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

L M Jackson, K C Wu, Y R Mahida, D Jenkins, C J Hawkey

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_2 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 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_2 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_2 production.**

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

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Endogenous prostaglandins are important for protection of the gastric mucosa against a wide variety of insults.[1 2] Non-steroidal anti-inflammatory drugs (NSAIDs) cause gastric injury by inhibiting synthesis of prostaglandins (PGs) via cyclooxygenase (COX) enzymes.[3 4] Two isoforms of cyclooxygenase have been recognised. COX-1 is a constitutively expressed enzyme in many tissues, including the gastrointestinal tract[5 6] while COX-2 is an inducible enzyme predominantly expressed at sites of inflammation.[7–11] 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,[12] 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.[13–15] Specific inhibitors of COX-2 have been reported to delay healing of erosions and ulcers in mice and rats.[13 15] 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 immunohisto-

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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chemistry. All sections of gastric mucosa were deparaffinised and heated in citrated buffer at 102°C for 12 minutes. Immunohistochemistry was performed on an automatic machine (Dako TechMate 500) using the Dako ChemMate peroxidase labelled streptavidin/biotin technique. Sites of peroxidase activity were visualised using 3,3'-diaminobenzidine tetrahydrochloride (Dako ChemMate, Dako). The primary antibodies to COX-1 (prostaglandin H synthase 1 (ovine) polyclonal serum, Cat. No. 160107) and COX-2 (prostaglandin H synthase 2 (human) polyclonal antibody, Cat. No. 160107) were purchased from Cayman Chemical Co (Ann Arbor, Michigan, USA).

A mouse monoclonal antibody to human milk fat globule 2 (HMFG2; Novacastra Laboratories Ltd, Newcastle upon Tyne, UK) was also used. This antibody has recently been described as a specific marker of parietal cells because of its reactivity with antigen in canalicular membranes.[16] Following serial titration studies to determine optimal dilutions, COX-1, COX-2, and HMFG were routinely used at 1:500, 1:500, and 1:75 dilutions, respectively. Antigen absorption studies were performed with the sections treated in the same manner except that 95.4 μg/ml of purified ovine COX-1 antigen and 98.7 μg/ml of human COX-2 antigen (both from Cayman Chemical Co.) were added to the antibodies and incubated at 4°C for 48 hours before application. Slides were examined microscopically and scored for relative staining intensity for COX-1 and COX-2 (0–3).

Immunoelectron microscopy

Sections (15 μm) were immunostained as above and postfixed with 1% OsO_4 for one hour at room temperature. Sections were then dehydrated in serial ethanol and embedded in epoxy resin. Ultrathin sections were prepared and observed under a Jeol 1200 EX transmission electron microscope.

PREPARATION OF ISOLATED GLANDULAR EPITHELIAL CELLS

Gastric mucosal cells were obtained using a modification of a method previously described from this unit.[17] Gastric mucosal tissue was obtained from a surgical resection specimen of a 70 year old male patient undergoing surgery (*H pylori* positive, not receiving NSAIDs) for gastric cancer. Fresh specimens of non-malignant tissue taken at least 5 cm from the tumour were transferred to the laboratory and mucosal strips were dissected. Epithelial cells were removed from the basement membrane by five sequential 30 minute incubations in 1 mmol/l ethylenediaminetetra-acetic acid (EDTA; Sigma, St Louis, Missouri, USA) with continuous stirring at 37°C following a 15 minute incubation period in 1 mmol/l dithiothreitol (Sigma) at room temperature. Between each incubation, mucosal samples were washed with calcium and magnesium free Hank's balanced salt solution (HBSS; Gibco/BRL, Life Technologies, Paisley, UK). Epithelial cells detached from the mucosal samples in the last EDTA incubation were collected and washed with HBSS.

