patients participating in the prospective study.

MICRODISSECTION. In the retrospective group formalin fixed paraffin-embedded biopsies were obtained and 10-μm sections were made. In the prospective group biopsies were immediately snap frozen and stored in liquid nitrogen, and were subsequently embedded in optimal cutting temperature (OCT) compound (Sakura Finetek USA, Inc., Torrance, CA) and 20-μm sections were made. Sections were mounted on uncoated glass slides. The OCT sections were stored at −80°C while paraffin sections were kept at room temperature. For histological confirmation sections from the beginning, middle, and end of each tissue block were stained with hematoxylin and eosin (H&E) and examined by a single expert gastrointestinal pathologist (PTC).

Before microdissection, paraffin sections were deparaffinized in xylene for 10 min, rehydrated with 100%, 95%, and finally 70% ethanol, and were washed in H2O for 30 s. The OCT frozen specimens were air-dried, fixed in 70% ethanol for 3 min and washed in H2O for 2 min. Afterward, both paraffin and OCT sections were stained with nuclear fast red (NFR, American MasterTech Scientific, Inc., Lodi, CA) for 30 s and washed in H2O for 30 s. Samples were then dehydrated in 70%, 95%, and 100% ethanol for 30 s each, followed by incubation in xylene for 10 min and complete air-drying. Samples were dissected from the slides using a scalpel if the histology was homogeneous and contained more than 90% squamous epithelium. All other sections were selectively isolated by laser capture microdissection (P.A.L.M. Microsystem, Leica, Wetzlar, Germany) according to a standard procedure (10). The dissected flakes of tissue were transferred to a reaction tube containing 400 μL of RNA lysis buffer.

RNA ISOLATION AND CDNA SYNTHESIS. Tissue samples to be extracted were placed in a 0.5-mL thin walled tube containing 400 μL of 4 M diethiothreitol (DTT)-GITC/sarc (4 M guanidinium isothiocyanate, 50 mM Tris-HCl, pH 7.5, 25 mM EDTA) (Invitrogen; #15577-018). The samples were homogenized and an additional 60 μL of GITC/sarc solution was added. They were heated at 92°C for 30 min and then transferred to a 2 mL centrifuge tube. A measure of 50 μL of 2M sodium acetate was added at pH 4.0, followed by 600 μL of freshly prepared phenol/chloroform/isoamyl alcohol (250:50:1). The tubes were vortexed for 15 s, placed on ice for 15 min and then centrifuged at 13,000 rpm for 8 min in a chilled (8°C) centrifuge. The upper aqueous phase (250–350 μL) was carefully removed and placed in a 1.5-mL centrifuge tube. Glycogen (10 μL) and 300–400 μL of isopropanol were added and the samples vortexed for 10–15 s. The tubes were placed at −20°C for 30–45 min to precipitate the RNA. The samples were then centrifuged at 13,000 rpm for 7 min in a chilled (8°C) centrifuge. The supernatant was poured off and 500 μL of 75% ethanol was added. The tubes were centrifuged at 13,000 rpm for 6 min in a chilled (8°C) centrifuge. The supernatant was carefully poured off so as not to disturb the RNA pellet and the samples were quick-spun for 15 s at 13,000 rpm. The remaining ethanol was removed with a 20-μL pipette and the samples air-dried for 15 min. The pellet was resuspended in 50 μL of 5 mM Tris. Afterward, cDNA was prepared as previously described (11). Known positive and negative RNA controls were run in parallel with all study samples to ensure appropriate isolation, and a separate RNA control was used to confirm reverse transcription.

REAL-TIME PCR QUANTIFICATION OF mRNA EXPRESSION. Quantification of Cox-2 and an internal reference gene (β-actin) was performed using a fluorescence based real-time detection method [ABI PRISM 7900 Sequence detection System (TaMan®) Perkin-Elmer (PE) Applied Biosystem, Foster City, CA, USA]. The PCR reaction mixture consisted of 1,200 nM of each primer, 200 nM probe, 0.4 U of AmpliTaq Gold Polymerase, 200 nM each dATP, dCTP, dGTP, dTTP, 3.5 mM MgCl2, and 1× Taqman® Buffer A containing a reference dye, to a final volume of 20 μL (all reagents from PE Applied Biosystems). Cycling conditions were 50°C for 2 min, 95°C for 10 min, followed by 46 cycles at 95°C for 15 s and 60°C for 1 min. The primers and probes used are listed in Table 1.

