Cyclooxygenase-1 and an alternatively spliced mRNA in the rat stomach: effects of aging and ulcers

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AGING IS ASSOCIATED WITH FUNCTIONAL AND MORPHOLOGICAL CHANGES IN THE STOMACH (15). IN PARTICULAR, PROSTAGLANDINS (PGS), WHICH PROTECT THE GASTRIC MUcosa AGAINST INJURY, DECREASE PROGRESSIVELY WITH AGE IN HUMANS (4, 5) AND RATS (22). THE SUPPRESSION OF PG SYNTHESIS BY NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs) THROUGH THE INHIBITION OF THE ENZYME CYCLOOXYGENASE (COX) IS A MAJOR COMPONENT UNDERLYING GASTRIC DAMAGE (39). EPIDEMIOLOGICAL DATA SUGGEST THAT AGING MAY BE AN INDEPENDENT RISK FACTOR FOR THE DEVELOPMENT OF NSAID-INDUCED GASTRIC INJURY (2, 21, 36). TWO ISOFORMS OF COX HAVE BEEN IDENTIFIED, COX-1 AND COX-2, WHICH ARE PRODUCTS OF DIFFERENT GENES SHARING 60% IDENTITY AT THE AMINO ACID LEVEL (11). COX-1 IS CONSIDERED TO BE THE CONSTITUTIVE ISOFORM, AND PROSTANOIDS SYNTHESIZED VIA THE COX-1 PATHWAY ARE RESPONSIBLE FOR A VARIETY OF PHYSIOLOGICAL FUNCTIONS, INCLUDING CYTOPROTECTION OF THE STOMACH (42). IN CONTRAST, COX-2 IS AN INDUCIBLE IMMEDIATE-EARLY GENE ASSOCIATED WITH INFLAMMATION (40, 43), OVULATION (7), AND CARCINOGENESIS (33). IT IS, HOWEVER, IMPORTANT TO EMPHASIZE THAT BOTH COX-1 AND COX-2 mRNA ARE CONSTITUTIVELY EXPRESSED IN A VARIETY OF HUMAN TISSUES, INCLUDING THE STOMACH, SMALL INTESTINE, KIDNEY, AND BRAIN (18, 27, 31, 44). THERE IS ALSO EVIDENCE OF COX-1 mRNA INDUCIBILITY IN VITRO AND IN VIVO (3, 10).

STANDARD NSAIDS (PREDOMINANT COX-1 INHIBITORS) ARE INITIATORS OF GASTRIC ULCERATION AND DELAY ULCER HEALING (34, 36). SELECTIVE COX-2 INHIBITORS SPARE THE GASTROINTESTINAL TRACT FROM INJURY (24), BUT, LIKE THEIR COX-1 COUNTERPARTS, THEY IMPAIR ULCER HEALING (25). TO ELUCIDATE THE ROLE OF COX IN GASTRIC DAMAGE, INVESTIGATORS HAVE DEMONSTRATED COX-1 AND COX-2 EXPRESSION IN ACETIC ACID-INDUCED RODENT GASTRIC ULCERS. ELEVATED LEVELS OF COX-2 mRNA AND PROTEIN WERE FOUND IN ACUTE ULCERS, WHICH CORRELATED WELL WITH THE DEGREE OF MUCOSAL INJURY (25). THIS SUGGESTS THAT PGs PRODUCED THROUGH COX-2 ARE IMPORTANT IN PROMOTING WOUND HEALING. INTERESTINGLY, A ROLE FOR COX-1 IN THE REGULATION OF STEM CELL PROLIFERATION AND WOUND REPAIR WAS INDICATED WHEN COX-1, BUT NOT COX-2, mRNA EXPRESSION INCREASED IN REGENERATING MOUSE INTESTINAL STEM CELLS AFTER RADIATION INJURY (3).