PREPARATION OF LAMINA PROPRIA CELLS

The gastric mucosal biopsy samples, completely denuded of epithelial cells, were subsequently cultured at 37°C in RPMI 1640 (Gibco/BRL) containing 10% fetal calf serum (Gibco/BRL) in 60 mm tissue culture dishes (Costar Corp., Cambridge, Massachusetts, USA) for 24 hours.[16] The cells that had migrated out of the biopsy sample and were in suspension or on the bottom of the dishes were collected by vigorous pipetting and centrifugation following incubation at 4°C for one hour. A 10^7 aliquot of these cells was resuspended in 2 ml of HBSS and incubated with fresh human serum treated zymosan (50 μg; Sigma) for 30 minutes.

Cytospin preparations of all of the above cell populations (≈50 000 cells/slide) were subsequently made, fixed with 10% buffered formalin (for epithelial cells) or acetone (for lamina propria cells), and stored at −70°C until required for immunohistochemistry.

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), glycerol (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 1× 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

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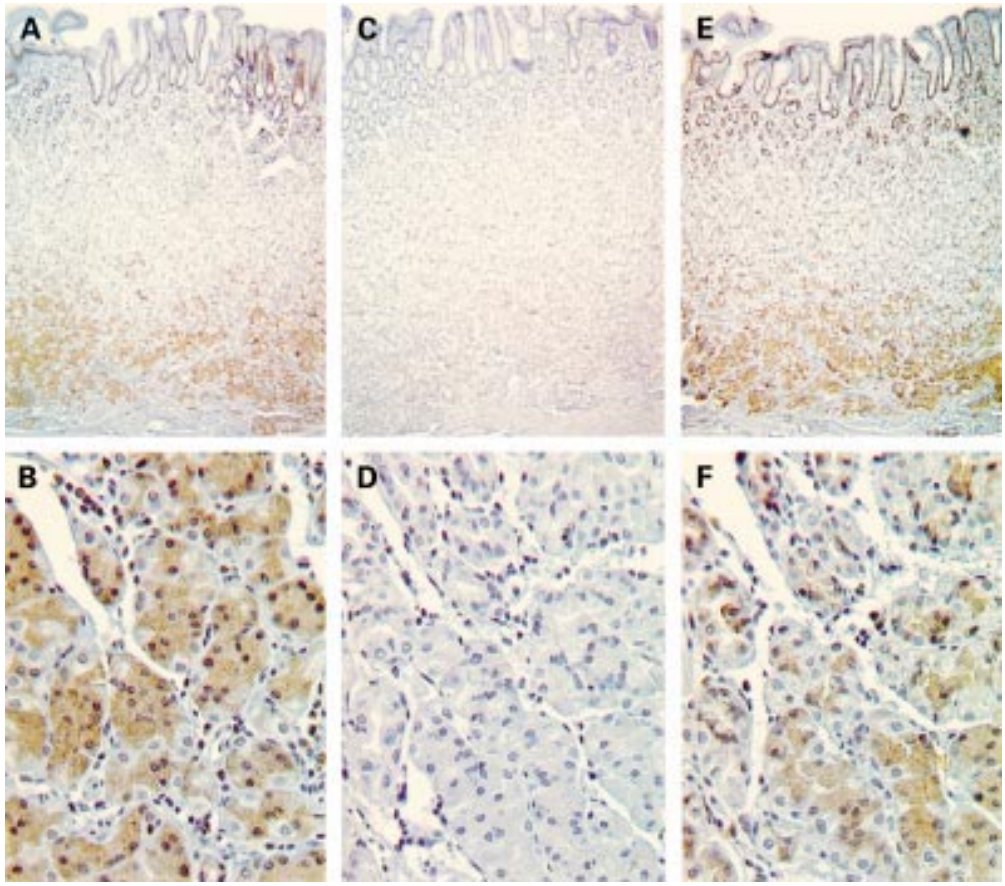

Figure 1 Immunohistochemistry of cyclooxygenase (COX)-1 and COX-2 enzymes in normal human gastric mucosa. Immunoperoxidase activity was positive in glandular epithelial cells when incubated with antibodies against COX-1 (A) and COX-2 (B), but negative when incubated with an anti-COX-1 antibody preabsorbed with purified COX-1 (C) and an anti-COX-2 antibody preabsorbed with purified COX-2 (D). Preincubation of COX-1 antibody with COX-2 antigen (E) and COX-2 antibody with COX-1 antigen (F) did not block staining. Original magnification: ×25 for A, C, E; ×160 for B, D, F (note, sections are not contiguous).

