in vivo *26*: 381-394 (2012)

Review

The Role of Nonsteroidal Antiinflammatory Drugs and Cyclooxygenase-2 Inhibitors on Experimental Colitis

ANA PAULA RIBEIRO PAIOTTI[1], PATRÍCIA MARCHI[1], SENDER JANKIEL MISZPUTEN[2], CELINA TIZUKO FUJIYAMA OSHIMA[1], MARCELLO FRANCO[1] and DANIEL ARAKI RIBEIRO[1,3]

Departments of [1]Pathology, [2]Division of Gastroenterology, and [3]Biosciences, Federal University of São Paulo, UNIFESP, SP, Brazil

Abstract. *The nonsteroidal anti-inflammatory drugs (NSAIDs) have been widely used in the management of pain and inflammation. Unfortunately, they are associated with dose-dependent gastrointestinal (GI) adverse events ranging from dyspepsia to symptomatic and complicated ulcers. The mechanism of NSAID action is attributed to the cyclooxygenase (COX) inhibition. New anti-inflammatory drugs have been synthesized, such as selective COX-2 inhibitors, however, these drugs may present side effects, such as modification of the epithelial barrier. Inflammatory bowel disease (IBD) is a common chronic gastrointestinal disorder characterized by alternating periods of remission and active intestinal inflammation. A possible association between the use of NSAIDs and the relapse of IBD has been repeatedly suggested. For this reason, many studies are conducted with the use of COX-2 in experimental models. The objective of this review is to describe the role of NSAIDs and COX-2 inhibitors in different experimental models of colitis. We reviewed controlled trials, original articles, case reports and reviews. The role of selective inhibition of COX-2 in the inflammatory process and the course of experimental and human colitis is controversially discussed. In conclusion, the relative role of COX-2 selective inhibitors on human and experimental colitis remains to be explored. Thus, the use of COX-2 inhibitors in IBD should be considered with caution.*

Since the introduction of acetylsalicylic acid (aspirin) as the first nonsteroidal anti-inflammatory drug (NSAID) in 1897, NSAIDs have been widely used in the management of pain and inflammation (1-3). Today, they are classified as nonsteroidal antiinflammatory drugs (NSAIDs), characterized by differing degrees of antiinflammatory, analgesic and antipyretic activity. NSAIDs are among the most widely used medicines in the world. Unfortunately, they are associated with dose-dependent gastrointestinal (GI) adverse events ranging from dyspepsia (10-20%) to symptomatic and complicated ulcers (1-4%) (4, 5). The mechanism of NSAID action is attributed to cyclooxygenase (COX) inhibition (1). Cyclooxygenase is a key rate-limiting enzyme that exists in at least two isoforms: COX-1 is constitutively expressed in various tissues, whereas COX-2 does not appear to be expressed except at very low levels in most tissues and is rapidly up-regulated in response to growth factors and cytokines. More recently, COX-2 has been implicated in several distinct cellular mechanisms, such as angiogenesis, proliferation and the prevention of apoptosis (6). Selective COX-2 inhibitors have been synthesized, however, these drugs may present side effects, such as the ability to modify the epithelial barrier (6).

Inflammatory bowel disease (IBD) is a common chronic gastrointestinal disorder characterized by alternating periods of remission and active intestinal inflammation. The precise etiology of IBD, including Crohn's disease (CD) and ulcerative colitis (UC), remains unclear. However, environmental factors, immunological disturbances, genetic influences and the presence of certain chemical mediators (cytokines) have been established as putative participants in the pathogenesis of the disease (7, 8). A possible association between the use of NSAIDs and the relapse of IBD has been repeatedly suggested. IBD patients seek relief in NSAIDs for non-IBD-related pain (arthralgia, arthritis) and these drugs are also prescribed for the symptons of extraintestinal manifestations of IBD, such as peripheral arthritis, sacroiliitis, ankylosing spondylitis, and osteoporosis-related fractures. NSAIDs are considered to be the first-line

Correspondence to: Daniel Araki Ribeiro, DDS, Ph.D., Departamento de Biociências, Av. Ana Costa, 95, Vila Mathias, Santos – SP, 11060-001, Brazil. Tel: +55 1332218058, Fax: +5513 32232592, e-mail: daribeiro@unifesp.br

Key Words: NSAIDs, COX-2 inhibitors, IBD, experimental colitis, cyclooxygenase, review.

treatment for the abnormalities just mentioned (to relieve pain and treat inflammation), although immunosuppressive and biological agents [methotrexate (MTX), thalidomide, tumor necrosis factor alpha (TNF-α) antigen] have also been used (9-11).

In the last few decades, the development of experimental models for studying IBD has greatly contributed to enhance understanding of the immunological mechanisms involved, such as changes in the gut epithelial barrier (12, 13). IBD seems to occur when luminal antigens from the bacterial flora stimulate the immune system in the gut barrier towards an exacerbated, genetically defined response. Patients with IBD present an increase in the amount of intestinal bacterial antigen compared to healthy individuals (14). In particular, some human and animal studies have shown the prime importance of gut epithelial barrier integrity and changes that lead to deregulation of the immune system as a result of the loss of intestinal homeostasis (15).

It has been reported that CD is associated with gut barrier dysfunction and that some patients express an instestinal barrier hyperresponsiveness to NSAIDs (16). Thus, clinicians are concerned that treatment with NSAIDs could increase the risk of aggravation and relapse in patients in which IBD is controlled. A large number of people suffering from IBD take NSAIDs, including COX-2 inhibitors, for various reasons, as the efficiency of these drugs in pain control seems to be unquestioned. In some patients, exacerbation of their disease occurs; however, it is uncertain whether NSAIDs are implicated in IBD relapse or whether COX-2 inhibitors are safer than general NSAIDs.

NSAIDs have been implicated in the onset or the exacerbation of IBD in a number of studies and case reports, whereas in other studies, no relationship has been found between NSAID use and an increase in significant disease flares. On the other hand, COX-2 inhibitors have a lower incidence of toxicity to the small bowel or colon, as recent studies indicate that COX-2 inhibitors are prescribed more often than NSAIDs in patients who are older, sicker, and have risk factors associated with NSAID gastropathy (17-20). Is the concept that the use of NSAIDs is associated with relapse of IBD true? Many studies have been conducted with the use of COX-2 in experimental models. The objective of this review is to describe the role of COX-2 inhibitors on different experimental models of colitis.

COX-1/ COX-2 Concept, Biochemistry and Functions

Cyclooxygenase (COX), or prostaglandin H 2 (PGH) synthase is the enzyme that catalyzes the first two steps in the biosynthesis of prostaglandins (PGs) from arachidonic acid (AA). These are the oxidation of AA to the hydroxyendoperoxide PGH 2. The PGH 2 is transformed by a range of enzymes and nonenzymic mechanisms into the primary prostanoids, PGD 2, PGE 2, PGF 2α, PGI 2 and thromboxane A 2 (TXA 2) (1) (Figure 1).

COX activity has long been studied in preparations from sheep seminal visicles, and this enzyme was cloned by three separate groups in 1988 (21-23). The discovery of a second form of COX in the early 1990s was the most important event in prostanoid biology in almost 20 years. Induction of this isoform, COX-2, by several stimuli associated with cell activation and inflammation assured the relevance of this finding to inflammatory disease in general. A clear sign of the therapeutic value of this discovery is that in the relatively short time of about five years, several highly effective anti-inflammatory agents and new therapeutic areas have become subjects of investigation (1, 24-27).

The inducible enzyme COX-2 is very similar in structure and catalytic activity to the constitutive COX-1. The biosynthetic activity of both isoforms can be inhibited by aspirin and other NSAIDs (1). Both isoforms have a molecular weight of 71 k and are almost identical in length, with just over 600 amino acids, of which 63% are identical in sequence. However, the human COX-2 gene at 8.3 kb is a small immediate early gene, whereas human COX-1 originates from a much larger 22-kb gene. The gene products also differ, with the mRNA for the inducible enzyme being approximately 4.5 kb and that of the constitutive enzyme being 2.8 kb (1, 24, 26).

The three-dimensional X-ray crystal structure of human or murine COX-2 can be superimposed on that of COX-1 (28-30); the residues that form the substrate binding channel, the catalytic sites, and the residues immediately adjacent are all identical except for two small variations. In these two positions, the same substitutions occur: Ile in COX-1 is exchanged for Val in COX-2 at positions 434 and 523 (the residues in COX-2 are given the same number as their equivalent aminoacids in COX-1).

In spite of this structural identity, there are clear biochemical differences between the isoforms in substrate and inhibitor selectivity. For example, COX-2 will accept a wider range of fatty acids as substrates than will COX-1 (1, 24). Thus, although both enzymes can utilize AA and dihomo-γ-linolenate equally well, COX-2 oxygenates other fatty acid substrates, such as eicosapentaenoic acid, γ-linolenic acid, α-linolenic acid, and linoleic acid more efficiently than does COX-1. Furthermore, COX-2 acetylated by aspirin on Ser 530 will still oxidize AA but to 15-HETE, whereas similarly acetylated COX-1 will not oxidize AA at all (31-33). In addition (see below), inhibitors will differentiate between COX-2 and COX-1, with more than 1000-fold selectivity (27, 34).

