Review

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.[1] The main indications were osteoarthritis (119 million) and rheumatoid arthritis (32 million). The use of NSAIDs, and in particular aspirin, has since been extended to prophylaxis of cardiovascular disease.[2] Additionally, colonic neoplastic disease[3] and the prophylaxis of Alzheimer's disease[4] are potential applications of NSAIDs. Dyspepsia interferes with quality of life in over 30% of chronic NSAID users and serious side effects occur in up to 5% of all subjects, mainly affecting the gastrointestinal tract and/or the kidneys. Chronic administration of NSAIDs produces gastroduodenal mucosal erosions in 35–60% of patients, ulcerations in 10–25%, and severe haemorrhages or perforations in <1%.[5] Epidemiological studies have established that overall, NSAIDs enhance the risk of severe ulcer complications such as bleeding, perforation, hospitalisation, and death by approximately 3–10-fold.[5–7] The majority of these complications occur in patients who do not have preceding side effects.[8] 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[9] (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 topically derived and systemic mucosal damage in the stomach and especially intestinal blood loss are amplified by NSAID induced inhibition of platelet aggregation.[10] Erosions can promptly be repaired through the process of restitution and adaptation.[11] 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

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 antiulcer 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

Data from McCarthy DM. *Clin Perspect Gastroenterol* 1999;**2**:219–26.

Table 2 Type, severity, and localisation of non-steroidal anti-inflammatory drug (NSAID) toxicity

Side effects	*Onset and development*
Mild side effects	
• Dyspepsia	Within hours, often regressive
• Gastrointestinal erosions (stomach > duodenal bulb)	despite maintenance of treatment
Moderate side effects	
• Iron-deficiency anaemia	
• Gastrointestinal ulcers (stomach and intestine)	Rare <6 weeks
• Scarring (antrum and duodenal bulb)	
Serious complications	
• Severe gastrointestinal bleeding (stomach >duodenal bulb >oesophagus, small, and large intestine)	Rare <6 weeks of treatment. Progressive increase in a near linear fashion during long term treatment
• Acute perforation (duodenal bulb >colon)	
• Gastric outlet obstruction	

The predominant localisations are listed in parentheses.

Abbreviations used in this paper: COX, cyclooxygenase; NSAID, non-steroidal anti-inflammatory drug; PGE_2, prostaglandin E_2; NO, nitric oxide; bFGF, basic fibroblast growth factor; BrdU, bromodeoxyuridine; ERK-2, extracellular signal regulated kinase 2.