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 purposes could be used for functional assessment. Subsequently, ex vivo PGE_2 synthesis was assessed in 30 randomly selected dyspeptic patients found to be ulcer free at routine endoscopy whose *H pylori* status was established 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 PGE_2 synthesis

Three pairs of endoscopic antral biopsy specimens were preincubated in Tris saline 0.15 M, the COX-1 preferential inhibitor SC58560[18] (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 vortexing 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 inhibition by NSAIDs and aspirin.[21 22] NS-398 is a selective COX-2 inhibitor with a reported COX-2:COX-1 IC_{50} ratio in transfected CHO cells of 8333.3.[19] SC58560 is a selective COX-1 inhibitor with a reported COX-2:COX-1 IC_{50} ratio in a recombinant enzyme system of 0.0014.[18] PGE_2 in the supernatant was measured by ELISA. In initial dose ranging studies, concentrations of 10^{-7} M and 10^{-5} M for each inhibitor were used. Based on these results, concentrations of 10^{-5} M for each drug were used in the studies relating specificity of inhibition to *H pylori* status.

CYCLOOXYGENASE ACTIVITY IN CELLS ISOLATED FROM THE GASTRIC MUCOSA

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 concentration of 5×10^6 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 PGE_2 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 PGE_2 production. The Student's *t* test or Mann-Whitney test

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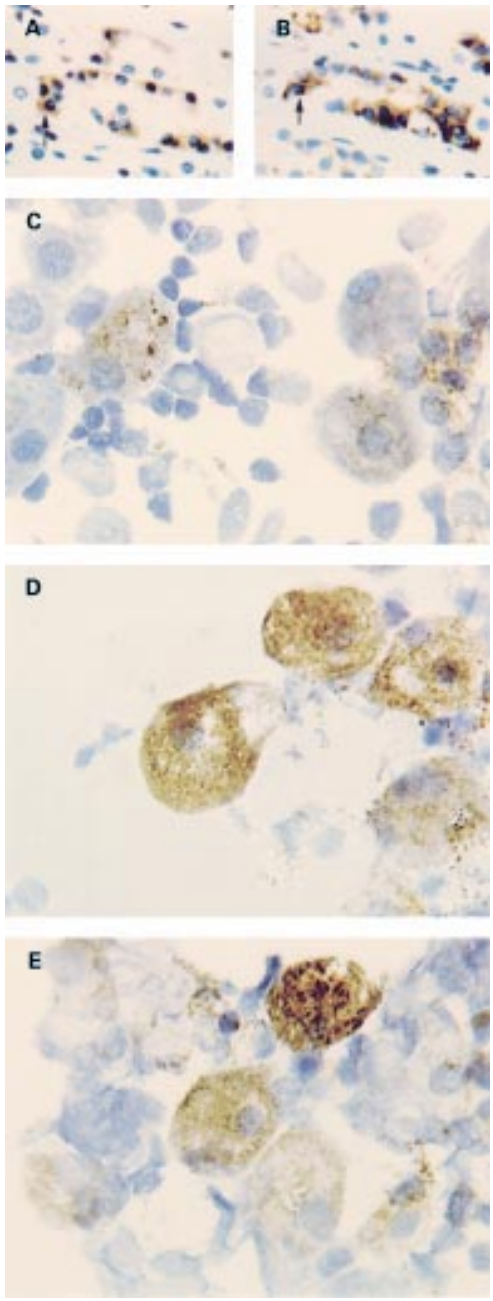

Figure 2 Immunohistochemical analysis of human gastric mucosa with antibodies to cyclooxygenase (COX)-1 (A) and human milk fat globule 2 (HMFG2) (B). Immunopositive cells are represented with morphological features of parietal cells (arrows). In cytospin preparations, cells with the morphological features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

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.

