

Gastrointestinal safety of novel nonsteroidal antiinflammatory drugs: selective COX-2 inhibitors and beyond

Gabriella Coruzzi, Nicola Venturi, Silvana Spaggiari

Department of Human Anatomy, Pharmacology and Forensic Medicine, Section of Pharmacology, University of Parma, Parma, Italy

Abstract. Nonsteroidal antiinflammatory drugs (NSAIDs) are frequently associated with adverse reactions, related to inhibition of cyclooxygenase (COX) in tissues where prostaglandins exert physiological effects, such as gastric mucosal defense and renal homeostasis. The discovery of two COX isoforms, namely COX-1 constitutively expressed in most tissues and COX-2 induced at sites of inflammation, led to the development of selective COX-2 inhibitors (“coxibs”), with the hope of significantly reducing the gastrointestinal toxicity associated with acute and chronic NSAID use. However, the increased knowledge of physiological roles of COX-2 enzyme in a variety of tissues, including stomach and kidney, together with the withdrawal from the market of rofecoxib and valdecoxib because of cardiovascular toxicity, have challenged the benefits of selective COX-2 inhibition. As a consequence, the interest for novel approaches has re-emerged; new therapeutic options, still under clinical evaluation, are represented by dual COX and 5-lipoxygenase (5-LOX) inhibitors, synthetic lipoxins, nitric oxide (NO)-releasing NSAIDs and, more recently, by NSAIDs releasing hydrogen sulphide (H₂S). This review focuses upon the gastrointestinal (GI) safety of selective COX-2 inhibitors and of novel therapeutic strategies, in comparison with traditional NSAIDs. (www.actabiomedica.it)

Key words: Nonsteroidal antiinflammatory drugs, cyclooxygenase, lipoxygenase, nitric oxide, lipoxins, gastrointestinal damage

Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs) are among the most widely used drugs worldwide and represent a mainstay in the therapy of acute and chronic pain. However, their use is frequently associated with a broad spectrum of adverse effects, which are related to the inhibition of prostaglandin (PG) synthesis in tissues where PGs are responsible for physiological homeostasis (1-3). In the early 1990s two structurally related isoforms of cyclooxygenase (COX) have been identified, namely COX-1, constitutively expressed in most mammalian tissues, and COX-2, which is usually undetectable under resting conditions and is rapidly induced at sites of inflammation in re-

sponse to noxious stimuli (4). This has led to the theory that COX-1 isoenzyme produced PGs which exert house-keeping functions, including gastric mucosal defense and renal homeostasis, whereas COX-2 synthesizes detrimental PGs which are responsible for inflammation and pain (5). As a consequence, considerable resources have been invested by pharmaceutical companies to develop highly selective COX-2 inhibitors, with the hope of an improved tolerability profile. Due to the great expectation, these drugs, also referred to as “coxibs”, were rapidly introduced in the market and gained an impressive success (6-10). In recent years, however, animal data have challenged the initial paradigm, unravelling the constitutive expression of COX-2 in normal tissues, together with new

physiologic roles of this isoenzyme, including gastric mucosal defense, renal homeostasis and endothelial PGI₂ production (for review see 11-15). Furthermore, serious cardiovascular effects of some selective COX-2 inhibitors emerged from clinical studies and pharmacosurveillance, forcing the drug companies to withdraw from the market rofecoxib and, soon afterwards, valdecoxib (16-18). Although clinical trials gave conflicting results, partly due to the influence of pharmaceutical manufacturers (19), pharmacological evidence seems to support the concept that cardiovascular toxicity of selective COX-2 inhibitors may be a class effect (20). This has raised serious concerns about the risk of thrombotic events during treatment with coxibs, marking off the therapeutic benefits that could be expected from COX-2 selective inhibition and questioning the need of more selective compounds (21-22).

Following the withdrawal of rofecoxib, which has been considered the most serious disaster after talidomide, the search for safe NSAIDs has found a renewed interest and novel strategies have emerged to improve the therapeutic efficacy and tolerability of these drugs. The rationale underlying the development of dual inhibitors of COX and 5-lipoxygenase (5-LOX) was based on both the proinflammatory activity of leukotrienes (LTs) (Fig. 1) and their deleterious effects in the gastric mucosa; furthermore, these compounds appear to be the major arachidonate products of the gastric mucosa under COX inhibition (23, 24). As such, dual COX/5-LOX inhibitors should theoretically display enhanced antiinflammatory effects and improved gastric tolerability (25-27). Recently, great interest has emerged for lipoxins, which can be considered as counter-regulatory arachidonic acid

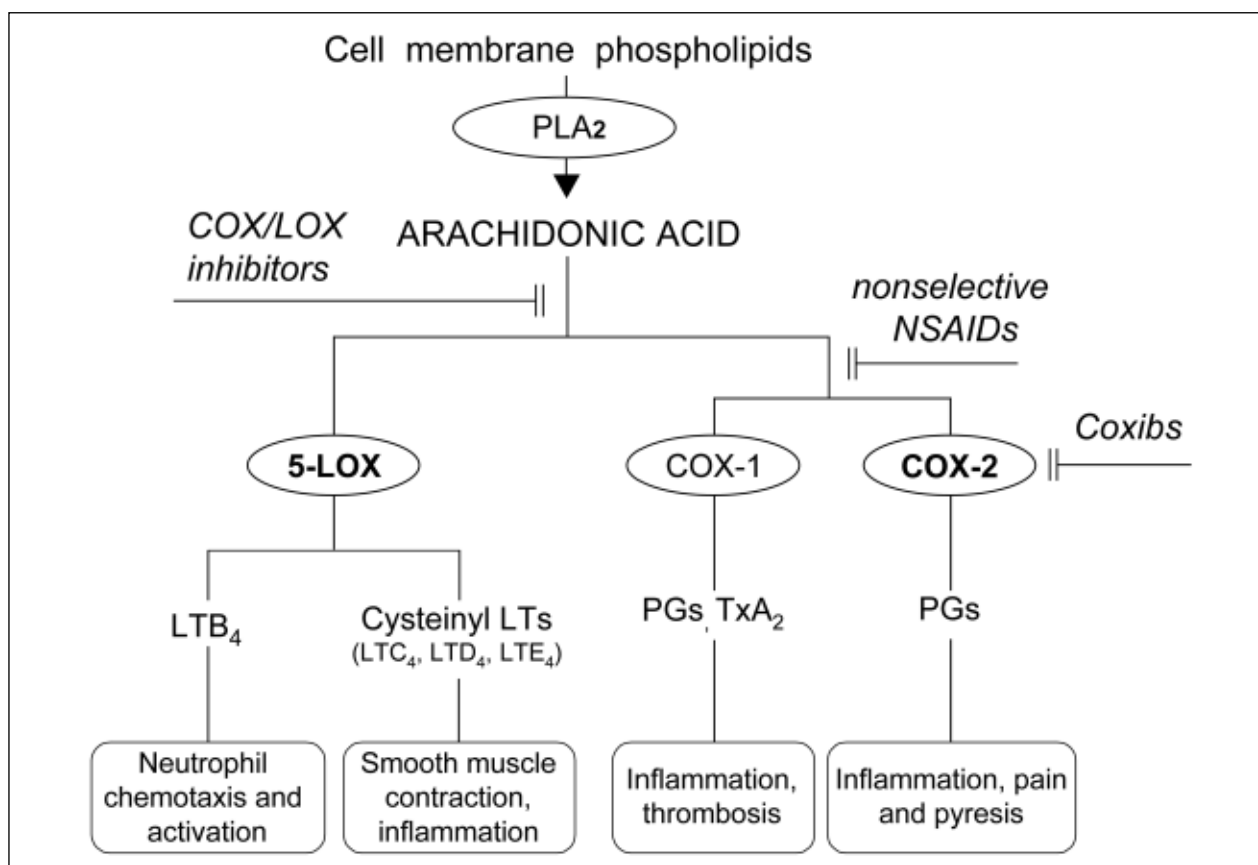


Figure 1. General scheme representing the main metabolic pathways leading to arachidonic acid products involved in the inflammatory process. Targets of antiinflammatory drugs are also shown. COX = cyclooxygenase; 5-LOX = 5-lipoxygenase; LTs = leukotrienes; PGs = prostaglandins; PLA2 = phospholipase A2; TxA₂ = thromboxane A₂.

products, responsible for the resolution of inflammation (28) (Fig. 1) and for gastroprotection (29).

An alternative strategy to limit the risk of GI damage induced by NSAIDs is to enhance the protective mechanisms of the gastric mucosa. This can be pursued by association of conventional NSAIDs with antisecretory and/or protective drugs (30) or by generating hybrid NSAID molecules, chemically-modified with groups capable of releasing protective mediators. The increased knowledge of the key role of nitric oxide (NO) in gastric mucosal defense (31-33) has generated NO-donor NSAIDs (34-36), also known as COX-inhibiting NO donors (CINODs) (37). However, despite excellent pharmacological premises based on experimental animals, the development of these new NSAIDs has suffered a delay in clinical research and, only recently, progress in this area has restarted.

