Selective Cyclooxygenase-2 Blocker Delays Healing of Esophageal Ulcers in Rats and Inhibits Ulceration-Triggered c-Met/Hepatocyte Growth Factor Receptor Induction and Extracellular Signal-Regulated Kinase 2 Activation

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Nonsteroidal anti-inflammatory drugs, both nonselective and cyclooxygenase-2 (COX-2) selective, delay gastric ulcer healing. Whether they affect esophageal ulcer healing remains unexplored. We studied the effects of the COX-2 selective inhibitor, celecoxib, on esophageal ulcer healing as well as on the cellular and molecular events involved in the healing process. Esophageal ulcers were induced in rats by focal application of acetic acid. Rats with esophageal ulcers were treated intragastrically with either celecoxib (10 mg/kg, once daily) or vehicle for 2 or 4 days. Esophageal ulceration triggered increases in: esophageal epithelial cell proliferation; expression of COX-2 (but not COX-1); hepatocyte growth factor (HGF) and its receptor, c-Met; and activation of extracellular signal-regulated kinase 2 (ERK2). Treatment with celecoxib significantly delayed esophageal ulcer healing and suppressed ulceration-triggered increases in esophageal epithelial cell proliferation, c-Met mRNA and protein expression, and ERK2 activity. In an ex vivo organ-culture system, exogenous HGF significantly increased ERK2 phosphorylation levels in esophageal mucosa. A structural analog of celecoxib, SC-236, completely prevented this effect. These findings indicate that celecoxib delays esophageal ulcer healing by reducing ulceration-induced esophageal epithelial cell proliferation. These actions are associated with, and likely mediated by, down-regulation of the HGF/c-Met-ERK2 signaling pathway. (Am J Pathol 2002, 160:963–972)
gical mucosa has not been studied and it is not known whether the COX-2 selective inhibitors affect esophageal ulcer healing.

Various growth factors, including epidermal growth factor and hepatocyte growth factor (HGF), have been implicated in the stimulation of epithelial proliferation during gastric ulcer healing. Suppression of HGF production has been suggested as a key factor involved in the inhibitory action of NSAIDs on gastric ulcer healing. However, the role of endogenous HGF in the healing of esophageal ulcers remains unexplored. In regard to the esophagus, previous studies demonstrated that exogenous HGF is the most potent stimulator of proliferation and restitution of esophageal epithelial cells in vitro, suggesting that it might be involved in the repair process of esophageal mucosal damage.

In previous studies we have demonstrated that the extracellular signal-regulated kinases (ERKs) and their upstream kinase, Raf-1, which mediate the mitogenic effects of growth factors, are activated during gastric ulcer healing and that interruption of this signaling pathway dramatically delays the healing process. However, the signaling pathways involved in esophageal ulcer healing, and stimulation of esophageal epithelial cell proliferation essential for the healing, remain unknown.

The present study was aimed to: 1) explore molecular events associated with esophageal ulcer healing: expression of COX-1, COX-2, HGF and its receptor c-Met, and ERK2 phosphorylation levels and activity; and, 2) determine whether the selective COX-2 inhibitor, celecoxib, affects esophageal ulcer healing, ulceration-triggered cell proliferation and the above stated molecular events.

Materials and Methods

The RNeasy Mini Kit was purchased from Qiagen (Valencia, CA), the bicinchoninic acid protein assay kit was purchased from Pierce Chemical (Rockford, IL), and the enhanced chemiluminescence Western blotting detection reagents were purchased from Amersham Life Science (Arlington Heights, IL). Monoclonal mouse anti-COX-1 and polyclonal rabbit anti-COX-2 antibodies were purchased from Cayman Chemical Co. (Ann Arbor, MI), polyclonal rabbit anti-HGF-α, anti-c-Met p140, anti-ERK2 antibodies and monoclonal mouse anti-pERK antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), monoclonal mouse anti-proliferating cell nuclear antigen (PCNA) antibody and 3,3′-diaminobenzidine tetrahydrochloride were purchased from DAKO (Carpinteria, CA). [γ-32P]ATP was purchased from Du pont NEN Research Products (Boston, MA). Keratinocyte basal media was purchased from Clonetics (San Diego, CA). All other chemicals were purchased from Sigma Chemical Company (St. Louis, MO).

Induction of Esophageal Ulcers

This study was approved by the Subcommittee for Animal Studies of the Long Beach Department of Veterans Af-
excised and fixed in formalin for immunohistochemical staining.