COX-1 AND COX-2 IN *H PYLORI* GASTRITIS: IMMUNOSTAINING

In contrast with normal gastric mucosa, in 8/10 sections of *H pylori* gastritis there was a relative increase in the intensity of staining of epithelial cells of the proliferative zone with both COX-1 (fig 4A) and COX-2 (fig 4B) antibodies. In all sections of *H pylori* associated gastritis there was an increase in the proportion of cells in the lamina propria which stained positively with both antibodies (fig 4A, B).

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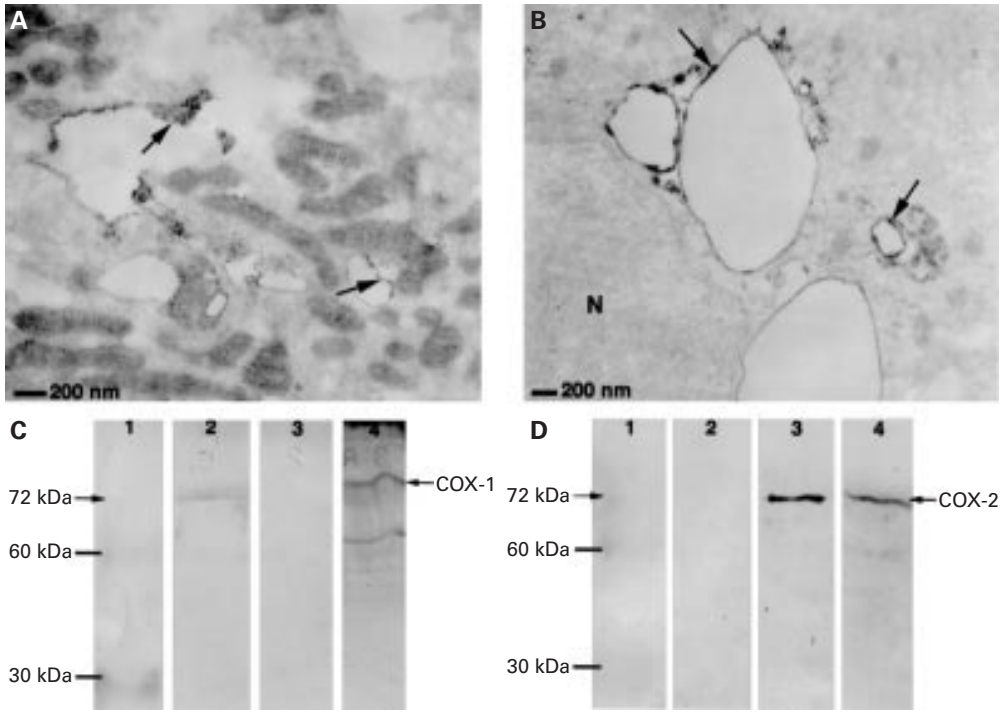

Figure 3 Electron photomicrography of human gastric glands stained with antibodies to cyclooxygenase (COX)-1 (A) and COX-2 (B). Immunoreactivity was localised to intracellular membrane structures (arrows), likely to be smooth endoplasmic reticulum or canicular membranes. (C, D) Western blot analysis of COX-1 and COX-2 proteins in human gastric epithelial cells. Cell lysate (10 µg, lane 4) was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and immunoblotted with (C) anti-COX-1 and (D) anti-COX-2 antibodies. Purified COX-1 and COX-2 standards (0.5 µg) were treated in the same way as the cell lysate and are shown on lane 2 and lane 3, respectively. Molecular weight marker for protein is shown on lane 1 with 60 kDa and 30 kDa bands present. An estimated 72 kDa protein band is seen on lanes with either COX standard or epithelial cells.

