REVIEW

Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs

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Dual 5-LOX/COX inhibitors are potential new drugs to treat inflammation. They act by blocking the formation of both prostaglandins and leucotrienes but do not affect lipoxin formation. Such combined inhibition avoids some of the disadvantages of selective COX-2 inhibitors and spares the gatrointestinal mucosa.

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Inflammation is a complex process occurring through a variety of mechanisms, leading to changes in local blood flow and the release of several mediators. These mediators account for local effects at the site of inflammation—that is, vasodilatation, increased vascular permeability, and migration of leucocytes into the affected area, and for general phenomena in the vascular systems of the body, including the cardiovascular system and the renal apparatus.

> "Leucotrienes have a major role in the inflammatory process"

The mediators arising from the cyclo-oxygenase (COX) cascade and the role of biologically active prostaglandins in the inflammatory process and body homoeostasis have been extensively studied. In addition, there is a growing body of evidence that the complex pathway of arachidonic acid metabolism in inflammation involves a variety of mediators other than the COX, all of which have a role in the overall process. Leucotrienes, which are the second main family of arachidonic acid derivatives, are synthesised from the activity of 5-lipoxygenase (5-LOX) and have a major role in the inflammatory process (fig 1).

The discovery of two different COX isoforms, COX-1 and COX-2, and evidence that arachidonic acid derivatives other than those formed in the classical COX pathway (for example, leucotrienes and lipoxins) exhibit or modulate inflammatory properties, has contributed to a better understanding of the cell mediators and effects triggered during an inflammatory response. As a consequence, there has been a drive for a better understanding of these pathways and development of new drugs to intervene in them. Hence, new dual 5-LOX/COX inhibitors are now being studied as potential new drugs, post-COX-2 selective inhibitors, to treat the inflammatory processes.

THE SYNERGISTIC FUNCTIONS OF COX-1 AND COX-2

Cyclo-oxygenase converts arachidonic acid to prostaglandin H_2 (PGH_2) which, in turn, is transformed into a series of final active products in different cell types and with different expressions. For example, thromboxane A_2 (TXA_2) is a potent vasoconstrictor and a stimulator of platelet aggregation, prostacyclin (PGI_2) is expressed in the vascular endothelium and inhibits platelet aggregation, PGE_2 and PGI_2 are potent vasodilators which account for the increased blood flow in inflamed areas.[1] On the other hand, the vasodilating properties of PGE_2 and PGI_2 contribute to increasing mucus secretion and reducing acidity and pepsin content in the stomach, thus protecting the integrity of the gastric mucosa.[2] In the kidneys, PGE_2 and PGI_2 increase blood flow in response to factors inducing vasoconstriction and contribute to modulating the glomerular filtration rate in the event of volume depletion.[3]

Two COX isoforms, COX-1 and COX-2, have been identified. They are encoded by two different genes,[4] and it has been postulated that while COX-1 is expressed in mammalian cells, particularly in endothelium, platelets, and kidneys in physiological conditions, COX-2 is inducible in pathological conditions by inflammatory stimulation.[5 6] It has, therefore, been suggested that constitutive COX-1 is involved in homoeostatic processes, whereas COX-2 is the isoform that plays a major part in the inflammatory process and the pain associated with it. On the basis of this assumption, selective COX-2 inhibitors were developed; these were intended to have the anti-inflammatory properties of classical non-steroidal anti-inflammatory drugs (NSAIDs) but without altering renal function and without affecting the integrity of the gastric mucosa. Nevertheless, there is accumulating evidence that COX-1 and COX-2 have overlapping actions and that both isoforms are involved in homoeostasis processes, just as both are modulators of inflammatory reactions.

Recent findings have shown that COX-2 is constitutively expressed in some tissues, including the brain and kidneys.[7 8] In addition, COX-2

