Editorial

Pros and Cons of Selective Inhibition of Cyclooxygenase-2 versus Dual Lipoxygenase/Cyclooxygenase Inhibition: Is Two Better than One?

This year will celebrate the 30th anniversary of the publication by John Vane and colleagues of those fundamental papers describing the inhibition of prostaglandin biosynthesis as the main mode of action of aspirin and related nonsteroidal anti-inflammatory drugs (NSAID)[1-3]. Since then the search for novel and safer antiinflammatory drugs has never faded, with moments of both excitement and disappointment. Because of the importance of prostaglandins in this process, the metabolism of arachidonic acid has been dissected to identify novel targets, namely enzymes and receptors, for pharmacological intervention. The story of these ongoing efforts indicates that the identification of new possible targets has led to a decrease in the interest in previous ones even before obtaining complete answers on the role of the latter. For example, the discovery of the lipoxygenase pathways in the early 1980s was accompanied by a diminished interest in the "classic" prostaglandins, leading to the development of selective lipoxygenase inhibitors as well as "dual" lipoxygenase/cyclooxygenase inhibitors. In turn these lines of research have lost momentum after the development of leukotriene receptor antagonists and selective inhibitors of the inducible cyclooxygenase.

As many excellent general reviews have been published recently in this field[4-7], this article will focus on two targets of drug action, cyclooxygenase and lipoxygenase, examining both the effects and the clinical implications of the inhibition of the single enzymes versus the combined dual inhibition of the two enzymes.

ARACHIDONIC ACID METABOLISM IN INFLAMMATION

Arachidonic acid (AA) is a member of the ω-6 series of essential fatty acids contained in membrane phospholipids. Activation of the enzyme phospholipase A_2 (PLA_2) releases AA, which can be further metabolized by several enzymatic complexes. Recently a non-enzymatic metabolism of AA has also been described. As a detailed discussion of these metabolic pathways is beyond the scope of this review, I will focus on those enzymatic activities generating the metabolites acting as mediators of many important facets of the inflammatory process (Figure 1).

Prostaglandin-H synthase. This enzyme consists of two related catalytic functions, a cyclooxygenase activity that catalyzes the formation of prostaglandin G_2 (PGG_2) and a peroxidase activity catalyzing a two-electron reduction of PGG_2 to PGH_2. The unstable PGH_2 is then transformed into prostanoids like PGI_2, thromboxane A_2 (TXA_2), PGE_2, $PGF_{2\alpha}$ by specific synthases in different cells. For the sake of simplicity in this article the PGH synthase will be referred to as cyclooxygenase (COX). In recent years two different COX have been described, a constitutive enzyme (COX-1) expressed in most cells and an inducible enzyme (COX-2), whose expression can be stimulated in many cells by inflammatory stimuli[4,8] (see below).

5-lipoxygenase (5-LOX). Through a 5-hydroperoxy intermediate this enzyme produces the leukotrienes (LT), so called for their occurrence in leukocytes and a characteristic conjugated triene structure. The first compound to be formed is LTA_4, an unstable metabolite containing an epoxide moiety. Derived from LTA_4 are LTB_4 by enzymatic hydrolysis and LTC_4 by addition of the tripeptide glutathione catalyzed by glutathione-S-transferase. From LTC_4, LTD_4 and LTE_4 are produced. The three compounds form the group of the cysteinyl-leukotrienes[9].

The biological effects of both prostanoids and leukotrienes are consistent with the role of mediators of the main phenomena of inflammation such as vascular changes, increase in body temperature, and leukocyte migration. Importantly, during inflammatory processes these mediators are synthesized and released by resident and migrated cells in concentrations sufficient to cause their biological effects.

