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Gastrointestinal side-effects of traditional non-steroidal anti-inflammatory drugs and new formulations

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SUMMARY

Although adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs) occur in only a small proportion of users, the widespread use of these drugs has resulted in a substantial overall number of affected persons who experience serious gastrointestinal complications. Dyspeptic symptoms are estimated to occur in 10–60% of NSAID users and lead to discontinuation of treatment in 5–15% of rheumatoid arthritis patients taking NSAIDs. It is now well established that the point prevalence of peptic ulcer disease in patients receiving conventional NSAID therapy ranges between 10 and 30%, representing a 10–30-fold increase over that found in the general population. One of 175 users of conventional NSAIDs in the USA will be hospitalized each year for NSAID-induced gastrointestinal damage. The mortality of hospitalized patients remains about 5–10%, with an expected annual death rate of 0.08%. The selective COX-II inhibitors (rofecoxib, celecoxib, parecoxib, etoricoxib, valdecoxib, lumiracoxib) show consistently comparable efficacy to that of conventional non-steroidal anti-inflammatory drugs (NSAIDs) in patients with rheumatoid arthritis and osteoarthritis, but have a significantly reduced propensity to cause gastrointestinal toxicity. In many cases, the gastric effects of therapeutically active doses of COX-II inhibitors are indistinguishable from placebo. The safety benefits of COX-2 inhibitors given alone appear similar to combined therapy with conventional NSAIDs and gastroprotective agents. These findings warrant the consideration of COX-II inhibitors as first-line therapy in patients requiring long-term pain control.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed drugs, and consumption is projected to increase because of the ageing population and more widespread use in cardiac and cerebrovascular disease. NSAIDs rank highly among the most frequently prescribed drugs in Italy and in the world. In 2001, this class of drugs ranked sixth as far as total direct medical costs are concerned ($15 664 billion), but came second when considering the proportional increase in direct medical costs (+ 15%), compared to 2000.[1]

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In the 1980s, serious NSAID-induced gastrointestinal complications resulted in approximately 100 000 hospital admission[2] and 16 000 deaths annually in the USA.[3] Thus, although adverse effects of NSAIDs occur in only a small proportion of users, their widespread use has resulted in a substantial overall number of affected persons who experience gastrointestinal complications.

It is estimated that 10–60% of NSAID users experience dyspeptic symptoms with a relative risk, at least in the elderly, of 1.6 and 1.8 for non-acetyl salicylic acid (ASA) NSAIDs and ASA consumers, respectively. Approximately 5% to 15% of rheumatoid arthritis (RA) patients taking NSAIDs are expected to discontinue medication because of dyspepsia.[4, 5] It is now well established that the point prevalence of peptic ulcer disease in patients receiving conventional NSAID

therapy ranges between 10 and 30%, which is a 10–30-fold increase over that found in the general population.[6] As far as major gastrointestinal complications are concerned, the odds ratio for gastrointestinal haemorrhage in non-selective NSAIDs consumers was 4.2 (CI 95% 3.9–4.5) in case–control studies and 2.74 (IC 95% 2.54–2.97) in randomized controlled trials. In a study that examined the prevention of NSAID-related ulcer complications in 8843 arthritis patients over a 6-month trial period, 0.76% of patients (or 1.5% annually) experienced upper gastrointestinal complications.[7] The US Food and Drug Administration estimates similarly that 2–4% of patients taking conventional NSAIDs for a year experience symptomatic ulcer or potentially life-threatening ulcer complications.[8] The Arthritis, Rheumatism and Ageing Medical Information Systems (ARAMIS) reported that the overall annual incidence of hospitalization for gastrointestinal events events was 1.3%; the rate was six times higher in patients with RA who were taking NSAIDs than in those who were not.[9] Despite a reduction in the rate of hospitalization[3, 9] it has been established that one of 175 users of conventional NSAID in the USA will be hospitalized each year for NSAID-induced gastrointestinal damage.[10] The mortality of hospitalized patients remains about 5–10%, with an expected annual death rate of 0.08–0.22%.[9]

