Cyclooxygenase 2—implications on maintenance of gastric mucosal integrity and ulcer healing: controversial issues and perspectives

**Summary**
Cyclooxygenase (COX), the key enzyme for synthesis of prostaglandins, exists in two isoforms (COX-1 and COX-2). COX-1 is constitutively expressed in the gastrointestinal tract in large quantities and has been suggested to maintain mucosal integrity through continuous generation of prostaglandins. COX-2 is induced predominantly during inflammation. On this premise selective COX-2 inhibitors not affecting COX-1 in the gastrointestinal tract mucosa have been developed as gastrointestinal sparing anti-inflammatory drugs. They appear to be well tolerated by experimental animals and humans following acute and chronic (three or more months) administration. However, there is increasing evidence that COX-2 has a greater physiological role than merely mediating pain and inflammation. Thus gastric and intestinal lesions do not develop when COX-1 is inhibited but only when the activity of both COX-1 and COX-2 is suppressed. Selective COX-2 inhibitors delay the healing of experimental gastric ulcers to the same extent as non-COX-2 specific non-steroidal anti-inflammatory drugs (NSAIDs). Moreover, when given chronically to experimental animals, they can activate experimental colitis and cause intestinal perforation. The direct involvement of COX-2 in ulcer healing has been supported by observations that expression of COX-2 mRNA and protein is upregulated at the ulcer margin in a temporal and spatial relation to enhanced epithelial cell proliferation and increased expression of growth factors. Moreover, there is increasing evidence that upregulation of COX-2 mRNA and protein occurs during exposure of the gastric mucosa to noxious agents or to ischaemia-reperfusion. These observations support the concept that COX-2 represents (in addition to COX-1) a further line of defence for the gastrointestinal mucosa necessary for maintenance of mucosal integrity and ulcer healing.

**Introduction**
NSAIDs are among the most widely used drugs as they have a particularly broad application. A world review revealed that in 1989, 458 million NSAID prescriptions were filled. The main indications were osteoarthritis (119 million) and rheumatoid arthritis (32 million). The use of NSAIDs and in particular aspirin, has since been extended (NSAID) toxicity

<table>
<thead>
<tr>
<th>Side effects</th>
<th>Onset and development</th>
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<tr>
<td>Mild side effects</td>
<td>Moderate side effects</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>Gastrointestinal erosions (stomach &gt; duodenal bulb)</td>
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<tr>
<td>Gastrointestinal ulcers (stomach and intestine)</td>
<td>Scarring (antrum and duodenal bulb)</td>
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<tr>
<td>Gastric outlet obstruction</td>
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The predominant localisations are listed in parentheses.

**Abbreviations used in this paper:** COX, cyclooxygenase; NSAID, non-steroidal anti-inflammatory drug; PGE, prostaglandin E; NO, nitric oxide; bFGF, basic fibroblast growth factor; BedU, bromodeoxyuridine; ERK-2, extracellular signal regulated kinase 2.

**Table 1. Risk evaluation for non-steroidal anti-inflammatory drug (NSAID) toxicity**

- Age and sex
- Disease for which NSAID is indicated
- Disease severity
- Comorbidity
- Previous gastrointestinal ulcers, bleeding, or perforation
- Previous antacid drug use
- Symptoms with previous NSAID
- Dose, type, and duration of NSAID therapy
- Co-therapy with anticoagulants, corticosteroids, or aspirin
- Perioperative use
- Use of over the counter medication


patients who do not have preceding side effects. Endoscopic monitoring has given a broad insight into the development and nature of gastroduodenal lesions during prolonged NSAID treatment and has established a series of risk factors (table 1). Gastric erosions are the most common endoscopic abnormalities related to acute exposure to NSAIDs (table 2). The acute mucosal damage induced by aspirin in humans occurs within 60 minutes and is visualised as extensive intramucosal petechial haemorrhage and erosions. It has been hypothesised that the topical and systemic mucosal damage in the stomach and especially intestinal blood loss are amplified by NSAID induced inhibition of platelet aggregation. Erosions can promptly be repaired through the process of restitution and adaptation. Endoscopic differentiation between a large erosion and a superficial ulcer is not always possible. Lesions larger than 3 mm, especially with a distinct margin and whitish base, are commonly regarded
as ulcers, although only histological examination including visualisation of the muscularis mucosae can differentiate between ulcers and erosions. Conflicting results have been obtained on the predictive value of the initial number of erosions on later ulcer development. In a large multicentre study extending for more than six months, gastroduodenal ulcers were found in 37% of patients and gastric ulcers were more frequent than duodenal ulcers. Among other factors (table 1), advanced age, osteoarthritis, duration of current NSAID treatment, previous ulcer disease, and current co-treatment with corticosteroids have been shown to be associated with an increased risk of NSAID induced ulcers. Another risk factor appears to be female sex. Overt upper gastrointestinal bleeding was observed in 41% of patients taking aspirin or NSAIDs and appears not to be higher following aspirin intake. In a large prospective study, the occurrence of relevant complications such as peptic ulcers was very rare before six weeks of onset of NSAID treatment but steadily increased thereafter in a near linear fashion and more than 20% of the study participants developed peptic ulcers within 24 weeks (table 2). It is now increasingly being recognised that the occurrence of NSAID induced complications is not restricted to the stomach and duodenum but can also occur in the small and large bowel.