THE COX-1 GENE EXPRESSES AN ALTERNATIVELY SPliced mRNA COX-1 splice variant (SV) THAT WAS CLONED FROM AN IMMORTALIZED RAT TRACHEAL EPITHELIAL CELL LINE (17). COX-1SV mRNA does not code for a full-length protein, and it is not known whether a truncated protein is synthesized. THIS ALTERNATIVELY SPliced mRNA IS THE SAME LENGTH AS THAT ENCODING COX-1 AND ONLY DISTINGUISHABLE IN THE FIRST 150 BP, IN WHICH A SEQUENCE THOUGHT TO ORIGINATE FROM THE SECOND INTRON IS SUBSTITUTED FOR THE FIRST TWO EXONS. IN RAT TRACHEAL EPITHELIAL CELLS, >90% OF TOTAL COX-1 mRNA WAS IDENTIFIED AS COX-1SV (17). NO OTHER STUDY HAS INVESTIGATED COX-1SV mRNA EXPRESSION. COX-1 mRNA EXPRESSION HAS BEEN EXAMINED IN THE RAT USING NORTHERN BLOT ANALYSIS, RNase PROTECTION, OR RT-PCR TECHNIQUES WITH PRIMERS AND/or PROBES DESIGNED DOWNSTREAM OF THE SPLICE JUNCTION (8–10, 16, 25, 37). HOWEVER, NONE OF THESE TECHNIQUES ALLOWS DISTINGUISHMENT BETWEEN COX-1 AND COX-1SV mRNA. IT IS WELL EStABLISHED THAT ALTERNATIVE SPlicing IS BIOLOGICALLY SIGNIFICANT (1). FURTHERMORE, IF COX-1SV mRNA IS PRESENT AND UNACCOUNTED FOR, IT WILL LEAD TO AN OVERESTIMATION OF THE PREDOMINANCE OF COX-1 mRNA.
functional COX-1 mRNA that codes for functional protein.

The objective of this study was to investigate the effect of aging on COX gene expression in the normal rat stomach. COX-1 is considered to be expressed constitutively. However, COX-1SV has never been identified or measured in vivo and it is not known whether the ratio of COX-1 to COX-1SV changes under different physiological or inflammatory conditions. Therefore, we also investigated the expression and inducibility of COX-1, COX-1SV, and COX-2 in experimentally induced acute gastric ulcers.

MATERIALS AND METHODS

Tissue collection. Experiments were approved by the Monash University Animal Experimentation and Ethics Committee. Male Sprague-Dawley rats of three different age groups, young (9 wk, 310 ± 6 g), adult (12 mo, 628 ± 19 g), and aged (24 mo, 717 ± 33 g) had access to rat chow (GR2 pellets; Clark King, Melbourne, Australia) and water ad libitum. Animals were maintained on 12:12-h light-dark cycles and fasted (24 h) before each experiment in which tissue was collected. Rats were anesthetized by intraperitoneal injection of pentobarbital (pentobarbitone) sodium, and acute gastric ulcers were induced in young and adult rats, as described previously (30). Briefly, the stomach was exposed via a midline incision and acetic acid (100%, 50 µl) was applied (25 s) to the serosal surface at the antral/corpus junction using a tube (6-mm internal diameter). The acid was blotted dry, and the incision was closed (day 0, ulcer induction). A macroscopically visible ulcer was present at day 2 (acute ulcer), and the defect was almost healed at day 12, ulcerated and completely healed at day 14. Animals were killed at day 2 or day 12, and the ulcerated area (mm²) was determined under a stereo microscope (Olympus, Tokyo, Japan). Stomachs from untreated (control) rats of three different age groups were also collected. Each stomach was removed, opened along the greater curvature, and rinsed with saline before tissue collection. Wedges of the stomach (mucosa, submucosa, muscle layer, and serosa) were collected from the antral/corpus junction. Normal or ulcerated tissue samples were divided into two samples; one was snap frozen in liquid nitrogen (stored at −80°C), and the other was fixed in formalin for 14 h before processing.

RNA extraction. Total RNA was isolated from samples with the RNeasy mini kit (Qiagen, Melbourne, Australia). After tissue homogenization with RNeasy lysis buffer, samples were passed through a 21-gauge syringe 10 times to shear chromosomal DNA. The concentration of each 4-µl RNA sample was determined by capillary spectrophotometry (Helix, San Diego, CA) using a Cary 1 spectrophotometer (Varian, Melbourne, Australia).