TaqMan® measurements yield Ct values that are inversely proportional to the amount of cDNA in the tube, i.e., a higher
STATISTICAL ANALYSIS. Cox-2 gene expression levels from biopsies taken in the squamous mucosa 3 cm proximal to the SCJ in patients before and after Nissen fundoplication were compared using the Wilcoxon signed ranks test. The Mann-Whitney U test was used to compare continuous variables and χ² was used to compare proportions. Spearman correlation analysis was performed to assess the relationship between time interval from surgery and change in Cox-2 gene expression levels. Statistical significance was set at p < 0.05. Cox-2 gene expression levels from the retrospective and prospective groups were evaluated separately since paraffin-embedded and fresh frozen tissues had a different magnitude of gene expression.

RESULTS

Retrospective Paraffin-Embedded Biopsy Group

Sixteen patients with matched pre- and post-Nissen biopsies from squamous mucosa 3 cm proximal to the SCJ met all of the criteria for retrospective assessment of Cox-2 gene expression, and in Table 2 the clinical characteristics and preoperative findings are summarized. Increased esophageal acid exposure on 24-h pH monitoring was present in all patients, but the endoscopic findings varied substantially. In three patients (18.9%) the endoscopic appearance of the esophagus was normal, and these patients were considered to have nonerosive reflux disease (NERD). Erosive esophagitis was present in three patients (18.9%), and Barrett’s esophagus was present in 10 (62.5%). All 16 patients underwent primary Nissen fundoplication (13 laparoscopic, 2 transthoracic, and 1 open abdominal).

The mean time interval from surgery to postoperative endoscopy and biopsy was 33 months (range 10–96 months). Preoperative reflux symptoms were completely eliminated in all but two patients (88%). These 2 patients noted occasional heartburn, but both had normal esophageal acid exposure on postoperative 24-h pH monitoring. A total of 7 patients underwent postoperative 24-h pH testing and all had normal esophageal acid exposure (median score 0.95). The fundoplication was intact by endoscopic evaluation and esophagitis was absent in all patients.

Cox-2 gene expression in the squamous mucosa of patients with reflux disease was significantly higher than in nonreflux controls (Fig. 1). After antireflux surgery Cox-2 gene expression levels decreased in 13 patients (81%) (Fig. 2). Median Cox-2 gene expression was significantly reduced after Nissen fundoplication and reached a level similar to the nonreflux control group (Fig. 3).

Prospective Frozen Biopsy Group

The clinical characteristics and preoperative findings of the 12 patients in the prospective group are shown in Table 2. All had increased esophageal acid exposure on 24-h pH monitoring, and on upper endoscopy 5 patients (41.7%) had NERD, 6 (50.0%) had erosive esophagitis, and 1 (8.3%) had Barrett’s. Primary Nissen fundoplication was performed in all 12 patients (7 laparoscopic, 4 transthoracic, and 1 open abdominal).

The mean time interval from surgery to postoperative endoscopy and biopsy was 21 months (range 14–33). Preoperative reflux symptoms were completely eliminated in all but one patient (92%). The fundoplication was intact by endo-
Control (n=15)  GERD (n=16)

Figure 1. Cox-2 gene expression in paraffin-embedded biopsies taken from the distal squamous esophagus in controls and GERD patients prior to antireflux surgery. Median Cox-2 expression was significantly higher in GERD patients (0.08 vs 0.165, \( p = 0.04 \)). The box demonstrates the 25th and 75th percentiles. Anoscopic evaluation and esophagitis was absent in all patients. None of these patients underwent postoperative 24-h pH monitoring.

Similar to the retrospective findings, median Cox-2 gene expression in the squamous mucosa from prospectively obtained frozen tissue was significantly higher in patients with reflux compared to nonreflux controls (Fig. 4). After antireflux surgery Cox-2 gene expression levels were reduced in 8 patients (66%), and the median expression reached a level similar to nonreflux controls (Fig. 5).

The Effect of Erosive Esophagitis and Barrett’s Esophagus on Cox-2 Expression

To determine whether the severity of reflux disease based on mucosal changes in the esophagus influenced Cox-2 gene expression we compared Cox-2 expression from the squamous mucosa in patients with NERD, erosive esophagitis, or Barrett’s esophagus. Compared to biopsies from patients with NERD, median Cox-2 gene expression in patients with endoscopic esophagitis was similar (median 0.12 vs 0.42, respectively, \( p = 0.25 \); frozen tissue prospective group). Similarly, Cox-2 gene expression was not significantly different whether Barrett’s was present or absent (median 0.16 vs 0.16, respectively, \( p = 0.43 \), paraffin-embedded retrospective group).