Mechanisms of NSAID injury to the gastrointestinal mucosa

For evaluation of the validity of new potentially less toxic NSAIDs it is mandatory to clearly understand the pathogenesis of NSAID induced ulceration (fig 1). Both aspirin and non-aspirin NSAIDs inhibit the COX pathway of prostaglandin synthesis. This represents the basis of anti-inflammatory action but is also responsible for the development of side effects in the gastrointestinal tract and kidney as well as inhibition of platelet aggregation. Inhibition of prostaglandin synthesis can exert injurious actions on the gastric and duodenal mucosa as it abrogates a number of prostaglandin dependent defence mechanisms.

Inhibition of COX leads to a decrease in mucus and bicarbonate secretion, reduces mucosal blood flow, and causes vascular injury, leucocyte accumulation, and reduced cell turnover, all factors that contribute to the genesis of mucosal damage. Within this broad spectrum of events, the microvascular damage appears to play a central role. Prostaglandins of the E and I series are potent vasodilators that are continuously produced by the vascular endothelium. Inhibition of their synthesis by an NSAID leads to vasoconstriction. Furthermore, inhibition of prostaglandin formation results in a rapid and significant increase in the number of neutrophils adhering to the vascular endothelium in both gastric and mesenteric venules. Adherence is dependent on expression of the β2 integrin (CD11/CD18) on neutrophils and intercellular adhesion molecule on the vascular endothelium. Neutrophil adherence in turn causes microvascular stasis and mucosal injury through ischaemia and release of oxygen derived free radicals and proteases. The severity of experimental NSAID gastropathy was markedly reduced in rats rendered neutropenic by pretreatment with antineutrophil serum or methotrexate. Recently, Wallace and colleagues provided evidence for an isoenzyme specific role of COX in the homeostasis of the gastrointestinal microcirculation. Thus in rats, the selective COX-1 inhibitor SC-560 decreased gastric mucosal blood flow without affecting leucocyte adherence to mesenteric venules. In contrast, the selective COX-2 inhibitor celecoxib markedly increased leucocyte adherence but did not reduce gastric mucosal blood flow. Only concurrent treatment with the COX-1 and COX-2 inhibitor damaged the gastric mucosa, suggesting that reduction of mucosal blood flow and increase in leucocyte adhesion have to occur simultaneously to interfere with mucosal defence.

Inhibition of prostaglandin synthesis thus plays a key role in induction of mucosal injury but does not represent the only pathway by which NSAIDs can damage the gastrointestinal mucosa. NSAIDs can also induce local damage at the site of their contact with the gastrointestinal

![Figure 1](https://example.com/figure1.png)
mucosa. Topical application of NSAIDs increases gastrointestinal permeability allowing luminal aggressive factors access to the mucosa. Aspirin and most non-aspirin NSAIDs are weak organic acids. In the acidic milieu of the stomach, they are converted into more lipid soluble unionised acids that penetrate into the gastric epithelial cells. There, at neutral pH, they are reionised and trapped within the cell causing local injury.33 Having entered gastric epithelial cells, NSAIDs uncouple mitochondrial oxidative phosphorylation. This effect is associated with changes in mitochondrial morphology34 and a decrease in intracellular ATP and therefore a reduced ability to regulate normal cellular functions such as maintenance of intracellular pH.35 This in turn causes loss of cytoskeletal control over tight junctions and increased mucosal permeability.34 35 The ability of NSAIDs to uncouple oxidative phosphorylation stems from the extreme lipid solubility and position of a carboxyl group that acts as a proton translator.35 A further mechanism involved in the topical irritant properties of NSAIDs is their ability to decrease the hydrophobicity of the mucus gel layer of the gastric mucosa. Lichtenberger et al have demonstrated that the surface of the stomach is hydrophobic and that this represents a defence mechanism which can be reduced by various pharmacological agents, including NSAIDs.36–39 NSAIDs can convert the mucus gel from a non-wettable to a wettable state and in experimental animals this effect has been shown to persist for several weeks or months after discontinuation of NSAID administration.36 37

