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Commentary

Dual inhibitors of cyclooxygenase and 5-lipoxygenase. A new avenue in anti-inflammatory therapy?

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Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a mainstay in the treatment of inflammatory disease and are among the most widely used drugs worldwide. They are anti-inflammatory, antipyretic, and analgesic and are prescribed as first choice for the treatment of rheumatic disorders and, in general, inflammation. The main limitation in using NSAIDs consists in their side-effects, including gastrointestinal ulcerogenic activity and bronchospasm. The mechanism of action of these drugs is attributed to the inhibition of cyclooxygenase (COX), and, consequently, the conversion of arachidonic acid into prostaglandins. It is hypothesized that the undesirable side-effects of NSAIDs are due to the inhibition of COX-1 (constitutive isoform), whereas the beneficial effects are related to the inhibition of COX-2 (inducible isoform). Arachidonic acid can also be converted to leukotrienes (LTs) by the action of 5-lipoxygenase (5-LOX). LTC_4, LTD_4, and LTE_4 are potent bronchoconstrictors, whereas LTB_4 is chemotactic for leukocytes and plays an important role in the development of gastrointestinal ulcers by contributing to the inflammatory process. Thus, developing dual inhibitor compounds that will simultaneously inhibit COX and 5-LOX could enhance their individual anti-inflammatory effects and reduce the undesirable side-effects associated with NSAIDs, especially of the gastrointestinal tract. The most promising COX/5-LOX inhibitor is ML3000 ([2,2-dimethyl-6-(4-chlorophenyl)-7-phenyl-2,3-dihydro-1H-pyrrolizine-5-yl]-acetic acid), now in Phase III clinical trials. This new approach will certainly help to unravel the mechanisms at the root of the undesirable effects of NSAIDs and to develop safer NSAIDs.

Keywords: COX; 5-LOX inhibitors; Leukotrienes; COX inhibitors; 5-LOX inhibitors; Prostaglandins; NSAIDs

1. Introduction

NSAIDs are a mainstay in the treatment of inflammatory disease and are among the most widely used drugs worldwide [1]. They are anti-inflammatory, antipyretic, and analgesic and are prescribed as first choice in the treatment of rheumatic disorders and other degenerative inflammatory joint diseases. The common mechanism of action of this class of drugs can be attributed to the inhibition of PGG/H synthase, colloquially known as COX, and, consequently, the conversion of arachidonic acid into PGs [2]. The main limitation in using NSAIDs consists in their side-effects, including gastrointestinal ulcerogenic activity and bronchospasm [3,4]. It is now well established that COX exists as two isoforms in mammals, a constitutive form (COX-1), and an inducible form (COX-2). COX-1 is expressed in most mammalian cells under physiological conditions [5], and particularly large amounts are produced by endothelial cells, platelets, and kidney tubule cells. COX-2 is induced by pro-inflammatory stimuli such as cytokines, bacterial lipopolysaccharide, growth factors, and tumour-promoting agents [6,7]. However, COX-2 also has been shown to be constitutively expressed in the cells of the reproductive tract and in the nervous system [8]. It is currently hypothesized that the undesirable side-effects of NSAIDs are due to COX-1 inhibition, whereas the beneficial effects, such as the reduction of swelling and analgesia, are related to COX-2 inhibition [9,10]. Nevertheless, both COX-1 and COX-2 have the same substrate, i.e. arachidonic acid, from which are formed not only PGs but also LTs. The LTs are formed from arachidonic acid by the action of 5-LOX.

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Abbreviations: NSAIDs, nonsteroidal anti-inflammatory drugs; COX, cyclooxygenase; LT, leukotriene; 5-LOX, 5-lipoxygenase; PG, prostaglandin; DFU, 5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsuphonyl)-phenyl-2(5H)-furanone; and DFP, diisopropyl fluorophosphate.

