Cyclooxygenase (COX) 1 and 2 in normal, inflamed, and ulcerated human gastric mucosa

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Abstract

Background and aims—Constitutive cyclooxygenase (COX) 1 is believed to mediate prostaglandin dependent gastric protection. However, gastric mucosa contains cells capable of expressing inducible COX-2. We therefore investigated COX-1 and COX-2 expression, localisation, and activity in normal and abnormal human gastric mucosa.

Methods—COX-1 and COX-2 distribution was investigated by light and electron microscopic immunohistochemistry and by western blot analysis, and their contribution to prostaglandin (PG)E synthesis using selective enzyme inhibitors.

Results—There was strong parietal cell COX-1 and COX-2 immunoreactivity in all sections and isolated cells, with macrophage and myofibroblast reactivity in some sections. Immunostaining was specifically abolished by antigen absorption. Western blot analysis confirmed COX-1 and COX-2 expression. COX-1 and COX-2 immunostaining was increased in Helicobacter pylori gastritis, particularly the mid glandular zone and lamina propria inflammatory cells. This was associated with increased ex vivo PGE synthesis (62.4 (13.5) pg/mg v 36.3 (15.5) pg/mg in uninflamed mucosa; p=0.017) which was significantly inhibited by COX-1 but not COX-2 inhibition. Increased COX-2 immunostaining in macrophages, endothelial cells, and myofibroblasts (with reduced epithelial expression) was seen at the rim of ulcers.

Conclusion—COX-2, as well as COX-1, is expressed by normal human gastric mucosa and is increased at the rim of ulcers. Although both are increased with H pylori, COX-1 contributes more than COX-2 to gastric PGE production.

Keywords: stomach; gastric mucosa; cyclooxygenases; Helicobacter pylori; ulceration; prostaglandins

Endogenous prostaglandins are important for protection of the gastric mucosa against a wide variety of insults. Non-steroidal anti-inflammatory drugs (NSAIDs) cause gastric injury by inhibiting synthesis of prostaglandins (PGs) via cyclooxygenase (COX) enzymes. Two isoforms of cyclooxygenase have been recognised. COX-1 is a constitutively expressed enzyme in many tissues, including the gastrointestinal tract, while COX-2 is an inducible enzyme predominantly expressed at sites of inflammation. This had led to the development of selective COX-2 inhibitors with the aim of reducing PG dependent inflammation while leaving protective gastric mucosal PG synthesis intact.

However, COX-2 is induced by many inflammatory and mitogenic stimuli, and there is no reason to believe that this would not also occur in the stomach. In animal models, there is increasing evidence that COX-2 expression can be induced by mucosal injury. Specific inhibitors of COX-2 have been reported to delay healing of erosions and ulcers in mice and rats. Moreover, COX-2 is induced by inflammation, making it possible that this would occur with Helicobacter pylori infection. The aim of this study was to investigate expression, localisation, and activity of both COX-1 and COX-2 enzymes in normal gastric mucosa in H pylori associated gastritis and near to the rim of ulcers.

Materials and methods

HISTOLOGICAL STUDIES

Patients

Archival specimens from 30 patients with histologically confirmed gastric ulcers were retrieved. These comprised 20 specimens from gastrectomies performed for complicated ulcer disease, bleeding, or perforation and 10 from patients with active gastric ulcers at endoscopy. Eleven patients (10 surgical, one endoscopic biopsy sample) were recorded in the hospital notes as receiving NSAIDs. Surgical or endoscopic biopsy specimens were obtained both from the region adjacent to an ulcer rim and an area at least 1 cm away. Normal gastric mucosa was also obtained prospectively at routine upper gastrointestinal endoscopy from 25 patients whose drug usage and H pylori status (C14 urea breath test, histology, and culture) were defined prospectively (15 H pylori positive, 10 H pylori negative). Patients studied prospectively gave informed consent for these biopsy samples. All specimens for immunohistochemistry were fixed in 10% buffered formalin, embedded in paraffin, and processed for routine histology.

Immunohistochemistry

Serial 4 µm thick sections were cut and mounted on capillary gap microscopic slides (Dako, High Wycombe, UK) for immunohistochemistry. The slides were mounted on a glass slide for light microscopy.

Abbreviations used in this paper: COX, cyclooxygenase; NSAIDs, non-steroidal anti-inflammatory drugs; PG, prostaglandin; HMFG2, human milk fat globule 2; EDTA, ethylenediaminetetra-acetic acid; HBSS, Hank’s balanced salt solution; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

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from this unit.17 Gastric mucosal tissue was modified of a method previously described.