Gastric mucosal lesions can also occur in a non-acidic milieu, such as following rectal application.19 20 With oral administration, gastric acid however appears to enhance NSAID induced damage. More extensive and deeper erosions occur at low pH and an elevation in gastric pH above 4 is necessary to prevent this acid related component.40

Prostaglandins do not represent a unique pathway to protect the gastric mucosa. Nitric oxide (NO) has the potential to counteract potentially noxious effects of inhibition of prostaglandin synthesis, such as reduced gastric mucosal blood flow and increased adherence of neutrophils to the vascular endothelium of the gastric microcirculation.41 NO has well characterised inhibitory effects on neutrophil activation/adherence demonstrated in various tissues.42 43

Interference of non-selective NSAIDs with ulcer healing
NSAIDs such as indomethacin or diclofenac delay gastric ulcer healing both in experimental animals and humans.44–46 In an experimental gastric ulcer model allowing detailed analysis of the healing kinetics45 (fig 2A, B), indomethacin caused reduction of healing velocity predominantly in the second week after ulcer induction while its action was marginal in the first week. Reversibility of NSAID induced healing delay by high dose omeprazole treatment indicates the important role of gastric acid in the interference by NSAIDs with ulcer healing probably at various steps of the underlying processes. Blockade of acid abates the NSAID induced noxious effects on epithelial cell migration/cell proliferation and angiogenesis, which are of prime importance in ulcer healing.46–50 Angiogenesis and blood flow are essential for nutrient and oxygen supply to the healing site47 52 and are considered to play a central role in ulcer healing (fig 3). Exogenous basic fibroblast growth factor (bFGF), a potent promoter of angiogenesis (and healing promoter of ulcers in the absence of NSAID co-treatment), cannot by itself prevent the NSAID induced healing delay in the same experimental model45 indicating the complexity of NSAID interference with ulcer healing. Whether exogenous prostaglandins directly affect ulcer healing is not established. Clinical trials with prostaglandins have clearly shown that in contrast with protective effects even in the absence of coadministration of NSAIDs, these drugs can only promote ulcer healing at doses inhibiting gastric acid secretion.51 This suggests that different mechanisms underlie the acceleration of healing and mucosal protection against noxious agents.45–50

Novel NSAIDs and their toxicity in acute models
Numerous strategies have been used in recent years to develop new anti-inflammatory and analgesic drugs that
damage produced in the cryoulcer model used by the Halter group may interfere with ulcer healing more profoundly than that of the acetic acid model used by Elliott and colleagues.60

Further development of NO-NSAIDs may have lost some of its momentum after it had been recognised that COX exists in two biologically different isoforms (COX-1 and COX-2), the latter being involved primarily in inflammation.

Role of COX-1 and COX-2 in the physiology and pathology of the gastrointestinal tract

In the early 1990s it was established that COX, the enzyme that catalyses the conversion of arachidonic acid to prostaglandins, exists in two isoforms, commonly referred to as COX-1 and COX-2.67,68 The genes for these two isoforms are located on separate chromosomes. Prostaglandins and thromboxanes generated via the COX-1 and COX-2 pathways are identical molecules and therefore have identical biological effects. COX-1 and COX-2 however may generate a different pattern and different amounts of eicosanoids, hence activation of COX-1 and COX-2 may result in different biological responses. Differences in the tissue distribution and regulation of expression of the two isoforms are considered crucial for the physiological role and beneficial and adverse effects of COX inhibitors. The generally held concept (classical COX hypothesis) is that COX-1 is expressed constitutively in most tissues whereas COX-2 is the inducible enzyme triggered by immediate early genes. Prostaglandins produced in normal gastric tissue are primarily derived from COX-1. This COX isoform is considered to exert housekeeping functions in the gastric mucosa essential for gastric physiology—for example, regulation of acid secretion and mucosal protection. Accordingly, COX-1 mRNA and protein are abundant in the gastric mucosa. Induction of COX-2 expression occurs in certain cell types by pro-inflammatory or mitogenic agents, including cytokines, endotoxins, and tumour promoters, as well as by growth factors and in response to tissue injury.69,70 COX-2 mRNA and protein and in inflamed tissues are mainly located in inflammatory cells but are also expressed in endothelial and fibroblast-like cells, and epithelial cells. COX-2 is thus considered to mainly mediate inflammation. Most conventional NSAIDs used in clinical therapy tend to be predominantly COX-1 selective except meloxicam and nimesulide. At low doses, the latter drugs show higher inhibitory activity against COX-2 than COX-1 (3–77-fold depending on the assay system used). At higher doses however the drugs may lose COX-2 selectivity and also inhibit COX-1.