Primers. For the specific detection of COX-1 mRNA, which is translated to functional COX-1 protein and COX-1SV using competitive PCR, primers must be designed to span the splice junction located in exons 1 and 2. We designed one set of primers downstream of the splice junction to detect total COX-1 mRNA (functional COX-1 and COX-1SV). A second set of primers were designed specifically to span the COX-1SV splice junction as demonstrated by Kitzler et al. (17). For each RNA sample, functional COX-1 mRNA was determined by subtracting COX-1SV mRNA from total COX-1 mRNA.

All primers and competitive templates were designed using Oligo 5.0 Primer Analysis software (National Biosciences, Plymouth, MI) and synthesized by Bresatec (Adelaide, Australia). The COX-1 primers were CACTTCCGTGTGCCAGATTAC (upper primer) and TCAACCGCAGGATGTCAC (lower primer), which amplify a 645-bp product. The COX-1SV primers were AGCAGGCCCAACCGGAGATG (upper primer) and CTGTTCTTTCTCTCTTGGTC (lower primer), which amplify a 445-bp product. The COX-2 primers were TGCCGTTGATGTTCTGTA (upper primer) and TTGTTAGGATTGGA (lower primer), which amplify a 535-bp product. PCR products were verified by sequencing and analysis using Basic Local Alignment search tool (National Centre for Biotechnology Information). COX-1SV primers gave a PCR product of predicted size, which was sequenced in both directions. The sequence downstream of the splice junction was identical to that of rat COX-1, and the sequence upstream of the splice junction matched that reported by Kitzler et al. (17).

Construction of competitors. Competitive templates for COX-1, COX-1SV, and COX-2 were designed by PCR-mediated mutagenesis. Each competitive template is 20–30% shorter yet otherwise identical to the target sequence, to allow distinction and quantitation of the amplified sequences. Briefly, for the COX-1 competitor, reverse transcription of 1 µg RNA in 20 µl was performed as for samples, except that 0.75 µM of the COX-1 lower primer was utilized instead of 2.5 units random hexamers. A composite primer, CCTTCCGTGTGCCAGATTACCTTGCTTTCCCTTGGTTC (lower primer), which incorporated the COX-1 upper primer, was designed to create a 197-bp deletion. The COX-1 composite primer was used in the primary PCR with 2 µl of cDNA and a thermocycling program of 94°C for 8 min, then 35 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, 72°C for 7 min, and 4°C for 1 min. A 1-µl aliquot of the 448-bp PCR product was diluted 1/100, and a secondary PCR was performed with COX-1 upper and lower primers with the previous thermocycling program for 40 cycles. The final PCR product was purified using the QIAquick PCR purification kit (Qiagen) and quantitated using a capillary spectrophotometer. The COX-1 competitor (448 bp) was diluted in 10 mM Tris·HCl, pH 7.5, to the required competitor copies. COX-1SV (445 bp) and COX-2 (357 bp) competitors were made in the same way. The COX-1SV composite primer, AGCAGGCCCAACCGGAGATGCTGCTCCCACTGTA, which incorporated the COX-1SV upper primer, was designed to create a 108-bp deletion. The COX-2 composite primer, TGCCGTTGATGTTCTGTAAGCTGACCCAGACCTGCTTT, which incorporated the COX-2 upper primer, was designed to create a 197-bp deletion.

Reverse transcription. Up to 1 µg of RNA was reverse transcribed in the presence of 20 µl containing 1 × GeneAmp PCR buffer, 5 mM MgCl₂, 1 mM dNTP, 1 unit RNase inhibitor, 2.5 units murine leukemia virus RT, and 2.5 units random hexamers (all from Perkin-Elmer). Reactions were incubated at 15°C for 1 min, and the temperature was increased to 42°C over 9 min followed by 42°C for 1 h, 85°C for 5 min, and 4°C for 1 min in a thermal cycler (M Research, Watertown, MA).

Competitive PCR. Each aliquot of cDNA used in the three competitive PCR assays originated from the same reverse transcription reaction. Competitive PCR was performed using 2 µl (for COX-1), 4 µl (for COX-1SV), and 2 µl (for COX-2) cDNA in a total volume of 50 µl per reaction. The reaction consisted of 2 units AmpliTaq Gold, 1 × GeneAmp PCR Buffer, 2.5 mM MgCl₂, and 200 µM dNTP (all from Perkin-Elmer), 0.2 µM of upper and lower primers, and 1,000 (COX-1), 100 (COX-1SV), or 10,000 (COX-2) copies of competitor. The thermocycling program was 94°C for 8 min, then 40 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, 72°C for 7 min, and 4°C for 1 min. The PCR products were visualized on ethidium bromide-stained 2% agarose gels.
and then scanned using a FluorImager 575 (Molecular Dynamics, Sunnyvale, CA). PCR product band volumes were quanti-
tated with ImageQuantNT software (Molecular Dynamics). 
Native-competitor product ratios were calculated, and the 
levels of gene expression were reported as copies of mRNA per 
microgram of total RNA.

