Enzyme Mediated Inflammation: Epidemiology and Therapeutic implications

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ABSTRACT

The identification of various enzymes participating in the arachidonic acid metabolism and recognition of inflammatory metabolites led to systematic studies for the development of anti-inflammatory drugs. Starting with the stimulatory role of phospholipases to release arachidonic acid from phospholipids, over-expression of cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) enzymes cause more than normal production of the respective metabolites. Excessive production of prostaglandins and leukotrienes leads to the manifestation of acute to chronic inflammation in humans. The consequent ailments are expressed in the form of asthma, atherosclerosis, irritable bowel syndrome, arthritis and cancer. Targeting the induced isoform of cyclooxygenase viz. cyclooxygenase-2 (COX-2) and 5-LOX enzyme has opened new aspects for capping the enzyme mediated inflammation. A number of such aspects have been discussed in this comprehensive review.

Keywords: COX; Cyclooxygenase; LOX; Lipoxygenase; Arachidonic acid

INTRODUCTION

According to a recent survey of the World Health Organization (WHO), cancer causes around 13% of the total deaths worldwide and if it continues rising, it is estimated to cause 13.1 million deaths in 2030 [1]. Moreover, the reason for about 15% of the total cancer deaths has been reported [2] to be associated with chronic inflammation. Hence, an obvious way out to minimize cancer deaths is to put control over the inflammatory conditions. The pioneering work of Sune K. Bergström, Bengt I. Samuelsson and John R. Vane for discovering prostaglandins and related biological substances, which led them to win Nobel Prize in Physiology/Medicine 1982, enabled the scientific community to make extensive exploration of the working of arachidonic acid (AA) pathway and solving several issues related to inflammatory diseases like rheumatoid arthritis and asthma. Dr. J. R. Vane discovered the mechanism of aspirin [3] which was used as a former Non-steroidal anti-inflammatory drug (NSAID) for relieving pain, inflammation and fever by slowing down the production of prostaglandins associated with them. He also discovered prostacyclins [4-6] that relax blood vessels and work for heart and blood vessel diseases and finally led to the development of cyclooxygenase-2 (COX-2) inhibitors. This work further inspired the exploration of arachidonic acid metabolism [7] leading to the production of various prostanoids including prostaglandins, prostacyclins, thromboxanes and leukotrienes.

DISCUSSION

Biochemistry of Arachidonic Acid Cascade

AA is a polyunsaturated fatty acid present in various phospholipids like phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositides of body cell membranes. It is found abundantly in brain, liver and muscles. Besides the way out of various signalling enzymes [8] it is a key intermediate in inflammatory processes [9,10]. AA generated for signalling purposes appears to be derived by the action of a phosphatidylcholine specific cytosolic phospholipase A2 (cPLA2) [11] whereas inflammatory AA is generated by the action of a low molecular weight secretory PLA2 (sPLA2) [12]. It is metabolized generally by three types of enzymes viz.
Cyclooxygenases, lipooxygenases and cytochrome P450 but the fate of the substrate is preferentially decided by cyclooxygenases and lipooxygenase enzymes (Chart 1, 2). Cyclooxygenase has two isomeric forms viz. cyclooxygenase-1 (COX-1) and COX-2 [13]. A milestone discovery with the recognition of prostaglandin synthase was made in 1991 when it was established that cyclooxygenase enzyme exists in two discrete isoforms viz. COX-1 and COX-2. Even though both of the isoforms of cyclooxygenase have high homology in amino acid sequence (60%), but the presence of Valine523, Arginine513 and Valine434 in COX-2 in place of isoleucine523, Histidine513 and isoleucine434 in COX-1 leads to 25% larger active site of COX-2 than that of COX-1. Besides, this presence of the smaller valine523 residue in COX-2 allows access to a hydrophobic side pocket accompanying the active site.

![Chart 1](chart1.png)

**Chart 1:** The design of selective COX-2 inhibitors is inspired from this structural dissimilarity around the active site of COX-1 and COX-2 isozymes

The presence of a bulkier group (sulphonyl) constrains the entry of the drug in the active site of COX-1 isozyme. Whereas no such restriction is countered by the same drug while its entry into the active site of COX-2 isozyme. Another isoform cyclooxygenase-3 (COX-3) [14,15] has also been discovered but is not much explored. Out of COX-1 and COX-2, the former is involved in the desired conversion of AA to homeostatic prostaglandins as an innate immunity mechanism of the body and is known as the housekeeping enzyme while the later one is involved in the production of pathophysiological prostaglandins which leads to the augmentation of severe inflammation.