Determination of COX-1, COX-2, and c-Met mRNAs by RT-PCR

RNA was isolated using the RNeasy Mini Kit according to the manufacturer’s instructions. Reverse transcription (RT) and polymerase chain reaction (PCR) were performed to determine mRNA levels. RT was performed using a GeneAmp RNA PCR kit and a DNA thermal cycler (Perkin Elmer, Norwalk, CT), which were also used for PCR. Total RNA (0.3 μg) was used as the template to synthesize complementary DNA (cDNA) with 2.5 U of Moloney murine leukemia virus reverse transcriptase in 10 μl of buffer containing 10 mmol of Tris-HCl, pH 8.3, 50 mmol KCl, 5 mmol random hexamer, 1.4 U of ribonucleases inhibitor. RT was performed at 42°C for 15 minutes. The resulting cDNA was used as a template for subsequent PCR. The specific primer set for rat COX-1 was 5’-ACGCCCTCATTCACCCATTT-3’ (sense) and 5’-CAGGAACGCTGTCTCAAGG-3’ (antisense) and for COX-2 was 5’-TGTTACGGTGCTCTGATGTG-3’ (sense) and 5’-GGAATGGGTTCTGATACTG-3’ (antisense), and the sizes of amplified fragments were 561 bp for COX-1 and 253 bp for COX-2, respectively.25 The primers for rat c-Met were 5’-GGAGACTAATTCACTGC-3’ (sense) and 5’-GGAGACGCCCTCATTCACCCATTT-3’ (antisense), and the size of the amplified fragment was 242 bp.26 The PCR for β-actin was used as a positive control and an internal standard. The specific primer set for rat β-actin was purchased from Clontech Laboratories, Inc., Palo Alto, CA. The PCR was performed in 50 μl of buffer containing 10 mmol Tris-HCl, pH 8.3, 2 mmol MgCl₂, 50 mmol KCl, 0.2 mmol each of deoxyribonucleoside triphosphates, 0.4 μmol of each of primer, 2 U of Taq DNA polymerase. For the amplification of COX-1, COX-2, and rat c-Met cDNAs, 35 cycles of 1 minute at 94°C for denaturing, 1 minute at 55°C for annealing, and 2 minutes at 72°C for extension were performed. Nine-μl aliquots of the PCR products were subjected to electrophoresis on a 1.25% agarose gel, and the DNA was visualized by ethidium bromide staining. Location of the products and their sizes were determined using a 100-bp ladder (Life Technologies, Inc., Gaithersburg, MD). The gel was then photographed under UV transillumination. For the quantitative assessment of the PCR products, a computerized video analysis system (Image-1/FL, Universal Imaging Corp.) was used. The results are expressed as target cDNA/β-actin ratio.

Protein Extraction

Esophageal tissues were homogenized with a Polytron homogenizer (Kinematica, Littau, Switzerland) in a lysis buffer containing 62.5 mmol ethylenediaminetetraacetic acid, 50 mmol Tris, pH 8.0, 0.4% deoxycholic acid, 1% Nonidet P-40, 0.5 mg/ml leupeptin, 0.5 mg/ml pepstatin, 0.5 mg/ml aprotinin, 0.2 mmol phenylmethylsulfonyl fluoride, and 0.05 mmol aminoethyl benzene sulfonyl fluoride. The homogenates were then centrifuged at 14,000 rpm for 10 minutes at 4°C. The protein concentration of the supernatant was determined by the bicinchoninic acid protein assay kit.

Determination of COX-1, COX-2, HGF, and c-Met Protein Levels by Western Blotting

Equal amounts of protein (0.15 mg) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The membranes were incubated with specific antibodies at room temperature for 1 hour. The membranes were washed and incubated with corresponding IgG peroxidase conjugates at room temperature for 1 hour. The signal of the bound antibody was visualized using enhanced chemiluminescence Western blotting detection reagents. Protein expression was measured using a computerized video analysis system (Image-1/FL, Universal Imaging Corp.). COX-1 and COX-2 protein levels were determined using monoclonal mouse anti-COX-1 antibody and polyclonal rabbit anti-COX-2 antibody (Cayman Chemical Co.) diluted 1:1000. For the determination of HGF and c-Met protein expression, polyclonal rabbit anti-HGF-α antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and polyclonal rabbit anti-c-Met p140 antibody (Santa Cruz Biotechnology) were used at 1:250 dilution.