Validation of competitive PCR. To ensure that competitor 
and target sequences compete equally in the PCR, increasing 
concentrations of each competitor were titrated against a 
constant aliquot of target cDNA known to be positive for the 
gene of interest.

Immunohistochemistry. Deparaffinized sections 3-µm thick 
were treated with 3% (vol/vol) H₂O₂ for 5 min and, after 
washing, were incubated with 20% (vol/vol) normal rabbit 
serum (NRS) in PBS for 30 min. The sections were incubated in PBS and 1% NRS with primary antibodies at 1:2,000 for 
COX-1 or COX-2 (polyclonal antiserum raised in a goat 
against the mouse COX-1 protein or the rat COX-2 protein; 
Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C.

On each slide two sections were present; one was exposed to 
primary antiserum and one was exposed to PBS with 1% 
(vol/vol) NRS. After washing in PBS three times for 5 min, 
sections were incubated with rabbit anti-goat IgG (Dako) for 1 
h at room temperature, washed with PBS, and incubated for a 
further 30 min with goat peroxidase-anti-peroxidase complex 
(1:60 in PBS and 1% NRS). After washing, sections were 
incubated with diaminobenzidine tetrahydrochloride (Sigma, 
St. Louis, MO) for 10 min, rinsed in distilled water for 5 min, 
counterstained with Harris hematoxylin (Sigma) for 15 s, and 
then rehydrated and mounted.

Statistical analysis. Values are expressed as means ± SE. 
Differences between groups were compared using the Mann-
Whitney U nonparametric test.

RESULTS

Competitive PCR: quantitative analysis. When increasing 
amounts of COX-2 competitor were titrated against a constant aliquot of target cDNA (Fig. 1A), the 
changing ratio generated from the experimental data closely followed the expected values (Fig. 1B). Thus 
both the COX-2 competitor and the COX-2 target sequence show similar amplification efficiencies and 
compete equally in the PCR. COX-1 (Fig. 1C) and 
COX-1SV (Fig. 1D) changing input ratio experiments 
showed similar results.

For each of the competitive PCR assays, a known 
number of competitor molecules were coamplified with 
the target sequence, and mRNA quantitation was 
achieved by comparing the ratio of competitor vs. the 
target sequence, as previously described (12). 

Determination of gene copy number allows the mRNA expres-
sion per microgram of RNA of different genes or splice 
variants to be compared, provided that the cDNA used

![Fig. 1. Competitive PCR: a quantitative analysis. A: an agarose gel showing increasing amounts of cyclooxygen-
ase (COX)-2 competitor (338 bp; from left to right) titrated against a constant aliquot of target cDNA (535 
bp). Quantitation of native/competitor ratios for COX-2 
(B), COX-1 (C), and COX-1SV (D) are shown. In each 
case, experimental data closely follow expected values. 
■, 40 PCR cycles; △, 45 PCR cycles.](https://example.com/image.png)
in each competitive PCR originates from the same reverse transcription reaction. Using this technique, the RNA copy numbers of COX-1, COX-1SV, and COX-2 were determined in each sample studied.

Representative samples from normal and ulcerated stomach tissue were subjected to competitive PCR with the addition of a known copy number of COX-1 (1,000 copies; Fig. 2A), COX-1SV (100 copies; Fig. 2B), or COX-2 (10,000 copies; Fig. 2C) competitor.

COX mRNA expression in the normal rat stomach with increasing age. COX-1 was significantly reduced in the aged rat stomach (mean = 1.1 × 10^4 copies/µg RNA; P < 0.001) compared with young (mean = 1.96 × 10^4 copies/µg RNA) and adult (mean = 2 × 10^4 copies/µg RNA) rats (Fig. 3A). COX-1SV (Fig. 3B) was significantly higher in the adult rats (mean = 1.6 × 10^3 copies/µg RNA; P < 0.05) compared with young and aged rats (mean = 6 × 10^2 copies/µg RNA).