Recently, drugs have been developed with several hundred-fold higher selectivity for COX-2—for example, L-745,337, NS-398, SC-5863 (celecoxib, Celebrex), and MK 996 (rofecoxib, Vioxx). It has been suggested that the term COX-2 specific inhibitor should be used to describe agents which inhibit COX-2 but have no effect on COX-1 over the whole range of doses used and concentrations achieved in clinical usage.71 Selective COX-2 inhibitors have minimal acute gastric toxicity in animals.75–77

Celecoxib and rofecoxib were recently introduced in the USA market and in some European countries. Phase III clinical trials indicate that these drugs are as effective as the non-selective NSAIDs in relieving pain in osteoarthritis and pain and inflammation in rheumatoid arthritis. Less gastrointestinal ulceration and bleeding have been observed with the selective COX-2 inhibitors compared with conventional NSAIDs.78–82 Moreover, selective COX-2 inhibitors have been shown not to interfere with platelet thromboxane A₂ formation, which could be a contributory factor in the
reduced incidence of gastrointestinal haemorrhage. Another not widely recognised advantage is their lack of interference with mitochondrial oxidative phosphorylation as they are not propionic acid derivatives. Taking this into account it has recently been pointed out by Palmer in a letter to Gastroenterology that it may be an oversimplification to unilaterally attribute the better gastrointestinal tolerance of the so-called COX-2 selective NSAIDs to the fact that there is no interference with the COX-1 enzyme.

**Is the classical COX hypothesis (constitutive COX-1 versus inducible COX-2) flawed?**

Increasing evidence indicates that the classical COX hypothesis is oversimplistic. COX-2 appears to play a more complex biological role than simply mediating pain and inflammation. Although it is accepted that COX-2 is primarily an inducible enzyme, there is ample evidence of its constitutive presence in normal non-inflamed tissues such as the macula densa and interstitial cells of the rat kidney and brain.

Of particular importance is the observation that in healthy humans COX-2 is the main source of systemic prostacyclin that plays a key role in the regulation of vasodilatation and inhibition of platelet aggregation. Furthermore, in human blood vessels induction of COX-2 by proinflammatory cytokines has been demonstrated and an anti-inflammatory role of vascular COX-2 at the level of cellular proliferation, adhesion receptor molecule expression, and cytokine release has been suggested. Concerns have therefore been raised regarding the cardiovascular safety of selective COX-2 inhibitors. Moreover, COX-2 inhibitors cannot replace aspirin as a cardioprotective drug due to lack of inhibition of platelet thromboxane A2 formation.

The classical COX-2 hypothesis has downplayed the role of COX-2 expression in the gastrointestinal mucosa. While in normal gastric mucosa COX-1 is the predominant COX isoenzyme, there is increasing evidence that detectable amounts of COX-2 mRNA and protein are both constitutively expressed and inducible in specific locations of the gastric mucosa both in animals and humans (table 3). Successful ex vivo and in vitro identification of the COX-2 protein is of particular importance as detection of mRNA only does not necessarily indicate message translation into COX proteins or functional COX activity. Immunohistochemical studies have yielded conflicting results regarding the cellular localisation of the COX-2 protein. Positive COX-2 immunolocalisation has been found in various cell types such as mesenchymal inflammatory cells, endothelial cells, surface epithelial cells, and parietal cells (table 3). In vitro studies have confirmed expression of both COX-2 mRNA and protein in epithelial cells derived from healthy rat gastric mucosa. Their upregulation by growth factors occurs through the extracellular signal regulated kinase 2 (ERK-2) signalling pathway.

More studies are necessary to define the situations where small amounts of constitutively expressed COX-2 protein play a relevant physiological role in the normal gastrointestinal mucosa. Recent studies in rats have shown that whereas selective inhibition of COX-1 or COX-2 is not ulcerogenic, combined inhibition of both COX-1 and COX-2 induces severe lesions in the stomach and small intestine comparable with the effect of NSAIDs suggesting an important contribution of COX-2 to the maintenance of gastrointestinal mucosal integrity. Furthermore, upregulation of COX-2 expression can be induced by various growth factors and cytokines. Conditions where significant overexpression of COX-2 occurs in the gastric mucosa are: *Helicobacter pylori* infection, stress damage to the gastric mucosa, ischaemia/reperfusion, and gastric ulcer healing.