Six COX-2 selective inhibitors (rofecoxib, celecoxib, parecoxib, etoricoxib, valdecoxib and lumiracoxib) have been launched recently. Also available are NSAIDs with preferential COX-II selectivity (e.g. meloxicam and etodoloc).[11] The National Institute for Clinical Excellence (NICE) guidance on COX-II-selective inhibitors for osteoarthritis (OA) and rheumatoid arthritis outlined that COX-II-selective inhibitors have equivalent efficacy to nonselective NSAIDs, and that there is evidence that all coxibs are associated with fewer gastrointestinal adverse events than NSAIDs,[12] From indirect comparisons there was no evidence to suggest that any one drug was clinically superior to any other. However, such comparisons should be viewed with caution because of the heterogeneity of these trials. Only rofecoxib and celecoxib have been investigated in large, long-term trials designed specifically to assess their effects on serious upper gastrointestinal complications. In the VIGOR trial[13] 8076 patients with rheumatoid arthritis were randomized to receive either 50 mg rofecoxib daily or 500 mg naproxen twice daily. Both drugs had a similar efficacy, but over a 9-month period rofecoxib was associated with fewer gastrointestinal events than naproxen (1.38% of patients in the rofecoxib group vs. 3.00% of the naproxen group; RR 0.46, 95% CI 0.34–0.63; number needed to treat (NNT) 62). Complications (bleeding, perforation, obstruction) were also less frequent with rofecoxib (0.40% vs. 0.92%; RR 0.43 CI 0.24–0.77; NNT 191). Patients taking low-dose aspirin were excluded from the VIGOR trial. In the CLASS trial,[14] patients with rheumatoid arthritis or osteoarthritis received randomly celecoxib 400 mg twice daily, ibuprofen 800 mg three times daily or diclofenac 75 mg twice daily. After 6 months, significantly lower upper gastrointestinal events were seen in the celecoxib group (annualized incidence 2.08% vs. 3.54%; RR 0.59 CI 0.38–0.94, NNT 68). However, gastrointestinal complications alone were not reduced significantly with celecoxib: 0.76% vs. 1.45% (RR 0.53, IC 0.26–1.11).

Unlike patients in the VIGOR trial, those in the CLASS study[14] were allowed to take aspirin for cardiovascular benefits, and approximately 20% took up to 325 mg/day. Sub-group analysis showed that this had a dramatic effect on the results. Patients not taking aspirin had significantly fewer gastrointestinal complications with celecoxib than with comparator drugs; but in the aspirin users these benefits were completely negated.

Later on, it became apparent that the CLASS trial was, in fact, two trials, both of which continued for longer than the 6-month initially reported. One trial (vs. diclofenac) continued for 12 months and the other (vs. ibuprofen) for 15 months. The longer-term data suggest that, by 12 months, many of celecoxib's gastrointestinal benefits were lost, with similar levels of serious gastrointestinal complications seen in the celecoxib and NSAID groups. Almost all the ulcer complications that occurred in the second half of the trial were in patients taking celecoxib, and there is concern that COX-II selective inhibitors could interfere with ulcer healing, as COX-II expression is increased in the margin of healing ulcers.[15] However, a recent review suggested that, compared with non-selective NSAIDs, celecoxib signficantly reduces upper gastrointestinal events and healthcare resource utilization (Figure 1).[16] On the basis of the available data, guidance on COX-II selective inhibitors for osteoarthritis and rheumatoid arthritis was suggested by NICE[12] and by a recently published Canadian Technology Report on the cost-effectiveness of rofecoxib and celecoxib in OA or RA patients.[17] The suggested guidelines were that rofecoxib and celecoxib[1] are not cost-effective in patients at average risk of upper

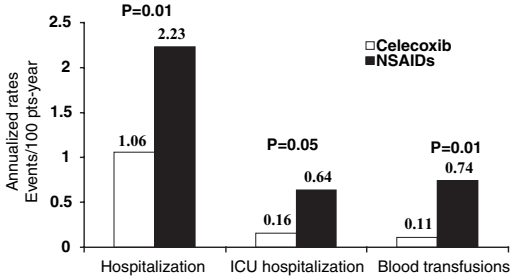

Figure 1. Celecoxib lowers the Healthcare Resource Utilization, when compared with non-selective NSAIDS (data from Reference 17).

gastrointestinal events or in a population with a typical mix of average- and high-risk patients;[2] are cost-effective in patients who are at high risk because they have a history of upper gastrointestinal events;[3] become less cost-effective in high-risk patients as the rate of co-prescription of proton pump inhibitors increases;[4] become cost-effective for patients without additional risk factors over the age of 76 years for rofecoxib and 81 years for celecoxib.