COX-2 mRNA was expressed in all normal tissue, and although expression appeared to increase with increasing age, no significant differences were observed between the three age groups (Fig. 3C). COX-2 mRNA expression varied considerably between animals in the same group, for instance, in young rats (mean = 2.8 × 10^4 copies/µg RNA, range = 471 to 4 × 10^4 copies/µg RNA).

COX mRNA expression in day 2 and day 12 ulcers. Young and adult rats developed acute ulcers of similar size when examined two days after ulcer induction (mean = 20 ± 4.3 mm²). Twelve days after induction, ulcers from young and adult rats were undergoing healing and repair, with a size of 2 ± 0.95 mm². In day 2 ulcers, COX-1 mRNA levels from young and adult rats were similar to those observed in controls (mean = 2 × 10^4 copies/µg RNA), whereas in day 12 ulcers a significant (3.5-fold) elevation in COX-1 mRNA (mean = 6.8 × 10^4 copies/µg RNA) was observed (Fig. 4A). COX-1SV mRNA levels did not change in ulcer tissue (Fig. 4B). However, adult rats expressed significantly more COX-1SV than young rats, consistent with the results observed in control rats (Fig. 3).

COX-2 mRNA levels were significantly elevated in acute ulcers (mean = 7.5 × 10^5 copies/µg RNA), a 37-fold elevation compared with normal gastric tissue (mean = 2.8 × 10^4 copies/µg RNA; Fig. 4C). In healing ulcers the levels declined (mean = 1.1 × 10^5 copies/µg RNA) and were still significantly higher (fourfold) than controls. There were no age-related differences in COX-2 expression in adult and aged rats with acute ulcers (data not shown). Furthermore, young and adult rats express similar levels of COX-2 in healed ulcers (data not shown).
Control (gray bar) or day 12 means after ulcer induction, expressed as mRNA copies/µg RNA. Values are significantly higher COX-2 levels in day 2 controls; * day 12 ulcers; ** day 12 ulcers compared with day 2 controls; * day 12 ulcers are significantly elevated C: COX-1, adult rats express significantly more COX-1SV than young rats; * * * COX-2 levels were variably expressed, whereas COX-2 mRNA remained unchanged. COX-2-positive cells were demonstrated immunohistochemically in the infiltrating cells and were absent in the gastric glands of the ulcer margin (Fig. 5A), whereas COX-1-immunopositive cells were absent at this stage (Fig. 5B). Adjacent to the lesion, we observed immunoreactive COX-1 in the gastric glands and mucous neck cells, as demonstrated by Iseki (13) in the normal gastric mucosa, whereas COX-2-immunopositive cells were located in the fibroblasts of the serosa (data not shown).

Twelve days later, ulcers histologically resembled human peptic ulcers undergoing healing and repair. Fibrous granulation and scar tissue were observed in the ulcer base, and the regenerating epithelium contained glands that were cystic and hyperproliferative. The muscularis mucosa around the ulcer margin was replaced by granulation tissue. Externally the serosa of day 12 ulcers was thickened and exhibited fibrous adhesions to the underlying liver. Strongly staining COX-1-immunopositive cells were present in the gastric glands (Fig. 5C) adjacent to the regenerating glands. Immunoreactive COX-2 was predominantly located in the fibroblasts of the granulation tissue (Fig. 5D); similar staining with the COX-1 antibody was observed (data not shown). In sections in which the primary antibody was absent, no immunostaining was observed (Fig. 5E).

**DISCUSSION**

We have reported the mRNA expression of COX-1, COX-1SV, and COX-2 in the rat stomach using competitive PCR. In histologically normal specimens from rats of three different age groups, COX-1 and COX-1SV levels were variably expressed, whereas COX-2 mRNA remained unchanged. This is the first study that has distinguished COX-1 and COX-1SV mRNA in vivo, and although COX-1SV levels were somewhat lower than those observed for COX-1, previous studies would have reported additive results. Our findings also demonstrate that reduced COX-1 mRNA may contribute to reduced levels of gastric mucosal cytoprotection in aged rats. Surprisingly, COX-1 and COX-2 mRNA were expressed at similar copy numbers in the stomach of young rats. Furthermore, only COX-1 and COX-2 mRNA levels were differentially expressed during acute gastric ulceration and healing, indicating that both isoforms contribute to this process. Both COX isoforms were also immunolocalized to the fibroblasts of the granulation tissue in ulcers undergoing healing and repair.