Supporting evidence is strongest from the work on COX-2-selective inhibitors; mutation of Ile 523 to Val in the COX-1 protein allows COX-2-selective inhibitors to bind and

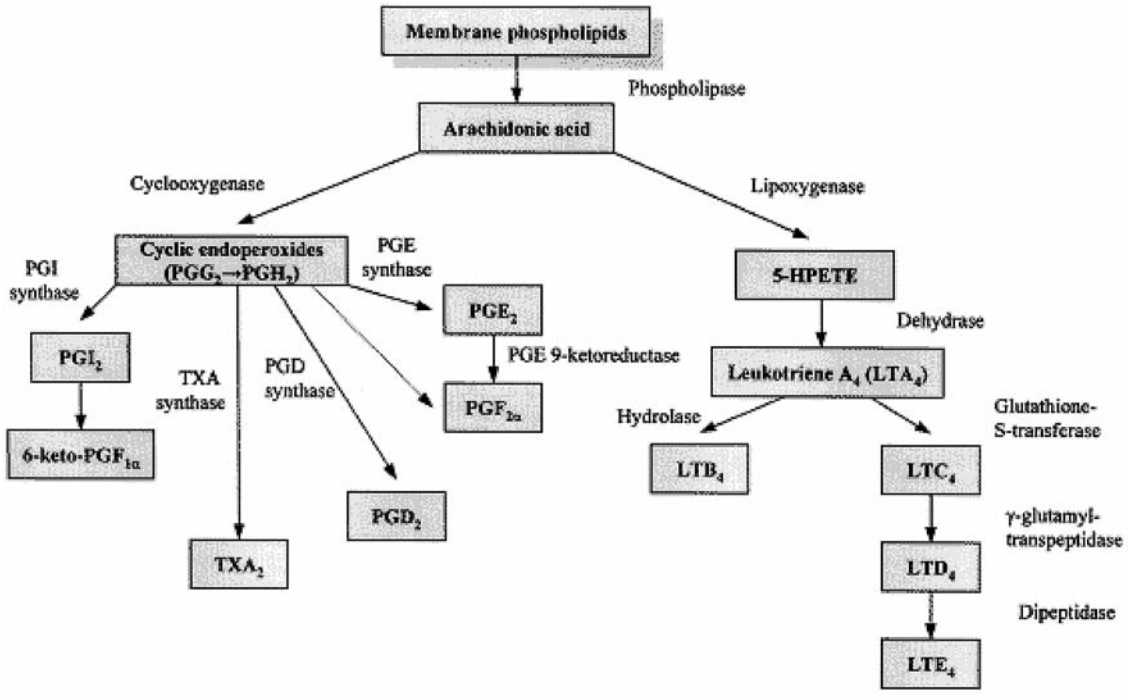

Figure 1. *The arachidonic acid cascade. PG: Prostaglandin; LT: Leukotriene.*

inhibit PGH 2 formation without altering the binding constant *Km* for AA, and the reverse mutant of COX-2 in which Val 523 is exchanged for Ile shows inhibitor binding and selectivity profiles comparable to those of wild-type COX-1 (35-37). The structural basis for this has been shown clearly in the crystal analyses of COX-2, which have used either the human or the murine protein, each bound to a nonselective COX-1 or COX-2 inhibitor. The smaller size of Val 523 allows the inhibitor access to a side pocket off the main substrate channel in COX-2 access that is denied sterically by the longer side chain of Ile in COX-1. Selective inhibitors of COX-2 do not bind to Arg 120, which is used by the carboxylic acid of the substrate AA and by the COX-1-selective and nonselective NSAIDs, all of which are carboxylic acids (38, 39).

Another striking structural difference between the isoforms, but of unknown significance, is the absence of a sequence of 17 amino acids from the *N* terminus and the insertion of a sequence of 18 amino acids at the *C* terminus of COX-2 in comparison to COX-1. This accounts for the different numbering for the analogous residues in the two isoforms (*e.g.* the acetylatable serine is Ser 530 in COX-1 but Ser 516 in COX-2). The C-terminal insert in COX-2 does not alter the last four amino acid residues, which in both proteins form the signal for attachment to the membrane of the endoplasmic reticulum (ER). However, COX-2 is located on the nuclear membrane as well as on the ER, while COX-1 is found attached only to the membranes of the ER. The reason for this selective localization may lie in the different sequence of the *C* terminus. It is relevant that in the X-ray structural analysis of both isoforms, the three-dimensional structures of the last 18 C-terminal residues in COX-1 and the last 30 residues in COX-2 were not resolved, implying a marked flexibility in this region of the proteins even in the crystalline form (40-44). Although emphasis has been placed here on the differences between isoforms, the extensive overall structural and biochemical similarity between COX-1 and COX-2 must be reiterated. Both use the same endogenous substrate, AA, and form the same product by the same catalytic mechanism. Their major difference lies in their pathophysiological functions.

Chronic inflammation is an excellent example of a disease that represents a malfunction of normal host defense systems. Thus, rather than classifying PG biosynthesis into physiological and pathological, it may be better to use the classification applied to the COX isoforms: either constitutive or induced. COX-1 activity is constitutive, present in nearly all cell types at a constant level; COX-2 activity is normally absent from cells, and when induced, the protein levels increase and decrease in a matter of hours after a single stimulus (1, 24, 26).

The main reason for labeling COX-1 and COX-2 as physiological and pathological, respectively, is that most of the stimuli known to induce COX-2 are those associated with

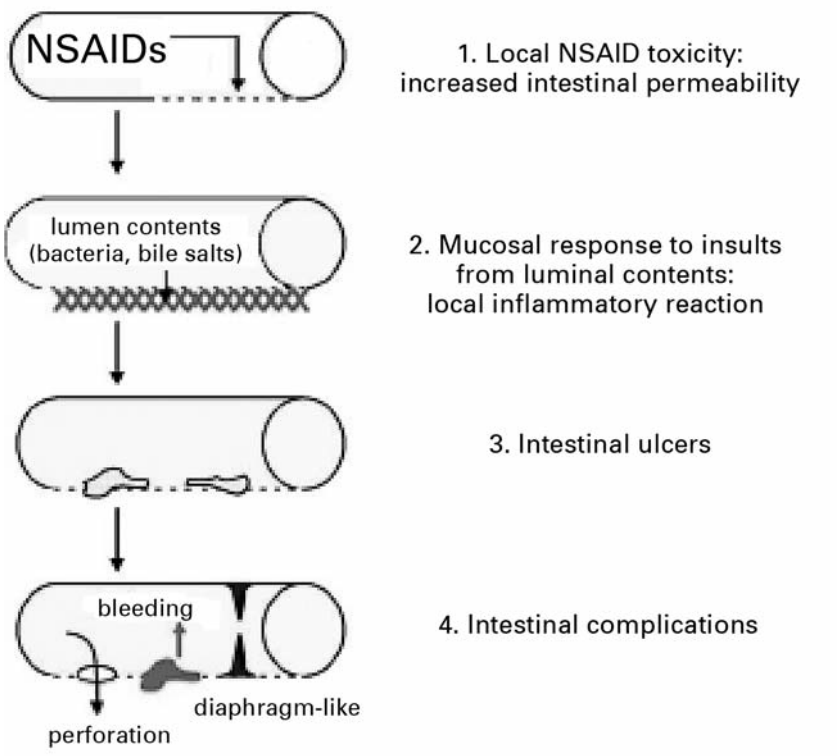

Figure 2. *Pathogenesis of NSAID-induced intestinal lesions - Taken from Thiéfin & Beaugerie, 2005 (49).*

inflammation, for example, bacterial lipopolysaccharide (LPS) and cytokines such as interleukin (IL)-1, IL-2, and tumor necrosis factor alpha (TNF-α). The anti-inflammatory cytokines, IL-4, IL-10, and IL-13, will decrease induction of COX-2, as do the corticosteroids (24, 27). The physiological roles of COX-1 have been deduced from the deleterious side-effects of NSAIDs, which while inhibiting PG biosynthesis at inflammatory sites, also inhibit constitutive biosynthesis. Thus, COX-1 provides PGs in the stomach and intestine to maintain the integrity of the mucosal epithelium and its inhibition leads to gastric damage, hemorrhage and ulceration.