As far as the newer coxibs (etoricoxib, valdecoxib, parecoxib and lumiracoxib) are concerned, the available data suggest that these drugs are as effective as non-selective NSAIDs in the treatment of osteoarthritis and rheumatoid arthritis as well as for acute pain.[18–20] Only a study by Matsumoto *et al.* has shown that is more effective than naproxen in the treatment of rheumatoid arthritis.[21] However, it should be noted that these trials were designed to see if coxibs and NSAIDs have equivalent effiacy, but not to evaluate differences between treatments. In addition, the clinical endpoints used in these trials are not to demonstrate small differences in efficacy which might reflect the involvement in inflammation of prostanoids deriving from COX-1.[22]

Data on gastrointestinal safety of newer coxibs have been extrapolated from clinical efficacy trials. From three multicentric studies conducted on a total of 1480 patients with OA of the knee and hip and with RA treated for up to 12 weeks, valdecoxib, at doses of 5–40 mg, proved safe in chronic treatment.[23–25] The most common adverse effects were gastrointestinal symptoms (abdominal pain, diarrhoea, dyspepsia and nausea), headache and upper respiratory infection, with an incidence of about 5% during the 12-week treatment period. The incidence of gastroduodenal ulcers evaluated in the study by Sikes *et al.*[26] was comparable among patients who had received 10 or 20 mg/day of valdecoxib or placebo for 12 weeks, and was significantly higher in patients who had received ibuprofen or diclofenac ($P < 0.05$) vs. placebo. When valdecoxib, 10 or 20 mg/day, was administered with low doses of aspirin, the incidence of ulcer was significantly lower than with ibuprofen, 2400 mg/day, or diclofenac, 150 mg/day, administered with low doses of aspirin ($P < 0.014$).[26]

The gastrointestinal safety of parecoxib was compared to that of ketorolac, a conventional NSAID used widely for preoperative analgesia. The incidence of gastroduodenal and gastric ulcers or erosions was higher in the group treated with ketorolac ($P < 0.05$ vs. parecoxib and vs. placebo).[27] However, this trial was limited by the small number of patients ($n = 92$) and by the short duration of treatment (up to 7 days).

For etoricoxib, gastrointestinal safety was evaluated by combined analysis of 10 clinical trials in which a total of 3142 patients were enrolled.[18, 28] This study suggested that compared with nonselective NSAIDS, etoricoxib halves both perforations and confirmed and unconfirmed bleeding (PUB). In addition, another combined analysis suggested that etoricoxib significantly reduces the need for gastroprotective agents and gastrointestinal medications and the number of discontinuations due to gastrointestinal adverse effects.[29, 30] The incidence of endoscopically detected gastric/duodenal ulcers was evaluated in 742 patients with OA and RA treated with etoricoxib (120 mg/day), naproxen (500 mg b.i.d.) or placebo for 12 weeks. The incidence of ulcers with a diameter of 3 mm or greater was significantly higher in the group treated with naproxen (25.3%) than in the group treated with etoricoxib (7.4%) or placebo (1.4%, $P < 0.01$); the results for ulcers with a diameter of 5 mm or greater were similar.[31]

A long-term study (12 weeks) conducted on 1042 patients with OA treated with lumiracoxib (200–400 mg/day), celecoxib (400 mg/day) or ibuprofen (2400 mg/day) showed that the cumulative incidence of gastroduodenal ulcers was comparable among patients receiving lumiracoxib and celecoxib, and was reduced significantly in patients treated with both coxibs compared with those treated with ibuprofen.[32] In another study, in 65 healthy volunteers receiving 200 mg b.i.d. of lumiracoxib, placebo or naproxen 500 mg b.i.d. for 8 consecutive days, naproxen was associated with a higher incidence of duodenal erosions compared with lumiracoxib and placebo.[33] However, the results of this study are limited by the small numbers and short duration of treatment.

RISK FACTORS

Non-selective NSAIDs

The risk of adverse gastrointestinal events associated with NSAID use is significantly greater in patients aged over 60 years in whom the relative risk is 5.5 (CI 95% 4.6–6.6) compared to 1.7 (CI 95% 1.1–2.5) in younger patients. The relative risk increases with advancing age: from 3.5 in patients between 60 and 75 years of age to 8.9 in patients over 75 years, compared to 1.8 in younger patients.[34]

There is evidence from a number of studies that a previous history of ulcer may increase the risk of further gastrointestinal problems. Evidence for this has come from a number of studies. One meta-analysis indicated the relative risk of the first gastrointestinal event as 2.4 (CI 95%2.2–2.7), and the risk of a subsequent gastrointestinal event if given NSAIDs again as 4.8 (CI95% 4.0–5.6).[35]