Determination of ERK2 Activity and Phosphorylation Levels

ERK2 activity was determined as described previously.6 Briefly, 50 μg of protein from tissue lysates was added to a conjugate of protein A Sepharose and 1 μg of polyclonal rabbit anti-ERK2 antibody and mixed at 4°C for 2 hours. The conjugates were then pelleted by centrifugation and washed four times. After the final wash, buffer was removed completely and 40 μl of protein kinase assay mixture (10 mmol/L HEPES, pH 7.5, 10 mmol/L MgCl₂, 50 μmol/L ATP, 30 μg myelin basic protein, and 4 μCi [γ-32P]ATP) were added to each sample. The samples were incubated at 30°C for 20 minutes and the reaction was terminated by the addition of sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer. The samples were then electrophoresed on 15% acrylamide gels. After electrophoresis, the gels were stained with Coomassie brilliant blue and dried. The gels were autoradiographed; the myelin basic protein bands were cut out and the radioactivity was counted in a scintillation counter. The activity was expressed as pmol of [γ-32P]ATP incorporated into 1 mg of MBP (pmol/mg). ERK2 and phosphorylated ERK (pERK) protein levels were determined by Western blot analysis using polyclonal rabbit anti-ERK2 antibody and monoclonal mouse anti-pERK antibody. The ERK2 phosphorylation levels were expressed as a percentage of total ERK2 protein levels.

Localization of COX-2 and c-Met Protein Expression by Immunohistochemical Staining

Immunohistochemical staining with specific antibodies was performed to determine localization of COX-2 and
c-Met expression in normal and ulcerated esophageal sections. Deparaffinized sections were incubated overnight at 4°C with polyclonal rabbit anti-COX-2 antibody diluted 1:300 in phosphate-buffered saline (PBS) or polyclonal rabbit anti-c-Met antibody diluted 1:50 in PBS. After washing, sections were then incubated for 30 minutes with rabbit fluorescein-conjugated IgG diluted 1:100 in PBS. Immunofluorescence signal was evaluated under a Nikon Optiphot epifluorescence microscope with B-filter composition (Nikon, Garden City, NY).

**Evaluation of Esophageal Epithelial Cell Proliferation**

Expression of PCNA in formalin-fixed paraffin-embedded esophageal tissue sections was determined by the enhanced polymer one-step staining method.27 Deparaffinized sections were incubated with monoclonal mouse anti-PCNA antibody for 1 hour at room temperature. The color was developed with 3,3′-diaminobenzidine tetrahydrochloride (DAKO) and the sections were counterstained with Mayer’s hematoxylin. Coded specimens were evaluated quantitatively under ×400 microscopic magnification by two investigators unaware of the code. Cell nuclei that stained dark brown were considered as labeled. Labeled cells were counted in the epithelium above the edge of interrupted muscularis mucosa at each ulcer margin corresponding to 700 μm of mucosal section length, in the epithelium distant from the ulcer, and in normal esophageal epithelium of SO rats. The length of the basement membrane was measured on the photographed images of corresponding hematoxylin and eosin (H&E) sections using a computerized video analysis system (Image-1/FL, Universal Imaging Corp.) and the number of PCNA-labeled cells per 100-μm length of the basement membrane was calculated. The results are expressed as percentage of increase in the number of labeled cells in the epithelium distant from the ulcer. Sections from six rats per group were evaluated and the mean ± SD was calculated.

**Assessment of Angiogenesis in Granulation Tissue at the Ulcer Bed**

To assess angiogenesis, enhanced polymer one-step immunostaining with monoclonal mouse anti-factor VIII-related antigen antibody (DAKO) that visualizes endothelial cells of vessels was used. Five days after ulcer induction, only a few endothelial cells forming microvessels were present in granulation tissue at the ulcer bed. Therefore, microvessels were counted only in sections obtained 7 days after ulcer induction. Coded specimens were evaluated quantitatively under ×200 microscopic magnification by two investigators unaware of the code. Microvessels with distinct lumen were counted in granulation tissue below the regenerating epithelium of the ulcer margin at each side. The results are expressed as a number of microvessels (mean ± SD) per ×200 microscopic field. Sections from six rats per group were evaluated.