A further development in the field of gastroprotective NSAIDs has come from the discovery of the gastroprotective actions of hydrogen sulphide (H₂S), a new endogenous gaseous mediator which is constitutively produced by the gastric mucosa (38). This has led to the synthesis of chemically-modified H₂S-NSAIDs which are able to release H₂S in the stomach, thus preventing gastric injury associated with COX inhibition (39).

In this review we focus upon the GI safety of a) selective COX-2 inhibitors, b) drugs interfering with the biosynthesis of PGs and LTs, c) lipoxins and d) hybrid NSAIDs endowed with protective activity, including NO- and H₂S-donor NSAIDs, in comparison with conventional NSAIDs.

Selective COX-2 inhibitors (“coxibs”)

As previously said, the rationale for developing selective COX-2 inhibitors was the concept that selective inhibition of COX-2 isoenzyme may induce antiinflammatory and analgesic effects comparable to nonselective COX inhibitors, with considerably less damage to the gastric mucosa. However, over the recent years, the knowledge on the location and function of COX isoform in resting and pathological conditions has greatly expanded (12, 13, 40, 41); this enzyme was found constitutively expressed in a variety of

Table 1. Proposed beneficial roles of COX-2 enzyme

CNS	Brain development and neuronal homeostasis
Heart	Myocardial protection
Vessels	PGI ₂ production, endothelial protection
Airways	Protection against allergens
Kidney	Development, salt and water homeostasis, renin synthesis, regulation of blood flow
Stomach	Ulcer healing
Intestine	Mucosal homeostasis
Bone	Bone formation and fracture healing
Reproductive system	<i>Female</i> Ovulation, implantation, parturition <i>Male</i> Erection (?)

tissues, including brain, kidney, vascular endothelium, reproductive system; moreover, novel physiologic and protective functions of COX-2 enzyme have been unravelled, challenging the concept that selective COX-2 inhibition would not impair tissue homeostasis (40). A list of COX-2-mediated roles in the regulation of tissue function is reported in Table 1. Particular concerns have emerged from the serious cardiovascular effects of rofecoxib; the cardiovascular toxicity of coxibs is outside the scope of this review and the reader is referred to excellent editorials on this topic (19, 42-44) and to pharmacosurveillance database (www.farmacovigilanza.org).

Physicochemical properties of coxibs

Several compounds endowed with high selectivity for COX-2 enzyme were synthesized and up to 6 drugs entered the market worldwide (Table 2). These compounds differ for their chemical structure: celecoxib, valdecoxib and parecoxib (a water-soluble pro-drug of valdecoxib) are sulphonamides; rofecoxib and etoricoxib are methylsulphones, whereas lumiracoxib is a phenylacetic acid derivative (Fig. 2).

Differences in chemical structures and physicochemical properties of the available COX-2 inhibitors may be of interest in the choice of drugs in different clinical settings (for review see 45). Sulphonami-

Table 2. Selective COX-2 inhibitors

Drug	Brand name and Producer		
Celecoxib	Artilog	Pharmacia Italia	
	Artrid	Sefarma	
	Celebrex	Pharmacia Italia	
	Solexa	Pfizer	
Rofecoxib	Vioxx*	Merck Sharpe & Dohme	withdrawn from the market (September 30, 2004)
Valdecoxib	Bextra*	Pfizer	withdrawn from the market (April 7, 2005)
Parecoxib	Dynastat	Pfizer	prodrug of valdecoxib; injectable formulation
Etoricoxib	Algix	Ist. Gentili	
	Arcoxia	Merck Sharpe & Dohme	
	Tauxib	Addenda Pharma (Sigma-Tau)	
Lumiracoxib	Prexige	Novartis	not marketed in Italy

* For details see the website www.farmacovigilanza.org

des, such as celecoxib, valdecoxib and parecoxib, may have the potential risk of allergic reactions (12); moreover, differences in the molecule acidity may contribute to the drug tolerability profile, by altering the direct irritant effect on the gastric mucosa; only lumiracoxib is a phenylacetic derivative, whereas all the other compounds have the carboxylic group protected

and should be theoretically less damaging than lumiracoxib and other acidic NSAIDs (46). A recent *in vitro* study (47) has suggested that coxibs with sulphone moiety, namely rofecoxib and etoricoxib, have pro-oxidant properties, unrelated to their COX activity; this may be relevant to the cardiovascular safety profile.

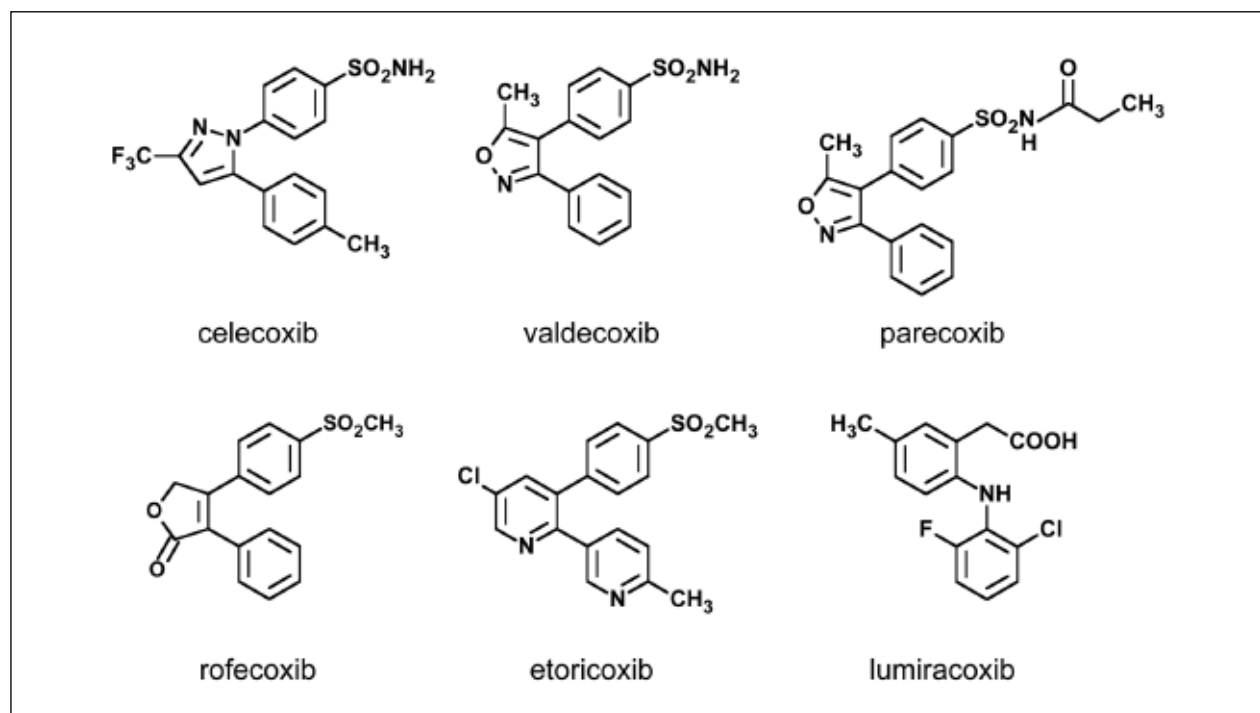


Figure 2. Chemical structures of selective COX-2 inhibitors.

Table 3. Pharmacological differences among selective COX-2 inhibitors

Compound	COX-1:COX-2 IC ₅₀ * ratio	t _{1/2} (h)	Metabolic pathway
<i>First generation</i>			
Celecoxib	6 ⁴⁹	6-12	2C9, 3A4
Rofecoxib	38 ⁵⁰	15-18	Reductase
<i>Second generation</i>			
Valdecoxib	28 ⁵¹	6-10	2C9, 3A4
Etoricoxib	105 ⁵²	20-26	3A4(2C9, 2D6, 1A2) 3A4 (2C9, 2D6, 2C9 (2C8, 2C19)
Lumiracoxib	515 ⁵³	2-6	

* the concentration required to inhibit COX activity by 50%, assessed using the human whole blood assay

Whereas all coxibs are lipophilic and reach high concentrations in the brain, the acidic nature of lumiracoxib accounts for its high distribution in blood, kidney, liver and inflamed tissue, but low in the CNS. Coxibs are slowly eliminated by liver metabolism, involving different isoforms of cytochrome P450 system, such as CYP3A, CYP2C9, CYP2D6 and CYP1A2 (Table 3); rofecoxib undergoes first step metabolism by cytoplasmic reductases, thus being potentially less susceptible to drug-drug interactions. However, all compounds appear to interfere with the metabolism of other drugs; in particular, celecoxib has recently been shown to inhibit the metabolism of the CYP2D6 substrates metoprolol, sedatives, SSRIs, antidepressants and some antiarrhythmic drugs (45, 48).