The use of corticosteroids (at doses greater than 10 mg prednisolone daily) and anticoagulants may lead to significant gastrointestinal complications. The relative risk of damage with co-administration of corticosteroids ranges from 1.8 to 14.6[36] and there is evidence that anticoagulants increase bleeding significantly from a relatively minor lesion induced initially by NSAIDs.[37]

Dose levels of NSAIDs, multiple NSAIDs administration, the presence of other debilitating diseases (chronic heart or lung diseases), type and severity of arthritis undisputedly magnify the risk of bleeding peptic ulcer in patients taking NSAIDs. There is also strong evidence that invidual NSAIDs vary in the risk associated with them (Figure 2). Earlier studies suggested that risk was higher in the first 3 months of NSAID use than later, but more recent trials have failed to support this. There is a debate as to whether the indications for NSAID use, sex of the patients, smoking or alcohol history or *Helicobacter pylori* status are risk modifiers[38, 39] (Table 1).

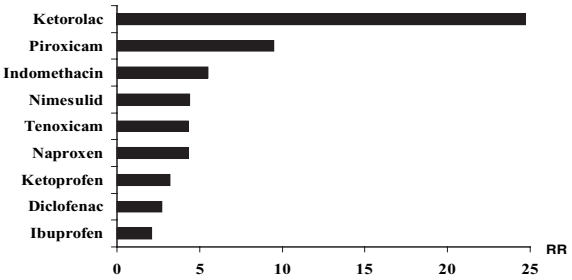

Figure 2. Relative risk (RR) of gastrointestinal complications related to the effects of individual NSAIDs (data from Reference 38).

COX-2 selective inhibitors

Analysis of the final results of trials regarding rofecoxib shows that the relative risk of confirmed upper gastrointestinal events is significantly smaller with rofecoxib than with naproxen in patients with no risk

Table 1. Influence of potential risk factors on cumulative incidence of upper gastrointestinal events (data from References 35–44)

Potential risk factors	Non-selective NSAIDs RR	Rofecoxib RR	Celecoxib RR
History of gastrointestinal ulcers	17.1 (10.0–29.6)	3.8 (1.4–10.6)	1.8 (1.38–2.3)
Ulcer complications			
First	2.4 (2.2–2.7)		
Subsequent	4.8 (4.0–5.6)		
Age			
> 60 years	5.5 (4.6–6.6)	5.9 (1.9–18.3)	1.13 (0.86–1.48)
60–75 years	3.5 (2.5–4.1)		1.0 (0.74–1.3)
> 75 years	8.9 (4.0–10.2)		(0.79–1.26)
		1.0	
Concomitant steroids	2.2 (1.8–14.6)		
Concomitant anticoagulants	6.4 (2.8–14.6)		
Cardiovascular disease	1.8 (1.1–3.2)		
High-dose NSAID	9.0 (5.7–14.2)		
Low-dose ASA			1.4 (1.1–1.8)
Multiple NSAIDs	7.0 (5.2–9.6)		
Baseline gastroduodenal erosions		5.0 (1.9–13.5)	

factors except rheumatoid arthritis (relative risk 0.12), as well as for patients with ≥ one risk factor (relative risk 0.49). The risk factors taken into account were age > 65 years, *H. pylori* positive status, haemorrhagic upper gastrointestinal events and use of corticosteroids. Although the difference in gastrointestinal safety of coxibs and nonselective NSAIDs is significant, physicians need to investigate the patient's age, previous history of any gastrointestinal events or complicated gastrointestinal events, and whether the patient is taking any concomitant medication (in particular steroids and anticoagulants), before initiating coxib therapy.[39–41] The risk factors for ulcer complications and symptomatic ulcers with celecoxib identified by univariate analysis in some studies were age > 75 years, aspirin use and a history of upper gastrointestinal bleeding, gastroduodenal ulcer, NSAID intolerance and cardiovascular disease[42, 43] (Table 1).

MECHANISMS OF NSAID GASTROPATHY

NSAIDs cause gastrointestinal damage through a variety of mechanisms. Some of these detrimental effects are due to the topical irritant actions of these compounds, but the vast majority are due to the main pharmacological effect, i.e. inhibition of cyclo-oxygenase (COX) activity. However, although the concept has developed that there are two main routes of mucosal injury (topical toxicity and inhibition of prostaglandin synthesis), it can in practice be difficult to quantify the relative contribution of each or to state with certainty whether all NSAIDs possess prostaglandin-independent topical toxicity. Moreover, an increasing body of evidence suggests that COX-independent effects, such as up-regulation of adhesion molecules, inhibition of nuclear transcription factor κB (NFκb) and MAP kinases or release of TNF-α might be important.