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Abbreviations: AUC, area under the curve; COX, cyclo-oxygenase; FLAP, 5-lipoxygenase activating-protein; GI, gastrointestinal; IL, interleukin; 5-LOX, 5-lipoxygenase; LT, leucotriene; LX, lipoxin; NO, nitric oxide; NSAIDs, non-steroidal anti-inflammatory drugs; PG, prostaglandin; PMN, polymorphonuclear cells; TXA$_2$, thromboxane A$_2$
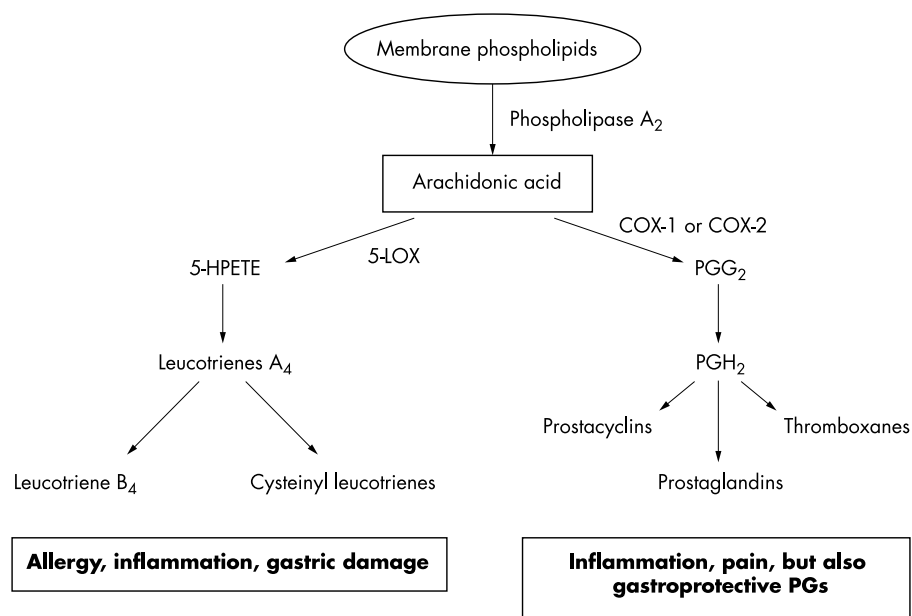
Figure 1 Products and enzymes of arachidonic acid metabolism involved in the inflammatory process.

exhibits a protective role in asthma[9] and in bronchoconstriction of aspirin sensitive subjects,[10] thereby accounting in part for the asthmogenic effects of a variety of NSAIDs in susceptible patients. COX-2 may also play an important part in the homoeostasis of other functions and body areas, including the kidneys, gastric tissue repair, cardiovascular system, and bone repair.[11–16] COX-2 is also involved in the process of ovulation, implantation, and neonatal development[17 18]; it is particularly essential for normal renal development.

The kidneys exhibit abundant expression of both COX-1 and COX-2. Whereas COX-1 is primarily expressed in the vascular smooth muscle and collecting ducts in the kidneys, COX-2 is predominantly expressed in the macula densa, in the interstitial cells of the medulla, and in the cortical thick ascending limb.[19] While COX-1 expression does not exhibit dynamic regulation, COX-2 derived prostanoids mediate renin release in the macula densa and are involved in tubular control of sodium, potassium, and water excretion.[20] At the renal level, COX-2 activity leads to the synthesis of renal prostaglandin I_2 (PGI_2) and PGE_2. These two PGs have been shown to influence vascular homoeostasis and the regulation of normal blood flow and glomerular filtration rate.[13 21] Finally, an important and debated argument about the potential weaknesses of selective COX-2 blockage is that reducing the synthesis of PGI_2, which causes vasodilation and inhibits platelet aggregation, leaves the production of thromboxane A_2 (TXA_2) which causes vasoconstriction and stimulates platelet aggregation.[22] Both are vital for the normal functioning of the cardiovascular system.

The effect of COX-2 inhibitors on the incidence of vascular diseases requires scrutiny, particularly considering that COX-2 is a major source of PGI_2 biosynthesis in humans.[22] COX-2 specific inhibitors could potentially increase the thrombotic risk, because they block the production of PGI_2, a potent anti-aggregating agent, but, as they do not inhibit the COX-1, the only isoform expressed in platelets, they do not block the formation of TXA_2 released from platelets, favouring a prothrombotic state. Further studies will be necessary to evaluate whether these compounds are contraindicated in patients at risk for cardiovascular events.[23–25]

Conversely, the role of COX-1 is not only physiological. In particular, it has been noted that the anti-inflammatory effects of selective COX-2 inhibitors cannot be seen if the dose is not increased above levels which also inhibit COX-1

activity,[26] suggesting that this latter isoform has a significant role in the synthesis of proinflammatory PGs. In addition, data have been produced suggesting that COX-1 may be induced at the site of inflammation[27 28] and, in fact, mice lacking the gene for COX-1 exhibited diminished inflammatory responses in comparison with wild-type controls. All these pieces of evidence explain why the simple concept that only COX-2 is involved in inflammatory processes and that only COX-1 contributes to homoeostatic processes needs to be revised.

The functions, physiological and/or pathological, of the two COXs can be divided into functions that depend solely or primarily on COX-1, functions that depend solely or primarily on COX-2, and functions in which both COXs are involved (table 1). The precise contributions of the two isoforms, in particular those of the two COX genes, to various pathophysiological processes was difficult to assess, but studies performed in animals suggest that there are processes in which each isoenzyme is uniquely involved (for example, platelet aggregation for COX-1, ovulation for COX-2) and others in which both isoenzymes function together (for example, carcinogenesis, inflammation). As COX-2 is induced during the resolution of an inflammatory response, it then produces anti-inflammatory, not proinflammatory PGs.[29] Indeed, cellular infiltration and oedema are present for longer in COX-2 deficient mice than in wild-type mice,[30] indicating that during the resolution phase of inflammation COX-2 may have a more significant role. There are also physiological events in which one COX normally functions but for which the other can compensate when the first is lacking (for example, parturition and remodelling of ductus arteriosus).[30] The mechanisms by which compensatory responses normalise prostanoid biosynthesis remain to be elucidated.