Mechanisms of NSAID Injury to the Gastrointestinal Mucosa

For evaluation of the validity of new potentially less toxic NSAIDs, it is mandatory to clearly understand the pathogenesis of NSAID-induced ulceration (Figure 2). Both aspirin and non-aspirin NSAIDs inhibit the COX pathway of PG synthesis (1, 40, 41). This represents the basis of anti-inflammatory action but is also responsible for the development of side-effects in the gastrointestinal tract and kidney as well as the inhibition of platelet aggregation. Inhibition of PG synthesis can exert injurious actions on the gastric and duodenal mucosa as it abrogates a number of PG dependent defence mechanisms. Inhibition of COX leads to a decrease in mucus and bicarbonate secretion, reduces mucosal blood flow, and causes vascular injury, leucocyte accumulation, and reduced cell turnover, all factors that contribute to the genesis of mucosal damage. Within this broad spectrum of events, microvascular damage appears to play a central role. Prostaglandins of the E and I series are potent vasodilators that are continuously produced by the vascular endothelium. Inhibition of their synthesis by an NSAID leads to vasoconstriction (45). Furthermore, inhibition of PG formation results in a rapid and significant increase in the number of neutrophils adhering to the vascular endothelium in both gastric and mesenteric venules. Adherence is dependent on expression of the α2 integrin (CD11/CD18) on neutrophils and intercellular adhesion molecule on the vascular endothelium (46-48).

The severity of experimental NSAID gastropathy was markedly reduced in rats rendered neutropenic by pretreatment with antineutrophil serum or methotrexate. Wallace *et al*. provided evidence for an isoenzyme specific role of COX in the homeostasis of the gastrointestinal microcirculation (48). Thus in rats, the selective COX-1 inhibitor SC-560 reduced gastric mucosal blood flow without affecting leucocyte adherence to mesenteric venules. In

contrast, the selective COX-2 inhibitor celecoxib markedly increased leucocyte adherence but did not reduce gastric mucosal blood flow. Only concurrent treatment with both COX-1 and COX-2 inhibitor damaged the gastric mucosa, suggesting that reduction of mucosal blood flow and increase in leucocyte adhesion have to occur simultaneously to interfere with mucosal defence. Inhibition of PG synthesis thus plays a key role in the induction of mucosal injury but does not represent the only pathway by which NSAIDs can damage the gastrointestinal mucosa (49). NSAIDs can also induce local damage at the site of their contact with the gastrointestinal mucosa (50, 51). Topical application of NSAIDs increases gastrointestinal permeability allowing luminal aggressive factors access to the mucosa. Aspirin and most non-aspirin NSAIDs are weak organic acids. In the acidic milieu of the stomach, they are converted into more lipid-soluble unionised acids that penetrate into the gastric epithelial cells. There, at neutral pH, they are reionised and trapped within the cell causing local injury. Having entered gastric mucosal epithelial cells, NSAIDs uncouple mitochondrial oxidative phosphorylation. This effect is associated with changes in mitochondrial morphology and a decrease in intracellular ATP and therefore a reduced ability to regulate normal cellular functions, such as maintenance of intracellular pH. This in turn causes loss of cytoskeletal control over tight junctions and increased mucosal permeability. The ability of NSAIDs to uncouple oxidative phosphorylation stems from their extreme lipid solubility and the position of a carboxyl group that acts as a proton carrier (52, 53). A further mechanism involved in the topical irritant properties of NSAIDs is their ability to reduce the hydrophobicity of the mucous gel layer of the gastric mucosa. NSAIDs can convert the mucous gel from a non-wettable to a wettable state and in experimental animals this effect has been shown to persist for several weeks or months after discontinuation of NSAID administration (52, 53). Gastric mucosal lesions can also occur in a non-acidic milieu, such as following rectal application. With oral administration, gastric acid, however, appears to enhance NSAID-induced damage. More extensive and deeper erosions occur at low pH and an elevation in gastric pH above 4 is necessary to prevent this acid related component. PGs do not represent a unique pathway to protect the gastric mucosa (50).

Chronic IBD and NSAIDs

Although inhibition of PG production is useful to relieve symptoms of extraintestinal manifestations of IBD, there is no role for NSAIDs in the treatment of IBD. However, various studies have shown that conventional NSAIDs trigger more frequent relapse of pre-existing intestinal inflammation than inducing *de novo* colitis (54, 55). In experimental models with animals, the damage was initiated by various combinations of the three biochemical actions common to all conventional NSAIDs, such as COX-1 and COX-2 inhibition and local effect. The latter was thought to involve an NSAID surface-membrane phospholipid interaction and an effect on mitochondrial energy metabolism. These effects were consequent to the physicochemical properties of conventional NSAIDs, namely, acidity and lipophilicity (51, 56, 57). Clinical relapse of IBD after treatment with NSAIDs was associated with escalating intestinal inflammatory activity similar to the clinical course seen in patients with active IBD not taking NSAIDs. It was suggested that the relapse might be triggered by dual inhibition of the enzymes, and it was clear that the nonselective NSAIDs were associated with clinical relapse. There is increasing evidence that the main pathophysiological consequence of COX-1 inhibition is impaired mucosal microcirculatory blood flow, whereas the COX-2 enzyme might have an immunomodulatory role in the gastrointestinal tract (58-60).

The potential role for PGs in the inflammatory process underlying chronic IBD has been a focus of controversy. Under the hypothesis that PGs may be protective, treatment with exogenous prostaglandins was investigated but found to exacerbate the diarrhea. The possibility that proinflammatory mechanisms might be involved prompted trials of NSAID therapy. In keeping with these early findings, some reports suggested a deleterious effect of NSAIDs on the course of IBD (61, 62). The magnitude of the risk, however, remains controversial. Some studies including original papers, case reports, reviews, controlled trials and databases about exacerbation of IBD associated with the use of NSAIDs are published in the literature (61-64).

Takeuchi *et al*. demonstrated that conventional NSAIDs cause clinical relapse within a few days of ingestion in 18% of asymptomatic patients with IBD (56). Patients who tolerate NSAIDs for a week did not seem to be at serious risk of clinical relapse. Another study conducted by Meyer *et al*. retrospectively reviewed the records of IBD patients and showed that the use of NSAIDs was associated with relapse (65). Case–control studies showed that treatment with NSAIDs increased the risk of a new relapse of IBD (62, 63). Bonner *et al*. in a prospective study, found that the administration of high doses of NSAIDs was associated with a higher numerical Disease Activity Index score among patients with CD with colonic involvement, but this was not reflected by an increase in significant disease deteriorations (18). A case–control study which included 60 patients with IBD and 62 with irritable bowel syndrome (IBS) receiving NSAIDs demonstrated that at least 31% of all the IBD patients who used NSAIDs had onset or an exacerbation of IBD, whereas only 2% of the IBS population who used NSAIDs had an apparent provocation of their disease (62). Thus, they suggested that patients with a history of IBD

should avoid using NSAIDs whenever possible. To elucidate the nature of these associations, more studies should be carried out using a broader spectrum of cases of colitis encountered in clinical practice.

Development of the COXIBs

The identification of the COX-2 isoenzyme opened the door to development of NSAIDs which selectively inhibit COX-2, the main goal of which was to reduce the gastrointestinal toxicity. The first generation of selective COX-2 inhibitors came from animal models in which compounds were sought that were potent anti-inflammatory agents with minimal side-effects on the stomach (Nimesulide, etodolac and meloxicam) (63). The discovery of the specificity of these products was in reality found after their sale, being due, mainly in clinical and experimental observations to reduce incidence of gastrointestinal side-effects, and subsequently confirmed by *in vitro* studies. Nimesulide is considered an aberrant example of NSAIDs, with good power in *in vivo* inflammatory models, but with weak inhibition in *in vitro* preparations of COX. Nimesulide displays specificity of action on COX-2, and it has other effects that further enhance their anti-inflammatory activity, as inhibition of neutrophil activation and antioxidant properties. Based on *in vitro* studies initially suggested that meloxicam selectively inhibited COX-2. However, when tested *in vivo*, in humans, its specificity for COX-2 was only about ten times higher than that for COX-1, with further platelet inhibition (66). The molecular modification of these drugs, especially those of nimesulide, in order to increase its COX-2 selectivity resulted in structures without a carboxylic group and the presence of a sulphonamide or sulphone group, resulting in specific inhibitors in the second generation of products. This group includes celecoxib, rofecoxib, valdecoxib, parecoxib (pro-drug of valdecoxib), o-(acetoxyphenyl)hept-2-ynyl sulfide (APHS) and etoricoxib (67, 68).

COXIBs spare COX-1 and inhibit COX-2 function, therefore reducing but not eliminating NSAID-associated gastrointestinal toxicity and are efficacious as traditional NSAIDs in relieving pain. Data from large outcome studies have characterized the gastrointestinal effects of COXIBs. The Celecoxib Long-term Arthritis Safety Study (CLASS Study) that compared high dose celecoxib (400 mg *bid*), diclofenac (75 mg *bid*), and ibuprofen (800 mg 3 times daily) showed that symptomatic ulcers were significantly less common among celecoxib users than traditional NSAIDs users; however, ulcer complication rates were not significantly different (which was probably due to the confounding factor of concomitant low-dose aspirin in 22% of patients) (70). However, a recent meta-analysis of available trials of the Cochrane collaboration confirms that celecoxib at any dose was associated with statistically fewer gastrointestinal events (71). Moreover, the results of another large outcome study, celecoxib *vs*. naproxen and diclofenac in osteoarthritis patients (SUCCESS I Study) confirmed the significantly better safety profile of celecoxib compared with traditional NSAIDs (72). The Vioxx Gastrointestinal Safety of Rofecoxib trial (VIGOR Study) concluded that rofecoxib users had 50% fewer GI events compared with naproxen users (73). Later, the comparison of lumiracoxib with naproxen and ibuprofen in the Therapeutic Arthritis Research and Gastrointestinal Event Trial (TARGET), showed a 75% decrease in adverse gastrointestinal events with the coxib (74). It is important to emphasise that although the incidence of adverse gastrointestinal events increased in relation to the presence of risk factors, the differences from NSAIDs were maintained in subgroups of patients with and without risk factors (75).