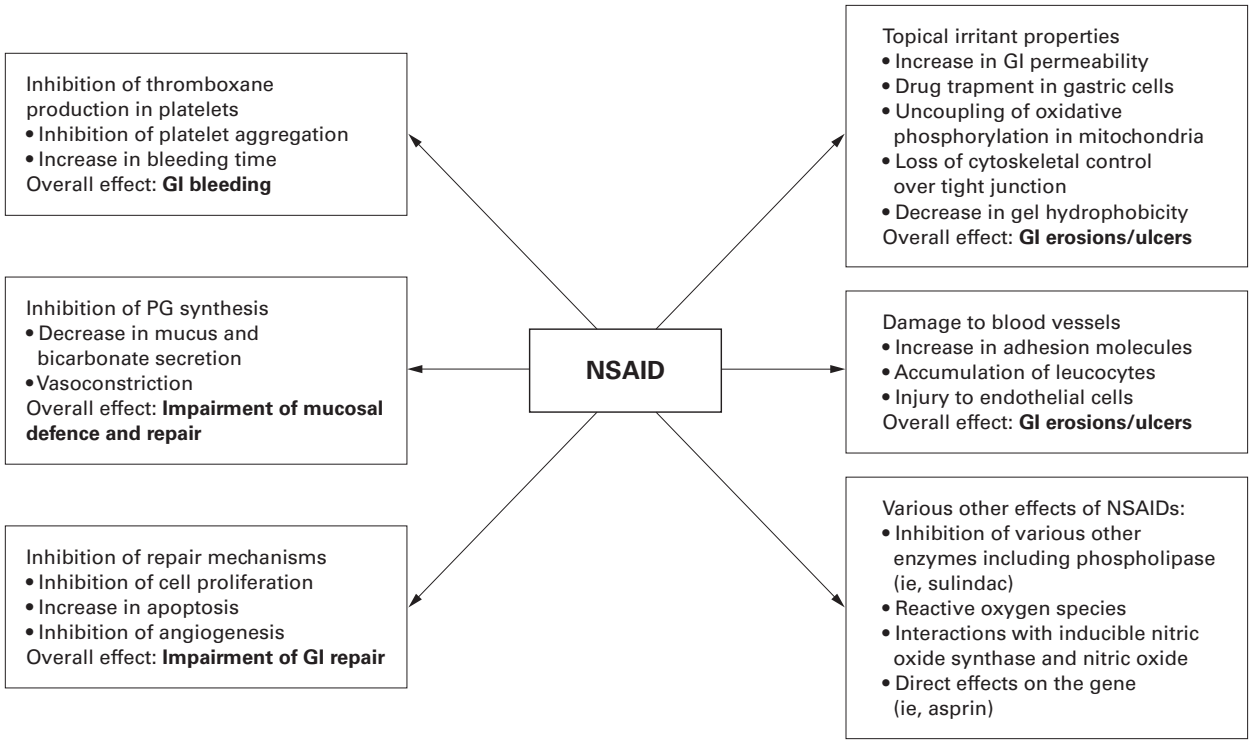

Figure 1 Diagrammatic presentation of the mechanisms of non-steroidal anti-inflammatory drug (NSAID) injury to the gastrointestinal tract.

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.[12–14] 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.[15] 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.[15 16] Another risk factor appears to be female sex.[15] Overt upper gastrointestinal bleeding was observed in 41% of patients taking aspirin or NSAIDs and appears not to be higher following aspirin intake.[17] 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[18] (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.[19 20]

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.[21–23] 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.[24] 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.[25] 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.[26–28] Adherence is dependent on expression of the β2 integrin (CD11/CD18) on neutrophils and intercellular adhesion molecule on the vascular endothelium.[28] Neutrophil adherence in turn causes microvascular stasis and mucosal injury through ischaemia and release of oxygen derived free radicals and proteases.[29] The severity of experimental NSAID gastropathy was markedly reduced in rats rendered neutropenic by pretreatment with antineutrophil serum or methotrexate.[30 31] Recently, Wallace and colleagues[32] 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