EFFECTS OF LEUCOTRIENES IN INFLAMMATION

Leucotrienes (LTs) play a major part in the inflammatory process (fig 2).[31] They are synthesised via the 5-LOX. However, this enzyme requires the presence in intact cells of the 5-LOX activating-protein (FLAP). Indeed, the activity of 5-LOX is stimulated by calcium in intact cells, whereas this is not required in in vitro experiments with broken cells.[32] Likewise, although the drug MK-866 inhibits 5-LOX in intact human leucocytes, it failed to inhibit its activity in broken cells.[32] This was believed to be related to the ability of MK-866 to block

Table 1 Specific physiological/pathological functions of COX-1 and COX-2

Physiological/pathological process	COX-1	COX-2	PGs involved
Ovulation	Not essential	Essential	PGE_2
Implantation	Not essential	Essential	PGI_2
Parturition	Essential	Compensatory	$PGF_{2\alpha}$
Inflammatory signs	Essential in some kind of inflammation	Essential	Not specified
Inflammation resolution	Not essential	Essential	PGD_2, 15-deoxy-PGI_2
Platelet aggregation	Essential	No role	TXA_2
Perinatal kidney development	Not essential	Essential	Not specified
Kidney function	Essential	Essential	PGE_2
Ductus arteriosus remodelling	Compensatory	Essential	TXA_2/ PGH_2
T cell development	Stage specific	Stage specific	PGE_2
GI mucosa protection	Both essential under inflammatory condition		Several PGs
Gastric ulceration	Inhibition of both isoforms necessary		Several PGs
Ulcer healing	Not essential	Essential	PGE_2
Intestinal cancer	Essential	Essential	Several PGs
Crypt stem cell survival	Essential	Compensatory	PGE_2

membrane association of 5-LOX.[33] A radiolabelled analogue of MK-866 in neutrophils was used to identify an 18 kDa protein,[34] and complementary DNAs, isolated from rat and human, of an encoded novel protein, FLAP. This protein, FLAP, has three membrane spanning regions and two hydrophilic loops. Both 5-LOX and FLAP are required for LT production in cells stimulated by the ionophore A23187 (without exogenous arachidonic acid addition).[35]

"Inhibition of COX may lead to a shunt of the arachidonic metabolism towards the leucotriene pathway"

The final and biologically active metabolites of the 5-LOX cascade are LTB_4 and the so-called cysteinyl LTs (LTC_4, LTD_4, and LTE_4), formerly known as a slow reacting substance related to anaphylaxis, which are derived from the unstable intermediate LTA_4.[36] Leucotrienes are potent mediators of inflammation; table 2 summarises their specific functions. The target of the biological effects of LTB_4 has been found to be primarily inflammatory cells: LTB_4 is a potent stimulator of leucocyte activation, and adhesion of these cells to vascular endothelium, elicits chemokinetic and chemotactic responses.[37] During brief exposure to LTB_4, polymorphonuclear leucocytes (PMN) are predominantly recruited,[37] whereas during prolonged exposure, as probably occurs when LTB_4 is formed in vivo, other granulocytes, including neutrophils and eosinophils, are found in tissues and exudates.[38 39] Furthermore, LTB_4 has been shown to be involved in the pathogenesis of a variety of inflammatory diseases.[40] It has been observed that this LT stimulates the production and release of proinflammatory cytokines from macrophages and lymphocytes, and, recently, from synovial membrane.[41 42]

Cysteinyl LTs (LTC_4, LTD_4, and LTE_4) have long been known to be involved in the pathogenesis of bronchial asthma,[36] but their role in other inflammatory conditions has always been ignored. However, their proinflammatory effects in conditions other than asthma have recently been reconsidered.[43] It has been shown that neutrophil activation causes synthesis of a significant proportion of LTC_4 from the intermediate metabolite LTA_4.[44] Additionally, the derived cysteinyl LTs can induce significant functional and morphological changes, consisting of endothelial cell contraction leading to oedema and smooth muscle contraction, as shown in isolated heart in animal models.[45 46] Furthermore, experimental trials have demonstrated that cysteinyl LTs may participate in the damage of gastric mucosa[47] by inducing mucosal microvascular injury and gastric vessel vasoconstriction, promoting breakdown of the mucosal barrier and stimulating the secretion of gastric
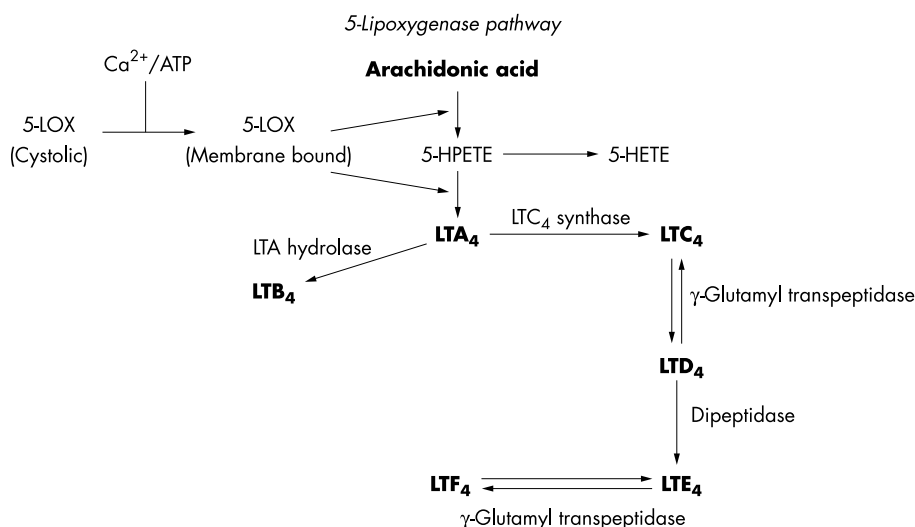