Topical effects

(a) Ion trapping. In a study published in 1964, Davenport[44] showed that aspirin induces topical injury in the dog stomach; subsequently Fromm[45] reported that acidic NSAIDs can also directly damage gastric epithelium by intracellular accumulation. In the stomach this effect is described as breaking the mucosal barrier. Indeed, this was one of the earliest identified toxic effects. As a rough generalization, acidic NSAIDs are more toxic than those with a neutral pK. Acidic NSAIDs and aspirin at the usual acidic gastric pH are unionized which allow them to be freely lipid soluble. The unionized and lipid soluble NSAIDs penetrates the cell membrane and accumulate in the mucosal epithelial cells where the pH is 7.4. At this pH value, aspirin and NSAIDs become ionized and trapped within the cells, a phenomenon defined as 'ion trapping'. Trapping of the molecules in the ionized form causes cell damage and thus vascular damage. Release of oxygen radicals in the damaged area has a chemotactic action which can contribute to worsening of the damage by promoting neutrophil margination in the gastric microcirculation, which results in a reduced blood flow due to white thrombi occluding the microvessels. In practice, technical difficulties have limited direct demonstration of this phenomenon to aspirin and salicylic acid, both of which appear to be concentrated 2-fold. High mucosal concentrations (generally presumed rather than empirically demonstrated for non-aspirin NSAIDs) not only enhance the ability of NSAIDs to inhibit prostaglandins synthesis but could also bring into play other properties of NSAIDs that have been demonstrated at relatively high concentrations.[38] These include a direct effect on enzyme activity, uncoupling of oxidative phosphorylation and inhibition of fatty acid metabolism. The contribution of these effects of NSAID, together with their relationship to the concept of breaking of the gastric mucosal barrier, are controversial, but the fact that aspirin is clearly much more toxic than salicylic acid implies a prominent role of inhibition of prostaglandin synthesis. In addition, the fact that enteric-coated formulation, pro-drugs or systemic administration of NSAIDs did not reduce the frequency of gastroduodenal ulceration implies a minor role for topical injury compared to systemic effect.[46]

(b) Proliferation and apoptosis. In principle, mucosal integrity is a balance between proliferation and apoptosis. Animal and *in vitro* data support the concept that NSAIDs administration enhances gastric epithelial cells apoptosis through a mechanism that involves activation of pro-apoptotic caspases.[47, 48] A sustained up-regulation of gastric cysteine endoprotease was shown in animals after prolonged exposure to NSAIDs. It has been suggested that TNF-α, a potent extracellular modulator of pro-apoptotic caspases *in vitro*, may play an important role in regulating gastric epithelial cells apoptosis in NSAID-treated rats. This finding may be of clinical relevance, because an increased rate of apoptosis might be the mechanism underlying the gastric epithelial cell

loss encountered in chronic atrophic gastritis, a common feature in chronic NSAIDs consumers.

The enhanced proliferation seen with NSAIDs may be a response to increased apoptosis or desquamation, rather than a primary effect of NSAIDs. The net effect of these changes may vary in time-course and model, with both mucosal erosion and mucosal hyperplasia being reported.[49]

Mucosal lesions, erosions and petechiae induced by acute NSAID administration are common, but of little clinical relevance, and their pathogenesis is due largely to vascular damage. On the contrary, the pathogenesis of lesions defined as clinically significant (that is, chronic gastric and/or duodenal ulcers) is less well known and in many cases can be attributed to patient-related factors, such as *H. pylori* status, previous history of ulcers, etc.

(c) Alteration of surface hydrophobicity of the gastric mucus gel layer. The gastric mucosal has a hydrophobic, lipidic surface, due mainly to secretion of a surfactant-like phospholipid into the gastric mucus gel layer. The phospholipid content of the gastric surfactant layer is enriched by gastroprotective agents, such as prostaglandins, and is attenuated rapidly by NSAIDs which reduce surface hydrophobicity by combining chemically with and destabilizing phspholipids within the mucus gel layer, in particular phophotidylcholine.[50] Recently, newer NSAIDs have been developed in which the native NSAID moiety is coupled with synthetic phosphatidylcholine (PC). The results of studies of PC-NSAIDs in animals and human beings indicate that these agents are associated with a reduction in gastrointestinal ulceration and with faster ulcer healing in the face of continued NSAID exposure.[51]

(d) Motility. NSAIDs have been associated with altered gastroduodenal motility, although the results of studies have varied and the most recent study found no effects in humans.[52] NSAIDs appear to reduce oesophageal sphincter pressure and lead to motility derangements. Deranged motility could be a key abnormality leading to NSAID-associated gastro-oesophageal reflux and to other subtypes of NSAID-associated dyspepsia.