COX-2 selectivity

All coxibs selectively block COX-2 isoenzyme, although with different COX-1/COX-2 selectivity ratios, being lumiracoxib the most selective for COX-2 enzyme (49-54) (Table 3). The re-evaluation of COX-selectivity of conventional NSAIDs have shown that some compounds, including diclofenac, nimesulide and nabumetone, display the same selectivity as that of celecoxib (1, 20). It must be considered, however, that the selectivity ratio for different COX inhibitors is highly variable, depending on the assay and experimental conditions used; furthermore, differences in selectivity observed in isolated assays may not correlate with therapeutic efficacy after dosing; in line with this, a recent study has shown that plasma concentrations following therapeutic doses of rofecoxib (25-50

mg) and etoricoxib (60 mg) are proper to inhibit more than 80% COX-2 activity; celecoxib (100-200 mg) and valdecoxib (10 mg) plasma concentrations are two- to four-fold lower, whereas those of lumiracoxib (400 mg) are 30-fold higher than those necessary to inhibit by 80% COX-2 (20). This may explain the clinical efficacy of 400 mg lumiracoxib when administered once daily, despite its short half life (Table 3).

Gastric effects of coxibs

Several clinical studies have confirmed that selective COX-2 inhibitors are associated with less gastroduodenal damage and risk of GI bleeding, in comparison with conventional NSAIDs, by sparing COX-1 enzyme in the gastric mucosa and in platelets (for review see 55). However, in recent years the classical concept that only COX-1 is physiologically important to maintain gastric mucosal integrity has been challenged and considered an oversimplification of a more complex picture. Although COX-1 is the predominant isoenzyme in normal gastric mucosa, there is increasing evidence that COX-2 mRNA and protein are either constitutive or inducible in specific areas of the stomach of animals and humans (9, 56-60). One of the first observations suggesting that COX-2 has a role in gastric mucosal defense came from experiments in COX-1-deficient mice (61). In these animals, while no evidence was found of spontaneous gastric injury, despite the absence of COX-1-derived PGs, the administration of NSAIDs induced gastric damage, invariably related to COX-2 inhibition. In accordance, Wallace et al. (62) demonstrated that in the rat

stomach the inhibition of both COX-1 and COX-2 is required for the development of gastric lesions; this was based on the observation that neither a COX-1 inhibitor nor a COX-2 inhibitor caused gastric damage when given alone at doses that selectively inhibit the target enzyme *in vivo*; however, the combination of both inhibitors invariably resulted in gastric erosion development. COX-2 levels were found to be elevated in ulcerated mucosa (63, 64) and following a variety of stimuli, such as mild irritants (65), ischemia-reperfusion (66), acid instillation (67), lansoprazole-induced hypergastrinemia (68), *Helicobacter pylori* infection (63, 69), aspirin (65, 70) and cold-induced stress (71). In the gastric mucosa COX-2 immunoreactivity is increased in the region of maximal repair activity at the ulcer margin (55, 57, 71), suggesting that COX-2 inducible enzyme may act as a second line of defense for ulcer healing and repair. In line with this, a variety of studies in rodents have shown that selective COX-2 inhibitors, similarly to traditional NSAIDs, delayed gastric ulcer healing (56, 67, 72-79). On the light of these data, it is reasonable to hypothesize that COX-2 enzyme does not play a major role in mucosal defense under resting conditions, but seems to gain importance in face of pending injury, by assisting COX-1 in safeguarding gastric mucosal integrity (15, 23, 66). As a consequence, the gastric damaging effect of

coxibs is absent in healthy gastric mucosa, becoming evident when gastric mucosal defence is impaired (Fig. 3). In accordance to this concept and in line with animal studies, human studies have confirmed a better GI profile of coxibs (7, 55); however, selective COX-2 inhibitors lose their benefits in patients assuming low-dose aspirin and run the same risk of GI bleeding, as conventional NSAIDs (80-83); thus, in these patients or in patients at risk, a protection with proton pump inhibitors is mandatory, as in the case of standard NSAIDs.

Intestinal effects of coxibs

Until recently the tolerability profile of NSAIDs has focused on the deleterious effects on the upper GI tract; however, it has been clearly documented that chronic ingestion of NSAIDs is a risk factor for a number of adverse effects to the bowel, including bleeding, perforation, ulceration, strictures and obstruction (for review see 84-86). Moreover, it is widely recognized that NSAIDs aggravate both ulcerative colitis and Crohn's Disease (85, 86).

Conversely from the stomach, the role of cyclooxygenase inhibition in NSAID-induced enteropathy is debated (87). Among pathogenetic factors, local epithelial injury, barrier dysfunction and mucosal

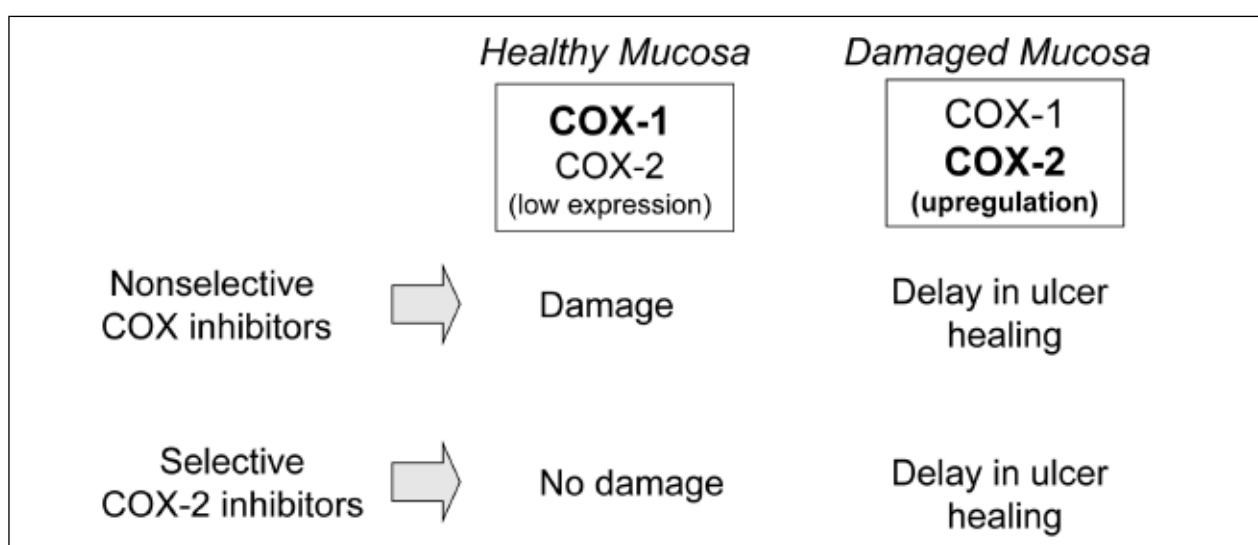


Figure 3. Gastric effects of nonselective and COX-2 selective NSAIDs in normal or damaged gastric mucosa. The different effects of nonselective or COX-2 selective inhibition are explained by different tissue expression and roles of COX isoenzymes.

invasion of enteric bacteria have been considered of primary importance; moreover, a key role has been attributed to the entero-hepatic recirculation of NSAID molecule (84, 88). As expected, the discovery of two COX isoforms have led to reassessment of the role of PG synthesis inhibition in NSAID-induced intestinal damage. In contrast to early data showing undetectable levels of COX-2 expression in the intestine (58), more recent findings have found constitutive COX-2 in enteric neurones and in smooth muscle of mouse and human colon (89); COX-2 amounts are significantly elevated in pathological conditions, including animal models of colitis (88), postoperative ileus (90, 91) and in human inflammatory bowel disease (IBD) (92). Mice lacking either COX-1 or COX-2 enzyme do not develop spontaneous intestinal inflammation (61); furthermore, only the combined administration of COX-1 and COX-2 inhibitors induced jejunal lesions, suggesting that both isoforms contribute to maintenance of small bowel integrity (87, 93, 94). This led to the concept that, similarly to the stomach (62), COX-2 enzyme has no role in intestinal homeostasis, but it may assist COX-1 in protecting the mucosa against damaging stimuli. A recent study from our laboratory carried out in rats showed that selective COX-1 or COX-2 inhibitors or the combination of both inhibitors were unable to damage the small intestine, suggesting that COX-independent mechanisms are relevant to the development of NSAID enteropathy; interestingly, also the nonselective NSAID ibuprofen was ineffective, whereas intestinal damage was observed following administration of indomethacin (95).

Controversial data concerning the intestinal effects of selective COX-2 inhibitors do not allow to conclude whether increased COX-2 expression in damaged mucosa has to be considered pathogenetic or protective (for review see 14, 85, 86). In animal experiments either induction (96) or lack (87, 93, 97, 98) of damage were observed following selective COX-2 inhibition. Likewise, coxibs either aggravated (99-101) or protected (102, 103) from experimental colitis induced by trinitrobenzenesulphonic acid (TNBS), a widely used animal model of human IBD. There have been a number of studies in humans indicating that selective COX-2 inhibitors exacerbate symptoms of

IBD in the same way as nonselective NSAIDs or reactivate it when taken by patients in remission (104, 105); on the other hand, in a recent meta-analysis (sponsored by Pfizer) coxibs were associated with lower intestinal injury compared with nonselective NSAIDs (106). Until further information is available, the use of coxibs in IBD patients should require careful consideration, as with standard NSAIDs.