Lumiracoxib is a novel, highly selective COX-2 inhibitor. Lumiracoxib differs structurally from other selective COX-2 inhibitors (Figure 3) (76, 77), in that it is a phenyl acetic acid derivative. It has the highest selectivity for COX-2 compared with COX-1 in the human whole blood assay (with a ratio of 515:1 in healthy subjects) and a fairly short plasm half-life (3-6 hours) compared with other COX-2-selective inhibitors (78). In endoscopic studies, lumiracoxib use was associated with a rate of acute gastric injury and chronic ulcer formation that did not differ from placebo and which was significantly lower than with the NSAID ibuprofen and with celecoxib (79-81).

Notwithstanding, it is important to note that three of the above commented outcome studies mentioned (CLASS, TARGET and SUCCESS) (71, 72, 74), and one endoscopic and several epidemiological studies have shown that the concomitant use of low-dose aspirin and COXIB or traditional NSAIDs increases the risk of upper gastrointestinal bleeding further and attenuates the gastrointestional advantage of use COXIB over a traditional NSAID (82, 83). A recent meta-analysis of randomized controlled trial has shown that COXIB plus low-dose aspirin use was associated with a lower risk of upper gastrointestinal complications when compared to non-selective NSAID plus low-dose aspirin (84). These gastrointestinal benefits have to be balanced against the known cardiovascular risks, particularly with long-term use. The VIGOR and Adenomatous Polyp Prevention on Vioxx Trial Investigators (APPROVe) studies showed that rofecoxib was associated with increased risk of cardiovascular events after 12 and 36 months of treatment when compared to naproxen (VIGOR) and placebo (APPROVe) (73, 85). Other outcome studies have also shown that celecoxib at doses of 400 mg *bid* or 200 mg *bid* (86), but not 400 mg once a day (87), is associated with increased risk of cardiovascular events. Observational studies have shown, however, that celecoxib at 200 mg/day was not associated with increased risk of

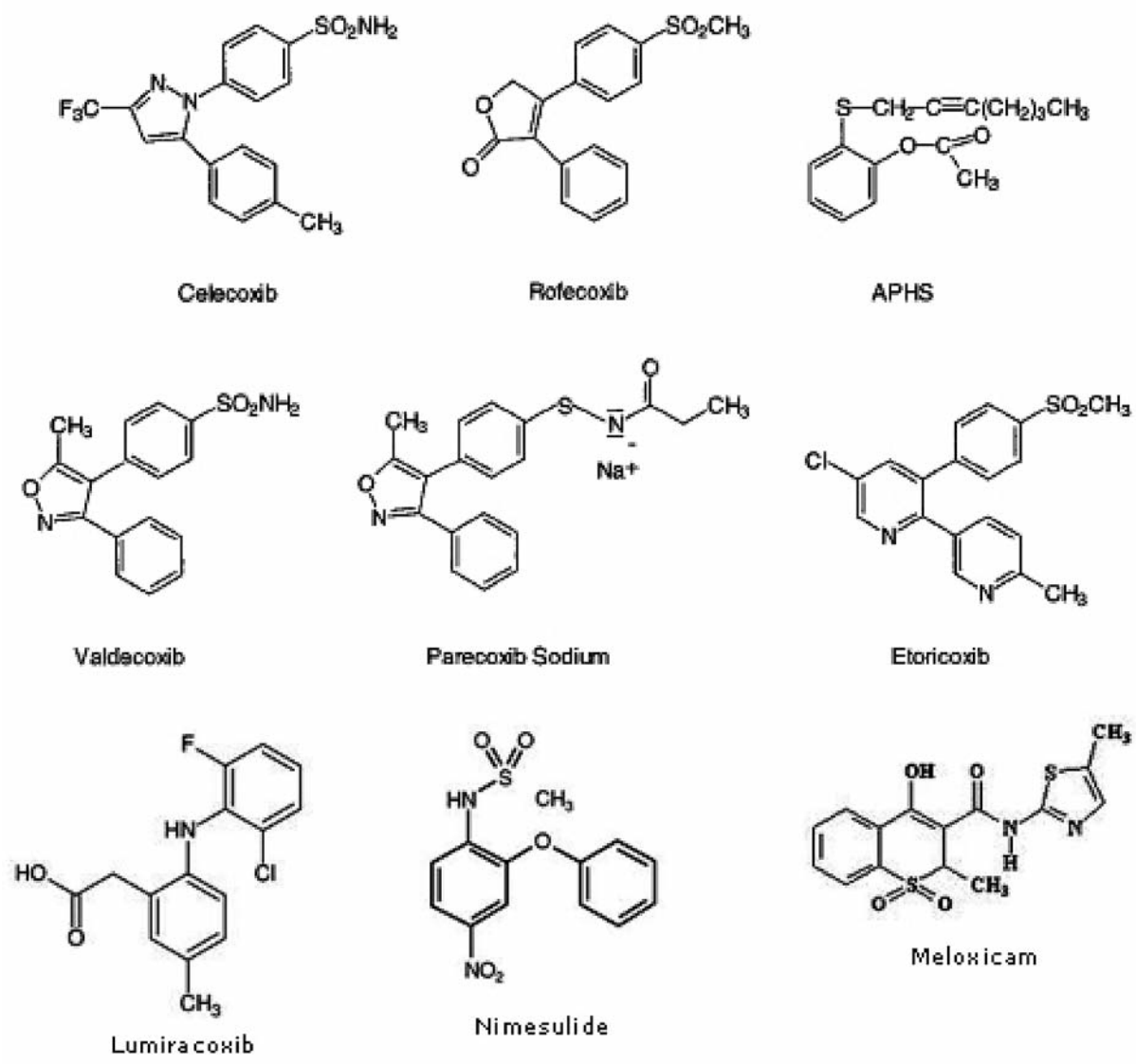

Figure 3. *The chemical structures of some COX-2 inhibitors. APHS: o-(acetoxyphenyl)hept-2-ynyl sulfide.*

cardiovascular events (70, 73). Recent observational studies have shown that the use of NSAIDs (including nonselective ones) may be associated with increased cardiovascular risk and this may be different for the different compounds, dose and length of treatment (83, 88-90). Of all traditional NSAIDs, diclofenac has been found to increase the cardiovascular risk the most (91). In the MEDAL program, etoricoxib at the dose of 60-90 mg/day was found not to be different from diclofenac in the incidence of cardiovascular events (92). The study also showed no differences in the incidence of upper gastrointestinal complications between these two drugs, although the total number of events (symptomatic ulcers and complications) was statistically lower in etoricoxib users (93). Lastly, both traditional NSAIDs and COXIB may also increase blood pressure and reduce kidney function.

COX-2 Inhibitors in Experimental Models of Colitis

The role of selective inhibition of COX-2 in the inflammatory process and the course of experimental and human colitis is controversially discussed, even though increased levels of PGs (PGE 2 and PGI 2) and other eicosanoids were detected in both colitis models and patients with chronic IBD, which correlates well with the disease

activity. PGE 2 is produced by mononuclear cells in the *lamina propria* and is dependent on COX-2 expression. It modulates the intestinal immune response, including the differentiation of T-cells and the production and release of proinflammatory cytokines. During the course of IBD and experimental colitis, some prostanoids are released and subsequently modulate the course of the disease.

Animal models are used extensively to study the pathogenesis and pathophysiology of IBD and to evaluate therapies. The more extensively used models were those induced using: acetic acid, dextran sodium sulphate (DSS) and 2,4,6'-trinitrobenzene sulphonic acid (TNBS). Acetic acid-induced colitis in rats resembles human UC in histology, eicosanoid production and excessive oxygen-derived free radical release by inflamed mucosa. DSS-induced UC is accompanied by erosion and ulceration as well as inflammatory cell infiltration, characteristics resembling those of human UC. TNBS-induced colitis is accompanied by marked thickening of the colonic wall, infiltration of polymorphonuclear leukocytes and ulceration, resembling the human CD (93-95). A number of animal studies have reported the positive effect of COX-2 inhibition and, others, its exacerbation of colitis.