(e) Role of acid. Gastric acid probably exacerbates NSAID injury by disrupting the basement membrane to produce deep injury[53] affecting platelet aggregation[54] and impairing[55] ulcer healing. Indomethacin has been shown to retard the proliferative response to epidermal growth factor at the ulcer edge and inhibit angiogenesis in the granulation tissue of rats gastric ulcers. This inhibitory effect was reversed partly by co-administration of omeprazole with indomethacin.[55]

Systemic effects

Although the mechanisms of NSAID-induced gastrointestinal damage are not fully understood, NSAID-induced injury generally correlates with inhibition of prostaglandin synthesis. Endogenous prostaglandins regulate mucosal blood flow, epithelial cell proliferation, epithelial restitution, mucosal immunocyte function, mucus and bicarbonate secretion and basal acid secretion. Inhibition of prostaglandins synthesis probably weakens the gastric mucosal defence to resist luminal irritants.

(1) COX-dependent effects. When cell injury or receptor activation occurs, arachidonic acid is released from membrane-bound phospholipids by the enzyme phospholipase A_2. In the following step, prostaglandin (PG) G/H synthase, a dual-role enzyme with cyclooxygenase (COX) and endoperoxidase activities, metabolizes arachidonic acid into the unstable PGs, G2 and H_2. PGH2 is transformed into stable prostanoids, such as PGE2, prostacyclin (PGI2), thromboxane (TX)A_2, PGD2 and PGF2α by tissue- and cell-specific isomerases and synthases. These lipid mediators activate specific cell-membrane receptors and play prominent roles in cellular functions.

Two COX isoforms have been identified and characterized: COX-1 and COX-2. The structures of the two molecules are very similar, each consisting of a long, narrow hydrophobic channel with a hairpin bend at the end. A single amino acid difference between the two isoforms, an isoleucine at position 523 in COX-1 and a valine residue in COX-2, proved to be critical in allowing the design of selective COX-2 inhibitory drugs. The smaller valine residue in COX-2 leaves a gap in the wall of the hydrophobic channel and thus gives access for selective drugs to bind to a site pocket.

Although homologous in protein structure and enzymatic activity, COX-1 and COX-2 are induced differently, and their expression pattern varies throughout the body tissue. The classical theory of COX expression is that COX-1 is expressed constitutively in most tissues and cell types, and that COX-2 expression is low or undetectable in most cells and is induced in inflammation. More recent studies have demonstrated that, despite having the characteristics of a 'housekeeping' protein, COX-1 is present together with COX-2 at sites of

inflammation in synovial tissue, its expression may also be regulated and COX-2 may be expressed constitutively.[56, 57] Thus, the classical theory, which distinguishes COX-1 as a constitutive enzyme and COX-2 as an inducible enzyme, no longer reflects the biological reality.

Among other tissues and cell types, constitutive COX-1 expression was shown in the gastrointestinal tract, platelets and endothelial cells, and in certain kidney tissues (renal medullary collecting ducts and interstitium). This isoform plays an essential role in homeostatic processes, such as platelet aggregation, gastroprotection and sodium and water balance. COX-2 is present under basal conditions in brain and kidney (macula densa, cortical thick ascending limb of the loop of Henle and medullary interstitium) (Figure 3).

Up-regulation of COX-2 expression by cytokines [interleukin (IL)-1β, IL-8, IL-18], TNF-α, interferon (IFN)-γ, endotoxins and growth factors at the inflammatory sites and in cancer indicates that COX-2 plays a role in inflammation and carcinogenesis.