A recent study in the isolated human colon showed that both COX-1 and COX-2 are involved in the negative modulation of cholinergic excitatory control of colonic motor activity, at pre- and postjunctional level, respectively (89). However, in healthy humans neither celecoxib nor rofecoxib did modify gastric emptying or intestinal transit (107).

In conclusion, the promise of a better safety profile of selective COX-2 inhibitors has been only partially fulfilled: a better GI tolerability, which occurs under some, but not all, circumstances must be balanced with a comparable renal toxicity as nonselective NSAIDs and, what's more, with a serious cardiovascular toxicity.

Dual COX/5-LOX inhibitors

In recent years it has been clarified that several mediators of the arachidonic acid metabolism are involved in the inflammatory process. Leukotrienes (LTs), which are the second main family of arachidonate products, are synthesized from the activity of 5-lipoxygenase (5-LOX) and have a major role in the inflammatory response (Fig. 1). LTs are extremely potent vasoactive and leucotactic compounds, that are in some respects more inflammogenic than PGs. LTB₄, in particular, induces recruitment of leukocytes to inflamed sites, lysosomal release in neutrophils, adhesion molecule expression and subsequent plasma leakage (23, 27, 108). This finding has suggested that dual inhibition of both LTs and PGs may lead to enhanced and wider antiinflammatory activity. Moreover, it can also be expected that combined COX and LOX inhibition may originate an improved GI safety profile, due to a number of adverse effects of LTs in the GI mucosa, which impair mucosal integrity and exacerbate the damaging effect of noxious stimu-

li (23, 109). In particular, reduction of mucosal blood flow, leukocyte-endothelial cell interaction and leukocyte infiltration are considered a prerequisite for NSAID-induced gastropathy (1). In line with this, several studies have demonstrated that 5-LOX inhibitors or LT receptor antagonists exert protective effects on acute and chronic gastric mucosal damage in various ulcer models, including NSAID-induced gastric lesions (23, 109, 110). These observations, along with the possibility that COX inhibition by NSAIDs can divert arachidonate to lipoxygenase pathway, led to the theory that excess LT production, combined with PG deficit, could contribute to NSAID-induced mucosal damage (111). In line with this, elevated production of LTB_4 by the human stomach has been documented in patients taking NSAIDs (112).

One compound in the 5-LOX/COX series of NSAIDs is licofelone (previously named ML3000), which in animal experiments and in clinical trials showed antiinflammatory effects comparable to conventional NSAIDs, but with an improved GI safety profile (113).

In conclusion, results with dual COX/5-LOX inhibitors seem to be promising; however, although the dual inhibition concept appears a rather logical approach, the lesson from COX-2 inhibitors demand to be cautious before drawing definite conclusions about the pharmacological profile of a new class of drugs. Large clinical trials will establish in the future whether theoretical expectations on safety and efficacy of these drugs are achieved.

Lipoxins, aspirin-triggered lipoxin and synthetic analogs

Lipoxin A_4 (LXA_4) and lipoxin B_4 (LXB_4) were first identified in 1984 by Serhan and colleagues (114) as 5- and 15-lipoxygenase interaction products of activated leukocytes (Fig. 4). It subsequently emerged that lipoxins are products of cellular co-operation (transcellular biosynthesis) that provide counter-regulatory signals during the inflammatory process, ultimately leading to resolution of inflammation (28, 115). Lipoxins have been found in tissues of patients with various immuno-inflammatory states, including asthma, rheumatoid arthritis, pneumonia and sarcoi-

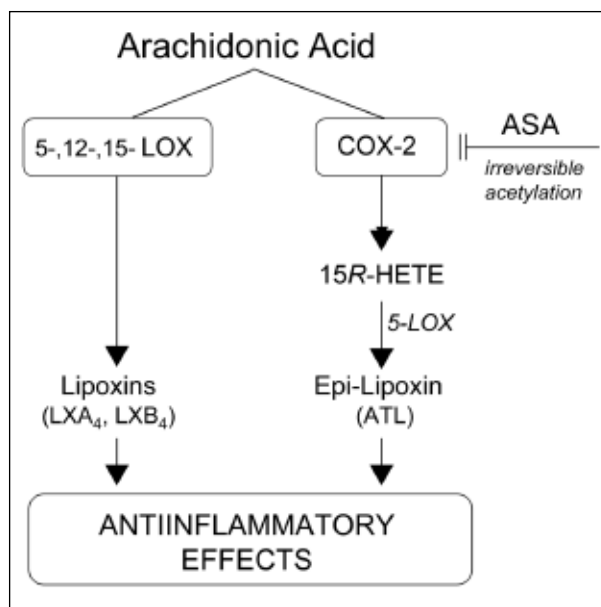


Figure 4. Synthesis of antiinflammatory lipoxins (LXs) from arachidonic acid. Irreversible acetylation of COX-2 enzyme by aspirin leads to the formation of a lipoxin epimer (Epi-lipoxin), also known as aspirin-triggered lipoxin (ATL) endowed with antiinflammatory properties. 15R-HETE = 15R-hydroxyeicosatetraenoic acid; LOX = lipoxygenase.

dosis. Lipoxin-induced effects are listed in Table 4. They exert potent antiinflammatory actions, acting as braking signals to limit neutrophil chemotaxis, release of proinflammatory cytokines and adhesion molecule expression (for review see 28, 115). Interestingly, the irreversible acetylation of COX-2 (Ser 516) by aspirin still allows the enzyme to retain a certain enzymatic activity; as a consequence, 15-hydroxy-5,8,11,13-eicosatetraenoic acid is formed which leads to the transcellular biosynthesis of epi-lipoxin A_4 , an isomer of LXA_4 , also known as aspirin-triggered lipoxin (ATL) (Fig. 4) (114). ATL virtually exerts identical actions to its epi-

Table 4. Biological effects of lipoxins

Vasodilation
Nitric oxide generation
Endothelial prostacyclin generation
Reversal of ET-1 contraction
Inhibition of eosinophil chemotaxis
Inhibition of PMN chemotaxis and degranulation
Downregulation of adhesion molecules

ET-1 = endothelin-1; PMN = polymorphonuclear cells

meric counterpart, LXA₄ (114). In the gastric mucosa LXA₄ and ATL reduced aspirin-induced injury and the associated increase in neutrophil adherence within the gastric microcirculation (115), possibly by the intervention of NO (29). ATL generation is not observed following administration of non-aspirin NSAIDs, such as indomethacin; moreover, it is completely inhibited by co-administration of a selective COX-2 inhibitor. A recent study in healthy volunteers showed that the co-administration of celecoxib, which by itself does not cause gastric damage, significantly increased the endoscopic damage induced by low-dose aspirin for 14 days and suppressed ATL levels in the urine (116). Whether or not lipoxins contribute to mucosal defense outside the context of aspirin administration has yet to be determined. All these observations, however, have provided the impetus to develop synthetic LXA₄, LXB₄ and ATL analogs, characterized by resistance to metabolism, as new approaches for antiinflammatory drugs, devoid of gastrotoxicity (28).

Chemically-modified NSAIDs

A second strategy to limit the GI damage of conventional NSAIDs is the incorporation in the NSAID molecule of chemical moieties releasing gastroprotective substances. This approach has led to the development of nitric oxide (NO)-releasing NSAIDs and, more recently, of NSAIDs releasing hydrogen sulphide (H₂S).

1. NO-releasing NSAIDs (NO-NSAIDs)

It is now recognized that NO, an ubiquitous signalling molecule, has a key role in the maintenance of gastric mucosal integrity (31-33). This has led to the concept (34) that the introduction of a NO-releasing moiety into the molecule of conventional NSAIDs, could overcome the deleterious effects due to PG inhibition (Fig. 5). Over the past ten years, a range of traditional NSAIDs, including aspirin, diclofenac, naproxen, ibuprofen and indomethacin have been coupled to a NO-donating moiety and their actions have been widely explored in several inflammation and GI ulceration models (for review see 35, 117-119).

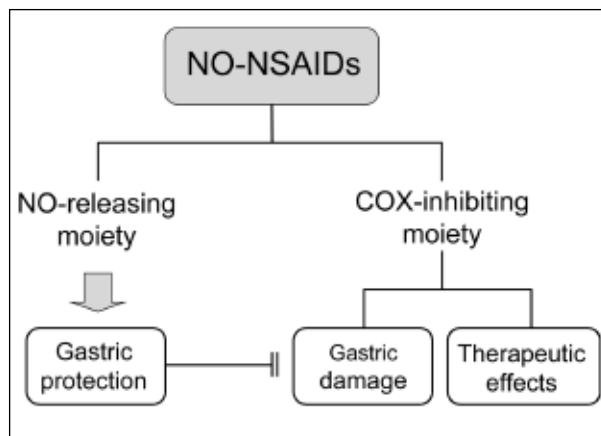


Figura 5. Gastric effects of nitric oxide (NO)-releasing NSAIDs. The gastroprotective effects of NO released from NSAID molecule may counteract the gastric damaging effect related to the COX inhibition.