Figure 2 Products and enzymes of the 5-LOX pathway.

Table 2 Specific functions of leucotrienes

Leucotriene	Function	Organ/cell
LTB$_4$	Chemotaxis	Neutrophils, eosinophils, lymphocytes, monocytes
	Aggregation	Neutrophils, eosinophils
	Recruitment, migration, adhesion	Granulocytes
	Degranulation with superoxide generation	Granulocytes
	Increase cytokine production and release	Granulocytes
	Increase cytotoxicity and cytokine production (IL5, IL6, IL8)	T cells, macrophages?
	Inhibition of transformation and secretion	T cells
	Increase of proliferation, activation, and immunoglobulin production	B cells
	Increase of permeability	Microvasculature
	Hyperalgesia	Afferent nerves
	Role in extracellular matrix synthesis	Bone tissue
	Increase bone resorption	Bone tissue
LTC$_4$, LTD$_4$, LTE$_4$	Contraction	Smooth muscle, coronary arteries distal segment of the pulmonary arteries, mesenteric vasculature, gastrointestinal muscle, bronchi
	Mucus secretion and oedema	Bronchi
	Increase permeability	Microvasculature
	Recruitment of inflammatory cells	Lymphocytes, eosinophils
	Stimulation of secretion of glycoproteins	Lung
	Airway remodelling in chronic allergic inflammation	Lung

LTC$_4$, LTD$_4$ are biologically equipotent, while LTE$_4$ is less active.

acid, as well as the production of interleukin 1 (IL1) and proinflammatory cytokines.[48] As confirmation of these findings, inhibition of neutrophil adhesion through modulation of nitric oxide (NO) formation[48] or through monoclonal antibodies[49] reduced the synthesis of cysteinyl LTs and prevented the effects on vascular tone and permeability induced by neutrophil activation.

Overall, these studies demonstrate that besides LTB$_4$, cysteinyl LTs induced by activation of neutrophilic 5-LOX may be responsible for vascular permeability changes occurring during acute inflammation and may play a part in NSAID induced gastrointestinal (GI) damage. Importantly, because the inhibition of COX may lead to a shunt of the arachidonic acid metabolism towards the 5-LOX pathway, treatment with NSAIDs may increase the formation of LTs, which can induce gastric damage and ulceration.[50–53] Such a shunt has been shown in vivo and an enhanced synthesis of LTB$_4$ was demonstrated in patients with rheumatoid arthritis or osteoarthritis taking NSAIDs for >3 months.[53] The authors also concluded that this increase is strongly associated with type C gastritis.

EMERGING ROLE OF LIPOXINS

Lipoxins (lipoxygenase interaction products (LXs)) are yet another group of lipid mediators formed during arachidonic acid metabolism. They are generated during the cellular interactions that occur as part of the multicellular host response to inflammation. Lipoxins are formed by transcellular metabolism from an intermediate derivative (5(6)-epoxytetraene), which gives rise to the metabolically active products, LXA$_4$, LXB$_4$, or 15(R)-HETE for 15-epi-LXs (aspirin triggered LXs) (fig 3). They are synthesised not only through the 5-LOX pathway, but also by the action of two other enzymes, 12-LOX and 15-LOX.[55–57]