Aging may be an independent risk factor for the development of gastric injury in humans and rodents (22, 36). After submucosal injections of acetic acid or aspirin, aged rats have increased susceptibility to mucosal injury and gastric ulcerations (22, 38). Gastric mucosal PG, but not leukotriene formation, decreases application. Acute inflammation was observed that was characterized by the production of a protein-rich fluid exude infiltrated by leukocytes (predominantly neutrophils). Necrotic tissue was also present at this stage, whereas gastric tissue around the ulcer margin remained unchanged. COX-2-positive cells were demonstrated immunohistochemically in the infiltrating cells and were absent in the gastric glands of the ulcer margin (Fig. 5A), whereas COX-1-immunopositive cells were absent at this stage (Fig. 5B). Adjacent to the lesion, we observed immunoreactive COX-1 in the gastric glands and mucous neck cells, as demonstrated by Iseki (13) in the normal gastric mucosa, whereas COX-2-immunopositive cells were located in the fibroblasts of the serosa (data not shown).
with increasing age in the rat stomach, indicating that mucosal content of arachidonic acid in membrane phospholipids, the common precursor for PG and leukotriene biosynthesis, is unlikely to alter with increasing age (22). We have shown that COX-1, but not COX-2, mRNA is significantly reduced in the aged stomach. Therefore, reduced PG formation in aged rats may occur as a direct result of reduced COX-1 activity.

We observed elevated levels of COX-1SV mRNA in adult rats, and alternative splicing has been demonstrated to change during aging in other physiological systems (23). A biological role for COX-1SV has not been established. COX-1SV mRNA can at best code for a truncated protein, since it is missing the open reading frame normally located in exon 1 (17). Attempts by these previous investigators to assay for COX-1SV protein by Western blotting were hindered by low levels of protein expression and the lack of a specific COX-1 antibody. In addition, induction of COX-1 mRNA levels by serum-treated 3T3 cells showed no concomitant increase in COX-1 protein, suggesting specific induction of COX-1SV (6). Recently, Western blot analysis of rat and mouse stomach lysates in three separate studies (11, 14, 25) have demonstrated two distinct COX-1 protein bands: a 72-kDa band corresponding to the full-length COX-1 protein and a second 50- to 60-kDa unidentified protein that may be a good candidate for COX-1SV. Alternative splicing for a large variety of cytokines and growth factors has been described (1). In chicken embryo fibroblasts, the COX-2 gene has a mRNA containing an unspliced intron, which on mitogenic stimulation is removed, resulting in fully spliced COX-2 mRNA (43). The human interleukin-4 (IL-4) gene expresses a splice variant, IL-4-462, that has been identified as an IL-4-receptor antagonist (12). Therefore, alternative splicing has potentially important biological roles in physiological systems. To date, the role of COX-1SV remains unknown and will only be established if COX-1SV mRNA codes for an alternative COX-1 protein.

We have demonstrated similar levels of COX-1 and COX-2 mRNA in young histologically normal rat stomachs. Iseki (13) demonstrated strong immunoreactivity for COX-1 in the mucous neck cells of the gastric gland, and COX-2 was immunolocalized to the surface mucous cells in both the fundic and pyloric regions of the stomach. Elsewhere, under physiological conditions or in unstimulated cell lines, COX-2 is considered to be undetectable or expressed at low levels. With the use of Northern blot analysis (37) and semiquantitative RT-PCR (25), COX-2 mRNA was not detected in the normal stomach of rodents yet was highly elevated in gastric ulcers. In situations where the mucosa is inflamed or damaged, COX-2 can increase to levels of 20-fold or
higher (29). When comparing samples with extremely varied COX-2 levels, low or undetectable mRNA expression in normal tissues may be attributed to the low sensitivity or the detection limit of the assay utilized. Interestingly, the COX-2 inhibitor NS-398 did not reduce PGE2 levels in the normal stomach of rats (37), suggesting that COX-1 is the predominant isoform. However, it is possible that NS-398 is reducing the PGE2 levels produced through COX-2 and at the same time increasing the levels of PGE2 produced through COX-1. Our study is in accord with previous findings demonstrating COX-1 and COX-2 mRNA transcripts (27) and proteins (13, 45) in the normal human and rat stomach. Together, these findings demonstrate that COX-1 is not the predominant isoform expressed and implicate a physiological role for COX-2 in the normal stomach.