Further, we found that the proportion of patients with decreased Cox-2 expression following antireflux surgery was similar regardless of the presence of mucosal injury on preoperative endoscopy. When the paraffin and frozen biopsy

Figure 2. Cox-2 gene expression in paraffin-embedded squamous biopsies from individual patients before and after antireflux surgery. Cox-2 gene expression decreased in 13 of 16 patients, with an increase in 2 and no change in 1. Interestingly, the 3 patients without a decrease in Cox-2 expression all had a very low preoperative level of Cox-2 expression.

Figure 3. Cox-2 gene expression in paraffin-embedded squamous biopsies from GERD patients before and after antireflux surgery compared to controls. Median Cox-2 gene expression decreased significantly in GERD patients after Nissen (0.165–0.075, \( p = 0.0003 \)) and reached a median value similar to controls (0.075 vs 0.08, \( p = 0.74 \)).

Figure 4. Cox-2 gene expression in fresh frozen biopsies from the squamous esophageal mucosa in controls and GERD patients prior to antireflux surgery. Median Cox-2 expression was significantly higher in GERD patients (0.07 vs 0.195, \( p = 0.029 \)).
The Effect of Time Interval After Surgery on Cox-2 Expression

In order to assess whether a longer time interval after surgery was associated with a greater likelihood that Cox-2 gene expression had decreased, Spearman correlation analysis was performed. We found no significant correlation between the time interval from surgery and the change in Cox-2 gene expression levels (Fig. 6). However, the shortest interval from surgery to biopsy in this study was 10 months.

DISCUSSION

GERD affects an estimated 19 million people in the United States and is a significant health problem (1). While many equate reflux with irritating symptoms of heartburn or regurgitation, there is also an ominous side to GERD. Barrett's esophagus develops as a consequence of long-standing reflux, and Barrett's is the precursor to esophageal adenocarcinoma, the fastest increasing cancer in the United States (12–14). Interestingly, patients with Barrett's may have only minor reflux symptoms, while patients with relatively severe symptoms may have a normal appearing esophagus on endoscopy. Thus, there is a disconnection between symptoms and endoscopic findings in many patients with reflux despite a shared pathophysiology.

The premalignant nature of Barrett's and the substantial increase in the incidence of esophageal adenocarcinoma have stimulated efforts to identify the genes and molecular mechanisms involved in the progression of Barrett's to adenocarcinoma. However, the development of esophageal adenocarcinoma is a multistep process that begins with the transformation of squamous esophageal mucosa to columnar mucosa. This first step is perhaps the most critical, since adenocarcinoma does not develop within squamous mucosa. Despite the importance of the squamous epithelium in reflux disease, little is known about the molecular changes that occur in this mucosa.

Previously, in one of the few studies looking at gene expression changes in squamous mucosa we attempted to correlate the expression of a panel of genes with the severity of esophageal acid exposure based on 24-h pH monitoring (7). From this panel only the expression of Cox-2 changed significantly with exposure of the distal esophagus to acidic reflux (7). Consequently, one purpose of this study was to compare in a quantitative fashion Cox-2 gene expression from esophageal squamous mucosal biopsies in patients with reflux versus controls to confirm that Cox-2 expression is increased in patients with reflux disease. We found that Cox-2 expression was significantly increased, and in fact was doubled in the squamous mucosa from patients with gastroesophageal reflux compared to controls. Thus, gastroesophageal reflux alters gene expression in squamous esophageal mucosa, and increased expression of Cox-2 is a potential molecular indicator of gastroesophageal reflux disease.

Interestingly, the increased Cox-2 expression in the squamous mucosa associated with reflux disease was independent of the mucosal abnormalities evident on endoscopy in these patients. We found that Cox-2 expression was similarly increased whether the patient had NERD, esophagitis, or Barrett's esophagus. Thus, the severity of mucosal changes did not significantly impact the expression of Cox-2 from distal esophageal squamous epithelium in patients with reflux disease. This suggests that the expression of Cox-2 and potentially other genes may be useful indicators of the presence of reflux in squamous mucosa, and that gene expression alterations may function as a common denominator in these patients with otherwise disparate manifestations of reflux disease. Further, increased expression of Cox-2 in squamous mucosa may represent an early change associated with gas-

Figure 5. Cox-2 gene expression in frozen squamous biopsies from GERD patients before and after antireflux surgery compared to controls. Median Cox-2 expression decreased significantly in GERD patients after Nissen (0.195–0.08, p = 0.032) and reached a value similar to control patients without reflux (0.08 vs 0.07, p = 0.25).