Prostanoids like PGI_2 and PGE_2 are powerful vasodilators in their own right and synergize with other autacoids like histamine and bradykinin. It is this combined dilator action on precapillary arterioles that contributes to the redness and increased blood flow in areas of acute inflammation. These prostanoids also potentiate the effect on vascular permeability by bradykinin and histamine, contributing to plasma exudation and the formation of inflammatory edema. PGI_2 and PGE_2 do not cause overt pain, but produce hyperalgesia by sensitizing afferent C fibers. Moreover, PGE_2 acts on neurones in the thermoregulatory network of the hypothalamus,

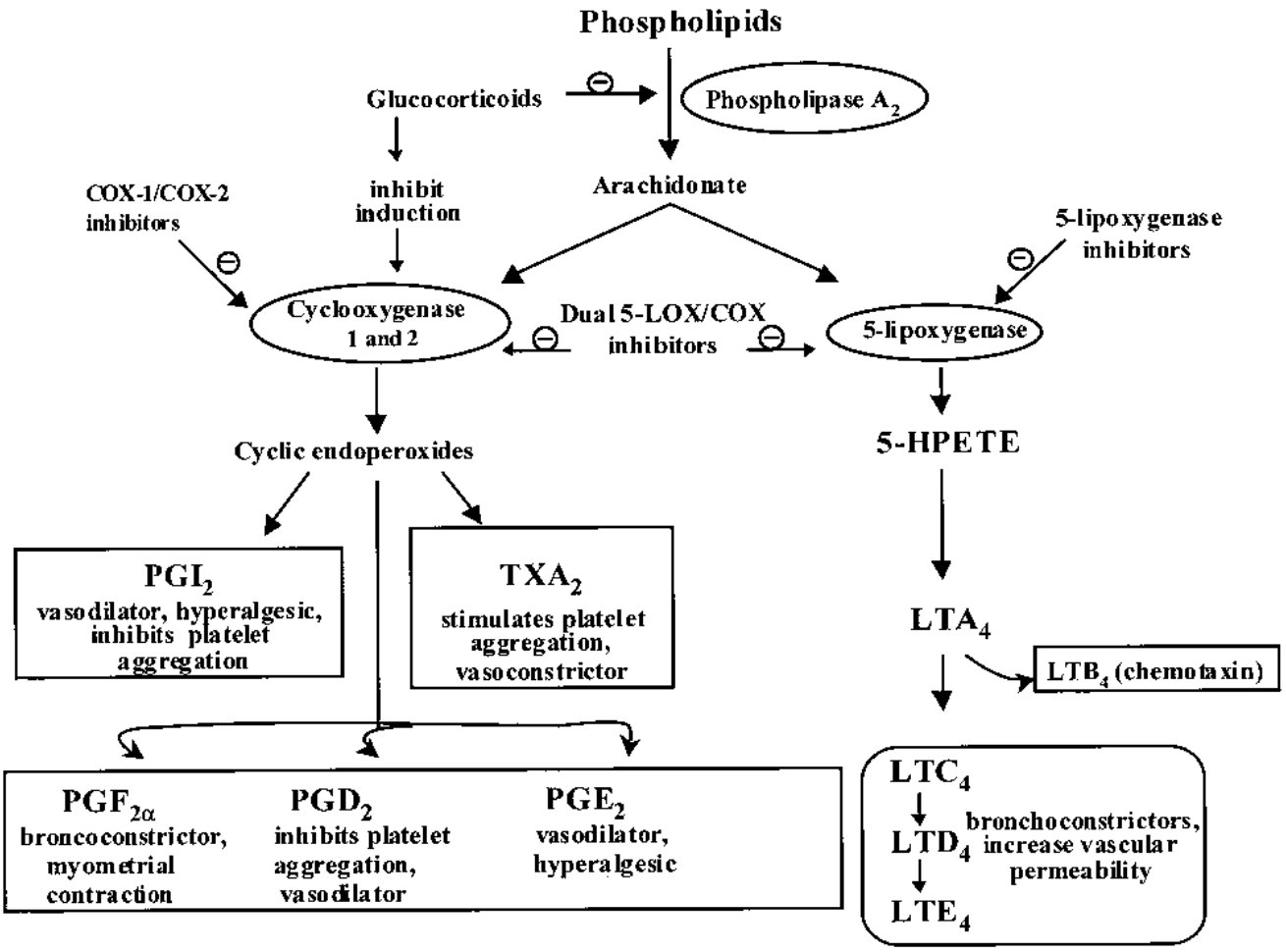

Figure 1. Metabolism of arachidonic acid in inflammation. Arachidonic acid released from phospholipids by the action of the enzyme phospholipase A_2 can be metabolized by either cyclooxygenase 1 and 2 or by 5-lipoxygenase. Cyclooxygenases generate prostanoids like prostaglandin I_2 (PGI_2) and PGE_2 that are involved in physiological processes including platelet aggregation and regulation of gastrointestinal and renal blood flow. However, both PGI_2 and PGE_2 are potent vasodilator agents that contribute to the vascular signs of the inflammatory reactions. 5-lipoxygenase forms leukotrienes (LT) such as LTB_4, a potent chemotaxin, and LTC_4, LTD_4, $LTDE_4$, which are potent bronchoconstrictor agents.