**Effects of Exogenous HGF on Esophageal Mucosal ERK2 Phosphorylation Levels**

Rats (n = 18) were euthanized and a 1-cm long segment of the lower esophagus was excised and opened longitudinally. The esophageal mucosa was stripped from its muscle layers by sharp dissection as described previously.28 Explants consisted of squamous epithelium, lamina propria, and muscularis mucosa as determined by H&E staining. Esophageal mucosal explants were incubated in serum-free keratinocyte basal medium at 37°C with 5% CO₂ and 95% air in a humidified incubator in the presence of 10 μmol/L or 100 μmol/L of SC-236 (Searle, Skokie, IL), a structural analog of celecoxib,29 or its vehicle (dimethyl sulfoxide) for 6 hours. SC-236 was used because of its better solubility compared to celecoxib. Then explants were treated with either human recombinant HGF (100 ng/ml) or its vehicle (PBS) and were incubated for an additional 30 minutes. Protein extraction and Western blotting for pERK2 and ERK2 were performed as described above.

**Statistical Analysis**

Values are expressed as the mean ± SD. Student’s t-test was used to determine the statistical significance of the differences. One-way analysis of variance followed by Bonferroni correction was used for multiple comparisons. A P value of <0.05 was considered statistically significant.

**Results**

**Esophageal Ulceration Triggers Increases in COX-2 (but not COX-1) mRNA and Protein Expression**

Three days after ulcer induction, COX-2 mRNA and protein levels in ulcerated esophageal tissue were increased ~2.5-fold and ~threelfold, respectively, versus normal esophageal tissue (Figures 1 and 2). COX-2 protein levels in ulcerated esophageal tissue were decreased at 7 days versus 3 days after ulcer induction, but remained elevated versus normal tissue. COX-1 mRNA and protein levels were not significantly affected by esophageal ulceration (Figures 1 and 2).

**COX-2 and c-Met Protein Localization by Immunohistochemical Staining**

In normal esophageal epithelium, faint COX-2 signal was detected predominantly in the cells of the basal zone (Figure 3A). Three days after ulcer induction, a strong COX-2 fluorescence signal was present in all epithelial cells constituting the ulcer margin (Figure 3B). In the
normal esophageal epithelium of sham-operated rats, c-Met signal was predominantly localized to the membranes of cells in basal and stratified zones (Figure 4A).

Three days after ulcer induction, c-Met signal was present in all epithelial cells constituting ulcer margin; cells in the stratified zone displayed strong membrane and cytoplasmic staining, whereas, basal cells showed mainly diffuse cytoplasmic staining (Figure 4B).

Esophageal Ulcer Healing Dynamics: Effects of Celecoxib

The mean ± SD area of the ulcers 3 days after ulcer induction was 7.1 ± 1.5 mm². Five days after ulcer induction, the mean ulcer area was slightly but significantly decreased (versus 3 days) in vehicle-treated but not in celecoxib-treated rats (Figure 5). Seven days after ulcer induction, the mean ulcer area was significantly decreased (versus 3 days) in both vehicle-treated and celecoxib-treated rats. However, the mean ulcer area in celecoxib-treated rats was nearly twofold larger than in vehicle-treated rats, demonstrating that celecoxib treatment significantly delays esophageal ulcer healing.

Esophageal Epithelial Cell Proliferation: Effects of Celecoxib

Seven days after ulcer induction, in the esophageal epithelium distant from the ulcer, PCNA staining was present only in cells of the basal zone (Figure 6, A and B). Epithelium of the ulcer margin in rats treated with vehicle showed a dramatic increase in the number of cells expressing PCNA (Figure 6C). This increase was significantly diminished in rats treated with celecoxib (Figure 6D). The quantitative data are shown in Figure 7. The number of PCNA-labeled epithelial cells in normal esophageal mucosa (mucosa of SO rats) was not significantly different between the lower (corresponding to the ulcer site in rats with ulcers) and upper (corresponding to the area distant from the ulcer) esophageal segments (18.5 ± 1.1 versus 17.9 ± 2.2, respectively; P = 0.501). Three days after ulcer induction, the number of PCNA-labeled epithelial cells in the mucosa distant from the ulcer (17.2 ± 2.1) was not significantly different from that in the mucosa of the upper esophageal segment of SO rats. Three days after ulcer induction, the number of PCNA-labeled epithelial cells at the ulcer margin was increased to 180% over that in the epithelium distant from the ulcer. In vehicle-treated rats, this increase reached 390% at 7 days after ulcer induction (Figure 7). In rats treated with celecoxib, the number of PCNA-labeled epithelial cells in the epithelium of the ulcer margin was also increased (versus epithelium distant from the ulcer), however, this increase was significantly reduced (versus vehicle-treated rats) both 5 and 7 days after ulcer induction (Figure 7).