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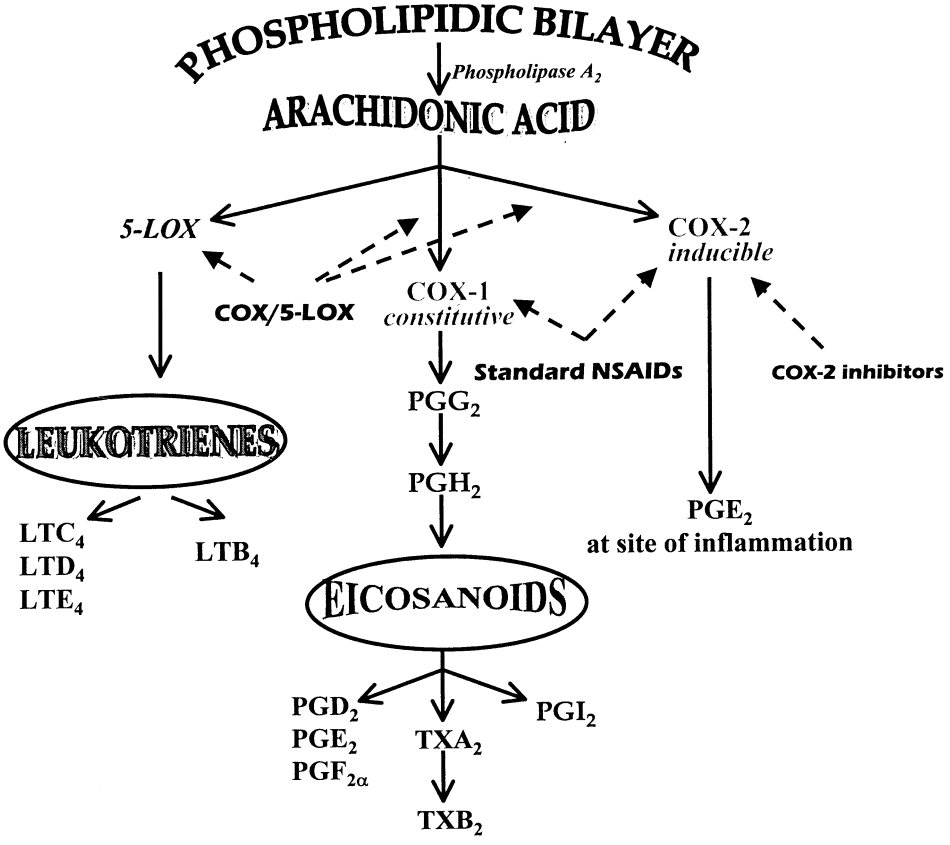

Fig. 1. Schematic pathways of the arachidonic cascade. Solid lines represent metabolite production; dotted lines represent inhibition.

While LTC_4, LTD_4, and LTE_4 are potent bronchoconstrictors [11], LTB_4 is a potent chemotactic agent for leukocytes, and it has been reported to play an important pathophysiological role in the development of gastrointestinal ulcers [12]. The current therapeutic approach and chemical design of NSAIDs are targeted to developing selective COX inhibitors (Fig. 1). However, it is intuitive that LTs, and in particular LTB_4, which is involved in leukocyte recruitment at the site of injury, also contribute and sustain the inflammatory process at the site of the injury. For this reason, developing compounds that will inhibit COX and 5-LOX at the same time could lead to an enhanced anti-inflammatory effect and reduce undesirable side-effects (Fig. 1).

2. COX-1 and COX-2 inhibition: a double-edged sword

One of the most important inflammatory diseases associated with COX-2 induction is arthritis. Indeed, in animal models of arthritis, COX-2 is strongly expressed, and it seems to be responsible for the increase in PG production [13]. COX-2 expression has also been found in human osteoarthritis [14], as well as in synovial tissue obtained from patients with rheumatoid arthritis [15]. In addition, several studies have also suggested a potential role for COX-2 in inflammatory pain, since highly selective COX-2 inhibitors (e.g. DFU) inhibit hyperalgesia in rats [16], and pain-inducing sensory stimulation leads to induction of COX-2 in the spinal cord, as shown in rats exposed to inflammatory stimuli [17,18]. In humans, selective COX-2 inhibitors, such as rofecoxib, have been shown to be analgesic in a post-dental surgery pain study [19]. All of these studies indicate selective COX-2 inhibitors as drugs that might, in theory, reduce the severity of adverse reactions of non-selective NSAIDs. However, recent studies have shown that the role played by COX-1 is more than physiological, just as the role played by COX-2 is not exclusively pathological. Recently, Warner and coworkers [20] using different *in vitro* assays investigated the relative potencies, as inhibitors of COX-1 and COX-2, of a wide range of NSAIDs as well as the newer COX-2 selective agents. The authors, on the basis of the results obtained, classified the agents tested into four main groups, which range from compounds that are full inhibitors of both COX-1 and COX-2 (group one) to compounds that selectively inhibit COX-2 with only weak activity against COX-1 (group four) [20]. Comparing these groups of NSAIDs to the epidemiological studies of NSAID-induced gastrointestinal toxicity [21], it has been found that compounds associated with the greatest gastrointestinal toxicity have the greatest COX-1 selectivity. It is of particular interest to note that this group