Gastric and intestinal effects of NO-NSAIDs

The development of NO-releasing NSAIDs was based on the protective effects of NO in the gastric mucosa (31-33). Similarly to PGs, NO was found to increase mucosal defence, by increasing protective factors, such as mucus and bicarbonate secretion, mucosal blood flow, epithelial proliferation and angiogenesis and decreasing aggressive factors, such as acid, leukocyte adherence to the endothelium and cytokine production. In line with this, classical NO donors, such as glyceryl trinitrate, reduced NSAID-induced gastric damage in rats (120, 121) and decreased GI bleeding in patients assuming low-dose aspirin (122). The amount of NO released in the gastric mucosa is a crucial point, since overproduction of NO may cause vasocongestion and cytotoxicity either directly or indirectly, through the generation of the highly toxic metabolite, peroxynitrite (32). Animal data have clearly shown that NO-NSAIDs are able to spare the gastric mucosa, despite reducing PG synthesis as do the parent compounds (123, 124). More interestingly, NO-NSAIDs accelerate ulcer healing, exhibiting a definite better tolerability profile in comparison with selective COX-2 inhibitors (124, 125). Furthermore, studies in arthritic rats have demonstrated that, whereas co-administration of celecoxib or naproxen with doses of ASA resulted in aggravation of gastric damage and

Table 5. Gastric effects of selective COX-2 inhibitors and NO-releasing NSAIDs in different experimental conditions

	Coxibs	NO-NSAIDs
Healthy mucosa	no damage	no damage
Ulcerated mucosa	aggravation	protection
Ulcer healing	delay	acceleration
Aspirin intake	loss of benefit	protection
Mucosal blood flow	decrease	increase

enhanced neutrophil accumulation, the NO-donor naproxen (compound HCT-3012) prevented gastric lesions and neutrophil recruitment into the gastric microcirculation (126).

Clinical studies have supported animal data demonstrating an excellent GI safety profile of NO-NSAIDs, in comparison with parent drugs (127); this holds true for NO-donor derivatives of aspirin (128), or of naproxen (129-131). In conclusion, NO-NSAIDs seem to provide a definitely better gastric profile in different experimental and clinical settings, when compared to selective COX-2 inhibitors (Table 5). Moreover, unlike conventional NO donors, small amounts of NO are released by NO-NSAIDs after drug metabolism and the release is very slow, with no effect on systemic blood pressure (127).

The effects of NO in the intestine are very complex and depend on the amount of NO produced (33). Likewise, conflicting data have been reported concerning the effects of conventional NO donors on experimentally induced colitis models (for review see 14). The effects of NO-releasing NSAIDs on intestinal functions have been less documented; despite some studies have shown that these drugs cause less mucosal damage in rats when acutely or chronically administered (118, 123, 132), further studies are needed to elucidate the effects of NO-NSAIDs in the intestinal mucosa.

Selective COX-2 inhibitors chemically modified with the incorporation of a NO-donating group have been recently synthesized, that retain the therapeutic activity of COX inhibition and the gastroprotective effects of NO (133). In experimental animals these compounds caused minimal gastric toxicity and did not enhance the damaging effect of aspirin; moreover, of particular interest, these drugs should theoretically compensate the cardiovascular risk associated with

COX-2 inhibition, through the NO-dependent inhibition of platelet aggregation and vasodilation (133). The promising results obtained in animals have to be confirmed in clinical trials.

2. *H₂S-donating NSAIDs*

In the recent years it has become clear that small gaseous molecules serve as endogenous mediators in the body; this is the case for NO, carbon oxide (CO) and H₂S (for review see 38). H₂S is produced in several tissues and exerts many physiological functions; in the digestive system, this molecule is constitutively produced in the gastric mucosa from sulphur-containing aminoacids (cysteine) *via* the action of two enzymes, cystathionine β -synthase (CBS) and cystathionine γ -lyase (CSE) (134). Recent studies have shown that H₂S is involved in the maintenance of mucosal integrity and in the regulation of blood flow, acting in concert with NO to protect the gastric mucosa from injury (38). It was therefore hypothesized that deficiency of H₂S synthesis might contribute to the pathogenesis of several GI disorders and, in particular, to NSAID-induced gastropathy. In line with this, the administration of NSAIDs, including aspirin, indomethacin, diclofenac and ketoprofen, resulted in a significant decrease in H₂S synthesis (38). Recently, a H₂S-releasing diclofenac derivative has been shown in rats to possess greatly reduced GI damaging effects as compared to the parent drug, despite a comparable antiinflammatory activity and suppression of PG synthesis (39). This could be an early indication that coupling H₂S-releasing moiety to conventional or COX-2 selective NSAIDs may represent an attractive approach for the development of gastroprotective NSAIDs.

Conclusions

Two different approaches were pursued in the search of GI sparing NSAIDs: a) define novel targets in the complex picture of the inflammatory process or b) modify classical NSAIDs by adding chemical moieties that release gastroprotective mediators. Selective COX-2 inhibitors, although demonstrating

established efficacy in the treatment of inflammation and pain and some advantages in terms of gastric tolerability when compared to traditional NSAIDs, may have similar gastrotoxicity in patients with active ulcer or taking aspirin; moreover, they have similar renal adverse effects and, most importantly, some coxibs are endowed with serious cardiovascular toxicity. Furthermore, the increased knowledge of physiologic roles of COX-2 enzyme in several tissues, makes doubtful the clinical advantage of developing compounds with increased COX-2 selectivity. Thus, these drugs do not represent a significant step forward in the therapeutic tolerability of COX inhibitors. Alternative promising approaches are represented by NO-releasing NSAIDs and dual COX/5-LOX inhibitors; however, these attractive compounds still need a validation in large clinical trials. The advent of new mediators (lipoxins, H₂S) may offer new key targets for safer antiinflammatory drugs.

References

- Whittle BJR. Gastrointestinal effects of nonsteroidal antiinflammatory drugs. *Fundam Clin Pharmacol* 2003; 17: 301-13.
- Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat New Biol* 1971; 231: 232-5.
- Rainsford KD. Profile and mechanisms of gastrointestinal and other side effects of nonsteroidal antiinflammatory drugs (NSAIDs). *Am J Med* 1999; 107: 27S-35S.
- Xie WL, Chipman JG, Robertson DL, Erikson RL, Simmons DL. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc Natl Acad Sci USA* 1991; 88: 2692-6.
- Vane JR, Botting RM. Mechanism of action of nonsteroidal antiinflammatory drugs. *Am J Med* 1998; 104: 2S-8S.
- Hawkey CJ. COX-2 inhibitors. *Lancet* 1999; 353: 307-14.
- Masferrer JL, Zweifel BS, Manning PT, et al. Selective inhibition of inducible cyclooxygenase 2 in vivo is antiinflammatory and nonulcerogenic. *Proc Natl Acad Sci USA* 1994; 91: 3228-32.
- Micklewright R, Lane S, Linley W, McQuade C, Thompson F, Maskrey N. Review article: NSAIDs, gastroprotection and cyclo-oxygenase-II-selective inhibitors. *Aliment Pharmacol Ther* 2003; 17: 321-32.
- Donnelly MT, Hawkey CJ. Review article: COX-II inhibitors—a new generation of safer NSAIDs? *Aliment Pharmacol Ther* 1997; 11: 227-36.
- Crofford LJ, Lipsky PE, Brooks P, Abramson SB, Simon LS, van de Putte LB. Basic biology and clinical application of specific cyclooxygenase-2 inhibitors. *Arthritis Rheum* 2000; 43: 4-13.
- Whittle BJ. COX-1 and COX-2 products in the gut: therapeutic impact of COX-2 inhibitors. *Gut* 2000; 47: 320-5.
- Warner TD, Mitchell JA. Cyclooxygenases: new forms, new inhibitors, and lessons from the clinic. *FASEB J* 2004; 18: 790-804.
- Gudis K, Sakamoto C. The role of cyclooxygenase in gastric mucosal protection. *Dig Dis Sci* 2005; 50: S16-23.
- Coruzzi G, Menozzi A, Dobrilla G. Novel non-steroidal antiinflammatory drugs: what we have learned from animal studies. *Curr Drug Targets Inflamm Allergy* 2004; 3: 43-61.
- Wallace JL. COX-2: a pivotal enzyme in mucosal protection and resolution of inflammation. *Scientific World Journal* 2006; 6: 577-88.
- Bresalier RS, Sandler RS, Quan H, et al. Cardiovascular events associated with rofecoxib in colorectal adenoma chemoprevention trial. *N Engl J Med* 2005; 352: 1092-102.
- Nussmeier NA, Whelton AA, Brown MT, et al. Complications of the COX-2 inhibitors parecoxib and valdecoxib after cardiac surgery. *N Engl J Med* 2005; 352: 1081-91.
- Solomon SD, McMurray JJV, Pfeffer MA, et al. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. *N Engl J Med* 2005; 352: 1071-80.
- Krumholz HM, Ross JS, Presler AH, Egilman DS. What have we learnt from Vioxx? *Br Med J* 2007; 334: 120-3.
- Capone ML, Tacconelli S, Di Francesco L, Sacchetti A, Sciulli MG, Patrignani P. Pharmacodynamic of cyclooxygenase inhibitors in humans. *Prostaglandins Other Lipid Mediator* 2007; 82: 85-94.
- Cairns JA. The coxibs and traditional nonsteroidal antiinflammatory drugs: a current perspective on cardiovascular risks. *Can J Cardiol* 2007; 23: 125-31.
- Spalding WM, Reeves MJ, Whelton A. Thromboembolic cardiovascular risk among arthritis patients using cyclooxygenase-2-selective inhibitor or nonselective cyclooxygenase inhibitor nonsteroidal antiinflammatory drugs. *Am J Ther* 2007; 14: 3-12.
- Atay S, Tarnawski AS, Dubois A. Eicosanoids and the stomach. *Prostaglandins Other Lipid Mediator* 2000; 61: 105-24.
- Celotti F, Laufer S. Antiinflammatory drugs: new multi-target compounds to face an old problem. The dual inhibition concept. *Pharmacol Res* 2001; 43: 429-36.
- Fiorucci S, Meli R, Bucci M, Cirino G. Dual inhibitors of cyclooxygenase and 5-lipoxygenase. A new avenue in antiinflammatory therapy? *Biochem Pharmacol* 2001; 62: 1433-8.
- de Leval X, Julemont F, Delarge J, Pirotte B, Dogné JM. New trends in dual 5-LOX/COX inhibition. *Curr Med Chem* 2002; 9: 941-62.
- Julémont F, Dogné JM, Pirotte B, de Leval X. Recent development in the field of dual COX / 5-LOX inhibitors. *Mini Rev Med Chem* 2004; 4: 633-8.
- Parkinson JF. Lipoxin and synthetic lipoxin analogs: an overview of antiinflammatory functions and new concepts