In LX formation, 15-LOX is a pivotal enzyme that initiates LX biosynthesis and converts the LT intermediate LTA$_4$ to LX.[58] 15-LOX is positively controlled by PGE$_2$. Indeed, Levy et al demonstrated that preincubation of PMN with PGE$_2$ switched the predominant LO activity of PMN from 5-LOX to 15-LOX after five hours. Moreover they showed that this switch is under the regulation of 15-LOX gene expression, meaning that 15-LOX is also an inducible enzyme.[59] Although it has been
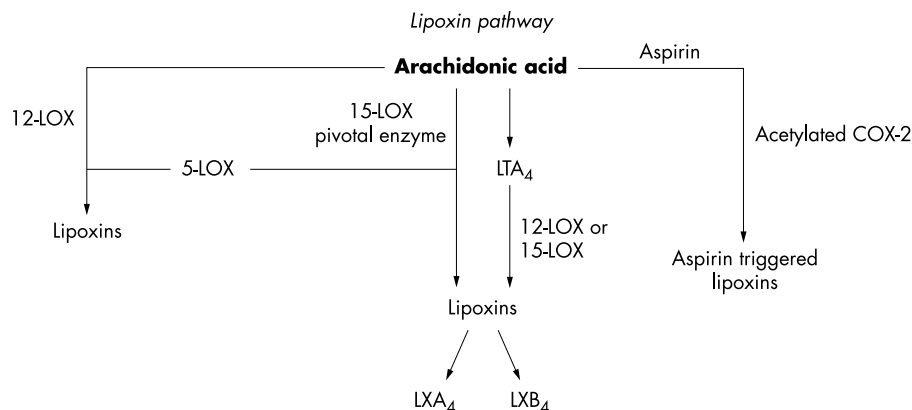

Figure 3 Products and enzymes of the LX pathway.

Table 3 Actions of lipoxins on human leucocytes

Lipoxin	Leucocytes	Action
LXA_4	PMN	Blocking of emigration, transmission, and chemotaxis
		Down regulation of CD 11/18, IP_3, Ca^{2+}
		Inhibition of PMN-endothelial cells and epithelial cell interactions
	Eosinophils	Inhibition of chemotaxis (PAF, FMLP)
	NK cells	Inhibition of cytotoxicity
	Monocytes	Stimulation of chemotaxis and adherence
		Stimulation of myeloid progenitors
LXB_4	PMN	Blocking of emigration, transmission, and chemotaxis
		Down regulation of CD 11/18, IP_3, Ca^{2+}
		Inhibition of PMN-endothelial cells and epithelial cell interactions
	NK cells	Inhibition of cytotoxicity
	Monocytes	Stimulation of chemotaxis and adherence
		Stimulation of myeloid progenitors

previously shown that LXA_4 can share binding to the LTD_4 receptor, a seven transmembrane G protein coupled LXA_4 receptor has recently been cloned from a human colonic adenocarcinoma cell line.[60] This receptor has also been identified in synovial fibroblasts, suggesting a potential local immunoregulatory role in those cells.[61]

LXs can be considered as stop-signal mediators, which possess anti-inflammatory effects: in particular, LXA_4 and LXB_4 have an inhibitory action on LT mediated effects in inflammation by reducing the production and chemotaxis of granulocytes and by stimulating monocyte activation. 15-Epi-LXA_4 and 15-epi-LXB_4 are formed after the administration of aspirin because the aspirin induced COX inhibition shunts arachidonic acid metabolism to form an LX intermediate. These LXs are potent blockers of neutrophil function and cell proliferation.[61 62] Table 3 summarises the actions of LXs on human leucocytes. Importantly, the inhibitory actions of aspirin triggered LXs are dependent on both tissue and the delivery site and can produce their anti-inflammatory effect at sites distant from the point of delivery.[63]

Based on this new information on the actions of LXs, it is reasonable to presume that their production can counteract the inflammatory effects of LTs and that the induction of aspirin triggered 15-epi-LX should provide additional anti-inflammatory benefits.

DUAL INHIBITORS: RATIONALE FOR USE
Both the conventional NSAIDs and the selective COX-2 inhibitors primarily exert their activity by reducing the production of PGs induced in the inflammatory process. In recent years, it has been clarified that PG synthesis is only one part of the arachidonic acid pathway, this precursor being a substrate that gives rise to many other lipid mediators, such as the LTs and the LXs. Leucotrienes themselves have a major role in the development and persistence of the inflammatory process, and it is now clear that PGs and LTs have complementary effects, whereas the production of LXs can counteract the inflammatory actions of LTs.

In view of these concepts, it has been suggested that blocking both LT and PG production might have synergistic effects and achieve optimal anti-inflammatory activity. In addition, taking into account the roles of LTB_4 and cysteinyl LTs (against which neither selective nor non-selective NSAIDs are effective) in the inflammatory process, dual inhibition of the COX and 5-LOX pathways could produce a wider spectrum of anti-inflammatory effects (fig 4).

Dual inhibition of COX and 5-LOX may limit the vascular changes seen during inflammation and leucocyte induced GI damage.