includes indomethacin, ketoprofen, naproxen, and ketorolac, which are among the most efficacious NSAIDs used as pain killers. In this respect, a recent paper has shed new light on the role played by COX-1 in pain perception, and it has confirmed this previous observation. By using knockout mice homozygous for COX-$1^{-/-}$ and for COX-$2^{-/-}$, it has been proposed that peripheral COX-1 mediates nociception in slowly developing pain, while spinal COX-1 is involved in rapidly transmitting pain [22]. However, compounds that selectively inhibit COX-2 (>50-fold COX-2 selective), such as rofecoxib and DFP, have been shown to be effective as anti-inflammatory drugs and to produce few serious gastrointestinal complications when used in the general population; preliminary reports indicate that rofecoxib has low gastrointestinal toxicity, but clinical trials are still in progress to confirm this finding [23]. Nevertheless, also concerning gastric safety, it has to be taken into account that studies in animals [24] and in humans [25] have shown that COX-2 selective inhibitors can delay the repair of existing gastrointestinal damage caused by ulcers, most likely by reducing the synthesis of PGs, produced by COX-2, that are thought to play an important role in the healing process. Thus, chronic use of COX-2 selective inhibitors may delay the healing of pre-existing ulcers. Another beneficial effect proposed for COX-2 is the protective role that this enzyme may play in asthma; in fact, it has been demonstrated that COX-2 is involved in controlling human airway smooth muscle cell proliferation [26]. In addition, it has also been shown that COX-2 is expressed in the airways of aspirin-sensitive patients [27,28], implying that COX-2 induction in airway cells in some way may limit the production of LTs [25]. Indeed, it is well known that aspirin and other NSAIDs can induce asthmatic symptoms in sensitive patients [29].

Thus, it appears that selective COX-2 inhibitors do not represent a complete answer to the need for safer and more effective drugs to be used in inflammatory disease therapy.

3. Role of 5-LOX

The activation of phospholipase A_2 induces the mobilization of fatty acids from the membrane lipid pool, in particular arachidonic acid, for the synthesis of lipidic mediators at the site of cellular damage. As discussed above, arachidonic acid is metabolized by two major pathways to pro-inflammatory mediators: the COX pathway and the 5-LOX pathway [30,31]. The first step in the 5-LOX cascade consists of activation of the enzyme by 5-LOX-activating protein (FLAP), which leads to the formation of the LTs [32] 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and 5-hydroxyeicosatetraenoic acid (5-HETE). LTC_4, LTD_4, and LTE_4 are known as "cysteinyl leukotrienes" because of the presence of a thioether-linked peptide, and there is some evidence supporting the role of sulphidopeptide-LT in the pathophysiology of asthma [33]. These LTs also induce the synthesis and release of other pro-inflammatory mediators such as interleukin (IL)-8 and platelet-activating factor [34]. LTB_4 has remarkable chemotactic activity on neutrophils [35] and eosinophils [36,37], and it also has been shown to be involved in the pathogenesis of a wide variety of human inflammatory diseases [38,39]. The role played by LTs in these pathologies has been addressed in recent years by using LT receptor antagonists [40,41]. Indeed, LT receptor antagonists have been shown to be effective in the maintenance treatment of patients with mild-to-moderate persistent asthma [34]. Evidence has accumulated in recent years for a key role for LTB_4 in the development of the inflammatory response. Recently, Haribabu and coworkers [42] demonstrated that deletion of the LTB_4 receptor in mice leads to a reduction in the vascular and cellular components of acute inflammatory responses, suggesting inhibition of LTB_4 activity as a therapeutic intervention in certain human inflammatory conditions [42]. In addition, the recent discovery of a new distinct receptor for LTB_4 in humans and mice may lead to the identification of other members of the LTB_4 receptor family and provide new knowledge concerning the pathophysiological role of LTB_4 [43].