Karmeli *et al*. reported that nimesulide, ameliorates the extent of tissue damage in acetic acid and iodoacetamide-treated rats (96). The decrease induced by nimesulide in the extent of colitis was accompanied by a significant decrease in mucosal (MPO) and NOS activities.

There is good evidence that enhanced formation of reactive oxygen species contributes to the pathophysiology of IBD (97, 98). Quantitatively, the principal free radical in tissues is superoxide anion (O_2^-), which is converted to H_2O_2 by SOD. Superoxide anion can be produced by activated neutrophils through NADPH oxidase, which reduces molecular oxygen to the O_2^- radical through the enzyme MPO. NO, a reactive free radical gas, is generated enzymatically in a variety of cells from the L-arginine pathway by three isoforms of NOs synthetase (99). In the gastrointestinal tract, NO can be either protective or damaging to tissues, depending on what type of NOS is involved in the pathological condition. In experimental colitis, NO derived from inducible NOS, together with other free radicals, contribute significantly to the inflammatory response in the colon. The mechanism for this inflammatory response is likely explained by the interaction of NO with superoxide to produce peroxynitrite, which is a strong oxidizing agent that initiates lipid peroxidation (100). Combination of rofecoxib and aminoguanidine hydrochloride has a protective effect on colonic injury by TNBS which is probably *via* mechanism of local inhibition of iNOS and COX-2 activity in colonic mucosa (101).

Cuzzocrea *et al*. have provided evidence for the potential protective effect of celecoxib in reducing the severity of colonic injury induced by dinitrobenzene sulfonic acid. They observed reduction of the degree of colonic injury, the MPO activity, hemorrhagic diarrhea and weight loss (102). Martin *et al*. have demonstrated that rofecoxib seems to have beneficial effects in TNBS-induced colitis in rats and in acute DSS-induced colitis in mice; probably by diminishing the initial stage of inflammation by a mechanism related to inhibition of PGE 2 by the COX-2 pathway, as well as by reducing neutrophil infiltration and inhibiting up-regulation of IL-1β (103, 104). The use of nimesulide in two different models (acetic acid and LTB4-induced IBD) significantly prevented development of inflammatory changes, reduced MPO activity, and also restored the altered contractility response of the isolated colon segment (105). In addition, El-Medany *et al*. showed that treatment with celecoxib and rofecoxib reduced the inflammation and subsequent tissue damage to the colon induced by acetic acid, as verified by macroscopic, histological and biochemical findings. They demonstrated that these drugs exert a significant attenuation of the extent and severity of the histological signs of cell damage, significant reduction in tissue PGE 2 production, as well as reduction in NOS activity (100).

The acute phase of TNBS colitis is characterized by a significant reduction of capillary blood flow, capillary density, diuresis and weight, and a significant increase in capillary permeability, leucocyte sticking and hematocrit. Kruschewski *et al*. demonstrated that the selective COX-2 inhibitor NS-398 leads to a significant improvement of all microcirculatory parameters and clinical findings compared to untreated colitis (106).

On the other hand, Reuter *et al*. reported that administration of three types of COX-2 inhibitor with moderate to high selectivity significantly exacerbated the severity of colonic damage in experimental colitis. Continued twice-daily administration of these compounds for one week resulted in perforation of the colon, leading to death in a substantial number of rats (107). Lesch *et al*. evaluated three highly selective COX-2 inhibitors (NS-398, SC-58125 and PD-138387) on TNBS-induced colitis and observed that these three compounds did not seem to have any beneficial effect in this model (108). Zhang *et al*. showed that celecoxib resulted in exacerbation of inflammation-associated colonic damage and even led to perforation, megacolon and death of the rats, with the mortality rate reaching 50% (109). Tsubouch *et al*. demonstrated that daily administration of indomethacin and rofecoxib significantly delayed the healing of colitis with deleterious influences on histological pattern, as well as mucosal inflammation (110). Okayama *et al*. showed that celecoxib aggravated the severity of colonic ulceration and inflammation, as represented by gross injury and the shortening of colon length, as well as the MPO activity on DSS-induced colitis (94).

Although lumiracoxib interacts with the COX-2 enzyme *via* mechanisms different from those of other COX-2-

selective inhibitors and is associated with improved gastrointestinal tolerability, Paiotti *et al*. showed this did not reduce inflammation-associated colonic injury in TNBS-induced colitis. They demonstrated that macroscopic and histopathological assessment on untreated TNBS-induced colitis and lumiracoxib-treated induced colitis were similar (111).

Conclusion

The ability of selective COX-2 inhibitors to significantly exacerbate colonic injury in different models of colitis suggests that PGs derived from COX-2 are beneficial in the setting of colonic inflammation. There is a strong body of evidence to suggest that PGs do indeed exert anti-inflammatory and mucosal protective effects in experimental colitis. It is known that PGE 2 inhibits inflammatory cytokines and stimulates mucus secretion in the gastrointestinal mucosa through activation of (EP4) receptors. Nitta *et al*. reported that a selective EP4 agonist reduced the levels of IL-1β and cytokine-induced neutrophil chemoattractant in the colorectal mucosa with marked down-regulation mRNA expression of the corresponding cytokine (113). They also found that the IL-10 concentration was higher following administration of the EP4 agonist. These findings may suggest that endogenous PGE 2 ameliorates the severity of DSS induced colitis, presumably by suppressing the induction of proinflammatory cytokines. PGs are capable of reducing the production of reactive oxygen metabolites and a number of inflammatory mediators suggested to contribute to the pathogenesis of human and experimental colitis, including leukotriene B4 and TNF-α. In addition, PGs increase the secretion of water and electrolytes into the intestinal tract and in the acute stage of UC and CD, activated monocytes promote an increased concentration of PG in the enteric mucosa, which in turn suppresses the effect of the Na^+, Ka^+-ATP enzyme and prevents the reabsorption of Na^+, resulting in diarrhea (112, 113). Some studies demonstrated that pretreatment with intraluminal PGE analogs (*e.g*. 16,16'-dimethyl PGE 2) caused a reduction in the severity of injury induced by TNBS and acetic acid (114-116).

In conclusion, the relative role of COX-2-selective inhibitors on human and experimental colitis remains to be explored. Thus, the use of COX-2 inhibitors in IBD should be considered with caution.

References

1 Botting RM: Vane's discovery of the mechanism of action of aspirin changed our understanding of its clinical pharmacology. Pharmacol Rep *62*: 518-525, 2010.

2 Vane JR, Flower RJ and Botting RM: History of aspirin and its mechanism of action. Stroke *21(Suppl 12)*: IV12-IV23, 1990.

3 Wallace JL: Nonsteroidal anti-inflammatory drugs and gastroenteropathy: the second hundred years. Gastroenterology *1112*: 1000-1016, 1997.

4 Scheiman JM: Unmet needs in non-steroidal antiinflammatory drug-induced upper gastrointestinal diseases. Drugs *66(Suppl 1)*: 15-21, discussion 29-33, 2006.

5 Wolfe MM, Lichtenstein DR and Singh G: Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N Engl J Med *340*: 1888-1899, 1999.

6 Dempke W, Rie C, Grothey A and Schmoll HJ: Cyclooxygenase-2: a novel target for cancer chemotherapy? J Cancer Res Clin Oncol *127(7)*: 411-417, 2001.

7 Barbieri D: Inflammatory bowel diseases. J Pediatr (Rio J) 76(suppl 1): S173-S180, 2000.

8 Podolsky DK: Inflammatory bowel disease. N Engl J Med *347(6)*: 417-429, 2002.

9 Generini S, Fiori G, Matucci CM: Therapy of spondyo-larthropathy in inflammatory bowel disease. Clin Exp Rheumatol *20(6 Suppl 28)*: S88-S94, 2002.

10 Korzenik JR and Podolsky DK: Selective use of selective nonsteroidal anti-inflammatory drugs in inflammatory bowel disease. Clin Gastroenterol Hepatol *4(2)*: 157-159, 2006.

11 Lanas A and Sopena F: Nonsteroidal anti-inflammatory drugs and lower gastrointestinal complications. Gastroenterol Clin North Am *38(2)*: 333-352, 2009.

12 Colpaert S, Liu Z, De Greef B, Rutgeerts P, Ceuppens JL and Geboes K: Effects of anti-tumour necrosis factor, interleukin-10 and antibiotic therapy in the indometacin-induced bowel inflammation rat model. Aliment Pharmacol Ther *15(11)*: 1827-1836, 2001.

13 Shorter RG, Huizenga KA, Spencer RJ and Guy SK: Inflammatory bowel disease. The role of lymphotoxin in the cytotoxicity of lymphocytes for colonic epithelial cells. Am J Dig Dis *17(8)*: 689-696, 1972.

14 Bonen DK and Cho JH: The genetics of inflammatory bowel disease. Gastroenterology *124(2)*: 521-536, 2003.

15 Élson CO, Sartor RB, Tennyson GS and Riddell RH: Experimental models of inflammatory bowel disease. Gastroenterology *109(4)*: 1344-1367, 1995.