causing increase in body temperature. There is general agreement that this prostaglandin mediates fever induced by endogenous pyrogens like interleukin 1 (IL-1)[10].

On the other hand prostaglandins, especially PGI_2 and PGE_2, have important cytoprotective effects on the gastrointestinal (GI) mucosa. Different mechanisms contribute to this protective action: inhibition of secretion of both acid and pepsin, stimulation of mucus formation and bicarbonate secretion, and improved mucosal blood flow[11]. Further, PGE_2 contributes to normal renal physiology by regulating the vascular tone and the normal blood flow[7], while PGI_2 is involved in vascular homeostasis and normal platelet functions[10].

LTB_4 is a powerful chemotactic agent for both neutrophils and macrophages. On neutrophils it also causes cell degranulation with release of lysosomal enzymes and upregulation of the membrane adhesion molecules. On macrophages and lymphocytes it stimulates the release of proinflammatory cytokines. The cysteinyl-leukotrienes (LTC_4, LTD_4, and LTE_4) are important mediators of bronchial asthma, causing constriction of bronchial tissue and edema mediated by augmented venular permeability. LTE_4 is less potent than LTC_4 and LTD_4, but its effect is much longer lasting. This allergic pulmonary inflammation results from the activation of cells with a full 5-LOX/LTC_4 synthase pathway, including monocytes, eosinophils, basophils, and mast cells[9].

CONSTITUTIVE AND INDUCIBLE CYCLOOXYGENASES — "GOOD" AND "BAD" PROSTAGLANDINS?

As discussed above it has become clear in recent years that cyclooxygenase exists in two isoforms. COX-1 can be detected in most tissues and is typically expressed at constant levels throughout the cell cycle. COX-2 is undetectable in most

mammalian tissues, but its expression can be rapidly induced in cells involved in inflammation such as fibroblasts, monocytes, and vascular endothelium in response to growth factors, tumor promoters, hormones, bacterial endotoxin, and cytokines. Therefore, COX-1 has become known as the constitutive isoform and COX-2 as the inducible one. This appears to be an oversimplification inasmuch as COX-1 expression can be inhibited in endothelial cells in response to acidic fibroblast growth factor and upregulated in mast cells by stem cell factor plus dexamethasone, while COX-2 is constitutively expressed in brain, testes, tracheal epithelia, and kidney[8]. However, the predominant constitutive nature of COX-1 together with the observations that expression of COX-2 can be upregulated by inflammatory stimuli and that prostanoids are produced by COX-2 in much larger amounts compared with COX-1 has led to the hypothesis of the existence of "good" versus "bad" prostaglandins. According to this hypothesis COX-1 generates good prostaglandins for physiological "housekeeping" functions, including platelet dependent homeostasis, gastric mucosal integrity, and regulation of renal blood flow; while COX-2 forms the bad prostaglandins involved in inflammatory reactions and responsible for inflammatory signs like fever, pain, capillary edema, vasodilatation, etc. As a direct consequence, the specific inhibition of COX-2 is expected to cause significant antiinflammatory relief without interfering with prostaglandin mediated physiological processes, especially GI and renal functions[7,10]. In other words the long dreamt hope of a drug having significant antiinflammatory action devoid of toxicity for the GI tract and for the kidney seemed imminent. Unfortunately, in spite of positive experimental and clinical data, recent findings suggest that it may be unwise to separate so sharply the functions of the prostaglandins produced by the two cyclooxygenases (Table 1).