Figure 6. Relationship between time and pattern of change in Cox-2 gene expression levels in squamous mucosa of the distal esophagus from all tissue samples (paraffin and frozen) following Nissen fun-
treatment of reflux disease, a second purpose of this study was to determine whether antireflux therapy would alter Cox-2 gene expression. To answer this question we selected patients with proven reflux and compared Cox-2 expression from distal esophageal biopsies in squamous epithelium before and after antireflux surgery. We choose to study patients undergoing antireflux surgery since the surgery was done in a standardized fashion that included reduction of any associated hiatal hernia, crural closure, and a 360° Nissen fundoplication, and all surgeries were performed by one group at a high-volume center. Following antireflux surgery concerns regarding acid-suppression medication dose, efficacy, timing, and regular usage that would have had to be addressed in medically treated patients were eliminated. We found that after Nissen fundoplication Cox-2 gene expression in the squamous esophageal mucosa of the majority of patients with GERD was significantly reduced, and in fact median expression was normalized to the level of control patients without reflux. The retrospective findings from paraffin-embedded tissue were subsequently confirmed in a prospective study using fresh frozen tissue.

To our knowledge this is the first time that alteration of gene expression has been demonstrated in a quantitative manner in humans following antireflux therapy. Previously Menges and colleagues noted that the overall pattern of gene expression by microarray assessment differed in biopsies of Barrett’s mucosa from patients before and after acid suppression therapy with a proton pump inhibitor, but individual genes were not assessed in a quantitative fashion (15). There are several important implications of our findings. First, we have clearly demonstrated that reflux-induced alterations in gene expression are not permanent, and that abnormal cellular gene expression can normalize if an injurious environmental stimulus is eliminated. The ability to favorably change the expression of an important gene known to be involved in gastrointestinal carcinogenesis supports the controversial concept that elimination of reflux may alter the natural history of reflux disease, and certainly warrants further investigation. Second, our findings open the door to the possibility of an entirely new way to monitor the efficacy of antireflux therapy. Traditional methods include the assessment of symptomatic response or evaluation of reflux based on the endoscopic appearance of the esophagus or the results of 24-h pH monitoring. While useful, none of these methods allow assessment of the impact of reflux at the cellular level, and it is at the cellular level where the most significant consequences of reflux originate. Monitoring of individual gene expression or a panel of relevant genes might provide a more sensitive and perhaps specific method to assess the impact of antireflux therapy in an individual patient.

Modulation of the expression of genes that regulate cellular processes in order to alter the natural history of a disease is a concept that has recently been proven in the laboratory with animals, and has epidemiologic support in humans. The potential role of Cox-2 in human esophageal carcinogenesis was suspected after epidemiologic studies noted that regular aspirin use was associated with a decreased risk for esophageal cancer, an effect that was also observed with selective Cox-2 inhibitors in a case control study (16, 17). Subsequently, models of severe reflux in rodents confirmed that inhibition of Cox-2 resulted in a reduced rate of intestinal metaplasia and cancer development (18, 19). These findings prompted the initiation of prospective human trials for chemoprevention of esophageal cancer using Cox-2 inhibitors in patients with Barrett’s (20, 21). However, these trials were halted after reports emerged that selective Cox-2 inhibitor medication use was associated with an increased risk for cardiovascular events (22–25). Thus, although the importance of Cox-2 in esophageal carcinogenesis has been demonstrated, pharmacologic inhibition of this enzyme appears to have significant detrimental side-effects. An important message from our study is that pharmacologic manipulation of Cox-2 may not be necessary to obtain the beneficial effects of reduced Cox-2 expression since we demonstrated that Cox-2 expression is usually normalized after a Nissen fundoplication.