The 3′-untranslated region of COX-2 contains several copies of the Shaw-Kamen instability sequence (9, 43). These sequences are found in many immediately early genes and confer enhanced mRNA degradation (35). Under normal physiological conditions, COX-2 mRNA transcripts are prone to degrading rapidly, whereas COX-1 mRNA is more stable, indicating that PGs derived from COX-1 may predominate even when transcripts from COX-1 and COX-2 are coexpressed. Both of the COX isoforms have been located in the endoplasmic reticulum (28). Additionally, COX-2 is located in the nuclear membrane, where it is ideally positioned to participate in mitogenesis under physiological or pathophysiological conditions (26, 32).

We also investigated the role of COX-1 and COX-1SV during ulcer induction, healing, and repair. Two days after experimentally induced gastric ulceration, acute inflammation occurred, which was characterized by the production of a protein-rich fluid exudate infiltrated by leukocytes (predominantly neutrophils) and COX-2, but no COX-1, immunopositive cells were present in the infiltrating cells. Consistent with previous studies (25, 37) we observed significantly elevated COX-2 mRNA levels in young rats with acute gastric ulcers. COX-1 and COX-1SV mRNA expression remained unchanged at this stage. When ulcers were undergoing healing and repair, COX-2 mRNA was reduced yet still above that of normal gastric tissue. This corresponded with an elevation of COX-1 mRNA in healing ulcers, with no alteration in COX-1SV mRNA. Immunoreactive COX-1 and COX-2 were localized to the fibroblasts of the granulation tissue, and COX-1 was also located in the gastric glands. Thus, like COX-2, COX-1 may contribute to the late stage of gastric ulcer healing. The differences observed between this and previous studies, which demonstrated constitutive COX-1 mRNA expression during ulcer healing and repair (25, 37), may be due to the distinct ulcer models used. Furthermore, the roles for COX-1 and COX-2 in chronic human peptic ulceration, in which the injurious agent (usually gastric acid) persists and hinders the repair sequence, remain to be determined. The results of the present study are consistent with the findings that predominant COX-1 inhibitors delay ulcer healing in rodents and humans (34, 36). A role for COX-1 in wound repair has also been demonstrated in regenerating mouse intestinal stem cells after radiation injury (3).

With gene knockout technology, mice unable to express either COX-1 or COX-2 have been developed and show surprisingly little gastrointestinal pathology (7, 20). The PGE2 levels in the stomach of COX-1-null mice represented <1% of the levels observed in wild-type mice. In this model compensation by COX-2 is unlikely, due to the low levels of PGE2 and the inability to detect COX-2 protein in the COX-1-deficient mice (20). This is in contrast to our demonstration of relatively high levels of COX-2 mRNA in the stomach, in which COX-2 mRNA appears to be increasing with age, whereas COX-1 mRNA is significantly decreased. It is important to note that knockout mice that lack a functional COX-1 or COX-2 gene in utero develop to adulthood without the gene of interest; thus the effects observed in such mice may be a result of adaptation or differences initiated during development. Conditional gene targeting may be a way to circumvent this issue. Furthermore, it is possible that mice heterozygous for one or both COX isoforms may more closely mimic the effects observed by NSAIWs.

The results of the present study suggest that both COX isoforms play a physiological role in the stomach and that decreased expression of COX-1 mRNA may contribute to the development of gastric injury in the aging rat. The exact mechanisms involved in the regulation of COX-1 alternative splicing have yet to be established. COX-1SV mRNA is differentially expressed in the aging stomach but is not induced after acute gastric injury. Finally, the differential expression of COX-1 and COX-2 during gastric ulceration and healing suggests that each enzyme makes a separate and important contribution in the concerted effort to heal a gastric ulcer.

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REFERENCES