CONFLICTING EXPERIMENTAL EVIDENCE

A number of laboratories have shown that COX-2 is induced during various experimental inflammatory processes[12] and in human rheumatoid synovial tissues[13], and that prostaglandins produced by COX-2 are indeed responsible for inflammatory signs[14]. Moreover, it has been shown that the selective inhibition of the inducible enzyme is antiinflammatory and nonulcerogenic in rat carrageenin induced inflammation[15] as well as in rat adjuvant arthritis[16]. The antiinflammatory efficacy and the relative low toxicity of selective COX-2 inhibition has been confirmed *in vitro* in human tissues[17,18].

However, other reports tell a different story. With some highly selective COX-2 inhibitors, a significant antiinflammatory effect is only observed after administration of doses that also inhibited COX-1, suggesting that this enzyme substantially contributes to prostaglandin synthesis in inflammation[6,19]. The role of COX-1 in inflammation has been confirmed by studies using mice in whom the *Ptgs1* gene encoding COX-1 was disrupted[20]. These animals showed a decreased ear inflammatory response to arachidonic acid. Interestingly, these mice were less sensitive to indomethacin induced stomach damage. Parallel studies on COX-2 deficient mice have shown that these animals have a normal inflammatory response, but develop a severe nephropathy and are susceptible to peritonitis[21,22]. Moreover, COX-2 deficient mice present multiple failures in female reproductive processes including ovulation, fertilization, implantation, and decidualization[23].

Other studies have investigated the time course of COX-2 expression in experimental inflammation. In carrageenin induced pleurisy in rats, two peaks of COX-2 activity were observed, at 2 and 48 hours. The second peak of expression, which was 350% greater than that at 2 hours, was associated with minimal PGE_2 synthesis and coincided with the resolution of the inflammatory reaction[24]. In contrast, at 48 hours a peak of synthesis of antiinflammatory prostanoids like 15deoxy$\Delta^{12\text{-}14}$prostaglandin J_2 was observed. Indomethacin and a selective COX-2 inhibitor reduced inflammation at 2 hours, but significantly exacerbated inflammation at 48 hours. The authors conclude that COX-2 may be proinflammatory during the early phases of the inflammatory process, but may contribute to its resolution at a later phase by generating antiinflammatory prostanoids[24,25].

All these data suggest that the relationship between COX activity, inflammation, and gastric and renal physiology is decidedly more complex than previously thought.

Table 1. Involvement of prostaglandins synthesized by COX-1 and COX-2 in physiological and pathological processes.

	Prostaglandins Formed by		
	COX-1	COX-2	References
Inflammatory signs	+	+++	19–21
Gastric mucosa protection (including adaptive cytoprotection)	+++	++	30–36
Platelet aggregation	+++	—	10
Renal function	++	++	4, 7, 22
Female reproductive processes	±	++	23
Endothelial PGI_2 synthesis	±	++	4, 37
Cancer development	+	+++	38–41

+, ++, +++: importance in function. —: no effect.

CYCLOOXYGENASE INHIBITORS

"Classical" NSAID like aspirin, indomethacin, ibuprofen, and naproxen have no enzyme selectivity and inhibit with similar potency both COX-1 and COX-2. According to the above hypothesis these drugs exert antiinflammatory action by blocking COX-2 activity and, at the same time, produce unwanted side effects, mainly GI and renal toxicity, due to the inhibition of COX-1 activity. Clinically, the beneficial effects of NSAID in rheumatic diseases have come with a price: according to the Arthritis, Rheumatism and Aging Medical Information System, 13 of every 1000 patients with rheuma-

toid arthritis (RA) who take NSAID for one year have a serious GI complication[26]. Moreover, 5–15% of patients with RA are expected to discontinue NSAID therapy because of dyspepsia within a 6 month period of treatment[26]. Due to the widespread use of NSAID, these toxicities are one of the most prevalent drug associated health risks. This has dramatically stimulated the search for selective inhibitors of COX-2 that promise to have the same antiinflammatory, antipyretic, and analgesic activities as classical NSAID, but are not expected to cause the same GI complications.

Two types of inhibitors have been developed[5]. The first type of drug includes compounds like meloxicam, nimesulide, and etodolac, which can be classified as selective COX-2 inhibitors. These compounds have a preferential effect on COX-2, although they are not devoid of COX-1 inhibitory activity. These compounds have shown a favorable safety profile over classical NSAID; however, large scale comparison studies are needed to confirm these observations — information on clinical trials with these drugs has recently appeared[6,27].