- in immunomodulation. *Inflamm Allergy Drug Targets* 2006; 5: 91-106.
29. Wallace JL, de Lima OM Jr, Fiorucci S. Lipoxins in gastric mucosal health and disease. *Prostaglandins Leukot Essent Fatty Acids* 2005; 73: 251-5.
 30. Goldstein JL. Challenges in managing NSAID-associated gastrointestinal tract injury. *Digestion* 2004; 69 (Suppl 1): 25-33.
 31. Whittle BJ. Cyclooxygenase and nitric oxide systems in the gut as therapeutic targets for safer antiinflammatory drugs. *Curr Opin Pharmacol* 2004; 4: 538-45.
 32. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991; 43: 109-42.
 33. Wallace JL, Miller MJS. Nitric oxide in mucosal defense: a little goes a long way. *Gastroenterology* 2000; 119: 512-20.
 34. Wallace JL, Cirino G. The development of gastrointestinal-sparing nonsteroidal antiinflammatory drugs. *Trends Pharmacol Sci* 1994; 15: 405-6.
 35. Wallace JL, Del Soldato P. The therapeutic potential of NO-NSAIDs. *Fundam Clin Pharmacol* 2003; 17: 11-20.
 36. Del Soldato P, Sorrentino R, Pinto A. NO-aspirins: a class of new antiinflammatory and antithrombotic agents. *Trends Pharmacol Sci* 1999; 20: 319-23.
 37. Hoogstraate J, Andersson LI, Berge OG, Jonzon B, Ojteg G. COX-inhibiting nitric oxide donors (CINODs) - a new paradigm in the treatment of pain and inflammation. *Inflammopharmacology* 2003; 11: 423-8.
 38. Fiorucci S, Distrutti E, Cirino G, Wallace JL. The emerging roles of hydrogen sulfide in the gastrointestinal tract and liver. *Gastroenterology* 2006; 131: 259-71.
 39. Wallace JL, Caliendo G, Santagada V, Cirino G, Fiorucci S. Gastrointestinal safety and antiinflammatory effects of a hydrogen sulfide-releasing diclofenac derivative in the rat. *Gastroenterology* 2007; 132: 261-71.
 40. Katori M, Majima M. Cyclooxygenase-2: its rich diversity of roles and possible application of its selective inhibitors. *Inflamm Res* 2000; 49: 367-92.
 41. FitzGerald GA. COX-2 and beyond: Approaches to prostaglandin inhibition in human disease. *Nat Rev Drug Discov* 2003; 2: 879-90.
 42. Zarraga IG, Schwarz ER. Coxibs and heart disease: what we have learned and what else we need to know. *J Am Coll Cardiol* 2007; 49: 1-14.
 43. Graham DJ, Campen D, Hui R, et al. Risk of acute myocardial infarction and sudden cardiac death in patients treated with cyclooxygenase 2 selective and nonselective nonsteroidal anti-inflammatory drugs: nested case-control study. *Lancet* 2005; 365: 475-81.
 44. McAdam BF, Catella-Lawson F, Mardini IA, Kapoor S, Lawson JA, FitzGerald GA. Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. *Proc Natl Acad Sci USA* 1999; 96: 272-7.
 45. Hinz B, Brune K. Pain and osteoarthritis: new drugs and mechanisms. *Curr Opin Rheumatol* 2004; 16: 628-33.
 46. Rainsford, KD. The ever-emerging anti-inflammatories. Have there been any real advances? *J Physiol Paris* 2001; 95: 11-9.
 47. Walter MF, Jacob RF, Day CA, Dahlborg R, Weng Y, Mason RP. Sulfone COX-2 inhibitors increase susceptibility of human LDL and plasma to oxidative modification: comparison to sulphonamide COX-2 inhibitors and NSAIDs. *Atherosclerosis* 2004; 177: 235-43.
 48. Savage R. Cyclo-oxygenase-2 inhibitors: when should they be used in the elderly? *Drugs Aging* 2005; 22: 185-200.
 49. Penning TD, Talley JJ, Bertenshaw SR, et al. Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzene sulfonamide (SC-58635, celecoxib). *J Med Chem* 1997; 40: 1347-65.
 50. Prasit P, Wang Z, Brideau C, et al. The discovery of rofecoxib, [MK 966, Vioxx, 4-(4'-methylsulfonylphenyl)-3-phenyl-2(5H)-furanone], an orally active cyclooxygenase-2-inhibitor. *Bioorg Med Chem Lett* 1999; 9: 1773-8.
 51. Talley JJ, Brown DL, Carter JS, et al. 4-[5-Methyl-3-phenylisoxazol-4-yl]- benzenesulfonamide, valdecoxib: a potent and selective inhibitor of COX-2. *J Med Chem* 2000; 43: 775-7.
 52. Riendeau D, Percival MD, Brideau C, et al. Etoricoxib (MK-0663): preclinical profile and comparison with other agents that selectively inhibit cyclooxygenase-2. *J Pharmacol Exp Ther* 2001; 296: 558-66.
 53. Esser R, Berry C, Du Z, Dawson J, et al. Preclinical pharmacology of lumiracoxib: a novel selective inhibitor of cyclooxygenase-2. *Br J Pharmacol* 2005; 144: 538-50.
 54. Padi SSV, Jain NK, Singh S, Kulkarni SK. Pharmacological profile of parecoxib: a novel, potent injectable selective cyclooxygenase-2 inhibitor. *Eur J Pharmacol* 2004; 491: 69-76.
 55. Cryer B. The role of cyclooxygenase selective inhibitors in the gastrointestinal tract. *Curr Gastroenterol Rep* 2003; 5: 453-8.
 56. Mizuno H, Sakamoto C, Matsuda K, et al. Induction of cyclooxygenase 2 in gastric mucosal lesions and its inhibition by the specific antagonist delays healing in mice. *Gastroenterology* 1997; 112: 387-97.
 57. Iseki S. Immunocytochemical localization of cyclooxygenase-1 and cyclooxygenase-2 in the rat stomach. *Histochem J* 1995; 27: 323-8.
 58. Kargman S, Charleson S, Cartwright M, et al. Characterization of Prostaglandin G/H Synthase 1 and 2 in rat, dog, monkey, and human gastrointestinal tracts. *Gastroenterology* 1996; 111: 445-54.
 59. Takahashi S, Shigeta J, Inoue H, Tanabe T, Okabe S. Localization of cyclooxygenase-2 and regulation of its mRNA expression in gastric ulcers in rats. *Am J Physiol* 1998; 275: G1137-45.
 60. Zimmermann KC, Sarbia M, Schror K, Weber AA. Constitutive cyclooxygenase-2 expression in healthy human and rabbit gastric mucosa. *Mol Pharmacol* 1998; 54: 536-40.
 61. Langenbach R, Morham SG, Tian HF, et al. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic

- acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell* 1995; 83: 483-92.
62. Wallace JL, Mcknight W, Reuter BK, Vergnolle N. NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroenterology* 2000; 119: 706-14.
 63. Jackson LM, Wu KC, Mahida YR, Jenkins D, Hawkey CJ. Cyclooxygenase (COX) 1 and 2 in normal, inflamed, and ulcerated human gastric mucosa. *Gut* 2000; 47: 762-70.
 64. To KF, Chan FK, Cheng AS, Lee TL, Ng YP, Sung JJ. Up-regulation of cyclooxygenase-1 and -2 in human gastric ulcer. *Aliment Pharmacol Ther* 2001; 15: 25-34.
 65. Davies NM, Sharkey KA, Asfaha S, Macnaughton WK, Wallace JL. Aspirin causes rapid up-regulation of cyclooxygenase-2 expression in the stomach of rats. *Aliment Pharmacol Ther* 1997; 11: 1101-8.
 66. Peskar BM. Role of cyclooxygenase isoforms in gastric mucosal defence. *J Physiol Paris* 2001; 95: 3-9.
 67. Gretzer B, Maricic N, Respondek M, Schuligoi R, Peskar BM. Effects of specific inhibition of cyclo-oxygenase-1 and cyclo-oxygenase-2 in the rat stomach with normal mucosa and after acid challenge. *Br J Pharmacol* 2001; 132: 1565-73.
 68. Tsuji S, Sun WH, Tsujii M, et al. Lansoprazole induces mucosal protection through gastrin receptor-dependent up-regulation of cyclooxygenase-2 in rats. *J Pharmacol Exp Ther* 2002; 303: 1301-8.
 69. Fu S, Ramanujam KS, Wong A, et al. Increased expression and cellular localization of inducible nitric oxide synthase and cyclooxygenase 2 in *Helicobacter pylori* gastritis. *Gastroenterology* 1999; 116: 1319-29.
 70. Konturek PC, Brzozowski T, Pierzchalski P, et al. Activation of genes for spasmolytic peptide, transforming growth factor alpha and for cyclooxygenase (COX)-1 and COX-2 during gastric adaptation to aspirin damage in rats. *Aliment Pharmacol Ther* 1998; 12: 767-77.
 71. Tanaka A, Hatazawa R, Takahira Y, Izumi N, Filaretova L, Takeuchi K. Preconditioning stress prevents cold restraint stress-induced gastric lesions in rats: roles of COX-1, COX-2, and PLA₂. *Dig Dis Sci* 2007; 52: 478-87.
 72. Schmassmann A, Peskar B, Stettler C, et al. Effects of inhibition of prostaglandin endoperoxide synthase-2 in chronic gastrointestinal ulcer models in rats. *Br. J. Pharmacol* 1998; 123: 795-804.
 73. Yamamoto H, Tanaka A, Kunikata T, Hirata T, Kato S, Takeuchi K. Inducible types of cyclooxygenase and nitric oxide synthase in adaptive cytoprotection in rat stomachs. *J Physiol Paris* 1999; 93: 405-12.
 74. Ehrlich K, Plate S, Stroff T, Gretzer B, Respondek M, Peskar BM. Peptidergic and cholinergic neurons and mediators in peptone-induced gastroprotection: role of cyclooxygenase-2. *Am J Physiol* 1998; 274: G955-64.
 75. Brzozowski T, Konturek PC, Konturek SJ, et al. Role of prostaglandins generated by cyclooxygenase-1 and cyclooxygenase-2 in healing of ischemia-reperfusion-induced gastric lesions. *Eur J Pharmacol* 1999; 385: 47-61.
 76. Maricic N, Ehrlich K, Gretzer B, Schuligoi R, Respondek M, Peskar BM. Selective cyclo-oxygenase-2 inhibitors aggravate ischaemia-reperfusion injury in the rat stomach. *Br J Pharmacol* 1999; 128: 1659-66.
 77. Laudanno OM, Cesolari JA, Esnarriaga J, et al. Gastrointestinal damage induced by celecoxib and rofecoxib in rats. *Dig Dis Sci* 2001; 46: 779-84.
 78. Berenguer B, Alarcon de la Lastra C, Moreno FJ, Martin MJ. Chronic gastric ulcer healing in rats subjected to selective and non-selective cyclooxygenase-2 inhibitors. *Eur J Pharmacol* 2002; 442: 125-35.
 79. Kato S, Ogawa Y, Kanatsu K, et al. Ulcerogenic influence of selective cyclooxygenase-2 inhibitors in the rat stomach with adjuvant-induced arthritis. *J Pharmacol Exp Ther* 2002; 303: 503-9.
 80. Bombardier C, Laine L, Reicin A, et al. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. *N Engl J Med* 2000; 343: 1520-8.
 81. Silverstein FE, Faich G, Goldstein JL, et al. Gastrointestinal toxicity with celecoxib vs nonsteroidal antiinflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: A randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. *JAMA* 2000; 284: 1247-55.
 82. Goldstein JL, Lowry SC, Lanza FL, Schwartz HI, Dodge WE. The impact of low-dose aspirin on endoscopic gastric and duodenal ulcer rates in users of a non-selective non-steroidal antiinflammatory drug or a cyclooxygenase-2-selective inhibitor. *Aliment Pharmacol Ther* 2006; 23: 1489-98.
 83. Henry D, McGettigan P. Selective COX-2 inhibitors: a promise unfulfilled? *Gastroenterology* 2007; 132: 790-4.
 84. Whittle BJR. Mechanisms underlying intestinal injury induced by antiinflammatory COX inhibitors. *Eur J Pharmacol* 2004; 500: 427-39.
 85. Fortun PJ, Hawkey CJ. Nonsteroidal antiinflammatory drugs and the small intestine. *Curr Opin Gastroenterol* 2005; 21: 169-75.
 86. Thiéfin G, Beaugerie L. Toxic effects of nonsteroidal antiinflammatory drugs on the small bowel, colon, and rectum. *Joint Bone Spine* 2005; 72: 286-94.
 87. Lanas A, Panes J, Piqué JM. Clinical implications of COX-1 and/or COX-2 inhibition for the distal gastrointestinal tract. *Curr Pharm Des* 2003; 9: 2253-66.
 88. Reuter BK, Davies NM, Wallace JL. Nonsteroidal antiinflammatory drug enteropathy in rats: role of permeability, bacteria, and enterohepatic circulation. *Gastroenterology* 1997; 112: 109-17.
 89. Fornai M, Blandizzi C, Colucci R, et al. Role of cyclooxygenases 1 and 2 in the modulation of neuromuscular functions in the distal colon of humans and mice. *Gut* 2005; 54: 608-16.
 90. Schwarz NT, Kalff JC, Turler A, et al. Prostanoid production via COX-2 as a causative mechanism of rodent postoperative ileus. *Gastroenterology* 2001; 121: 1354-71.
 91. Kalff JC, Turler A, Schwarz NT, et al. Intra-abdominal activation of a local inflammatory response within the human muscularis externa during laparotomy. *Ann Surg* 2003; 237: 301-15.