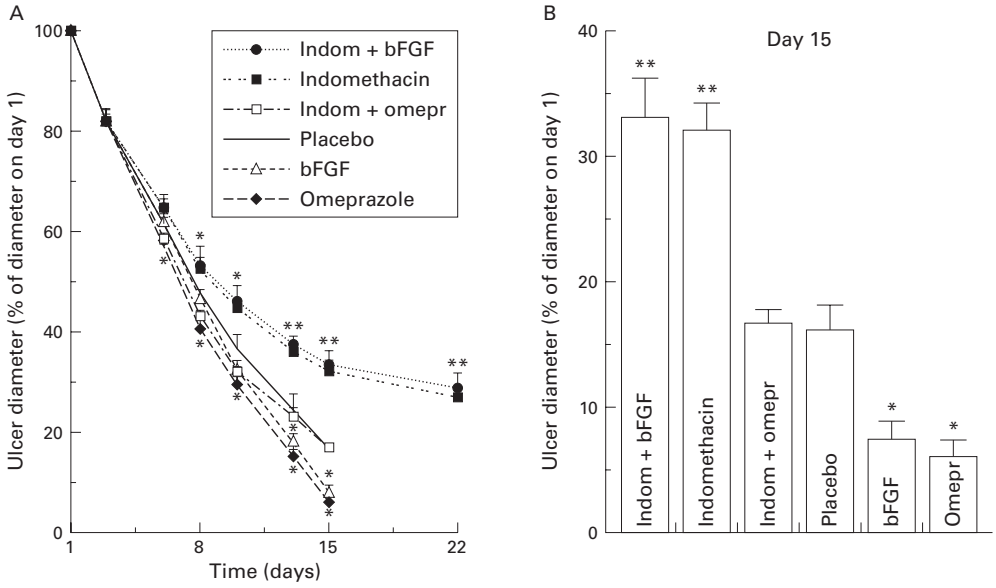

*Figure 2 (A) Ulcer healing curve assessed by video endoscopy. The data indicate mean (SEM) percentage residual ulcer size over the observation time points. Compared with placebo, omeprazole (omepr) and basic fibroblast growth factor (bFGF) treated rats showed significant reductions in ulcer diameter over days 6–15. Indomethacin (Indom) treated rats showed significantly increased ulcer diameters over days 8–22. Co-treatment with bFGF had no effect on the indomethacin induced increase in ulcer diameter. In contrast, omeprazole co-treatment reversed the deleterious effects of indomethacin. (B) Data from the same study, expressed as residual ulcer size on day 15 as a percentage of the initial ulcer size on day 1. Indomethacin plus omeprazole treated rats showed comparable ulcer diameters to placebo treated rats. *p<0.02, **p<0.005 versus placebo (from Schmassmann and colleagues[45] with permission).*

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 mucosal epithelial cells, NSAIDs uncouple mitochondrial oxidative phosphorylation. This effect is associated with changes in mitochondrial morphology[34] 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.[44–49] In an experimental gastric ulcer model allowing detailed analysis of the healing kinetics[45] (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 50–52] Angiogenesis and blood flow are essential for nutrient and oxygen supply to the healing site[51 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 model[45] 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.[53] This suggests that different mechanisms underlie the acceleration of healing and mucosal protection against noxious agents.[54–56]

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

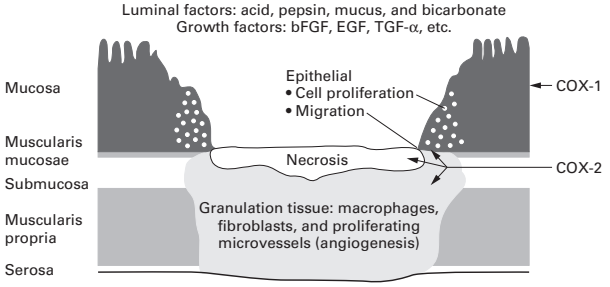

Figure 3 Diagrammatic presentation of ulcer healing and factors affecting ulcer healing. Healing of the ulcer is accomplished by filling the mucosal defect with cells migrating from the ulcer margin and by connective tissue, including microvessels originating from granulation tissue. Speed and quality of ulcer healing depend, among other factors, on (1) epithelial cell migration and proliferation in the mucosal ulcer margin, (2) angiogenesis in the ulcer bed, (3) maturation and contraction of the granulation tissue in the ulcer bed, and (4) quality of remodelling of epithelial and mesenchymal structures in the late healing phase. In the intact mucosa, cyclooxygenase 1 (COX-1) is the predominant COX isoform in the gastrointestinal tract. In contrast, during wound healing, expression of cyclooxygenase 2 (COX-2), but not COX-1, is strongly increased in the repair zone of ulcer healing (from Schmassmann and colleagues[46 76] with permission).

spare the gastrointestinal tract from injury. These strategies include the development of NSAIDs that predominantly inhibit lipoxygenase and/or which have antioxidant activity[57 58] or the coupling of a NO containing moiety to a standard NSAID.[59 60] In rats, NO-NSAIDs have markedly reduced ulcerogenic properties compared with the parent NSAID.[59] Furthermore, in an endoscopic study in healthy volunteers, NO donating flurbiprofen caused less gastric lesions than flurbiprofen.[61] Administration of NO-NSAIDs, but not conventional NSAIDs, has been shown to increase serum nitrite/nitrate levels indicating that NO is released from the drug.[59] NO has vasodilating properties and is involved in the maintenance of resting gastric mucosal blood flow[62] and gastric mucosal hyperaemic reactions.[63] Furthermore, NO inhibits neutrophil activation and adhesion. It has been confirmed in different organs, tissues, and species in vivo that NO induced inhibition of adherence and activation of leucocytes is not the consequence of decreased vascular damage or the effect of maintaining blood flow.[42 64 65] In addition, NO has been shown to increase gastric mucus gel thickness[66] and to reduce epithelial permeability in the small intestine,[67] effects which may contribute to or result from the reduced topical toxicity of NO-NSAIDs. While reduced bioavailability of the NSAID moiety has been considered as a contributory factor to the decreased toxicity of NO-NSAIDs, very high doses of NO-naproxen which produce serum naproxen levels higher than those of an ulcerogenic dose of naproxen do not damage the gastric mucosa in rats.[68]