COX-1 AND COX-2 IN *H PYLORI* GASTRITIS: FUNCTIONAL STUDIES

Whole biopsy

Dose dependent inhibition. In preliminary dose ranging experiments, mean PGE_2 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. PGE_2 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, PGE 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 PGE_2 production by gastric mucosa were *H pylori* infection and incubation with COX inhibitors. Overall, SC58560 10^{-5} M inhibited PGE_2 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 (PGE_2 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, PGE 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).

PGE_2 production by parietal cell enriched fraction of human gastric epithelial cells

Gastric epithelial cells enriched for parietal cells were cultured in vitro with or without selective COX inhibitors. As shown in fig 5C, the parietal cell enriched epithelial cells from fraction 5 synthesised more PGE_2 both from endogenous and exogenous arachidonic acid than those from fraction 1. Both SC5860 and NS-398 suppressed synthesis at concentrations of 10^{-6} M and 10^{-8} M.

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).

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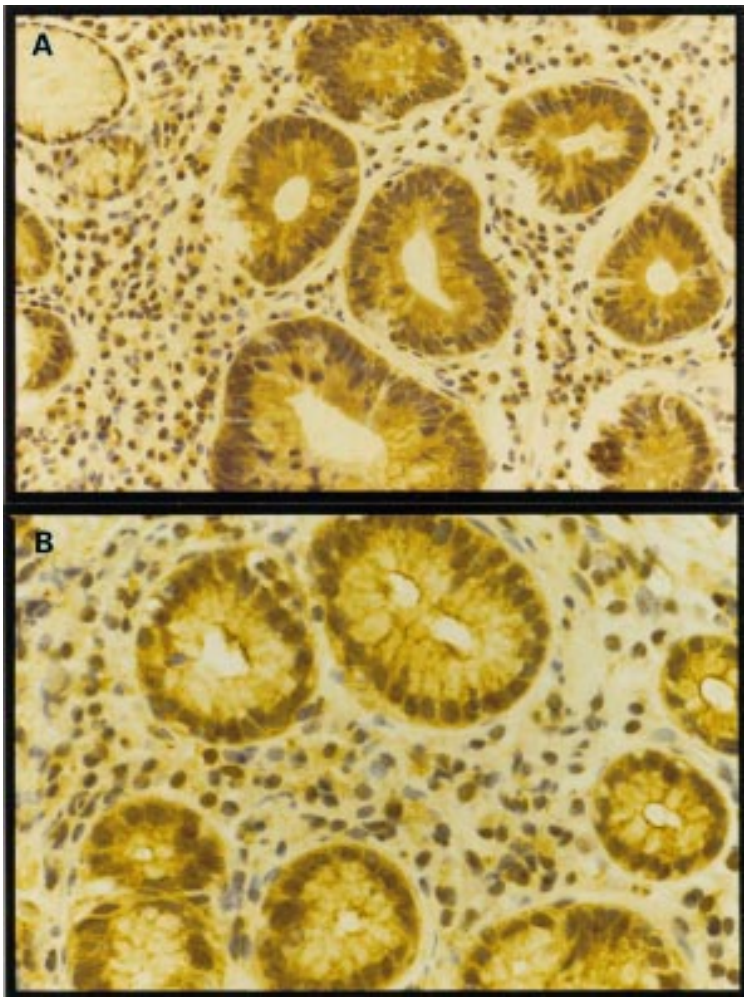

Figure 4 Immunohistochemistry of H pylori associated gastritis showed an increase in the relative intensity of staining of the proliferative zone of the epithelium with cyclooxygenase (COX)-1 (A) and COX-2 (B) antibodies. There was also an increase in the number of cells of the lamina propria which stained positively with COX-1 (A) and COX-2 (B) antibodies.