**Helicobacter pylori infection and COX-2 expression in the gastric mucosa**

*H pylori* colonisation of the stomach causes chronic gastritis and peptic ulcer disease. *H pylori* has been shown to increase the release of prostaglandin E (PGE), in MKN 28 gastric mucosal cells in vitro and to increase gastric mucosal PGE, formation in humans in vivo. Excess prostaglandin synthesis may at least in part stem from the induced COX-2 enzyme. The inducible form of COX is coexpressed with the inducible nitric oxide synthase.

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**Table 3: Immunohistochemical assessment of cyclooxygenase (COX) localisation in gastric tissue**

<table>
<thead>
<tr>
<th>Author</th>
<th>COX-1 localisation</th>
<th>COX-2 localisation</th>
<th>Antibody used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainsford 1995</td>
<td>Not detectable</td>
<td>Smooth muscle cells, inner circular muscle layer</td>
<td>Human polyclonal antibodies</td>
</tr>
<tr>
<td>Iseki 1995</td>
<td>Mucous neck cells</td>
<td>Surface mucus cells</td>
<td>Cayman antibody</td>
</tr>
<tr>
<td>Kargman 1996</td>
<td>Endothelial cells</td>
<td>Subset of macrophages</td>
<td>Cayman antibody</td>
</tr>
<tr>
<td>Tanwarasi 1996</td>
<td>Surface epithelial and some glandular cells</td>
<td>Endothelial cells of microvessels, submucosal macrophages</td>
<td>Cayman antibody</td>
</tr>
<tr>
<td>Tanwarasi 1997</td>
<td>Not examined</td>
<td>Endothelial cells of microvessels and basement membranes</td>
<td>Cayman antibody</td>
</tr>
<tr>
<td>Davies 1997</td>
<td>Not examined</td>
<td>”Superficial mucosa”</td>
<td>Cayman antibody</td>
</tr>
<tr>
<td>Donnelly 1997</td>
<td>Parietal cells</td>
<td>Myofibroblasts, endothelial cells, inflammatory mononuclear cells</td>
<td>Cayman antibody</td>
</tr>
<tr>
<td>Schmassmann 1998</td>
<td>Mucous neck cells</td>
<td>Monocytes, macrophages, fibroblasts, endothelial cells at ulcer margin</td>
<td>Cayman antibody</td>
</tr>
<tr>
<td>Zimmermann 1998</td>
<td>Smooth muscle cells</td>
<td>Smooth muscle cells, fibroblasts, endothelial cells</td>
<td>Dianova antibody (COX-1) Cayman antibody (COX-2)</td>
</tr>
<tr>
<td>Takahashi 1998</td>
<td>Not examined</td>
<td>Fibroblasts, mononuclear cells, macrophages, granulocytes</td>
<td>Not specified</td>
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<td>Fu 1999</td>
<td>Not examined</td>
<td>Mononuclear cells, myofibroblasts</td>
<td>“Primary rat antibody”</td>
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<tr>
<td>McCarthy 1999</td>
<td>Not examined</td>
<td>Surface epithelial cells, parietal cells</td>
<td>Cayman antibodies</td>
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<tr>
<td>Sawaoka 1998</td>
<td>Not examined</td>
<td>Mucous neck cells, mononuclear cells</td>
<td>Not specified</td>
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</tbody>
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**Table 4: Cyclooxygenase (COX) mRNA in normal and inflamed or damaged (ID) and healing (HE) gastric mucosa**

<table>
<thead>
<tr>
<th>Author</th>
<th>COX-1</th>
<th>COX-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>O’Neill 1993</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Tarnawski 1996</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Ferraz 1997</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Mirano 1997</td>
<td>Not examined</td>
<td>Not examined</td>
</tr>
<tr>
<td>Davies 1997</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Zimmerman 1998</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Kishimoto 1998</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Urano 1998</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Takahashi 1998</td>
<td>Not examined</td>
<td>Not examined</td>
</tr>
<tr>
<td>Fu 1999</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

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The increase in COX-2 mRNA in H. pylori infected subjects correlates positively with the degree of gastritis. COX-2 protein detected by immunohistochemistry was found to be abundant in mononuclear cells and fibroblasts of the lamina propria and in gastric epithelial cells, including parietal cells. Eradication of H. pylori reduced COX-2 protein expression proportionally to the reduction in mucosal inflammation. It has been suggested that while COX-2 may act to limit inflammation and injury in active gastritis it may also contribute to H. pylori associated neoplastic transformation. COX-2 mediated stimulation of cellular proliferation and inhibition of apoptosis in the gastrointestinal epithelium may indeed enhance mucosal defence and facilitate wound healing. When sustained, these effects have however the risk of neoplastic transformation. The polyp-cancer sequence in colonic epithelium may serve as a paradigm for the risk of developing cancer due to increased expression of COX-2.