16 Gornet JM, Hassani Z, Modiglian R and Lemann M: Exacerbation of Crohn's colitis with severe colonic hemorrhage in a patient on rofecoxib. Am J Gastroenterol *97(12)*: 3209-3210, 2002.

17 Bonner GF, Walczak M, Kitchen L and Bayona M: Tolerance of nonsteroidal antiinflammatory drugs in patients with inflammatory bowel disease. Am J Gastroenterol *95(8)*: 1946-1948, 2000.

18 Bonner GF, Fakhri A and Vennamaneni SR: A long-term cohort study of nonsteroidal anti-inflammatory drug use and disease activity in outpatients with inflammatory bowel disease. Inflamm Bowel Dis *10(6)*: 751-757, 2004.

19 Suenaert P, Bulteel V, Vermeire S, Noman M, Van Assche G and Rutgeerts P: Hyperresponsiveness of the mucosal barrier in Crohn's disease is not tumor necrosis factor-dependent. Inflamm Bowel Dis *11(7)*: 667-673, 2005.

20 Vane JR, Bakhle YS and Botting RM: Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol *38*: 97-120, 1998.

21 DeWitt DL and Smith WL: Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complementary DNA sequence. Proc Natl Acad Sci USA *85(5)*: 1412-1416, 1988.

22 Merlie JP, Fagan D, Mudd J and Needleman P: Isolation and characterization of the complementary DNA for sheep seminal vesicle prostaglandin endoperoxidase synthase (cyclooxygenase). J Biol Chem *263(8)*: 3550-3553, 1988.

23 Yokoyama C, Takai T and Tanabe T: Primary structure of sheep prostaglandin endoperoxidase synthase deduced from cDNA sequence. FEBS Lett *231*: 347-351, 1988.

24 Bakhle YS and Botting RM. Cyclooxygenase-2 and its regulation in inflammation. Mediat Inflamm *5(5)*: 305-323, 1996.

25 Herschman HR: Prostaglandin synthase 2. Biochim Biophys Acta *1299(1)*: 125-140, 1996.

26 Jouzeau J-Y, Terlain B, Abid A, Nédélec E and Netter P: Cyclooxygenase isoenzymes. How recent findings affect thinking about nonsteroidal anti-inflammatory drugs. Drugs *53(4)*: 563-582, 1997.

27 Luong C, Miller A, Barnett J, Chow J, Ramesha C and Browner MF: Flexibility of the NSAID binding site in the structure of human cyclooxygenase-2. Nat Struct Biol *3(11)*: 927-933, 1996.

28 Mancini JA, O'Neill GP, Bayly C and Vickers PJ: Mutation of serine-516 in human prostaglandin G/H synthase-2 to methionine or aspirin cetylation of this residue stimulates 15-R-HETE synthesis. FEBS Lett *342(1)*: 33-37, 1994.

29 Picot D, Loll PJ and Garavito RM: The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1. Nature *367(6460)*: 243-249, 1994.

30 Lecomte M, Laneuville O, Ji C, DeWitt DL and Smith WL: Acetylation of human prostaglandin endoperoxide synthase-2 (cyclooxygenase-2) by aspirin. J Biol Chem *269(18)*: 13207-13015, 1994.

31 Griswold DE and Adams JL: Constitutive cyclooxygenase (COX-1 and inducible cyclooxygenase (COX-2): rationale for selective inhibition and progress to date. Med Res Rev *16(6)*: 181-206, 1996.

32 O'Neill GP, Mancini JA, Kargman S, Yergey J, Kwan MY, Falgueyret JP, Abramovitz M, Kennedy BP, Ouellet M and Cromlish W: Overexpression of human prostaglandin G/H synthase-1 and -2 by recombinant vacinia virus: inhibition by nonsteroidal anti-inflammatory drugs and biosynthesis of 15-hydroxyeicosatetraenoic acid. Mol Pharmacol *45(2)*: 245-254, 1994.

33 Wong E, Bayly C, Waterman HL, Riendeau D and Mancini JA: Conversion of prostaglandin G/H synthase-1 into an enzyme sensitive to PGHS-2 selective inhibitors by a double His513 to Arg and Ile523 to Val mutation. J Biol Chem *272*: 9280-9286, 1997.

34 Gierse JK, McDonald JJ, Hauser SD, Rangwala SH, Koboldt CM and Seibert K: A single amino acid difference between cyclooxygenase-1 and -2 reverses the selectivity of COX-2 specific inhibitors. J Biol Chem *271(26)*: 15810-15814, 1996.

35 Guo Q, Wang L, Ruan K, and Kulmacz RJ: Role of Val509 in time-dependent inhibition of human prostaglandin H synthase-2 cyclooxygenase activity by isoform-selective agents. J Biol Chem *271(32)*: 19134-19139, 1996.

36 Bhattacharyya DK, Lecomte M, Rieke CJ, Garavito M and Smith WL: Involvement of arginine 120, glutamate 524 and tyrosine 355 in the binding of arachidonate and 2-phenylpropionic acid inhibitors to the cyclooxygenase active site of ovine prostaglandin endoperoxide H synthase-1. J Biol Chem *271(4)*: 2179-2184, 1996.

37 Mancini JA, Riendeau D, Falgueyret JP, Vickers PJ and O'Neill GP: Arginini 120 of prostaglandin G/H synthase-1 is required for the inhibition by nonsteroidal anti-inflammatory drugs containing a carboxylic acid moiety. J Biol Chem *270(49)*: 29372-29377, 1995.

38 Ren Y, Loose-Mitchell DS, Kulmacz RJ: Prostaglandin H synthase-1: evaluation of C-terminus function. Arch Biochem Biophys *316(2)*: 751-757, 1995.

39 Ren Y, Walker C, Loose-Mitchell DS, Deng J, Ruan KH and Kulmacz RJ: Topology of prostaglandin H synthase-1 in the endoplasmic reticulum membrane. Arch Biochem Biophys *323(1)*: 205-214, 1995.

40 Hudson N, Balsitis M, Everitt S and Hawkey CJ: Enhanced gastric leukotriene B4 synthesis in patients taking non-steroidal anti-inflammatory drugs. Gut *34(6)*: 742-747, 1993.

41 Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ and Vane JR: Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc Natl Acad Sci USA *90(24)*: 11693-11697, 1993.

42 Morita I, Schindler MS, Regier MK, Otto JC, Hori T, DeWitt DL and Smith WL: Different intracellular locations for prostaglandin endoperoxide H synthase 1 and 2. J Biol Chem *270(18)*: 10902-10908, 1995.

43 Otto JC and Smith WL: The orientation of prostaglandin endoperoxide synthases 1 and 2 in the endoplasmic reticulum. J Biol Chem *269(31)*: 19868-19875, 1994.

44 Regier MK, DeWitt DL, Schindler M and Smith WL: Subcellular localization of prostaglandin endoperoxide synthase-2 in murine 3T3 cells. Arch Biochem Biophys *301(2)*: 439-444, 1993.

45 Gana TJ, Huhlewych R and Koo J: Focal gastric mucosal blood flow in aspirin induced ulceration. Ann Surg *205(4)*: 399-403, 1987.

46 Asako H, Kubes P and Wallace JL: Indomethacin-induced leukocyte adhesion in mesenteric venules: role of lipoxygenase products. Am J Physiol *262*: G903-G908, 1992.

47 Asako H,Kubes P and Wallace JL: Modulation of leukocyte adhesion in rat mesenteric venules by aspirin and salicylate. Gastroenterology *103(1)*: 146-152, 1992.

48 Wallace JL, McKnight W, Miyasaka M, Tamatani T, Paulson J, Anderson DC, Granger DN and Kubes P: Role of endothelial adhesion molecules in NSAID-induced gastric mucosal injury. Am J Physiol *265*: G993-G998, 1993.

49 Thiéfin G and Beaugerie L: Review: Toxic effects of nonsteroidal antiinflammatory drugs on the small bowel, colon and rectum. Joint Bone Spine *72*: 286-294, 2005.

50 Lee M, Aldred K, Lee E and Feldman M: Aspirin-induced acute gastric mucosal injury is a neutrophil-dependent process in rats. Am J Physiol *263(6PT1)*: G920-G926, 1992.

51 Wallace JL, McKnight W, Reuter BK and Vergnolle N: NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. Gastroenterology *119*: 706-714, 2000.

52 Mahmud T, Rafi SS, Scott DL, Wrigglesworth JM and Bjarnason I: Nonsteroidal antiinflammatory drugs and uncoupling of mitochondrial oxidative phosphorylation. Arthritis Rheum *39*: 1998-2003, 1996.

53 Somasundaram S, Sigthorsson G and Simpson RS: Uncoupling of intestinal mitochondrial oxidative phosphorylation and inhibition of cyclooxygenase are required for the development of NSAID-enteropathy in the rat. Aliment Pharmacol Ther *14*: 639-650, 2000.

54 Puspok A, Kiener HP and Oberhuber G: Clinical, endoscopic, and histologic spectrum of nonsteroidal anti-inflammatory drug induced lesions in the colon. Dis Colon Rectum *43(5)*: 685-691, 2000.