The second type of inhibitor includes compounds like celecoxib and rofecoxib that were specifically designed to be exclusive COX-2 inhibitors with little or no COX-1 inhibitory activity — classified as highly selective COX-2 inhibitors. Clinical trials (phase III studies) have shown that celecoxib is as effective as ibuprofen and diclofenac in both osteoarthritis and RA in reducing arthritic pain and inflammation significantly better than placebo. The use of celecoxib was associated with a significantly lower incidence of symptomatic ulcers and/or ulcer complications compared with NSAID in non-aspirin users[28]. Other clinical studies have shown a similar pharmacological and safety profile for rofecoxib. This drug was as effective as naproxen in patients with RA; in addition the treatment with rofecoxib was associated with significantly fewer clinically important GI events than treatment with naproxen. The incidence of myocardial infarction was lower among patients taking naproxen than among those taking rofecoxib (0.1 vs 0.4%); however, the overall mortality rate and the rate of death from cardiovascular events were similar in the two groups[29].

IS REAL GOLD ALL THAT GLITTERS?

The available experimental and clinical evidence (although limited because of the novelty of the drugs) indicates that selective inhibition of COX-2 results in effective antiinflammatory action accompanied by a reduced risk of GI toxicity compared to classical NSAID. However, recent data have cast some doubts on effects of longterm treatments with highly selective COX-2 inhibitors. The above reported studies performed in COX deficient mice[20,22] suggest that prostaglandins formed by both cyclooxygenases are involved in the inflammatory response, while those generated by COX-2 contribute substantially to regulation of renal function and reproductive physiology. Recent clinical trials have shown that patients taking either rofecoxib or celecoxib had a slight increase in the incidence of edema, a condition often resulting from kidney dysfunction[28,29]. Classical NSAID are contraindicated in patients with renal insufficiency because of the risk of a complete renal failure. More clinical data are necessary to establish whether the same care will have to be taken in prescribing COX-2 inhibitors for patients with kidney diseases[4] (see also next section on postmarketing surveillance).

The hypothesis that COX-2 dependent prostanoids may act as regulators of physiological processes and not only as inflammatory mediators is confirmed in other settings. A recent editorial has pointed out that COX-2 may be involved in the so-called "adaptive cytoprotection" response in GI mucosa[30]. COX-2 is rapidly induced during GI ulcerative processes, where it generates large amounts of prostaglandins that contribute to the healing process. The administration of selective COX-2 inhibitors results in a reduction in mucosal PG synthesis and significant inhibition of gastric ulcer healing in both mice[31] and rats[32]. On a similar note, it has been shown that the selective inhibition of the induced COX-2 exacerbates both ischemia-reperfusion injury in the rat stomach[33] and inflammation associated injury in the rat colon[34]. Moreover, the induction of gastric injury in rats by NSAID requires inhibition of both COX-1 and COX-2[35]. Finally, a recent report describes the constitutive expression of COX-2 in healthy human and rabbit gastric mucosa[36]. The authors speculate that COX-2 may be an important enzyme generating vasodilatory and cytoprotective prostanoids in the gastric mucosa. All this evidence directly challenges the concept that the prostaglandins important for maintenance of GI mucosal integrity are produced solely via COX-1.

Another widespread concern for selective COX-2 inhibitors is that they may counteract the positive cardiovascular effect of aspirin[4]. It is well known that low dose aspirin blocks COX-1 dependent platelet synthesis of proaggregatory TXA_2 without affecting endothelial cell synthesis of antiaggregatory prostacyclin (PGI_2), so reducing the risk of thrombosis. Nonselective inhibitors do not affect the balance between TXA_2 and PGI_2, while selective COX-2 inhibitors have been shown to markedly reduce PGI_2 synthesis[37]. Since these compounds do not inhibit COX-1 they may bias vascular prostaglandin synthesis in favor of platelet TXA_2 production, a prothrombotic outcome. Further studies are required to ascertain whether the use of this type of drug is contraindicated in patients at risk of stroke or heart attacks.