It has been suggested that cysteinyl LTs, by inducing local vasoconstriction and thus reducing blood flow, can enhance the susceptibility of gastric mucosa to injury,[64] whereas LTB_4 can potentiate this damaging process by its effects on leucocyte infiltration.[65] However, it can be expected that blocking both pathways would limit the potential for COX-1/COX-2 inhibitors, NSAIDs, to produce an excess of LTs in a shunt process.[51–54] This would thereby exert a protective effect on GI mucosa. Moreover, a shunt effect has also been demonstrated in vitro on human osteoblasts from the subchondral bone. Indeed long term treatment of cells with a COX-2 specific inhibitor (NS-398, 10 μM) increased the level of LTB_4 fourfold. Interestingly, the PGE_2 levels were no more reduced than after a short treatment.[66] The concentration of NS-398 is well within the doses used in in vivo,[16 67 68] organ culture,[69] or in vitro experiments,[70 71] suggesting that within the physiological doses of COX-2 inhibitors the shunt observed with osteoblasts in vitro could be observed in vivo.

The biological properties of LTs, together with their formation in a variety of diseases, suggest that 5-LOX inhibitors should have a therapeutic potential in a range of allergic and inflammatory conditions, such as asthma, rheumatoid arthritis, ulcerative colitis, etc. However, the search for selective 5-LOX inhibitors has been on the whole rather disappointing,[72 73] because of their toxicity or lack of effect in vivo. Zileuton, an orally active 5-LOX inhibitor, significantly reduces allergen induced nasal congestion; it selectively blocks LT release in the nasal lavage fluids of patients with allergic rhinitis after challenge with specific allergens,[74] but in other studies in rheumatoid arthritis and in ulcerative colitis, the administration of this drug did not differ statistically from placebo.[75–77]

5-LOX/COX blockers have an excellent preclinical GI safety profile"

All these studies seem to indicate that the use of 5-LOX inhibitors might represent an insufficient single therapeutic model in inflammatory diseases other than asthma. Thus, the discovery of compounds that can inhibit both the main metabolic pathways of the arachidonic acid metabolism is worthy of interest. Moreover, dual inhibition of 5-LOX/COX does not block the 12-LOX and 15-LOX pathways, which contribute to the synthesis of metabolically active LXs; thus, the non-inhibited production of LXA_4, LXB_4, and 15-epi-LXs, continues to attenuate any remaining LT effects (fig 4).

In the past few decades, several compounds have been developed to block both COX and 5-LOX, but their use was abandoned owing to liver toxicity.[78] This side effect, however, was not ascribed to their pharmacological mode of action. However, common molecular features of these substances, either redox active features or the presence of the di-t-butyl moiety or hydroxamic acid, could be responsible for this side effect.

Prototype experimental dual inhibitors (such as BW 755C or SF&F 86002) have proved effective in preventing the production of both PGs and LTs and the consequent inhibition of migration and activation of inflammatory cells (mainly PMN and monocyte macrophages) into inflamed sites. Importantly, the inhibition of migration of inflammatory cells towards the affected sites has translated into a reduction of tissue damaging or necrosis in a model of tissue damage and foreign body rejection.[79] In this model subcutaneously implanted polyester sponges in rats were rejected after a mean of 12 days under basal conditions. Indometacin did not
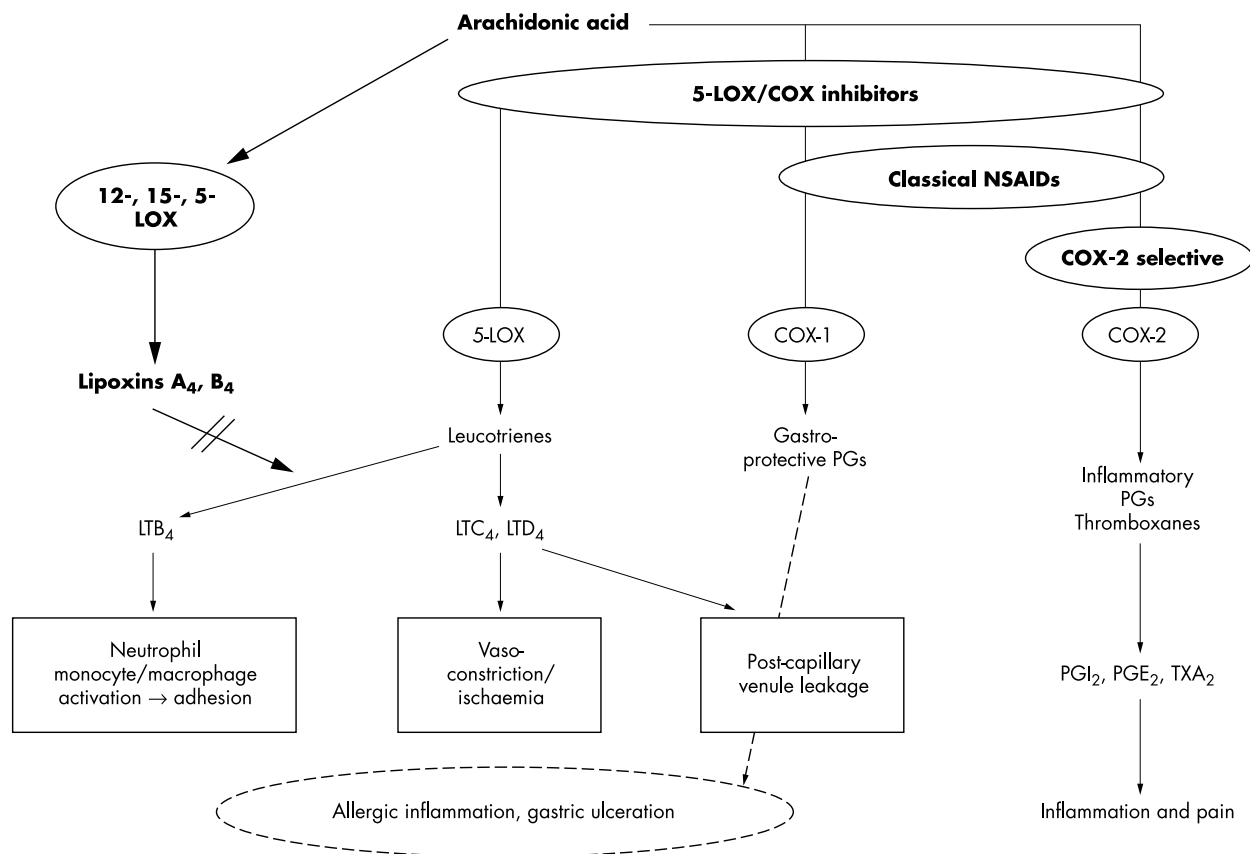
Figure 4 Inhibition of the different classes of anti-inflammatory drugs.