Gastric mucosal cells were obtained using a EPITHELIAL CELLS

PREPARATION OF ISOLATED GLANDULAR

EPITHELIAL CELLS

WESTERN BLOT ANALYSIS OF COX-1 AND COX-2

Glandular epithelial cells isolated from normal gastric mucosa, as described above, were assayed for COX-1 and COX-2 by western blotting. Cells were harvested in a proteinase inhibitor cocktail (2 mmol/l N-ethylmaleimide, 2 mg/ml aprotinin, 4 mg/ml pepstatin, 10 mg/ml leupeptin, and 2 mmol/l phenylmethylsulphonyl fluoride) and lysed by three cycles of prompt freezing and thawing. The supernatant samples for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) were solubilised in SDS-PAGE sample buffer containing Tris HCl (62.5 mmol/l), glycine (10%), SDS (2%), bromophenol blue (0.05%), and β-mercaptoethanol (5%) and the pH was adjusted to 6.8. Equal amounts of protein (10 µg) from each cell lysate were loaded onto 10% SDS polyacrylamide gels and electrophoresed at 200 V for two hours. The positive antigen controls were 0.5 µg of COX-1 and 0.5 µg of COX-2 (Cayman Chemical Co). After electrophoresis, the separated proteins were transferred to a nitrocellulose membrane (Amersham International, Little Chalfont, UK) in transfer buffer (48 mmol/l Tris, 39 mmol/l glycine, 20% methanol, pH 9.2) for two hours at a constant current of 20 amps and blocked in 1x Tris buffered saline containing 2.5% non-fat dry milk (Chiver and Sons Ltd, Coolock, Dublin, Ireland) for 30 minutes. The membranes were then incubated with the antibodies (diluted 1:1000) to COX-1 and COX-2 overnight at 4°C. An ABC peroxidase kit and peroxidase substrate kit (both from Vector Laboratories Ltd, Peterborough, UK) were used to detect the primary antibodies bound to the antigen.

CYCLOOXYGENASE ACTIVITY OF WHOLE MUCOSAL

BIOPSY SAMPLES

Patients

All patients gave informed consent for biopsy samples to be taken for research purposes. In
initial dose ranging studies, 10 antral mucosal biopsy samples were taken from eight subjects. 
*H pylori* was not defined in these subjects so that all samples permitted for research pur-
poses could be used for functional assessment. Subsequently, ex vivo PGE₂ synthesis was
assessed in 30 randomly selected dyspeptic patients found to be ulcer free at routine
endoscopy whose *H pylori* status was estab-
lished using the CLO test (15 negative, 15
positive). Patients with ulcers or erosions or
who had been taking NSAIDs or ulcer healing
agents in the previous 30 days were not
studied.

**Stimulation of PGE₂ synthesis**

Three pairs of endoscopic antral biopsy speci-
mens were preincubated in Tris saline 0.15 M,
the COX-1 preferential inhibitor SC5856018 (a
gift from Searle Skokie, Illinois, USA) and the
selective COX-2 inhibitor NS-398 (Cayman
Chemicals),19,20 for three, ten minute periods
before PG synthesis was stimulated by vortex-
ing for one minute.20 This method is derived
from one originally described in the rat 21 and
shown, as in humans, to be sensitive to inhibi-
tion by NSAIDs and aspirin.21,22 NS-398 is a
selective COX-2 inhibitor with a reported COX-
2:COX-1 IC₅₀ ratio in transfected CHO cells of
8333.3.19 SC58560 is a selective

**CYCLOOXYGENASE ACTIVITY IN CELLS ISOLATED**

In a single experiment, epithelial cells isolated
from normal human gastric mucosa were
cultured for 30 minutes at 37°C in RPMI 1640
containing 10% fetal calf serum at a concentra-
tion of 5×10⁶ cells/ml in the presence or
absence of SC58560 or NS-398. Culture
supernatant was obtained after centrifugation
at 13 000 rpm at 4°C for 10 minutes and stored
at −70°C until assayed for PGE₂ using a
specific enzyme linked immunosorbent assay
(Biotrak, Amersham International, Slough,
UK).

**STATISTICAL METHODS**

In functional studies, analysis of variance was
used to identify the influence of subject, drug,
dose, and *H pylori* status on PGE₂ production.
The Student’s *t* test or Mann-Whitney test
were used as appropriate for pairwise comparisons of both functional and immunohistochemical data.