The lower toxicity of NO-NSAIDs is not disputed but conflicting data have been reported on whether or not these drugs interfere with ulcer healing. Elliott and colleagues[60] reported that the NO releasing NSAID nitrofenac not only does not delay healing of experimental gastric ulcers but accelerates ulcer healing and scar formation. In contrast, Halter and colleagues[56 58] demonstrated that nitrofenac delayed ulcer healing similar to the parent drug diclofenac. These contradictory results may be largely due to differences in design and timing of the two studies. NSAID treatment was limited to one week in the study of Elliott and colleagues[60] while in the study of Halter and colleagues[56 58] the drug was given for two weeks. In this ulcer model, the inhibitory action of NSAIDs, including conventional NSAIDs, on experimental ulcer healing was predominantly evident during the second week after starting NSAID treatment[45] (see also fig 2A). In addition, the 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.[69 70] 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.[71–73] COX-2 mRNA and protein in inflamed tissues are mainly located in inflammatory cells but are also expressed in endothelial cells, 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.[74] 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 function because they do not inhibit platelet thromboxane A_2 formation, which could be a contributory factor in the

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

Author	*COX-1 localisation*	*COX-2 localisation*	*Antibody used*
Rainsford 1995[91]	Not detectable	Smooth muscle cells, inner circular muscle layer	Human polyclonal antibodies
Iseki 1995[92]	Mucous neck cells	Surface mucus cells	Cayman antibody
Kargman 1996[93]	Endothelial cells	Subset of macrophages	Cayman antibody
Tarnawski 1996[94]	Surface epithelial and some glandular cells	Endothelial cells of microvessels, submucosal macrophages	Cayman antibody
Tarnawski 1997[96]	Not examined	Endothelial cells of microvessels and basement membranes	Cayman antibody
Davies 1997[97]	Not examined	"Superficial mucosa"	"Primary rat antibody"
Donnelly 1997[98]	Parietal cells	Myofibroblasts, endothelial cells, inflammatory mononuclear cells	Cayman antibody
Schmassmann 1998[76]	Mucous neck cells	Monocytes, macrophages, fibroblasts, endothelial cells at ulcer margin	Cayman antibody
Zimmermann 1998[99]	Smooth muscle cells	Smooth muscle cells, fibroblasts, endothelial cells	Dianova antibody (COX-1) Cayman antibody (COX-2)
Takahashi 1998[101]	Not examined	Fibroblasts, mononuclear cells, macrophages, granulocytes	Not specified
Fu 1999[104]	Not examined	Mononuclear cells, myofibroblasts	"Primary rat antibody"
McCarthy 1999[105]	Not examined	Surface epithelial cells, parietal cells	Cayman antibodies
Sawaoka 1998[106]	Not examined	Mucous neck cells, mononuclear cells	Not specified

reduced incidence of gastrointestinal haemorrhage.[83] Another not widely recognised advantage is their lack of interference with mitochondrial oxidative phosphorylation[35] as they are not propionic acid derivatives. Taking this into account it has recently been pointed out by Palmer[84] 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.[85–87] 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 vasodilation and inhibition of platelet aggregation.[88] 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.[89] Concerns have therefore been raised regarding the cardiovascular safety of selective COX-2 inhibitors.[88 89] Moreover, COX-2 inhibitors cannot replace aspirin as a cardioprotective drug due to lack of inhibition of platelet thromboxane A_2 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[90–107] (tables 3, 4). 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.[108–110] Their upregulation by growth factors occurs through the extracellular signal regulated kinase 2 (ERK-2) signalling pathway.[110]

More studies are necessary to define the situations where small amounts of constitutively expressed COX-2 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[32 111 112] 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_2 (PGE_2) in MKN 28 gastric mucosal cells in vitro[108] and to increase gastric mucosal PGE_2 formation in humans in vivo.[113] 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.[104 114]

Table 4 Cyclooxygenase (COX) mRNA in normal and inflamed or damaged (ID) and healing (HE) gastric mucosa