4. Dual inhibitors of COX/5-LOX

Inhibition of 5-LOX is considered the ideal treatment for allergic disorders and asthma [44]. Indeed, it is known that COX inhibitors induce adverse reactions in patients with asthma either because of the shunting of LT synthesis due to increased availability of arachidonic acid to the 5-LOX pathway [44–46] or because of a reduction in vasodilatory prostaglandins PGE_2 and PGI_2 that have been shown to protect the airways [47–49]. A promising alternative to avoiding the adverse reactions of NSAIDs could be the development of drugs that inhibit both COX and 5-LOX (COX/5-LOX), leading to compounds with improved efficacy and reduced side-effects when compared with selective COX inhibitors [50]. In addition, dual inhibitors of the COX/5-LOX pathways may exhibit anti-inflammatory activity with a wider spectrum than that of classical NSAIDs by inhibiting 5-LOX product-mediated inflammatory reactions towards which NSAIDs are ineffective [51,52]. To this end, Inagaki *et al.* [53] recently found a novel antiarthritic agent, S-2474 (Fig. 2). This compound displayed a dual inhibition of COX/5-LOX with good selectivity toward COX-2 inhibition, like celecoxib. In rats, it exerted excellent anti-inflammatory activity without ulcerogenic effects and showed cytokine-modulating properties in THP-1 cells [53].

ER-34122, 5-{[1,5-bis(4-methoxyphenyl)pyrazol-3-yl] dimethoxymethyl}-2-chlorobenzamide (Fig. 2), is an orally active dual inhibitor of COX/5-LOX, and *in vivo* in mice this molecule has shown an enhanced anti-inflammatory effect, when compared with indomethacin, that has been linked to its ability to inhibit the generation of 5-LOX

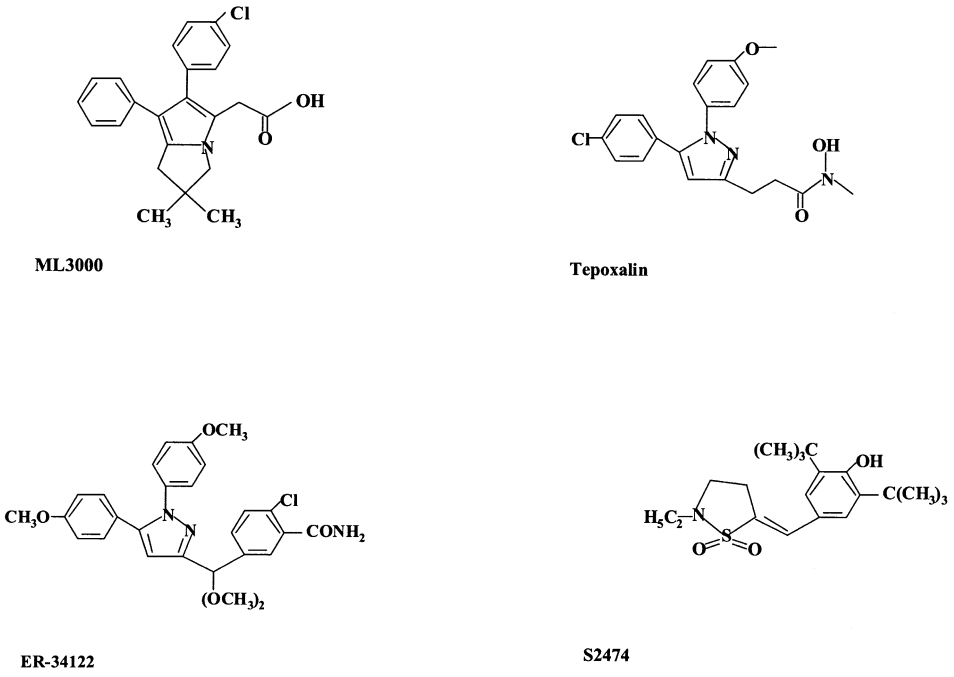

Fig. 2. Molecular structures of dual inhibitors

products [52]. These promising data have been further confirmed by the finding that ER-34122 suppresses polymorphonuclear neutrophil (PMN) infiltration, subsynovial soft tissue oedema, and multiplication of synovial lining cells in the early stage of arthritis in MRL/l mice [51]. Tepoxalin (Fig. 2), a dual inhibitor compound discontinued in clinical Phase II, significantly inhibited gastric LTB_4 synthesis in rats at a dose of 10 mg/kg, and at the same dose it markedly suppressed PG synthesis at a site of peripheral inflammation [54]. Tepoxalin also inhibited IL-2, IL-6, and tumor necrosis factor-α (TNFα) production with an IC_{50} of 10–12 mM [55]. Given orally, tepoxalin exhibited anti-inflammatory activity in rats with adjuvant-induced arthritis (ED_{50} = 3.5 mg/kg) [56]. This effect was also accompanied by potent analgesic activity in an acetic acid-induced abdominal constriction assay in the mouse (ED_{50} = 0.45 mg/kg) [56].