92. Roberts PJ, Morgan K, Miller R, Hunter JO, Middleton SJ. Neuronal COX-2 expression in human myenteric plexus in active inflammatory bowel disease. *Gut* 2001; 48: 468-72.
93. Sigthorsson G, Simpson RJ, Walley M, et al. COX-1 and 2, intestinal integrity, and pathogenesis of nonsteroidal antiinflammatory drug enteropathy in mice. *Gastroenterology* 2002; 122: 1913-23.
94. Tanaka A, Hase S, Miyazawa T, Ohno R, Takeuchi K. Role of cyclooxygenase (COX)-1 and COX-2 inhibition in nonsteroidal antiinflammatory drug-induced intestinal damage in rats: relation to various pathogenic events. *J Pharmacol Exp Ther* 2002; 303: 1248-54.
95. Menozzi A, Pozzoli C, Giovannini E, et al. Intestinal effects of nonselective and selective cyclooxygenase inhibitors in the rat. *Eur J Pharmacol* 2006; 552: 143-50.
96. Yokota A, Taniguchi M, Tanaka A, Takeuchi K. Development of intestinal, but not gastric damage caused by a low dose of indomethacin in the presence of rofecoxib. *Inflammopharmacology* 2005; 13: 209-16.
97. Leite AZ, Sipahi AM, Damiao AO, et al. Effect of a selective nonsteroidal antiinflammatory inhibitor of cyclooxygenase-2 on the small bowel of rats. *Braz J Med Biol Res* 2004; 373: 333-6.
98. Tibble JA, Sigthorsson G, Foster R, Bjarnason I. Comparison of the intestinal toxicity of celecoxib, a selective COX-2 inhibitor, and indomethacin in the experimental rat. *Scand J Gastroenterol* 2000; 35: 802-7.
99. Zhang L, Lu YM, Dong XY. Effects and mechanism of the selective COX-2 inhibitor, celecoxib, on rat colitis induced by trinitrobenzenesulfonic acid. *Chin J Dig Dis* 2004; 5: 110-4.
100. Singh VP, Patil CS, Jain NK, Kulkarni SK. Aggravation of inflammatory bowel disease by cyclooxygenase-2 inhibitors in rats. *Pharmacology* 2004; 72: 77-84.
101. Shafiq N, Malhotra S, Pandhi P, Nada R. Comparative gastrointestinal toxicity of selective cyclooxygenase (COX-2) inhibitors. *Indian J Exp Biol* 2005; 43: 614-9.
102. Martín AR, Villegas I, La Casa C, Alarcón de la Lastra C. The cyclooxygenase-2 inhibitor, rofecoxib, attenuates mucosal damage due to colitis induced by trinitrobenzene sulphonic acid in rats. *Eur J Pharmacol* 2003; 481: 281-91.
103. Cuzzocrea S, Mazzon E, Serraino I, et al. Celecoxib, a selective cyclooxygenase-2 inhibitor reduces the severity of experimental colitis induced by dinitrobenzene sulfonic acid in rats. *Eur J Pharmacol* 2001; 431: 91-102.
104. Biancone L, Tosti C, Geremia A, et al. Rofecoxib and early relapse of inflammatory bowel disease: an open-label trial. *Aliment Pharmacol Ther* 2004; 19: 755-64.
105. Matuk R, Crawford J, Abreu MT, Targan SR, Vasiliauskas EA, Papadakis KA. The spectrum of gastrointestinal toxicity and effect on disease activity of selective cyclooxygenase-2 inhibitors in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2004; 10: 352-6.
106. Laine L, Smith R, Min K, Chen C, Dubois RW. Systematic review: the lower gastrointestinal adverse effects of non-steroidal antiinflammatory drugs. *Aliment Pharmacol Ther* 2006; 24: 751-67.
107. Bouras EP, Burton DD, Camilleri M, Stephens DA, Thomforde GM. Effect of cyclooxygenase-2 inhibitors on gastric emptying and small intestinal transit in humans. *Neurogastroenterol Motil* 2004; 16: 729-35.
108. Rainsford KD. Aspirin and related drugs. Taylor & Francis, London, 2004.
109. Wallace JL, McKnight GW, Keenan CM, Byles NI, MacNaughton WK. Effects of leukotrienes on susceptibility of the rat stomach to damage and investigation of the mechanism of action. *Gastroenterology* 1990; 98: 1178-86.
110. Gyomber E, Vattay P, Szabo S, Rainsford KD. Effect of lipoxigenase inhibitors and leukotriene antagonists on acute and chronic gastric haemorrhagic mucosal lesions in ulcer models in the rat. *J Gastroenterol Hepatol* 1996; 11: 922-7.
111. Martel-Pelletier JM, Lajeunesse D, Reboul P, Pelletier JP. Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal antiinflammatory drugs. *Ann Rheum Dis* 2003; 62: 501-9.
112. Hudson N, Balsitis M, Everitt S, Hawkey CJ. Enhanced gastric mucosal leukotriene B4 synthesis in patients taking non-steroidal antiinflammatory drugs. *Gut* 1993; 34: 742-7.
113. Bias P, Buchner A, Klessler B, Laufer S. The gastrointestinal tolerability of the LOX/COX inhibitor, licofelone, is similar to placebo and superior to naproxen therapy in healthy volunteers: results from a randomized, controlled trial. *Am J Gastroenterol* 2004; 99: 611-8.
114. Serhan CN, Hamberg M, Samuelsson B. Lipoxins: novel series of biologically active compounds formed from arachidonic acid in human leukocytes. *Proc Natl Acad Sci USA* 1984; 81: 5335-9.
115. Schwab JM, Serhan CN. Lipoxins and new lipid mediators in the resolution of inflammation. *Curr Opin Pharmacol* 2006; 6: 414-20.
116. Fiorucci S, Santucci L, Wallace JL, et al. Interaction of a selective cyclooxygenase-2 inhibitor with aspirin and NO-releasing aspirin in the human gastric mucosa. *Proc Natl Acad Sci USA* 2003; 100: 10937-41.
117. Cirino G. Nitric oxide releasing drugs: from bench to bedside. *Dig Liver Dis* 2003; 35: S2-8.
118. Keeble JE, Moore PK. Pharmacology and potential therapeutic applications of nitric oxide-releasing non-steroidal antiinflammatory and related nitric oxide-donating drugs. *Br J Pharmacol* 2002; 137: 295-310.
119. Fiorucci S, Distrutti E. Cyclo-oxygenase (COX) inhibiting nitric oxide donating (CINODs) drugs: a review of their current status. *Curr Top Med Chem* 2007; 7: 277-82.
120. Lopez-Belmonte J, Whittle BJ, Moncada S. The actions of nitric oxide donors in the prevention or induction of injury to the rat gastric mucosa. *Br J Pharmacol* 1993; 108: 73-8.
121. Barrachina MD, Calatayud S, Canet A. Transdermal nitroglycerin prevents nonsteroidal antiinflammatory drug gastropathy. *Eur J Pharmacol* 1995; 281: R3-4.

122. Lanas A, Bajador E, Serrano P, et al. Nitrovasodilators, low-dose aspirin, other nonsteroidal antiinflammatory drugs, and the risk of upper gastrointestinal bleeding. *N Engl J Med* 2000; 343: 834-9.
123. Davies NM, Roseth AG, Appleyard CB, et al. NO-naproxen vs. naproxen: ulcerogenic, analgesic and antiinflammatory effects. *Aliment Pharmacol Ther* 1997; 11: 69-79.
124. Elliott SN, McKnight W, Cirino G, Wallace JL. A nitric oxide-releasing nonsteroidal antiinflammatory drug accelerates gastric ulcer healing in rats. *Gastroenterology* 1995; 109: 524-30.
125. Ukawa H, Yamakuni H, Kato S, Takeuchi K. Effects of cyclooxygenase-2 selective and nitric oxide-releasing nonsteroidal antiinflammatory drugs on mucosal ulcerogenic and healing responses of the stomach. *Dig Dis Sci* 1998; 43: 2003-11.
126. Fiorucci S, Di Lorenzo A, Renga B, Farneti S, Morelli A, Cirino G. Nitric oxide (NO)-releasing naproxen (HCT-3012 [(S)-6-methoxy-alpha-methyl-2-naphthaleneacetic acid 4-(nitrooxy)butyl ester]) interactions with aspirin in gastric mucosa of arthritic rats reveal a role for aspirin-triggered lipoxin, prostaglandins, and NO in gastric protection. *J Pharmacol Exp Ther* 2004; 311: 1264-71.
127. Burgaud JL, Ongini E, Del Soldato P. Nitric oxide-releasing drugs: a novel class of effective and safe therapeutic agents. *Ann NY Acad Sci* 2002; 962: 360-71.
128. Fiorucci S, Santucci L, Gresele, P, Faccino, RM, Del Soldato P, Morelli A. Gastrointestinal safety of NO-aspirin (NCX-4016) in healthy human volunteers: a proof of concept endoscopic study. *Gastroenterology* 2003; 124: 600-7.
129. Hawkey CJ, Jones JJ, Atherton CT, et al. Gastrointestinal safety of AZD3582, a cyclooxygenase inhibiting nitric oxide donor: proof of concept study in humans. *Gut* 2003; 52: 1537-42.
130. Jonzon B, Bjarnason I, Hawkey C, et al. The CINOD, AZD3582, exhibits an improved gastrointestinal safety profile compared with naproxen in healthy volunteers. *Inflammopharmacology* 2003; 11: 437-44.
131. Hill M, Sindet-Pederson S, Seymour RA, et al. Analgesic effects of the cyclooxygenase-inhibiting nitric oxide donor AZD3582 in postoperative dental pain: Comparison with naproxen and rofecoxib in two randomized, double blind, placebo-controlled studies. *Clin Ther* 2006; 28: 1279-95.
132. Mizoguchi H, Hase S, Tanaka A, Takeuchi K. Lack of small intestinal ulcerogenicity of nitric oxide-releasing indomethacin, NCX-530 in rats. *Aliment Pharmacol Ther* 2001; 15: 257-67.
133. Chegaev K, Lazzarato L, Tosco P, et al. NO-Donor COX-2 Inhibitors. New Nitrooxy-Substituted 1,5-Diarylimidazoles Endowed with COX-2 Inhibitory and Vasodilator Properties. *J Med Chem* 2007; 50: 1449-57.
134. Zanardo RC, Brancaleone V, Distrutti E, Fiorucci S, Cirino G, Wallace JL. Hydrogen sulfide is an endogenous modulator of leukocyte-mediated inflammation. *FASEB J* 2006; 20: 2118-20.

Accepted: 16th May 2007

Correspondence: Gabriella Coruzzi
Department of Human Anatomy,
Pharmacology and Forensic Medicine
Section of Pharmacology
Via Volturno 39
43100 Parma
Tel: 0039-0521 903851
Fax: 0039-0521 903852
E-mail: gabriella.coruzzi@unipr.it, www.actabiomedica.it