COX inhibitors cause regression of neoplastic polyposis and inhibit their formation. Of special interest in this context is the recent observation that COX-2 is constitutively expressed in normal oesophageal and duodenal mucosa and that its expression is upregulated in metaplastic and dysplastic epithelium of Barrett’s oesophagus and adenocarcinoma in a progressively increasing fashion. A further example of the tumorigenic potential of COX-2 comes from the observation that the selective COX-2 inhibitor celecoxib has chemopreventive properties in rat breast cancer.

The modest stimulatory effects of H. pylori on prostaglandin and nitric oxide synthesis are unlikely to confer significant protection in the presence of NSAIDs as H. pylori also induces a broad spectrum of pathophysiologic changes—for example, reduction of the viscosity of mucus gel facilitating back diffusion of hydrogen ions and reduction of mucosal blood flow which have the potential to diminish the resistance of the gastric mucosa to NSAID exposure. Moreover, Kishimoto and colleagues demonstrated that the presence of neutrophils in H. pylori gastritis is accompanied by an increased cumulative incidence of NSAID induced lesions. Additionally, gastric acid secretion, which is increased in the majority of H. pylori infected subjects, favours gastric mucosal damage as the severity of NSAID damage is dependent on gastric pH.

It is not currently known whether the subset of H. pylori infected patients suffering from pangastriitis associated with hypo- or achlorhydria are less sensitive to NSAID injury, similar to patients with longstanding rheumatoid arthritis associated with gastric atrophy.

In an endoscopic study with a high dose of aspirin (2 g/day), maximal gastric damage consisting of multiple microerosions occurred within three days and was independent of H. pylori infection. In uninfected subjects, gastric adaptation led to rapid reduction of lesions but in H. pylori positive subjects this damage was significantly maintained at similar levels up to day 14. This impairment of gastric adaptation was abated by eradication of H. pylori.

More data are available from studies performed in long term NSAID users. Of particular interest is the Hong Kong study in which a total of 100 patients were randomised to receive bismuth based H. pylori eradication or control treatment while starting naproxen (750 mg twice daily). After two months of treatment there was a striking reduction in gastric ulcers in those who had H. pylori eradication before treatment onset. This study has attracted large interest but has been criticised for using a highly preselected group and especially for its bismuth based H. pylori eradication strategy as the protective effects of bismuth persist within the body for a long period.

There are however case controlled studies that support the notion that H. pylori infection represents a risk factor for NSAID treatment. This notion was also confirmed in a meta-analysis and study based on 37 studies. Several studies however suggest that H. pylori infection does not render the gastric mucosa more vulnerable to NSAID treatment. In one study, H. pylori positive patients had reduced bleeding from gastric ulcers during NSAID treatment. Moreover, eradication of H. pylori in patients who had developed a peptic ulcer during NSAID therapy was found to have a negative effect on ulcer healing induced by high dose ranitidine or omeprazole. This is likely the consequence of eradication induced abolishment of the increased antisecretory efficacy of acid blockers in H. pylori positive subjects.

The controversial findings are likely related, at least in part, to differences between patients selected to these studies. Differences in acid output (with or without concurrent acid suppressive therapy), the degree of neutrophil infiltration of the gastroduodenal mucosa, environmental factors such as previous exposure to NSAIDs, past history of ulcer disease, age and underlying disease of the patient, and type of NSAID are likely to influence outcome. Moreover, study design and outcome measurements are likely to influence whether H. pylori infection is a risk factor for the individual patient during NSAID treatment.

Data concerning the impact of H. pylori infection on gastrointestinal safety of selective COX-2 inhibitors are limited. The incidence of gastrointestinal side effects was small and similar in H. pylori positive and negative subjects exposed to the selective COX-2 inhibitor treatment for 12 weeks. Clearly, more data based on prospective studies are necessary to determine whether eradication of H. pylori modifies gastrointestinal safety of long term treatment with COX-2 inhibitors.