A clear indication for selective COX-2 inhibitors is emerging in anticancer therapy. It has been suggested that COX-2 may be implicated in the promotion of colorectal polyposis and cancer. The precise role of COX-1 and COX-2 in carcinogenesis is still a matter of debate[38]; however, selective COX-2 inhibitors have been shown to have clear antitumor efficacy in rodent models of adenomatous polyposis[39]. These anticarcinogenic effects may not be confined to tumors of the large intestine. It has recently been shown that selective COX-

2 inhibitors suppress growth and induce apoptosis in human esophageal adenocarcinoma cells[40]. Moreover, celecoxib inhibits the development of urinary bladder cancer both in mice and rats[41].

POSTMARKETING SURVEILLANCE FOR SELECTIVE COX-2 INHIBITORS

The need of more comprehensive clinical investigations for selective COX-2 inhibitors to ascertain their potential side effects is reinforced by the first reports of postmarketing surveillance for rofecoxib. A newsletter from the Medicine Control Agency (MCA) and the Committee on Safety of Medicines (CSM), available on the Internet [www.medscape.com – 08 September 2000], reported that an estimated 557,100 prescriptions for rofecoxib have been dispensed in the UK up to the end of May 2000, and a total of 1120 reports of suspected adverse reactions to the drug were received by the agencies. Eleven deaths have been reported, 5 following GI reactions, 3 following cardiac failure, and 3 following myocardial infarction. GI adverse reactions accounted for almost half of the reports, of which the majority (84%) were nausea, dyspepsia, diarrhea, and abdominal pain. However, there have been 68 reports of upper GI perforations, ulcerations, and bleeds. Forty-four patients recovered, but 5 had a fatal outcome. There were 177 reports of suspected cardiovascular reactions, including 101 reports of edema and 31 of hypertension. The agencies also received 15 reports of cardiac failure, 3 of which had a fatal outcome, and 9 reports of myocardial infarction, 3 of them fatal. Adverse reactions common to classical NSAID were also reported with rofecoxib, including angioedema, bronchospasm or exacerbation of asthma, renal failure, and hepatic dysfunction.

In conclusion, the newsletter reminded us that rofecoxib is contraindicated in patients with active peptic ulceration, GI bleeding, and congestive heart failure. It also pointed out the need for caution in patients with a history of cardiac failure, left ventricular dysfunction, or hypertension and in patients with pre-existing edema for any other reason.

DUAL LIPOXYGENASE/CYCLOOXYGENASE INHIBITION

As discussed, prostaglandins, especially PGI_2 and PGE_2, contribute substantially to signs of acute inflammation such as increased vascular permeability, vasodilatation, formation of capillary edema, pain, and fever. On the other hand, the pathogenesis of chronic inflammatory conditions is closely associated with accumulation of phagocytic leukocytes. These cells release proteolytic enzymes, toxic oxygen metabolites, proinflammatory cytokines, and chemokines leading to the tissue damage occurring during chronic inflammatory processes. LTB_4 is a major component of inflammatory exudates and acts as a potent chemotaxin promoting the accumulation of leukocytes at sites of inflammation[42]. On the other hand the cysteinyl-leukotrienes (LTC_4, LTD_4, LTE_4) are thought to be important mediators of bronchial asthma. In addition to their well known potent broncho-constrictor properties, the cysteinyl-leukotrienes cause tissue edema, and stimulate both migration of eosinophils and airway secretions. They also stimulate cell cycling and proliferation of both smooth muscle cells and various hematopoietic cells[43]. It is therefore conceivable that dual inhibition of both cyclooxygenase and 5-lipoxygenase should counteract inflammatory vascular changes as well as leukocyte induced damage, resulting in a more profound antiinflammatory action than the mere blockade of cyclooxygenase. Further, dual 5-LOX/COX inhibitors may show therapeutic benefit in allergic inflammatory diseases like asthma.

ANTIINFLAMMATORY AND ANTIASTHMATIC ACTIVITY OF DUAL INHIBITORS

A number of reports have shown that dual 5-LOX/COX inhibitors exert marked antiinflammatory effects in different models of experimental inflammation. Compounds like BW755c[44] or SK&F 86002[45] are able to inhibit both fluid and cellular phases of the inflammatory response. It is noteworthy that the inhibition of leukocyte migration is accompanied by reduction of tissue damage, supporting the concept that the control of leukocyte accumulation could be an important factor in limiting tissue damage and necrosis[46]. However, one experimental compound, ML 3000, which is in phase III clinical development, blocked both acute and chronic models of inflammation[47]. It is also important to remember that selective and nonselective cyclooxygenase inhibitors, which do not affect the leukotriene pathway, have little effect on cell migration at doses that inhibit prostaglandin synthesis[44].