significantly change the time to rejection, but BW 755C prolonged rejection to a mean of 22 days as also did dexamethasone. No effects on cell migration have been observed with conventional doses of COX inhibitors.[80] An interesting activity profile has been also noted for ER-34122, an orally administered dual inhibitor. This compound had a more pronounced anti-inflammatory effect than indometacin at the usual pharmacological doses (0.3–3 mg/kg) in a model of arachidonic acid induced ear inflammation because, in addition to its COX inhibitory activity, it inhibited LOX product generation, as shown by the inhibition of oedema formation or PMN leucocyte infiltration.[81] ER-34122 also has anti-inflammatory activity in the early stage of spontaneous arthritis in MRL/MpJ-lpr/lpr mice (a mouse strain that develops a generalised autoimmune disease that is similar to human systemic lupus erythematosus). The compound suppresses progression of PMN infiltration, subsynovial soft tissue oedema, and multiplication of synovial lining cells in the early stage of arthritis, whereas indometacin (1 mg/kg) has no effect.[82] The effect of ER-34122 in this animal model may be due to the inhibition of LT production, and in fact arachidonic acid metabolites, including LTs, may be important in the pathogenesis of autoimmune disease in this mouse strain.[83]

Tepoxalin, an inhibitor of COX and 5-LOX, has a potent anti-inflammatory activity with an excellent gastric tolerance pattern in animal models treated for long periods at doses higher than those required to produce anti-inflammatory effects.[84] Pretreatment with this compound also prevented the GI side effects induced by normally gastrotoxic doses of indometacin.[85] Some studies in humans demonstrated that this compound inhibits COX and LOX after oral administration,[86] and that single doses up to 800 mg and multiple doses up to 400 mg are well tolerated.[87] Licofelone (formerly known as ML3000) is a novel dual 5-LOX/COX

inhibitor today in phase III clinical development that effectively inhibits leucocyte transcellular metabolism and adhesion, as well as the synthesis of cysteinyl LTs.[88] This compound is a pyrrolizine derivative and an arachidonic acid substrate analogue that inhibits both enzymes. This compound has shown a good pattern of tissue distribution, with highest tested levels being reached in the lung, liver, kidneys, heart, and large and small intestine.[89] Moreover, this compound did not show hepatotoxicity either in preclinical or clinical studies.[89–91]

In animal models, licofelone exhibits anti-inflammatory, analgesic, and antipyretic properties at a dosage that causes no GI damage.[92] The anti-inflammatory activity has been mainly studied in the rat model of adjuvant arthritis. This model showed that the reduction of the experimentally induced secondary lesions and paw swelling obtained with licofelone was similar to that seen with indometacin[89] and that the compound can reduce synovial cell proliferation, histological damage, and joint erosions in an affected ankle joint.[93] The effects of licofelone on experimentally induced osteoarthritic cartilage lesions were also studied in a dog model (osteoarthritis was induced by anterior cruciate ligament section); compared with placebo licofelone significantly reduced the severity of erosions and histological damage[94] by exerting an inhibitory effect on the production of collagenase-1 in cartilage, IL1β in synovial membrane, LTB₄ in synovium, and PGE₂ in synovial fluid. Licofelone was also shown in this model to be, in vivo, effective in reducing the level of chondrocyte death. This effect is likely mediated by a decrease in the level of caspase-3 activity, which may be related to the reduced production of two major factors involved in chondrocyte apoptosis, NO and PGE₂.[95 96]