	COX-1		*COX-2*	
Author	*Normal*	*ID, HE*	*Normal*	*ID, HE*
O'Neil 1993[90]	Positive	Not examined	Positive	Not examined
Tarnawski 1996[94]	Positive	Not examined	Positive	Not examined
Tarnawski 1997[96]	Not examined	Not examined	Positive	Overepressed
Ferraz 1997[73]	Positive	overexpressed	Positive	Overepressed
Mizuno 1997[95]	Positive	Pos. (stable)	Negative	Positive
Davies 1997[97]	Positive	Pos. (stable)	Positive	Overexpessed
Zimmermann 1998[99]	Positive	Not examined	Positive	Not examined
Kishimoto 1998[100]	Positive	Pos. (stable)	Low	Overepressed
Ukawa 1998[103]	Positive	Positive	Negative	Positive
Takahashi 1998[101]	Positive	Positive	Negative	Positive
Fu 1999[104]	Not examined	Not examined	Absent or low	Increased

The increase in COX-2 mRNA in *H pylori* infected subjects correlates positively with the degree of gastritis.[104] 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.[104–106] Eradication of *H pylori* reduced COX-2 protein expression proportionally to the reduction in mucosal inflammation.[105] 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.[104] 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.[115] 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.[3 116–118] COX inhibitors cause regression of neoplastic polyps and inhibit their formation.[3 116 118 119] Of special interest in this context is also 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.[120] 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.[121]

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 pathophysiological changes—for example, reduction of the viscosity of mucus gel facilitating back diffusion of hydrogen ions[122] and reduction of mucosal blood flow[123] which have the potential to diminish the resistance of the gastric mucosa to NSAID exposure. Moreover, Taha and colleagues[124] 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,[125] favours gastric mucosal damage as the severity of NSAID damage is dependent on gastric pH.[40]

It is not currently known whether the subset of *H pylori* infected patients suffering from pangastritis associated with hypo- or achlorhydria[126] are less sensitive to NSAID injury, similar to patients with longstanding rheumatoid arthritis associated with gastric atrophy.[127]

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*.[113]

More data are available from studies performed in long term NSAID users. Of particular interest is the Hong Kong study[128] 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[129] 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.[13 130] This notion was also confirmed in a meta-analysis study based on 37 studies.[131] Several studies however suggest that *H pylori* infection does not render the gastric mucosa more vulnerable to NSAID treatment.[132–135] In one study, *H pylor*i positive patients had reduced bleeding from gastric ulcers during NSAID treatment.[133] 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.[134 135] This is likely the consequence of eradication induced abolishment of the increased antisecretory efficacy of acid blockers in *H pylori* positive subjects.[136]

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.[137] 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[100] 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 ulceration 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 20% ethanol.[138 139] Furthermore, the selective COX-2 inhibitors NS-398 and DFU markedly aggravated gastric mucosal damage induced in rats by ischaemia-reperfusion.[140] On the other hand, indomethacin, but not a selective 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.[73] 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.[32 111 112] 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 prostanoid 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 prostanoid formation was only seen after intravenous injection of bradykinin or exogenous arachidonic acid.[141]