The relevance of cysteinyl-leukotrienes in asthma has prompted the search for selective antileukotriene drugs. Three receptor antagonists for cysteinyl-leukotrienes (montelukast, pranlukast, zafirlukast) and a 5-LOX inhibitor (zileuton) are currently available by prescription in many countries. Clinical data support their use in patients with chronic persistent asthma, whether it is mild, moderate, or severe. These drugs are also effective in aspirin induced asthma as well as helping to reduce nocturnal asthma in which there is a component of increased eosinophil recruitment and leukotriene release[43,48]. Clinical data are not yet available for dual inhibitors. However, one experimental compound blocked both the antigen induced bronchoconstriction and the airway hyperresponsiveness to aerosolized carbachol in experimental models of asthma[49]. On the other hand the use of COX inhibitors in asthma patients is contraindicated because of possible increased production of leukotrienes due to shunting of arachidonic acid to the 5-LOX pathway[50].

PROTECTIVE EFFECTS OF DUAL 5-LOX/COX INHIBITORS ON GI MUCOSA

It has been suggested that leukotrienes may contribute to GI damage[11]. The cysteinyl-leukotrienes, especially LTC_4, caus-

ing vasoconstriction may increase the susceptibility of the mucosa to injury by reducing mucosal blood flow[51]. LTB_4 potentiates GI damage probably by stimulating leukocyte infiltration[52]. Leukocytes might contribute to ulceration by occluding microvessels, thereby reducing mucosal blood flow, and by releasing mediators, proteases, and free radicals that cause tissue necrosis. Therefore it can be expected that dual inhibitors may have protective effects on GI mucosa. The dual inhibitor BW755C significantly reduced the extent of gastric damage caused by indomethacin[53], while an inhibitor of leukotriene synthesis, MK-886, accelerated healing in a rat model of colitis[54]. It is also noteworthy that the dual inhibitor ML 3000 reduced gastric prostaglandin synthesis without causing mucosal injury, although the mechanisms of this action are not completely clear[55].

PHOSPHOLIPASE A_2 INHIBITORS AND GLUCOCORTICOIDS

The concept that the inhibition of the synthesis of all proinflammatory eicosanoids would lead to a marked antiinflammatory effect is supported experimentally by the effects of PLA_2 inhibitors and clinically by the use of the antiinflammatory glucocorticoids. Since the inhibition of PLA_2 leads to a reduced supply of free AA and consequently to decreased formation of all eicosanoids, its antiinflammatory potential is very similar to the dual 5-LOX/COX inhibition. In this context the crucial question is: which phospholipase A_2 should we inhibit? In the past, attention has focused on secretory PLA_2 on the basis that increased levels of this enzyme can be detected in experimental inflammatory exudates as well as in synovial joint fluids of patients with RA. It was proposed that the secreted enzyme would interact with the membrane phospholipids of the same secreting cells or of adjacent cells to release arachidonic acid and start the eicosanoid cascade[56]. Many compounds have been identified, especially from marine sponges, that have potent anti-PLA_2 activity *in vitro*; however, none of these compounds has gone further in development due to the inconsistency of results in human application[57].

In a recent report specific inhibitors of cytosolic PLA_2 had therapeutic action in a model of chronic inflammation, the rat adjuvant arthritis model, and showed a number of *in vitro* antiinflammatory effects including inhibition of adhesion molecule expression and of cytokines like IL-1. Since in the same models inhibitors of secretory PLA_2 proved to be ineffective and also because the cytosolic PLA_2, unlike the secreted enzyme, is specific for arachidonyl phospholipids, cytosolic PLA_2 could be the predominant enzyme in inflammatory signalling[58].