The role of the COX-1 inhibition as the main cause of gastric mucosal lesions has been reconsidered recently in the light of the observation that disruption of the mouse PG synthase 1 gene (PTGS1), which encodes for COX-1, does not cause spontaneous development of gastrointestinal ulcers in homozygous mutant mice. Homozygous PTGS1 show less indomethacin-induced gastric ulceration than animals with an intact COX-1 gene. Although that the lack of gastric damage in COX-1 knock-out mice may be attributable to compensatory changes in mucosal defence in response to the reduced PG synthesis, the possibility that reduced COX-1 activity alone is not sufficient for mucosal lesions formation cannot be ruled out. On the other hand, the disruption of the mouse PG synthase 2 genes that encodes for COX-2 does not cause innate gastrointestinal pathology or development of spontaneous gastrointestinal ulcers. Many studies have also suggested that COX-2 can contribute to mucosal defence, at least in some circumstances. It has been suggested that COX-2 expressed in the stomach colonized by *H. pylori* may play a role in protecting the stomach against damage associated with the infection; furthermore, a role of COX-2 derived PGs in gastric ulcer healing is supported by studies in experimental models.

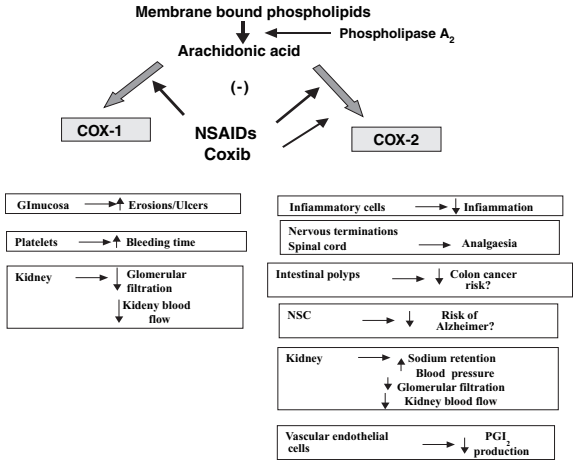

Figure 3. Pharmacological effects of COX-1 and COX-2 inhibition by nonselective NSAIDs and selective inhibitors of COX_2 (coxib)GI, GI, PGI_2, prostacyclin.

Thus, it is possible that inhibition of both COX-1 and COX-2 contributes to generation of erosions or ulcers. This hypothesis is supported by the observation that in rats, inhibition of both COX-1 and COX-2 is required to development of gastric erosions and that neither a selective COX-1 inhibitor nor a COX-2 inhibitor caused gastroduodenal damage when administered at doses that were proven to be effective in selectively inhibiting the target enzyme *in vivo*.

Despite the fact that suppression of PG synthesis is considered the major component of the mechanism underlying the gastric ulcerogenity of NSAIDs, results of recent studies show that there are distinct mechanisms through which inhibition of COX-1/COX-2 could contribute to erosions formation. For example, it has been suggested that the NSAID-induced adherence of neutrophils to the vascular endothelium within the gastric microcirculation contributes to the generation of mucosal injury. Interestingly, the selective COX-2 inhibitor, celecoxib, elicited significant leucocyte adherence in mesenteric venulas, perhaps by inhibiting prostacyclin synthesis. A decrease in gastric blood flow after NSAID administration has been documented in animals and humans, and has been suggested to contribute significantly to the pathogenesis of mucosal injury. The demonstration that the selective COX-1 inhibition produces a decrease in gastric blood flow in the rats suggests strongly that the effect of NSAIDs is due to suppression of COX-1.

In summary, it seems possible that inhibition of both COX isoforms is required for NSAID-induced damage to develop. COX-1 inhibition results in reduced gastric blood flow, whereas COX-2 inhibition leads to increased leucocyte adherence to the vascular endothelium.

In parallel with prostaglandins, the leukotrienes have been highlighted as another product of the COX pathway. These vasoconstrictive and pro-inflammatory molecules could contribute to mucosal injury by promoting tissue ischaemia and inflammation. Prostaglandins and leukotrienes share the same precursor, arachidonic acid, and the inhibition of COX isoenzymes may result in a diversion of arachidonic metabolism to the alternate 5-lipo-oxygenase pathway, resulting in leukotriene production. There is evidence that leukotriene levels are increased in the gastric mucosa of NSAID-treated animals, and that exposure of pre-stimulated gastric epithelial cells to NSAIDs results in concentration-dependent release of leukotriene-B4.