The gastric tolerance of licofelone given in single and repeated doses has been compared with that of indometacin[97 98] and with that of diclofenac[91] in animals. The

results showed that the drug generates minimal GI damage and is significantly better than ulcerogenic reference drugs. Unlike the comparator drugs, licofelone significantly reduced LTB_4 levels in the paw and did not induce leucocyte adherence,[98] thereby suggesting that inhibition of the 5-LOX pathway may explain the gastric sparing effects of dual inhibitors. Licofelone has also been directly compared with celecoxib in an assessment of the effects of these two drugs on the gastric mucosa in aspirin treated rats.[99] Celecoxib, but not licofelone, increased the gastric mucosal damage when given to aspirin treated rats and caused a marked increase of aspirin induced myeloperoxidase activity. It therefore seems that the effectors synthesised by the 5-LOX pathway provide a sustained contributory effect to gastric injury.

In summary, the 5-LOX/COX blockers have an excellent preclinical GI pharmacological safety profile. Moreover, recent findings in phase II and III studies of the clinical development of licofelone indicate that this drug also has an excellent GI profile, which was better than conventional NSAIDs such as naproxen and equivalent to specific COX-2 inhibitors. Tolerability was studied in healthy volunteers after 200 or 400 mg twice a day for four weeks in comparison with naproxen 500 mg twice a day: the gastric mucosa was completely normal in 93% (200 mg twice a day), 89% (400 mg twice a day), 90% (placebo), 37% (naproxen) of subjects.[74 100] In a study of osteoarthritic patients treated for 12 weeks with licofelone 200 mg twice a day or with naproxen 500 mg twice a day, licofelone showed a comparable efficacy to conventional NSAIDs but with excellent GI and general tolerability. No ulcers were present in either the licofelone or placebo group, while in the naproxen group ulcers occurred in 20%. GI adverse events were reported by 13% (licofelone) and by 26.3% (naproxen) of patients.[101] The combined inhibition of both COXs and of 5-LOX avoids some disadvantages of selective COX-2 inhibitors (thromboembolic risk), but spares also the GI mucosa.[101] A study of the pharmacokinetics, safety, and tolerability of licofelone (200 mg twice a day for five days with a final dose of 200 mg in the morning of day 6 after a standardised meal) was performed in young and elderly healthy volunteers.[102] Maximum plasma concentration was achieved 0.74–4 hours after the dose. After the first dose, Cmax (mean (SD)) was essentially identical for young (1665 (1151) ng/ml) and elderly (1637 (903) ng/ml) subjects. The mean area under the curve (AUC; 0–12) was about 23% higher in elderly than in subjects (5646 (2073) v 4582 (1927) ng.h/ml). Pharmacokinetic analysis in the steady state also demonstrated a similarity of Cmax,ss (young 1727 (829) ng/ml, elderly 1744 (616) ng/ml) and a 20% higher AUC in elderly subjects than in young subjects. A 20% higher value for the AUC in the elderly is not expected to be clinically significant in light of the minimal accumulation upon twice a day dosing. Licofelone was well tolerated in both study groups.

CONCLUSIONS

The pharmacological properties and unwanted side effects (GI ulcerogenic activity and bronchoconstriction) of classical NSAIDs and aspirin-like products which act, respectively, through non-reversible and reversible inhibition of COX activity, and the conversion of arachidonic acid into biologically active PGs and TXs, have been well established.[103 104]

Classical NSAIDs and selective COX-2 inhibitors block the cascade originating from arachidonic acid that leads to the production of PGs. Besides their inflammatory properties, PGs generated by COX-2 are also involved in several physiological functions, such as protecting the integrity of the gastric mucosa, homoeostasis of renal blood flow, and anti-aggregation of platelets. Consequently, the inhibition of COX-2 produces a lower rate of GI adverse effects, but may impair gastric ulcer healing and may result in altered glomerular function and platelet properties. Furthermore, COX inhibition probably shunts arachidonic acid metabolism towards an excess production of LTs.

The proinflammatory role of LTB_4 and cysteinyl LTs, their chemotactic action, and their recruitment of inflammatory cells have recently been elucidated. Furthermore, it has been shown that metabolically active LXs, also derived from arachidonic acid metabolism, have anti-inflammatory properties; 5-LOX blockage does not impair the synthesis of LXs, which are the products of the activation of other enzymatic pathways.

The dual 5-LOX/COX inhibitors act by blocking the formation of both PGs and LTs without affecting LX formation. The sparing effects on the gastric mucosa are probably due to the inhibition of the synthesis of 5-LOX products. It can, therefore, be expected that dual blockers induce an enhanced anti-inflammatory effect without damaging the GI mucosa.

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