Mucosal stress and COX-2 induction

Kishimoto and colleagues reported significant upregulation of COX-2 mRNA in rat gastric mucosa within six hours after acute ischaemia-reperfusion to levels similar to those produced by the constitutively expressed COX-1. This increased COX-2 expression regressed after reperfusion developed. There is increasing evidence that gastric COX-2 assists the housekeeping action of COX-1 in gastroprotection. The selective COX-2 inhibitors NS-398 and L-745,337 (but not dexamethasone) have been shown to counteract the mucosal protection against ethanol conferred by perfusion of the gastric lumen with peptone and to abolish the protection conferred by the mild irritant acidic ethanol reperfusion. On the other hand, indomethacin, but not a COX-2 inhibitor, abolished long term endotoxin induced gastric resistance to ethanol induced injury although expression of both COX-1 and COX-2 mRNAs was significantly increased. These observations suggest an important role of both isoenzymes in gastric cytoprotection. This is in line with recent observations that a selective COX-1 inhibitor does not damage the gastrointestinal mucosa and only simultaneous blockade of COX-1 and COX-2 induces mucosal injury. Overexpression of COX-2 protein itself does not equal the increase in enzyme activity and prostaglandin formation. Recently it has been shown that a substantial increase in prostanooid production in vivo did not occur after COX-2 induction alone but needed a second arachidonic acid liberating stimulus. Thus in lipopolysaccharide treated rats, a marked increase in prostanooid formation was only seen after intravenous injection of bradykinin or exogenous arachidonic acid.
Taken together, these observations suggest that gastroprotective prostaglandins can be derived from a constitutive as well as an induced COX-1 and from a constitutive as well as an induced COX-2. The same may also be true for prostaglandin generation during inflammation. Wallace and colleagues reported that a significant anti-inflammatory effect of selective COX-2 inhibitors can only be achieved at doses of the drugs that also inhibit the COX-1 pathway. Thus studies of inflammatory reactions and gastric mucosal defence do not confirm the exclusive role of COX-1 as a constitutive and COX-2 as an inducible enzyme. An interaction between COX-1 and COX-2 has also been postulated to play a role in the defence, maintenance of integrity, and function of large vessel endothelia, a role which was originally considered a main function of prostaglandins generated via the COX-1 pathway.

Upregulation of COX-2 expression during healing of experimental gastric ulcers

Studies of COX-2 mRNA and protein expression demonstrated that in rats COX-2 expression is strongly upregulated in the margins of healing gastric ulcers. The duration of this upregulation in vivo is dependent on the severity of the induced lesions and the duration of healing. Schmassmann and colleagues demonstrated that in rats COX-2 expression is strongly upregulated in the margins of healing gastric ulcers. The specific COX-2 inhibitor L-744,337 delayed ulcer healing to the same extent as diclofenac or indomethacin when administered at doses of similar anti-inflammatory potency (as tested in the carrageenan paw model) (from Schmassmann and colleagues with permission).

Figure 4 (A) Time sequence of immunoreactivity of cyclooxygenase (COX)-1, COX-2, and cell proliferation (as measured by bromodeoxyuridine (BrdU) incorporation) at the ulcer margin over 21 days. (B) Ulcer healing curve assessed by video endoscopy. The data indicate mean (SEM) percentage residual ulcer size over the observation period. The specific COX-2 inhibitor L-744,337 delayed ulcer healing to the same extent as diclofenac or indomethacin when administered at doses of similar anti-inflammatory potency (as tested in the carrageenan paw model) (from Schmassmann and colleagues with permission).

Prolonged COX-2 inhibition can induce intestinal perforation and exacerbation of colitis in experimental models

While treatment of rats with the selective COX-2 inhibitor L-745,337 limited to four days did not induce intestinal damage, small bowel perforation was frequently observed.
after 10 days of treatment with doses that inhibited gastric ulcer healing. 

This noxious effect cannot be attributed to inhibition of COX-1 activity because the specificity of L-745,337 as a COX-2 inhibitor had been clearly demonstrated. Similar observations were made by Reuter and colleagues in an experimental colitis model. In this model, one week treatment with L-745,337 resulted in exacerbation of colitis with perforations occurring in the majority of rats during the second week of treatment. As recently shown by Newberry and colleagues, COX-2 promotes tolerance of intestinal antigens and it is likely that the intestinal damage by COX-2 inhibitors is, at least in part, caused by their interference with the intestinal immune response.