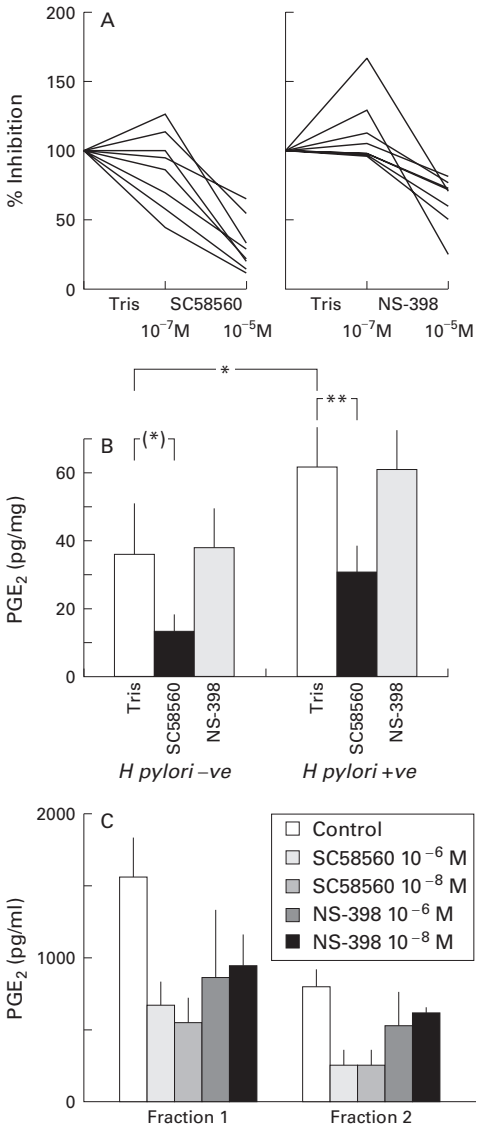

*Figure 5 (A) Dose dependent inhibition of prostaglandin E_2 (PGE_2) production by cyclooxygenase (COX)-1 inhibitor SC58560 and COX-2 inhibitor NS-398 at concentrations of 10^{-7} M and 10^{-5} M. (B) COX-1 and COX-2 inhibition of ex vivo PGE_2 production from whole biopsies by SC58560 and NS -398 at concentrations of 10^{-5} M in the presence or absence of H pylori. *p=0.017, **p=0.002, (*)p=0.13. (C) PGE_2 production by parietal cell enriched fraction of human gastric epithelial cells (control) and its inhibition by SC58560 and NS-398 at concentrations of 10^{-6} M and 10^{-8} M.*

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[6] and mRNA[24] 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.[6 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.[15] The human gastric mucosa contains many cells capable of expressing COX-2[26 27] and synthesising prostaglandins,[25] 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-

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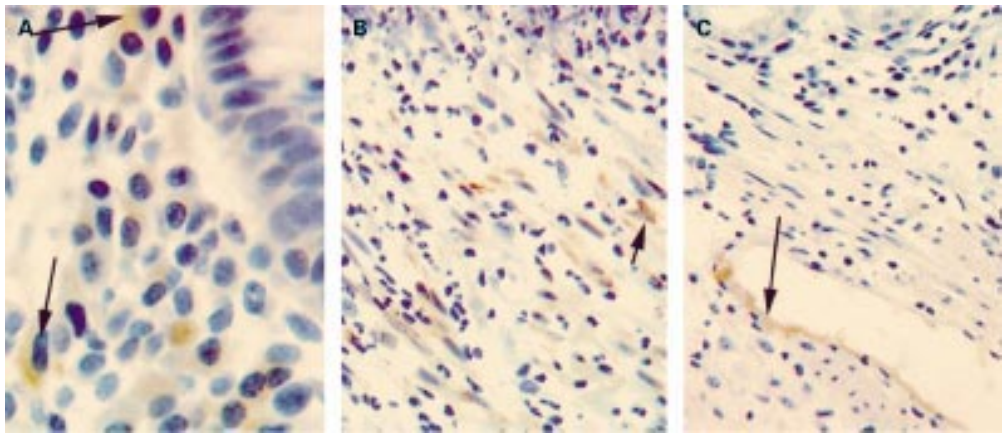