The glucocorticoids are potent antiinflammatory and immunosuppressive compounds widely used in inflammatory and immune disorders. These compounds interact with specific cytosolic receptors to regulate the expression of responsive genes, thereby either upregulating or downregulating the synthesis of specific proteins by the various target tissues. An important facet of glucocorticoid antiinflammatory action is upregulation of the expression of annexin (lipocortin)-1 (ANX-1), a protein that blocks the activation of PLA_2[59,60]. Again, which PLA_2? ANX-1 blocks the cytosolic enzyme in two ways, by direct enzyme inhibitor interaction[61] and by preventing the phosphorylation of the enzyme induced by activators like epidermal growth factor[62]. On the other hand, there is no experimental evidence of direct interaction between ANX-1 and secreted PLA_2. The activity of this enzyme may be only indirectly affected since ANX-1, which belongs to a family of phospholipid-binding proteins (annexins), can bind to the substrate, impairing the access of the enzyme[63]. These data confirm that the important enzyme to be blocked in inflammation is cytosolic PLA_2.

By means of these mechanisms both glucocorticoids and annexin-1 are indeed able to inhibit the *in vitro* and *in vivo* release of all metabolites of arachidonic acid. ANX-1 has potent antiinflammatory effects in animal models of experimental inflammation[64]. In addition, glucocorticoids inhibit the expression of both cytosolic PLA_2[65] and inducible cyclooxygenase[66]. All this evidence demonstrates that compounds able to interfere with PLA_2 activity and to reduce the formation of all eicosanoids are potent antiinflammatory agents in both experimental and clinical settings. Finally, the therapeutic equivalence between PLA_2 inhibition and dual 5-LOX/COX inhibition is supported by clinical and experimental data. Dual inhibitors, but not anti-COX drugs, may substitute for steroid treatment in brain edema that is caused by both prostaglandins and leukotrienes[67]. Another report has shown that in a model of mouse skin inflammation the combination of a dual 5-LOX/COX inhibitor with a glucocorticoid allows the use of very low drug concentrations, resulting in synergistic topical antiinflammatory activity without inducing skin atrophy[68].

CONCLUSION

1. Selective and highly selective COX-2 inhibitors, recently introduced in clinical practice, represent the result of an innovative approach to improve the benefit/risk ratio of antiinflammatory drugs. Several clinical studies show a reduction in the incidence of unwanted GI effects with the new drugs compared to classical nonspecific COX inhibitors. Also, severe side effects are generally rare and the compounds are well tolerated by the majority of patients.
2. There is growing evidence that the prostaglandins produced by COX-2, in addition to their undisputed proinflammatory properties, are also involved in a number of physiological housekeeping functions like adaptive cytoprotection in the GI mucosa, synthesis of antiaggregatory PGI_2 by endothelial cells, formation of vasodilatory PGE_2 in the kidney, and regulation of reproductive processes.
3. The first reports of postmarketing surveillance for a highly selective inhibitor like rofecoxib show that the rate of adverse reactions is about one per 500 prescriptions and that some of the adverse effects may be so severe as to have a fatal outcome.

4. We need more fundamental research on distribution and roles of COX-2 as well as more information from the clinical use of selective inhibitors in order to define precisely the place of these new drugs in the struggle against inflammatory diseases.
5. Dual 5-LOX/COX inhibitors are potent antiinflammatory drugs in experimental settings. By inhibiting the synthesis of both prostaglandins and leukotrienes these compounds counteract vascular changes and cellular infiltration occurring in inflammation and therefore may prove to be effective in chronic inflammatory processes. Moreover the use of these drugs may be indicated in allergic inflammatory diseases like asthma.
6. The pharmacological profile of dual 5-LOX/COX inhibitors is somewhat similar to that of antiinflammatory glucocorticoids, which inhibit PLA_2 and thereby prevent arachidonic acid metabolism by both COX and 5-LOX. On the other hand, dual inhibitors do not affect intermediate metabolism or endocrine functions and therefore are not expected to cause the severe side effects normally associated with the use of glucocorticoids.
7. Dual 5-LOX/COX inhibitors seem not to cause GI damage; rather, they show protective effects on GI mucosa, although this particular aspect awaits further experimental studies.
8. The experimental promise of dual 5-LOX/COX inhibitors of potent antiinflammatory action associated with fewer side effects needs confirmation from clinical studies.

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