Among dual inhibitors, a novel class of non-antioxidant compounds has been described; the most potent and well-balanced dual inhibitor of COX/5-LOX is ML3000 ([2,2-dimethyl-6-(4-chlorophenyl)-7-phenyl-2,3-dihydro-1H-pyrrolizine-5-yl]-acetic acid; see Fig. 2), a derivative of the phenyl moiety at the 6-position of the pyrrolizine ring [57], now in clinical Phase III. The inhibition of COX was determined in a bovine thrombocyte intact cell assay and inhibition of 5-LOX was assayed in intact bovine polymorphonuclear leukocytes (IC_{50} = 0.21 μM, COX; 0.18 μM, 5-LOX). ML3000 and indomethacin have been compared in a number of experimental models of inflammation. In carrageenan-induced rat paw oedema, the ED_{50} of ML3000 for oral administration was 17 mg/kg versus 3 mg/kg for indomethacin [58]. While indomethacin was more potent in this model, ML3000 produced significantly less gastric injury. ML3000 significantly inhibited whole blood thromboxane synthesis and, at doses which have anti-inflammatory activity, significantly suppressed gastric PG synthesis without producing significant mucosal injury [59]. In a guinea pig model of arachidonic acid bronchoconstriction, ML3000 was found to be highly effective and potent (ED_{50} = 0.2 mg/kg) [60]; when administered as an aerosol at a dose of 100 mg, 30 min before antigen challenge in allergic sheep, ML3000 provided significant inhibition against the early bronchial response, completely blocking the late antigen-induced bronchoconstriction and the airway responsiveness to aerosolized carbachol that occur 24 hr after antigen challenge in this model [49]. In the rat, oral administration of ML3000 at doses of 30–300 mg/kg produced no gastrointestinal damage [61]. After 11 days of daily dosing, some rats treated with ML3000 showed duodenal damage, but the level of damage caused by indomethacin was more severe and occurred at lower doses [60]. Thus, while clinical data are needed to confirm the same profile in humans, animal data suggest that COX/5-LOX inhibitors may represent a valid therapeutic alternative to standard NSAIDs and selective COX-2 inhibitors.

5. Concluding remarks

It is beyond question that in the NSAID field there is no need to develop more powerful drugs since they are already available. Indeed, the therapeutic demand has been always directed towards safer NSAIDs. The discovery of the inducible form of COX, i.e. COX-2, has given an enormous boost to research in this field, and COX-2 inhibitors have

been developed to fill the gap between therapeutic demand and the NSAIDs available in therapy. Thus, the concept of COX-2 selective inhibition being the key to new effective but safer NSAIDs has led to the development of very selective inhibitors that are now available on the market. The "drum beat" of companies that have developed these compounds has created the general feeling that the therapeutic problem linked to the side-effects of NSAIDs, which represent the major limitation to their use, is on its way to being solved. However, as often happens in science, things are never as simple as they appear. Indeed, a physiological role for COX-2 has been proposed, and a key role in pain perception for COX-1 has been put forward recently. Thus, COX-1 is not playing just a physiological role and COX-2 is not playing solely a pathological role, and this Ying-Yang theory has to be revised carefully. Inflammation is a very complex phenomenon, and several mediators play an important role in its development at the site of the injury. Currently, there are other approaches to this problem that are being developed to obtain NSAIDs that are effective but devoid of their major adverse side-effects. In this regard, the development of COX/5-LOX inhibitors may represent a new promising alternative. Indeed, by inhibiting both pathways these compounds may lead to a better anti-inflammatory effect accompanied by reduced gastric side-effects. In addition, since a key role in inflammation is also played by oxygen radicals, it must be taken into consideration that inhibition of the COX pathway increases their formation through the peroxidative cleavage of 5-HETEs and that 5-LOX inhibition would attenuate this effect. Certainly the development of these inhibitors, as well as of other different approaches, will help to unravel the complex mechanisms at the root of the undesirable effects of NSAIDs and aid in developing newer and safer NSAIDs.

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