### Possible role of additional COX independent gastroprotective mechanisms

Clearly, there are endogenous protective systems in the gastric mucosa which allow the maintenance of mucosal integrity independent of the prostaglandin system—for example, NO, calcitonin gene related peptide, and heat shock proteins. This was demonstrated in the acid challenged rat stomach where indomethacin did not cause acute damage during a 45 minute observation period when given alone but induced severe injury when endogenous NO formation was suppressed or calcitonin gene related peptide was depleted by afferent nerve denervation. Similar effects were observed when the activity of COX-2 was selectively inhibited. Although there was no detectable prostaglandin generation by the gastroduodenal mucosa in COX-1 deficient animals, there was no increased (versus normal controls) incidence of gastroduodenal ulcer. Likewise, COX-2 deficiency also did not cause spontaneous stomach ulcerations but caused severe kidney disease and led to spontaneous peritonitis in some animals. Interestingly, COX-1 deficient mice were more resistant to low dose indomethacin induced gastric damage than wild-type animals. These observations suggest a key role of the COX-2 gene in fetal development of the kidney whereas fetal disruption of the COX-1 or COX-2 gene can be compensated for in the gastrointestinal mucosa. The precise mechanisms compensating for COX-1 and COX-2 deficiency are not known. Kirtikara and colleagues have shown that lung fibroblasts from COX-1 or COX-2 deficient mice have enhanced expression of the remaining functional COX gene and cytosolic phospholipase A2, resulting in exaggerated basal and cytokine induced PGE2 synthesis. However, as prostaglandin formation was nearly absent in the gastric mucosa of COX-1 deficient mice, overexpression of COX-2 is unlikely to be the explanation for the lack of gastric damage in these animals. Prostaglandin independent systems of mucosal defence such as NO, calcitonin gene related peptide, or heat shock proteins may compensate for the lack of prostaglandins in COX deficient animals. Furthermore, it was shown that pharmacologically induced isolated suppression of COX-1 activity does not interfere with mucosal defence. Thus recent observations indicate that gastric and intestinal mucosal lesions do not develop in rats treated with a selective COX-1 inhibitor but only when additionally the activity of COX-2 is suppressed. These findings are much in line with the observation made in COX-1 knockout mice.

### Is extrapolation of the adverse effects noted in experimental animals to the situation of patients treated with selective COX-2 inhibitors justified?

Clearly, data derived from experimental animal models cannot directly be applied to humans as the mode of development of gastric ulcers in the animal model is different from that in humans. However, regardless of the cause of ulceration, once an ulcer develops the pattern of healing is similar in all species. Similarly, observations made in a colitis model may have implications in the treatment of inflammatory bowel disease in humans.

### Perspectives

The pharmaco-economic analysts have projected that due to their highly publicised gastrointestinal safety, COX-2 inhibitors could make a profit of $5 billion per year for their manufacturers in the USA alone. The Food and Drug Administration decided however that the two COX-2 inhibitors released on the USA market (celecoxib (Celebrex; Searle/Pfizer/Pharmacia) and rofecoxib (Vioxx, Merck, Sharp and Dohme)) have to carry the standard NSAID class warning about gastrointestinal complications until additional long term studies show that these drugs cause fewer serious gastrointestinal complications, such as bleeding and perforation, than those caused by conventional non-selective NSAIDs. Additional safety data have since been reported. The data showed that treatment for up to 24 weeks with celecoxib or rofecoxib had a lower incidence of clinically significant upper gastrointestinal side effects than treatment with the non-selective NSAIDs. There is evidence from long term studies that selective COX-2 inhibitors may have a favourable safety profile. Since the current human gastrointestinal ulcer data are based on small numbers performed on patients in whom a peptic ulcer has been excluded endoscopically prior to the initiation of medication, prospective studies are needed to assess whether selective COX-2 inhibitors delay ulcer healing in humans in a manner as demonstrated in experimental animals. There is a high probability that patients who previously have had poor tolerance to conventional NSAIDs or who had ulcers will be promptly switched to the selective COX-2 inhibitors. A substantial number of these patients will have ulcers before the new drug is given, especially as in controlled trials up to 80% of patients who had peptic ulcers on NSAID treatment did not have ulcer symptoms. The same may apply to severely ill patients in intensive care units with increased risk of initially asymptomatic stress ulcers. There is special concern based on observations that similarly to conventional NSAIDs, selective COX-2 inhibitors are generally less effective at inflamed sites, providing a rationale for the higher dose requirement in patients suffering from rheumatoid arthritis. The widespread use of the “super aspirin” and the higher costs compared with that of many “older” NSAIDs available as over the counter preparations is likely to make high risk patients the particular target of treatment with COX-2 inhibitors. Publication of case reports demonstrating severe gastrointestinal side effects attributed to selective COX-2 inhibitors as well as safety concerns have already started. There is no doubt that COX-2 selective inhibitors provide a clear advantage and progress over the non-selective NSAIDs. Also, it should be acknowledged that the classical COX hypothesis has led to significant progress in our knowledge of how NSAIDs interfere with the integrity of the gastrointestinal mucosa and with repair of mucosal damage. However, the full documentation of the gastrointestinal safety of COX-2 inhibitors requires additional extensive long term studies. In the meantime, particular precaution in high risk patients is fully justified. With the rapidly increasing knowledge that COX-2 is not only expressed in pathological inflammatory conditions outside the gastrointestinal tract but also plays an important role in the regulation of integrity, repair, growth, and healing (inclusive of tumour formation) of the gastrointestinal.
mucosa, caution should be exercised when regarding these compounds as “gastrointestinal safe” drugs.

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