Results
LOCALISATION OF COX-1 AND COX-2 IN NORMAL GASTRIC MUCOSA
Whole sections
In sections from normal human stomach, strong immunoreactivity was observed in the lower portion of the glandular epithelium for both COX-1 (14/15 cases) and COX-2 (12/15) (fig 1A, B). Antigen absorption studies showed that this staining was specific as preincubation with COX-1 antigen abolished COX-1 but not COX-2 immunoreactivity (fig 1C, F) while preincubation with COX-2 antigen abolished COX-2 but not COX-1 immunoreactivity (fig 1D, E). The position and morphology of the immunopositive cells in the glands suggested that these were parietal cells and this was supported by serial sections stained with haematoxylin and eosin and antibody to HMFG2 (fig 2A, B). However, some cells with apparent parietal cell position and morphology did not react with either HMFG2 or the cyclooxygenase antibodies. Electron microscopy also showed COX-1 and COX-2 immunoreactivity localised to parietal cells, demonstrated in smooth endoplasmic reticulum and canicular membranes but not in the nucleus or interior of cytoplasmic organelles such as mitochondria (fig 3A, B). Subcellular distribution of COX-1 and COX-2 enzymes in parietal cells was similar.

In the other layers of the stomach, there was limited immunoreactivity in the lamina propria (3/15 macrophages and 6/15 myofibroblasts) and submucosa (1/15 endothelial cells) but not the muscular layer. There was no significant difference in the proportion of immunopositive cells between antrum- and body-type mucosa for both COX-1 and COX-2. Weak positive immunoreactivity was occasionally observed in the cytoplasm of some mucus cells.

Isolated mucosal cells: immunostaining and western blot analysis
Sequential treatment with EDTA caused selective enrichment with cell, with the characteristics of parietal cells from 8% in fraction 1 to 50% in fraction 5. These cells were strongly labelled by HMFG2 antibody, supporting their identity as parietal cells (fig 2C), and that there was strong expression of COX-1 and COX-2 in the cytoplasm of the cells (fig 2D, E). Western blot analysis of lysates of the parietal cell enriched fraction showed protein bands at 72 kDa which comigrated with authentic COX-1 or COX-2 standard and reacted with antibodies to both COX-1 and COX-2 (fig 3C, D). The antibody used for COX-1 immunostaining bound to COX-1 but not COX-2 while the antibody used for COX-2 immunostaining bound to COX-2 but not COX-1. Gastric macrophages isolated from the lamina propria stimulated by in vitro phagocytosis of zymosan were shown to be immunopositive for both COX-1 and COX-2.

Figure 2 Immunohistochemical analysis of human gastric mucosa with antibodies to cyclooxygenase (COX)-1 (A) and human milk fat globule 2 (HMFG2) (B). Immunopositive cells are represented with morphological features of parietal cells (arrows). In cytospin preparations, cells with the morphological features of parietal cells demonstrated cytoplasmic immunoreactivity for HMFG2 (C), COX-1 (D), and COX-2 (E).
Whole biopsy

Dose dependent inhibition. In preliminary dose ranging experiments, mean PGE₂ production in Tris saline was 46.4 (SD 12.3) pg/mg (n=8). This was decreased by 13.4 (10.0) % by SC58560 10⁻⁷ M and by 70.0 (7.1) % by SC58680 10⁻⁵ M. PGE₂ synthesis was not inhibited by NS-398 10⁻⁷ M but decreased by 35.5 (6.6) % with NC398 10⁻⁵ M (fig 5A).

COX-1 and COX-2 inhibition in the presence or absence of H pylori. In samples whose H pylori status was defined, PGE₂ synthesis was 36.3 (16.0) pg/mg (n=14) in the absence of H pylori infection and increased to 62.4 (13.5) pg/mg/min (n=16, p=0.017) with H pylori infection. Analysis of variance showed that the two factors that significantly altered PGE₂ production by gastric mucosa were H pylori infection and incubation with COX inhibitors. Overall, SC58560 10⁻⁵ M inhibited PGE₂ synthesis by 58.1 (13.8) %, from 50.2 (10.3) pg/mg to 23.0 (5.6) pg/mg (p=0.002).

NS-398 10⁻⁵ M had no significant effect (PGE₂ synthesis 50.7 (9.9) pg/mg). In mucosa taken from H pylori infected individuals, SC58560 10⁻⁵ M reduced synthesis significantly from 62.4 (13.5) pg/mg to 31.0 (9.3) pg/mg (p=0.002) while with NS-398, PGE₂ synthesis was not significantly changed (61.7 (15.4) pg/mg) (fig 5B). A similar pattern was seen in H pylori negative individuals although the reduction by SC58560 10⁻⁵ M from 36.3 (15.5) to 14.0 (15.2) pg/mg was not statistically significant (p=0.13).