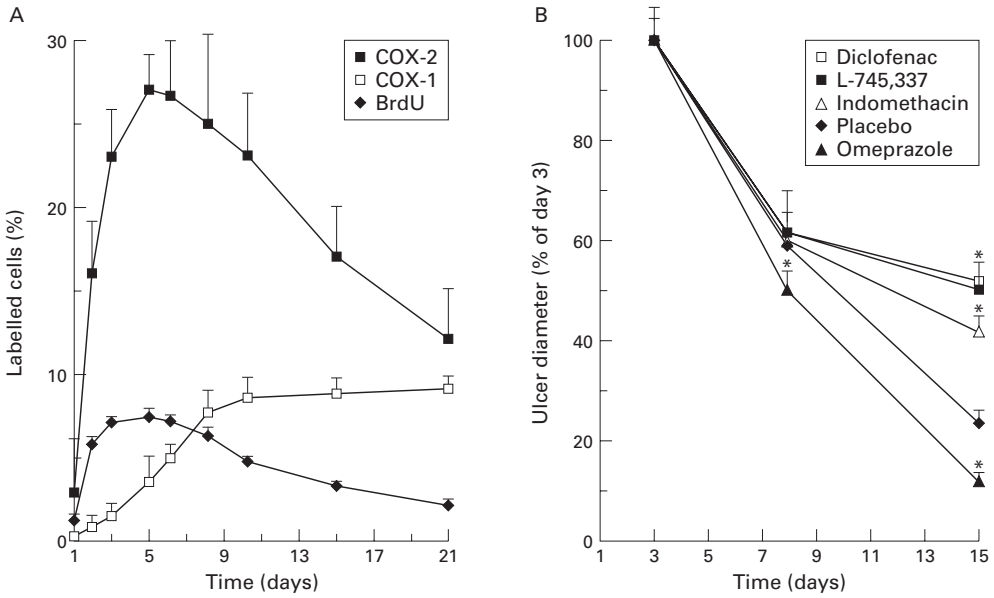

Figure 4 (A) Time sequence of immunoreactivity of cyclooxygenase (COX)-1, COX-2, and cell proliferation (as measured by bromodeoxyuridine (BrdU) incorporation) at the ulcer margin over 21 days. (B) Ulcer healing curve assessed by video endoscopy. The data indicate mean (SEM) percentage residual ulcer 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[76] with permission).

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[142] 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.[88 89]

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.[76 101 102] The duration of this upregulation in vivo is dependent on the severity of the induced lesions and the duration of healing. Schmassmann and colleagues[76] demonstrated that the increase in COX-2 immunoreactivity in monocytes, macrophages, fibroblasts, and endothelial cells at the ulcer margin shows close temporal and spatial correlation with the increase in cell proliferation (fig 4A). In contrast, COX-1 immunoreactivity (strongly expressed in normal gastric mucosa and localised to the mucus neck cells) was dramatically reduced in the early phase of ulcer healing and progressively reappeared over the 21 day observation period. At the site of ulceration COX-2 appears to be the primary contributor to prostaglandin synthesis. Similar observations were made in mice.[95] COX-2 thus appears to represent the second line of defence, which is activated during ulcer healing to compensate for the temporary loss of COX-1 occurring in the mucosa adjacent to the ulcer and assisting COX-1 in safeguarding gastric mucosal integrity. Interference of selective COX-2 inhibitors with healing dynamics of experimental gastric ulcers was demonstrated in the rat cryoulcer model.[76] Rats were treated for two weeks with the specific COX-2 inhibitor L-745,337 or the non-specific NSAIDs indomethacin or diclofenac which exert similar anti-inflammatory effects. Control groups were treated with either placebo or omeprazole given at a dose inducing near maximal acid inhibition. L-745,337 (COX-2 inhibitor) delayed ulcer healing similar to the two conventional NSAIDs (fig 4B), reduced to the same degree cell proliferation in the epithelial cells at the ulcer margin, and inhibited to the same degree angiogenesis and maturation of granulation tissue at the ulcer base. Omeprazole alone accelerated ulcer healing and significantly reversed most of the above inhibitory effects of NSAIDs on ulcer healing. The L-745,337 induced delay in gastric ulcer healing found in the above investigation has been confirmed by studies with the selective COX-2 inhibitor NS-398 in mouse and rat models of gastric ulcer.[95 103] Observations by Lesch and colleagues[143] also confirmed delay of ulcer healing in rats with acetic acid induced ulcers treated with NS-398. However, they found that PD 138387, a selective COX-2 inhibitor (and lipoxygenase inhibitor), did not inhibit ulcer healing. The importance of COX-2 as a regulator of angiogenesis and the molecular mechanisms involved have recently been further characterised in an in vitro study on endothelial cells.[144] Both selective COX-2 and non-selective COX inhibitors inhibited angiogenesis through direct effects on endothelial cells. This action involves inhibition of mitogen activated protein kinase (ERK-2) activity and interference with ERK nuclear translocation, and has prostaglandin dependent and independent components. This study also demonstrated for the first time that NSAIDs could directly inhibit kinase (phosphorylating enzyme) in addition to inhibiting cyclooxygenase.

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.[76] 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.[75 76] Similar observations were made by Reuter and colleagues[145] 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,[146] 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.[147] Similar effects were observed when the activity of COX-2 was selectively inhibited.[148] 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.[149] Likewise, COX-2 deficiency also did not cause spontaneous stomach ulcerations but caused severe kidney disease and led to spontaneous peritonitis in some animals.[150] 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[151] 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 A_2 resulting in exaggerated basal and cytokine induced PGE_2 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.[32 111 112] 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[145] 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.[1] 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, Merk, 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.[152 153] 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.[78–82] Thus there is evidence from long term studies that selective COX-2 inhibitors may have a favourable safety profile. Since the current human gastrointestinal safety data are based on studies 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.[8] 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.[141] The wide publicity about 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.[154–156] 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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