We recognize that patients with gastroesophageal reflux represent a diverse group, and disease severity varies among patients. One potential criticism of our findings is that we were merely assessing changes in inflammation with quantitative evaluation of Cox-2 gene expression. To minimize this possibility we included patients at each end of the spectrum of reflux—those with nonerosive disease all the way to those with Barrett’s esophagus. We also ensured that we were not measuring Cox-2 expression from inflammatory cells by utilizing laser capture microdissection to obtain only squamous mucosal cells. Importantly, the increased expression of Cox-2 in patients with reflux was independent of the presence or absence of endoscopic evidence of mucosal injury. Further, a significant decrease in Cox-2 gene expression following antireflux surgery occurred with similar frequency in patients with Barrett’s or esophagitis as it did in patients with NERD. Thus, it is unlikely that our findings merely reflect an overall reduction in inflammation after antireflux surgery.

Another potential criticism of our study is that patients with Barrett’s may represent a different group compared to patients with reflux without Barrett’s. Our study population in the retrospective group had a high percentage of patients with Barrett’s, in part because these patients returned for surveillance endoscopy after their antireflux surgery while fewer patients without Barrett’s underwent postoperative endoscopy. To avoid variability related to different mucosal types we evaluated gene expression only from squamous mucosal biopsies taken from a similar location before and after antireflux surgery in each patient. Although we considered evaluating Cox-2 gene expression from the Barrett’s mucosa, previous studies have raised concern that the biological behavior and gene expression of cells within a Barrett’s segment are not homogeneous. For example, Fitzgerald and colleagues reported that there is an expression gradient between the proximal and distal end of a Barrett’s segment (26). Further, our own preliminary studies have suggested that there can be...
a substantial variability in the gene expression from different levels of the Barrett’s mucosa in individual patients (data not shown). Thus, for this study we focused on an easily reproducible and clearly defined target—squamous mucosa 3 cm above the SCJ in every patient. To confirm that the presence of Barrett’s did not significantly alter Cox-2 expression we compared biopsies from squamous mucosa in patients with Barrett’s with similar biopsies from patients without Barrett’s and found no significant difference in Cox-2 gene expression. Further, patients with Barrett’s were just as likely to have a decrease in Cox-2 expression after antireflux surgery as patients without Barrett’s. Thus, we found no evidence that the presence of Barrett’s impacted Cox-2 gene expression in the squamous esophageal mucosa 3 cm proximal to the SCJ.

Overall we found a twofold decrease in median Cox-2 gene expression after antireflux surgery, and Cox-2 expression decreased in 75% of the patients in this study. However, in 7 patients Cox-2 expression was either unchanged or slightly increased after antireflux surgery. One potential explanation for the failure of Cox-2 to decrease in these patients was that the fundoplication, despite appearing intact on endoscopy, was not effectively preventing reflux. This is unlikely since 2 of these 7 patients had postoperative 24-h pH monitoring and both had normal esophageal acid exposure (composite scores of 0.8 and 1.0). Another possibility is that although we found no clear correlation with time since the fundoplication, perhaps with further follow-up the Cox-2 expression in these patients will decrease. Lastly, and perhaps most concerning, it is possible that these patients represent a group with permanent upregulation in Cox-2 gene expression. These patients may be those at highest risk for disease progression, and if so, these patients would be those selected for careful follow-up. Further studies in this group of patients likely will be very informative.

In conclusion, we report for the first time that Cox-2 gene expression in the squamous mucosa of patients with gastroesophageal reflux is significantly increased compared to patients without reflux. Further, the increased expression of Cox-2 in patients with reflux was independent of endoscopic evidence of mucosal injury. Therefore, increased Cox-2 expression may represent an early event and a common denominator in patients with reflux disease. Another important finding in this study was that effective antireflux therapy altered gene expression in most patients and was able to normalize median Cox-2 expression to the level of control patients without reflux. Consequently, in addition to symptom control, resolution of esophagitis, and improvement in quality of life, perhaps the assessment of gene expression alterations will be important in future studies assessing the efficacy of antireflux therapy.

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STUDY HIGHLIGHTS

- Cyclooxygenase 2 (Cox-2) has been shown to be an important gene in gastrointestinal malignancy, and altered Cox-2 gene expression has been demonstrated in association with Barrett’s and esophageal adenocarcinoma.
- This study has revealed that Cox-2 gene expression is already increased in the distal esophageal squamous mucosa of patients with gastroesophageal reflux disease (GERD).
- Furthermore this research has for the first time shown that antireflux therapy can normalize increased Cox gene expression in patients with GERD.
- This data would suggest that in addition to symptom control and improvement in quality of life, future studies assessing the efficacy of antireflux therapy—both medical and surgical—should focus on the impact of the therapy on gene expression in the distal esophagus.

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