Angiogenesis in Granulation Tissue at the Ulcer Bed: Effects of Celecoxib

Seven days after ulcer induction, the number of microvessels in granulation tissue at the ulcer bed was slightly, but not significantly, lower in celecoxib-treated
rats versus vehicle-treated rats (21.7 ± 1.7 versus 23.7 ± 3.0, respectively; $P = 0.18$).

**Esophageal Ulceration Induces c-Met mRNA and Protein Expression: Effects of Celecoxib**

Three days after ulcer induction, c-Met mRNA levels in ulcerated tissue were increased twofold versus normal esophageal tissue (Figure 8). In vehicle-treated rats, c-Met mRNA levels in ulcerated tissue remained high at 5 days and were decreased at 7 days versus 3 days after ulcer induction (Figure 8). Celecoxib significantly reduced (versus vehicle) c-Met mRNA expression in ulcerated tissue both 5 and 7 days after ulcer induction (Figure 8). Western blot analysis demonstrated that HGF protein levels in ulcerated tissue were significantly increased versus normal esophageal tissue 3 days after ulcer induction (Figure 9). In vehicle-treated rats, HGF protein levels remained high 5 and 7 days after ulcer induction. In celecoxib-treated rats, HGF protein levels in ulcerated tissue were significantly reduced versus vehicle-treated rats at both 5 and 7 days. Celecoxib treatment (versus vehicle treatment) significantly reduced c-Met protein levels in ulcerated tissue both 5 and 7 days after ulcer induction.

**Esophageal Ulceration Induces ERK2 Phosphorylation and Activity: Effects of Celecoxib**

Three days after ulcer induction, ERK2 phosphorylation levels and ERK2 activity were increased more than fourfold and fivefold, respectively, in ulcerated tissue versus normal esophageal tissue (Figure 10). In vehicle-treated rats, ERK2 phosphorylation levels and ERK2 activity remained high at both 5 and 7 days after ulcer induction. In celecoxib-treated rats, ERK2 phosphorylation levels and ERK2 activity in ulcerated tissue were significantly reduced versus vehicle-treated rats at both 5 and 7 days. ERK2 total protein levels were not significantly different between any of the groups studied, demonstrating that
celecoxib does not reduce ERK2 protein expression but inhibits its activation directly.

Exogenous HGF Increases ERK2 Phosphorylation Levels in Esophageal Mucosa Ex Vivo: Effects of SC-236

Dose response studies using 1 to 100 ng/ml of HGF revealed that the 100-ng/ml dose significantly increased ERK2 phosphorylation levels in esophageal mucosal explants. A thirty-minute incubation with 100 ng/ml of HGF significantly increased ERK2 phosphorylation levels in esophageal mucosal explants (Figure 11). For ex vivo studies we used a structural analog of celecoxib, SC-236, because of its better solubility compared to celecoxib. Pretreatment with both 10 μmol/L and 100 μmol/L SC-236 completely blocked the HGF-induced increase in ERK2 phosphorylation levels. Basal ERK2 phosphorylation levels were not significantly altered by 10 μmol/L of SC-236, however, they were slightly, but significantly reduced by 100 μmol/L of SC-236. ERK2 total protein levels were not significantly different between the studied groups.

Discussion

This study demonstrated for the first time that esophageal ulceration triggers overexpression of COX-2, HGF and its receptor c-Met, and induces ERK2 activation. Moreover, it demonstrated that the selective COX-2 inhibitor, celecoxib, significantly delays esophageal ulcer healing and reduces ulceration-triggered increases in esophageal epithelial proliferation, c-Met mRNA and protein expression, and ERK2 phosphorylation and activity.