55 Bjarnason I, Hayllar J, Macpherson AJ and Russell AS: Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine in humans. Gastroenterology *104(6)*: 1832-1847, 1993.

56 Takeuchi K, Smale S, Premchand P, Maiden L, Sherwood R, Thjodleifsson B, Bjornsson E and Bjarnason I: Prevalence and mechanism of nonsteroidal anti-inflammatory drug-induced clinical relapse in patients with inflammatory bowel disease. Clin Gastroenterol Hepatol *4(2)*: 196-202, 2006.

57 Darling RL, Romero JJ, Dial EJ, Akunda JK, Langenbach R and Lichtenberger LM: The effects of aspirin on gastric mucosal integrity, surface hydrophobicity, and prostaglandin metabolism in cyclooxygenase knockout mice. Gastroenterology *127(1)*: 94-104, 2004.

58 Tibble J, Teahon K, Thjodleifsson B, Roseth A, Sigthorsson G, Bridger S, Foster R, Sherwood R, Fagerhol M and Bjarnason I: A simple method for assessing intestinal inflammation in Crohn's disease. Gut *47(4)*: 506-513, 2000.

59 Roseth AG, Schmidt PN and Fagerhol MK: Correlation between faecal excretion of indium-111-labelled granulocytes and calprotectin, a granulocyte marker protein, in patients with inflammatory bowel disease. Scand J Gastroenterol *34(1)*: 50-54, 1999.

60 Newberry RD, Stenson WF and Lorenz RG: Cyclooxygenase-2-dependent arachidonic acid metabolites are essential modulators of the intestinal immune response to dietary antigen. Nat Med *5(8)*: 900-906, 1999.

61 Evans JM, McMahon AD, Murray FE, McDevitt DG and MacDonald TM: Non-steroidal anti-inflammatory drugs are associated with emergency admission to hospital for colitis due to inflammatory bowel disease. Gut *40(5)*: 619-622, 1997.

62 Felder JB, Korelitz BI, Rajapakse R, Schwarz S, Horatagis AP and Gleim G: Effects of nonsteroidal antiinflammatory drugs on inflammatory bowel disease: a case-control study. Am J Gastroenterol *95(8)*: 1949-19454, 2000.

63 Bonner GF: Exacerbation of inflammatory bowel disease associated with use of celecoxib. Am J Gastroenterol *96(4)*: 1306-1308, 2001.

64 Kafalakes H, Stylianides TJ, Amanakis G and Kolios G: Exacerbation of inflammatory bowel diseases associated with the use of nonsteroidal anti-inflammatpry drugs: myth or reality. Eur J Clin Pharmacol *65*: 963-970, 2009.

65 Meyer AM, Ramzan NN, Heigh RI and Leighton JA: Relapse of inflammatory bowel disease associated with use of nonsteroidal anti-inflammatory drugs. Dig Dis Sci *51(1)*: 168-172, 2006.

66 Carvalho WA, Carvalho RDS and Rios-Santos F: Specific cyclooxygenase-2 inhibitor analgesics: Therapeutic advances. Rev Bras Anestesiol *54(4)*: 448-464, 2004.

67 Panara MR, Renda G, Sciulli MG, Santini G, Di Giamberardino M, Rotondo MT, Tacconelli S, Seta F, Patrono C and Patrignani P: Dose-dependent inhibition of platelet cyclooxygenase-1 and monocyte cyclooxygenase-2 by meloxicam in healthy subjects. J Pharmacol Exp Ther *290(1)*: 276-280, 1999.

68 Fitzgerald GA and Patrono C: The coxibs, selective inhibitors of cyclooxigenase-2. N Engl J Med *345(6)*: 433-442, 2001.

69 Kulkarni SK, Jain NK and Singh A: Cyclooxygenase isoenzymes and newer therapeutic potential for selective COX-2 inhibitors. Methods Find Exp Clin Pharmacol *22(5)*: 291-298, 2000.

70 Silverstein FE, Faich G, Goldstein JL, Simon LS, Pincus T, Whelton A, Makuch R, Eisen G, Agrawal NM, Stenson WF, Burr AM, Zhao WW, Kent JD, Lefkowith JB, Verburg KM and Geis GS: Gastrointestinal toxicity with celecoxib *vs*. nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: a randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. J Am Med Assoc *284(10)*: 12447-12455, 2000.

71 Moore RA, Derry S, Makinson GT and McQuay HJ: Tolerability and adverse events in clinical trials of celecoxib in osteoarthritis and rheumatoid arthritis: systematic review and meta-analysis of information from company clinical trial reports. Arthritis Res Ther *7(3)*: R644-R665, 2005.

72 Singh G, Fort JG, Goldstein JL, Levy RA, Hanrahan PS, Bello AE, Andrade-Ortega L, Wallemark C, Agrawal NM, Eisen GM, Stenson WF, Triadafilopoulos G and SUCCESS-I Investigators: Celecoxib versus naproxen and diclofenac in osteoarthritis patients: SUCCESS-I Study. Am J Med *119(3)*: 255-266, 2006.

73 Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, Day R, Ferraz MB, Hawkey CJ, Hochberg MC, Kvien TK, Schnitzer TJ and VIGOR Study Group: Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. N Engl J Méd *343(21)*: 1520-1528, 2000.

74 Schnitzer TJ, Burmester GR, Mysler E, Hochberg MC, Doherty M, Ehrsam E, Gitton X, Krammer G, Mellein B, Matchaba P, Gimona A, Hawkey CJ and TARGET Study Group: Comparison of lumiracoxib with naproxen and ibuprofen in the Therapeutic Arthritis Research and Gastrointestinal Event Trial (TARGET), reduction in ulcer complications: randomised controlled trial. Lancet *364(9435)*: 665-674, 2004.

75 Skelly MM and Hawkey CJ: Dual COX inhibition and upper gastrointestinal damage. Curr Pharm Des *9(27)*: 2191-2195, 2004.

76 Brune K and Hinz B: Selective cyclooxygenase-2 inhibitors: similarities and differences. Scand J Rheumatol *33(1)*: 1-6, 2004.

77 Mangold JB, Gu H, Rodriguez LC, Bonner J, Dickson J and Rordorf C: Pharmacokinetics and metabolism of lumiracoxib in healthy male subjects. Drug Metab Dispos *32(5)*: 566-571, 2004.

78 Esser R, Berry C, Du Z, Dawson J, Fox A, Fujimoto RA, Haston W, Kimble EF, Koehler J, Peppard J, Quadros E, Quintavalla J, Toscano K, Urban L, van Duzer J, Zhang X, Zhou S and Marshall PJ: Preclinical pharmacology of lumiracoxib: a novel selective inhibitor of cyclooxygenase-2. Br J Pharmacol *144(4)*: 538-550, 2005.

79 Rordorf C, Kellett N, Mair S, Ford M, Milosavljev S, Branson J and Scott G: Gastroduodenal tolerability of lumiracoxib *vs*. placebo and naproxen: a pilot endoscopic study in healthy male subjects. Aliment Pharmacol Ther *18(5)*: 533-541, 2003.

80 Hawkey CJ, Svoboda P, Fiedorowicz-Fabrycy IF, Nasonov EL, Pikhlak EG, Cousin M, Gitton X and Hoexter G: Gastroduodenal safety and tolerability of lumiracoxib compared with ibuprofen and celecoxib in patients with osteoarthritis. J Rheum *31(9)*: 1804-1810, 2004.

81 Kivitz AJ, Nayiager S and Schimansky T: Reduced incidence of gastroduodenal ulcers associated with lumiracoxib compared with ibuprofen in patients with rheumatoid arthritis. Aliment Pharmacol Ther *19(11)*: 1189-1198, 2004.

82 Solomon SD, Mc Murray JJ and Pfeffer MA: Adenoma Prevention with Celecoxib (APC) study Investigators. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. N Engl J Med *352(11)*: 1071-1080, 2005.

83 Lanas A, Garcia-Rodriguez LA and Arroyo MA: Coxibs, NSAIDs, aspirin, PPIs and the risks of upper GI bleeding in common clinical practice. Gastroenterology *128*: 629, 2005.

84 Rostom A, Muir K, Dube C, Lanas A, Jolicoeur E and Tugwell P: Prevention of NSAID-related upper gastrointestinal toxicity: a meta-analysis of traditional NSAIDs with gastroprotection and COX-2 inhibitors. Drug Health Care Pat Saf *1*: 1-25, 2009.

85 Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, Lines C, Riddell R, Morton D, Lanas A, Konstam MA, Baron JA and Adenomatous Polyp Prevention on Vioxx (APPROVe) Trial Investigators: Adenomatous polyp prevention on Vioxx (APPROVe) trial investigators. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med *352(11)*: 1092-1102, 2005.

86 Laine L, Maller ES, Yu C, Quan H and Simon T: Ulcer formation with low-dose enteric-coated aspirin and the effect of COX-2 selective inhibition: a double-blind trial. Gastroenterology *127(2)*: 395-402, 2004.