(2) COX-independent effects. Neutrophil-mediated injury. Results from animal studies show strong evidence that leucocytes play an essential role in gastrointestinal damage. It has been suggested that leucocytes cause NSAID-induced damage by two major pathways. First, NSAID-induced accumulation (neutrophil margination) and NSAID-increased adherence of neutrophils to vascular endothelium (white thrombi) may critically reduce blood flow to the mucosa, predisposing it to injury. Secondly, activation of neutrophils can lead to the release of reactive oxygen metabolites and proteases damaging the microvascular endothelium.[58] It has been observed that: (i) NSAIDs administration increases the gastric expression of molecule adhesion involved in PMN adherence to the gastric endothelium. Expression of the adesion molecules involved in leucocyte margination is induced by cytokines (TNF-α, IL-1, IL-2, IL-4, IFN), by molecules having chemotactic action (C5a, platelet-activating factor, LT B_4), and by chemokines [IL-8, monocyte chemoattractant protein (MCP)-1, regulated upon activation normal T cell-expressed and secreted (RANTES) and other molecules]. The mechanisms through which NSAIDs induce neutrophil adhesion to endothelial cells are still not fully understood. Beside their ability to increase TNF-α levels in rats[59] a role of NSAIDs in the inhbition of nuclear translocation of NF-κB has been suggested.[60] This transcription factor modulates the expression of several adhesion molecules-1 (ICAM-1) and P-selectin as well as production of pro-inflammatory cytokines and chemokines.[61, 62] (ii) Pretreating rats with specific TNF-α synthesis inhibitors (glucocorticoids, oxpentifylline (pentoxifylline), lisofylline and thalidomide) or selective anti-TNF-α receptor monoclonal antibodies prevents gastric mucosal damage in NSAID-treated rats without interfering with PG metabolism.[63] (iii) In the murine model, neutrophil depletion and impairment of leucocyte recruitment [resulting from targeted disruption of fucosyltransferase VII (FT-VII/–)] resulted in a reduction of more than 50% in NSAID-induced injury. Leucocyte activation was required for NSAID-induced damage because the $gp91^{phox\ -/-}$ mice were less susceptible to NSAID injury than wild-type mice. (iv) Antibody blockade of CD18-, ICAM-1, E- and P-selectin reduces indomethacin-induced GI injury in rat and rabbit models and exogenous prostaglandins, NO, anti-TNF-α, antibody and leukotriene inhibitors block neutrophil adherence and alleviate the severity of NSAID damage in animal studies.[59, 64, 65] Unlike acute ulcers in animals, NSAID gastropathy in human beings is characterized by a lack of inflammatory cells unless *H. pylori* infection is present. Whether neutrophils initiate NSAID injury in humans is still unknown.

OTHER FACTORS INFLUENCING GASTROINTESTINAL DAMAGE

Impaired platelet function

It seems likely that the ability of NSAIDs to impair haemostasis by inhibiting platelet cyclo-oxygenase may play a role when patients present with bleeding ulcers, particularly (in view of its particular effects on haemostasis) when aspirin is used. Inhibition of TXA_2-dependent platelet function by aspirin may lead to prevention of thrombosis as well as to excess bleeding. The anti-thrombotic effect of aspirin is constant from 30 to 1300 mg, in keeping with the saturability of platelet COX-1 inhibition by aspirin at very low doses; in contrast the gastrointestinal toxicity of the drug appears to be consistent with dose and dosing-interval-dependent inhibition of COX-1 activity in the gastrointestinal mucosal cells. Thus, in theory, inhibition of platelet aggregation might to contribute marginally to ulcer bleeding. Human studies showed a doubling of acute biopsy-induced bleeding in patients taking low-dose aspirin, whereas the bleeding rate per erosion showed a significant dose-related increase with 600 mg of aspirin four times a day, suggesting that an increase in bleeding time itself is not a major contributor to bleeding.

Impaired mucosal adaptation and healing

After acute dose of aspirin, the gastric damage is much more widespread than that observed after several days

or weeks, suggesting that the mucosa possesses adaptative mechanisms that compensate for NSAID injury. This rapid repair is a complex process requiring stimulation by a range of cytokines and growth factors, including transforming growth factors (TGF)-β, epidermal growth factor receptor ligands, basic fibroblastic growth factor, human growth factor, IL-1β, IL-2, IFN-γ, adenosine and trefoil peptides.[66] The contribution of cyclo-oxygenase products is less certain. In contrast, human growth factor has been shown to enhance COX-2 expression in epithelial monolayers, resulting in enhancement of wound repair.[67] The failure of these healing mechanisms is operative in a small subset of individual exposed to NSAIDs. In these cases wounds (erosions and ulcers) develop that disrupt the basement membrane, thus predisposing to significant clinical events. NSAIDs impair cell replication and induce apoptosis, and COX-2 selective inhibitors reduce wound repair as it is by non-selective NSAIDs. The implication of these effects is that suppression of either healing or the physiological response to injury may be at least as important as the injury itself.

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