Epithelial cell proliferation is a crucial component of gastric ulcer healing because it provides the cells necessary to fill the mucosal defect and to restore the mucosa within the scar.2 It has been suggested that inhibition of COX activity and the resulting inhibition of prostaglandin synthesis may play a role in NSAID-induced inhibition of epithelial cell proliferation during gastric ulcer healing.4 Prostaglandins have been demonstrated to induce HGF expression in gastric fibroblasts in vitro19 and, conversely, inhibition of prostaglandin synthesis by indomethacin significantly reduced HGF production in these cells.20 Therefore, it has been suggested that effects of NSAIDs on gastric ulcer healing may be mediated by suppression of prostaglandin-dependent HGF expression.20 However, whether NSAIDs affect HGF protein levels in vivo in the stomach or in the esophagus remains unknown. In the present study, HGF protein levels were increased during esophageal ulcer healing but
were not affected by treatment with celecoxib, suggesting that effects of celecoxib on esophageal epithelial proliferation are not mediated via inhibition of HGF expression. On the other hand, there is evidence indicating an interaction between HGF and COX-2. Previously, we have demonstrated that HGF induces COX-2 expression in gastric epithelial cells in vitro.25 A recent in vivo study has also demonstrated that exogenous HGF up-regulates COX-2 expression in ulcerated gastric mucosa in rats.30 Moreover, the acceleration of gastric ulcer healing by HGF was significantly attenuated by selective COX-2 inhibitors, suggesting that COX-2 may mediate effects of HGF on gastric ulcer healing.30 However, the molecular mechanisms involved in this phenomenon remain unclear.

HGF elicits its biological effects by binding to and activating its receptor, c-Met,31 which is present on the majority of epithelial cells, including esophageal epithelial cells.22 The present study demonstrated that c-Met is expressed in esophageal epithelial basal and squamous cells and that its expression is induced by esophageal ulceration. Furthermore, we show that celecoxib significantly suppresses c-Met mRNA and protein expression during esophageal ulcer healing. Previous in vitro studies demonstrated that c-Met mRNA is inducible and that its expression is increased in response to HGF in cell lines derived from human lung adenocarcinoma and glioblastoma.32,33 Because inhibition of ERK activity completely prevented HGF-mediated c-Met induction in human glioblastoma cells,33 it is possible that celecoxib-induced inhibition of ERK2 activity is involved in the down-regul-
tion of c-Met mRNA expression during esophageal ulcer healing. Because c-Met mediates the mitogenic effects of HGF, suppression of its expression may be one of the mechanisms by which celecoxib inhibits epithelial proliferation during esophageal ulcer healing.

HGF binding and activation of its receptor, c-Met, triggers activation of ERKs and cell proliferation in many cell types. Our previous studies demonstrated that HGF activates ERK2 in gastric epithelial cells. Inhibition of enhanced epithelial proliferation during gastric ulcer healing by NSAIDs has been well documented in experimental models and in humans. Previous studies demonstrated that gastric ulceration activates epidermal growth factor/mitogen-activated protein kinase (ERK2) mitogenic signaling pathway, but no studies have evaluated the effects of NSAIDs on this pathway during gastric or esophageal ulcer healing. Our present study showed that ERK2 is also activated during esophageal ulcer healing. Moreover, it demonstrated for the first time that celecoxib suppresses ERK2 activity up-regulated by esophageal ulceration and that a structural analog of celecoxib, SC-236, inhibits basal and HGF-induced ERK2 phosphorylation in esophageal mucosal explants. Taken together, these results indicate that COX-2 inhibitors may directly or indirectly (via interference with upstream events of ERK signaling pathway) inhibit ERK2 activation in esophageal mucosa. Because activation of the ERK2 pathway is essential for cell proliferation, the inhibitory action of celecoxib on esophageal epithelial proliferation is likely mediated by suppression of the ulceration-triggered ERK2 activation.

Studies of experimental gastric ulcers demonstrated that NSAIDs, including COX-2-selective inhibitors, in addition to inhibition of epithelial cell proliferation at the ulcer margin, also interfere with angiogenesis (formation of new capillary blood vessels) in granulation tissue at the ulcer bed. In the present study, celecoxib, slightly (~8%) but not significantly, inhibited angiogenesis in granulation tissue during esophageal ulcer healing. This finding suggests that inhibition of epithelial proliferation may be more important for celecoxib-induced delay in esophageal ulcer healing than inhibition of angiogenesis. This finding is in contrast to NSAID-induced inhibition of gastric ulcer healing, whereas NSAIDs markedly inhibit angiogenesis and suggests that angiogenesis may be more important for healing of gastric ulcers than for healing of esophageal ulcers. However, more detailed sequential studies specifically assessing angiogenesis, as well as using angiogenic growth factors are necessary to determine the precise role of angiogenesis in esophageal ulcer healing.

In conclusion, esophageal ulceration triggers increases in esophageal epithelial proliferation; COX-2, HGF, and c-Met expressions; and ERK2 activity. A selective COX-2 inhibitor, celecoxib, delays healing of experimental esophageal ulcers and suppresses ulceration-induced increase in epithelial cell proliferation, c-Met induction, and ERK2 activation.

References