87 Arber N, Eagle CJ, Spicak J, Rácz I, Dite P, Hajer J, Zavoral M, Lechuga MJ, Gerletti P, Tang J, Rosenstein RB, Macdonald K, Bhadra P, Fowler R, Wittes J, Zauber AG, Solomon SD, Levin and ; PreSAP Trial Investigators: Pre-SAP Trial Investigators. Celecoxib for de prevention of colorectal adenomatous polyps. N Engl J Med *355(9)*: 885-895, 2006.

88 Chan AT, Manson JE, Albert CM, Chae CU, Rexrode KM, Curhan GC, Rimm EB, Willett WC and Fuchs CS: Nonsteroidal antiinflammatory drugs, acetaminophen, and the risk of cardiovascular events. Circulation *113(12)*: 1578-1587, 2006.

89 McHippisley-Cox J and Coupland C: Risk of myocardial infarction in patients taking cyclo-oxygenase-2 inhibitors or conventional non-steroidal anti-inflammatory drugs: population based nested case-control analysis. BMJ *330(7504)*: 1366, 2005.

90 Mc Gettigan P and Henry D: Cardiovascular risk and inhibition of cyclooxygenase: a systematic review of the observational studies of selective and non selective inhibitors of cyclooxygenase 2. J Am Med Assoc *296(13)*: 1633-1644, 2006.

91 Cannon CP, Curtis SP, FitzGerald GA, Krum H, Kaur A, Bolognese JA, Reicin AS, Bombardier C, Weinblatt ME, van der Heijde D, Erdmann E, Laine L and MEDAL Steering Committee: MEDAL Steering Committee. Cardiovascular outcomes with etoricoxib and diclofenac in patients with osteoarthritis and rheumatoid arthritis in the Multinational Etoricoxib and Diclofenac Arthritis Long-term (MEDAL) programme: A randomized comparison. Lancet 18; *368(9549)*: 1771-1781, 2006.

92 Laine L, Curtis SP, Cryer B, Kaur A, Cannon CP and MEDAL Steering Committee: MEDAL Steering Committee. Assessment of upper gastrointestinal safety of etoricoxib and diclofenac in patients with osteoarthritis and rheumatoid arthritis in the Multinational Etoricoxib and Diclofenac Arthritis Long-term (MEDAL) programme: A randomized comparison. Lancet *369(9560)*: 465-473, 2007.

93 Millar AD, Rampton DS, Chander CL, Claxson AW, Blades S, Coumbe A, Panetta J, Morris CJ and Blake DR: Evaluating the antioxidant potential of new treatments for inflammatory bowel disease using a mouse model of colitis. Gut *39(3)*: 407-415, 1996.

94 Okayama M, Hayashi S, Aoi Y, Nishio H, Kato S and Takeuchi K: Aggravation by selective COX-1 and COX-2 inhibitors of dextran sulfate sodium (DSS)-induced colon lesions in rats. Dig Dis Sci *52(9)*: 2095-2103, 2007.

95 Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR and Wallace JL: Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology *96(3)*: 795-803, 1989.

96 Karmeli F, Cohen P, and Rachmilewitz D: Cyclo-oxygenase-2 inhibitors ameliorate the severity of experimental colitis in rats. Eur J Gastroenterol Hepatol *12(2)*: 223-231, 2000.

97 Guo X, Wang WP, Ko JK, and Cho CH: Involvement of neutrophils and free radicals in the potentiating effects of passive cigarette smoking on inflammatory bowel disease in rats. Gastroenterology *117(4)*: 884-892, 1999.

98 Kruidenier L and Verspaget HW: Oxidative stress as a pathogenic factor in inflammatory bowel disease-radicals or ridiculous? Aliment Pharmacol Ther *16(12)*: 1997-2015, 2002.

99 Yue G, Pi-Shiang L and Kingsley Y: Colon epithelial cell death in 2,4,6 trinitrobenzene sulfonic acid induced colitis is associated with increased inducible nitric-oxide synthase expression and peroxynitrite production. J Pharmacol Exp Ther *297*: 915-925, 2001.

100 El-Medany Azza, Mahgoub Afaf, Mustafa ali, Arafa M and Morsi M: The effects of selective cyclooxygenase-2 inhibitors, celecoxib and rofecoxib, on experimental colitis induced by acetic acid in rats. Eur J Pharmacol *507(1-3)*: 291-299, 2005.

101 Dudhgaonkar SP, Tandan SK, Kumar D, Raviprakash V and Kataria M: Influence of simultaneous inhibition of cyclooxygenase-2 and inducible nitric oxide synthase in experimental colitis in rats. Inflammopharmacology *15(5)*: 188-195, 2007.

102 Cuzzocrea S, Mazzon E, Serraino I, Dugo L, Centorrino T, Ciccolo A, Sautebin L and Caputi AP: Celecoxib , a selective cyclooxygenase-2 inhibitor reduces the severity of experimental colitis induced by dinitrobenzene sulphonic acid in rats. Eur J Pharmacol *431(1)*: 91-102, 2001.

103 Martin AR, Villegas I, La-Casa C and 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 *481(2-3)*: 1-10, 2003.

104 Martin AR, Villegas I, and Alarcon de la Lastra C: The COX-2 inhibitor, rofecoxib, ameliorates dextran sulphate sodium induced colitis in mice. Inflamm Res *54(4)*: 145-151, 2005.

105 Singh VP, Patil CS, Jain NK, and Singh A and Kulkarni SK: Effect of nimesulide on acetic acid- and leukotriene-induced inflammatory bowel disease in rats. Prostaglandins Other Lipid Mediat *71(3-4)*: 163-175, 2003.

106 Kruschewski M, Anderson T, Burhr HJ, and Loddenkemper C: Selective COX-2 inhibition reduces leukocyte sticking and improves the microcirculation in TNBS colitis. Dig Dis Sci *51(4)*: 662-670, 2006.

107 Reuter BK, Asfaha S, Buret,A Sharkey KA and Wallace JL: Exacerbation of inflammatory associated colonic injury in rat through inhibition of cyclooxygenase-2. J Clin Invest *98(9)*: 2076-2085, 1996.

108 Lesch CA, Kraus ER, Sanchez B, Gilbertsen R and Guglietta A: Lack of beneficial of COX-2 inhibitors in an experimental model of colitis. Methods Find Exp Clin Pharmacol *21(2)*: 99-104, 1999.

109 Zhang L, Lu YM, and Dong XY: Effects and mechanism of the selective COX-2 inhibitor, celecoxib, on rat colitis induced by trinitrobenzene sulfonic acid. Chin J Dig Dis *5*: 110-114, 2004.

110 Tsubouch R, Hayashi S, Aoi Y, Nishio H, Terashima S, Kato S and Takeuchi K: Healing impairment effect of cyclooxygenase inhibitors on dextran sulfate sodium-induced colitis in rats. Digestion *74(2)*: 91-100, 2006.

111 Paiotti APR, Miszputen SJ, Oshima CTF, de Oliveira Costa H, Ribeiro DA and Franco M: Effect of COX-2 inhibitor after TNBS-induced colitis in wistar rats. J Mol Hist *40(4)*: 317-324, 2009.

112 Kabashima K, Saji T, Murata T, Nagamachi M, Matsuoka T, Segi E, Tsuboi K, Sugimoto Y, Kobayashi T, Miyachi Y, Ichikawa A and Narumiya S: The prostaglandin receptor EP4 suppresses colitis, mucosal damage and CD4 cell activation in the gut. J Clin Invest *109(7)*: 883-893, 1999.

113 Nitta M, Hirata I, Toshina K, Murano M, Maemura K, Hamamoto N, Sasaki S, Yamauchi H, and Katsu K: Expression of the EP4 prostaglandin E2 receptor subtype with rat dextran sodium sulphate colitis: colitis suppression by a selective agonist, ONO-AE1-329. Scand J Immunol *56*: 66-75, 2002.

114 Feng L, Sun W, Xia Y, Tang WW, Chanmugam P, Soyoola E, Wilson CB and Hwang D: Cloning two isoforms of rat cyclooxygenase: Differential regulation of their expression. Arch Biochem Biophys *307(2)*: 361-368, 1993.

115 Sasaki S, Hirata I, Maemura K, Hamamoto N, Murano M, Toshina K and Katsu K: Prostaglandin E2 inhibits lesion formation in dextran sodium sulphate-induced colitis in rats and reduces the levels of mucosal inflammatory cytokines. Scand J Immunol *51*: 23-28, 2000.

116 Tso JY, Sun X-H, Kao T-H, Reece KS and Wu R: Isolation and characterization of rat and human glyceraldehyde-3-phosphate dehydrogenase cDNAs: Genomic complexity and molecular evolution of the gene. Nuclei Acids Res *13*: 2485-2502, 1995.

Received February 15, 2012
Revised March 12, 2012
Accepted March 12, 2012