LOCALISATION OF COX-1 AND COX-2 FROM PATIENTS WITH GASTRIC ULCER

In mucosa adjacent to gastric ulcers, active inflammation was noted in all 30 samples, including 13 with some regenerative features. COX-2 immunoreactivity was intense in cells with the morphological appearance of macrophages (27/30) (fig 6A), myofibroblasts (28/30) (fig 6B), and vascular endothelial cells (18/30) (fig 6C) around the ulcer margin. Macrophages immunopositive for COX-2 (but not COX-1) were predominantly located in the lamina propria while myofibroblasts that were also immunopositive for COX-2 (but not COX-1) were localised predominantly to the ulcer base. Venular endothelial cells in the submucosa appeared to be the major type of endothelium to express COX-2 in gastric ulcer (fig 6C).
There was no significant change in COX-1 immunoreactivity in macrophages, myofibroblasts, or endothelial cells in ulcerated compared with normal tissue. However, when apparently healthy epithelial cells in intact mucosa immediately adjacent to the ulcerated area were examined, fewer were found to express COX-1 (43%) or COX-2 (43%) compared with normal tissue (93% and 80%, respectively; p<0.01 for both). Overall, NSAID use recorded in the hospital notes did not appear to be associated with changes in expression of COX-1 or COX-2.

Discussion

Prostaglandins are known to play a central role in protecting the gastric mucosa by virtue of their effects on mucus, bicarbonate, surface hydrophobicity, mucosal blood flow, and possible endothelial and epithelial cellular protection.23 Abundant animal and limited human data suggest that COX-1 protein and mRNA predominate in gastric tissue and may account for most endogenous prostaglandin synthesis.6 Consistent with these observations, selective COX-2 inhibitors reduced prostaglandin levels and oedema in areas of inflammatory lesions but had no effect on either gastric mucosal prostaglandin levels or integrity.25 However, other animal studies suggest that COX-2 is induced at the rim of gastric ulcers and that COX-2 inhibitors may retard healing of established ulcers.25 The human gastric mucosa contains many cells capable of expressing COX-226 27 and synthesising prostaglandins,29 raising the possibility that COX-2 could become a predominant source of prostaglandins in inflammatory gastric conditions, with the implication that COX-2 inhibitors would lose their target organ selectivity in the circumstances. Our results have confirmed that COX-1 is expressed in normal gastric mucosa where it appears to be localised principally to parietal cells. This is supported by colocalisa-
As with *H pylori* infection, COX-2 was also markedly upregulated in macrophages, myofibroblasts, and endothelial cells in granulation tissue adjacent to ulcers. These findings are consistent with previous studies on ulcerated gastric mucosa from rats and humans. Availability of material prevented functional assessment of the contribution of COX-1 and COX-2 to prostaglandin synthesis at the ulcer rim. However, it will be important to assess whether COX-2 becomes the predominant source of prostaglandins in this situation as observations from elsewhere suggest that COX-2 inhibitors may impair healing of established ulcers. Immunostaining suggested reduced cyclooxygenase expression in epithelial cells that could in theory also contribute to impaired ulcer healing.

One potential flaw with the notion that safe and effective anti-inflammatory drugs can be produced by selective COX-2 inhibition relates to the possible role of COX-1 in inflammation. In animal studies, Wallace and colleagues have reported that only doses of NSAIDs that are capable of inhibiting COX-1 have full anti-inflammatory effects. Conversely however, a highly selective COX-1 inhibitor was found not to reduce inflammation. Further work on both the role of COX-2 in gastric mucosal protection and of COX-1 in inflammation will be needed before the value of COX-2 inhibitors can be fully evaluated.
In this paper and elsewhere we have reported that macrophages, myofibroblasts, and endothelial cells isolated from human gastric mucosa readily express COX-2 and synthesise prostaglandins via this pathway when exposed to mitogenic or inflammatory stimuli.25,26 Myofibroblast infiltration into the ulcer base and/or rim is a prominent phenomenon seen in granulation tissue43 and myofibroblasts are believed to be important in the process of revascularisation of ulcer healing.50 High expression of cyclooxygenase in myofibroblasts may promote cell proliferation,27 differentiation, or production of the extracellular matrix28 which are required for wound healing. We have also reported elsewhere that human gastric endothelial cells isolated and established in culture express both COX-1 and COX-2, with increased COX-2 expression in response to mitogenic stimuli and production of PGE2, that can be inhibited by both COX-1 and COX-2 inhibitors.29 Prostaglandins synthesised by human gastric endothelial cyclooxygenases have potential to cause vasodilatation and angiogenesis that is inhibited by cyclooxygenase inhibitors.26

In summary, although there is some evidence for increased expression of COX-2 with H pylori infection, we were unable to show that this becomes a dominant source of prostaglandin production. Hence these results do not suggest that the gasroparing properties of COX-2 inhibitors would be lost in H pylori infection, and data from clinical trials confirm this.30 In contrast, whether the intense COX-2 expression seen in macrophages, myofibroblasts, and endothelial cells close to the edge of gastric ulcers is of sufficient functional importance for COX-2 inhibitors to delay gastric ulcer healing requires further evaluation.

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The UEGW abstract book (Gut 2000;47(suppl III)) has again been produced as a CD-ROM and can be found attached to the inside back cover of this issue.