Figure 6 Immunohistochemistry of cyclooxygenase (COX)-2 enzyme in ulcerated human gastric mucosa. Immunoreactivity is seen on (A) macrophages in lamina propria proximal to the ulcer rim, (B) myofibroblasts at the ulcer base or granulation tissue, and (C) vascular endothelial cells in the submucosa next to the ulcer.

tion to cells staining for HMFG2, and by western blot, immunohistochemical, and functional studies of a parietal cell enriched fraction. It has previously been shown that isolated parietal cells from rats,[28] dogs,[29 30] rabbits,[31 32] and humans[33] can synthesis and metabolise[34] PGE_2 which may act in an autocrine manner to inhibit acid secretion.[35]

In contrast with earlier studies,[6] however, we also observed COX-2 immunoreactivity in normal gastric mucosa. This had a similar distribution as COX-1. An obvious explanation for this colocalisation would be cross reactivity between the two cyclooxygenase isoforms. However, western blotting identified both COX-1 and COX-2 in the gastric mucosa. Moreover, in antigen absorption studies, immunoreactivity of COX-2 in mucosal sections from normal human stomach was completely abolished by preincubation of COX-2 antibody with COX-2 antigen but not with COX-1 antigen and vice versa. Constitutive expression of COX-2 enzyme has in fact been shown previously in the stomach[36–39] and other tissues.[6 40 41] COX-1 and COX-2 localisation was confined to the smooth endoplasmic reticulum of parietal cells, in agreement with earlier studies.[42 43] However, the perinuclear distribution of COX-2 reported in murine 3T3 and other cells[44] was not identified in our study with human stomach. Quantitative confocal fluorescence imaging microscopy may provide more information about the intracellular distribution of COX-2 in parietal cells.

When the effect of *H pylori* on cyclooxygenase and prostaglandin synthesis was investigated, surprising and somewhat conflicting results emerged. We confirmed our previous observations that *H pylori* infection was associated with significant increases in ex vivo PGE_2 synthesis.[45] This was associated with increased immunostaining for both COX-1 and COX-2 in epithelial cells of the proliferative zone, and for COX-2 in inflammatory cells of the lamina propria, a pattern that has previously been reported.[46] We do not know if this increase is due to true induction or expansion of a population of cells that normally express COX-1. Short term in vitro challenge studies should clarify this. As with normal parietal cell staining, the apparent colocalisation of the two enzymes leaves open the possibility that, despite the controls we used, these data arose non-specifically or by cross reactivity. More confidence can be placed in the increased immunostaining for COX-2 in inflammatory cells, which appeared to be specific for this isoform. Nevertheless, inhibitor studies showed that prostaglandin production was readily suppressed by a COX-1 but not by a COX-2 inhibitor. It is possible that this could reflect reduced diffusion by NS-398, although this has not been reported for this drug previously. Even if this were so, COX-1 remains a dominant source of prostaglandins in the presence of *H pylori*, as evidenced by the substantial inhibition achieved by the COX-1 inhibitor SC58560.

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.[13 15 38] 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.[13 15] 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.[47] Conversely however, a highly selective COX-1 inhibitor was found not to reduce inflammation.[18] 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.

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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.[26 27] Myofibroblast infiltration into the ulcer base and/or rim is a prominent phenomenon seen in granulation tissue[48 49] 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,[51] differentiation,[52] or production of the extracellular matrix[53] 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 PGE_2 that can be inhibited by both COX-1 and COX-2 inhibitors.[26] Prostaglandins synthesised by human gastric endothelial cyclooxygenases have potential to cause vasodilatation and angiogenesis that is inhibited by cyclooxygenase inhibitions.[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 gastroparing properties of COX-2 inhibitors would be lost in *H pylori* infection, and data from clinical trials confirm this.[54] 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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