![Chart 2](chart2.png)

**Chart 2:** Metabolites of arachidonic acid through cyclooxygenase pathway
On entering cyclooxygenase metabolic pathway, AA is first converted to the immediate substrate for various prostaglandin, prostacyclin and thromboxane synthases. This involves a two-step conversion [16] of AA to PGH2 via the formation of PGG2. The first step involves the addition of two oxygens into the AA molecule forming the bicyclic peroxide intermediate, PGG2. In the second step, the intermediate, PGG2 diffuses to the site where peroxidation takes place and gets converted to PGH2 which then gets metabolized by prostaglandin synthases, prostacyclin synthases and thromboxane synthases to various prostaglandins, prostacyclins and thromboxanes, respectively (Chart 2).

Lipoxygenases are a family of enzymes which catalyze the oxygenation of AA, each lipoxygenase forming a distinct hydroperoxy-eicosatetraenoic acid (HPETE). HPETEs may undergo a series of metabolic transformations - what is referred to as a lipoxygenase pathway (Chart 3) [17,18].

Therefore, AA metabolism results in the manifestation of (a) prostaglandins and prostacyclins which regulate the contraction and relaxation of smooth muscle tissues, (b) thromboxanes which acts as vasoconstrictor and effective hypertensive agents, and expedites platelet aggregation, and (c) leukotrienes (LTs) which activate contractions in smooth muscles lining the trachea, act as chemotactic agents while their over-production is a major cause of inflammation in asthma and allergic rhinitis. Basically, inflammation is an innate – immunity response of the body against harmful stimuli which is a protective endeavor by the organism to confiscate the injurious stimuli and to initiate the healing process [19]. It does not need to be suppressed but when the inflammation goes chronic, it can lead to a host of diseases, such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (e.g., gall bladder carcinoma) [20]. In 1863, Rudolf Virchow indicated that cancers tend to occur at the site of chronic inflammation [21]. Amongst many other factors responsible for initiation and propagation of cancer, it has now been well established after around 150 years of Virchow’s hypothesis, that inflammatory enzymes are over-expressed in cancer cells and a higher concentration of inflammatory metabolites are found in many cases of cancerous cells [22-25]. This confluence of cancer and inflammation has opened another front to combat cancer by targeting the inflammatory enzymes.

**Epidemiology of the leukotrienes and Prostaglandins**

Leukotrienes and Prostaglandins are culprits for the enzyme mediated inflammation in the humans. The preliminary symptoms of acute inflammation are symptomised by swelling, redness, loss of function and heat of the affected area. The situation might get chronic characterized by several diseased conditions in humans such as asthma, atherosclerosis, rheumatoid arthritis, inflammatory bowel diseases, neurodegeneration and cancer.
Asthma
Leukotrienes are the regulators of smooth muscle contraction during bronchoconstriction. But a lofty level of LTC4, LTD4 and LTE4 impedes the lung tissues. The main cause of asthma is due to the up-regulation of these mediators. CysteinyI leukotrienes contribute to the plasma leakage from post-capillary venules in respiratory tissues, which can lead to inflammatory edema. Apart from that, a higher level of prostaglandin PGD2 protects lower airways of the lungs from bronchoconstriction. Hence, with some cadence in the production of pro-inflammatory leukotrienes and prostaglandins, a prospective anti-asthma rehabilitation could be developed.

Atherosclerosis
Lipoxygenases participate in the oxidation of low density lipoproteins (LDLs) present in the macrophages to form foam cells. These cells develop plaques of atheroma and their accretion in the arteries leads to atherosclerosis. An increase in the cysteinyI LTE4 levels in urine and LTB4 in the atheroma were observed in patients with atherosclerosis. Hence, the reticence of lipoxygenase activity may endow with a treatment strategy against this inflammatory disease.

Rheumatoid Arthritis
High level of LTB4 in the synovial fluid of rheumatoid arthritis patients has been reported. This leukotriene is produced principally by neutrophils which are most bountiful leukocytes in rheumatoid joints. The inflammatory responses were reduced in mice with the deficiency of 5-LOX and leukotriene A4 hydrolase enzyme. This specifies the probability of development of a curable remedy against rheumatoid arthritis by the inhibition of lipoxygenase activity.

Inflammatory Bowel Disease
Patients with inflammatory bowel disease (IBD) confirm several fold enhancements of 5-lipoxygenase, FLAP and LTA4 hydrolase expression in the mucosa of colon and the rectum dialysates. The urinary excretion of LTE4 significantly increased in patients with IBD. Such information evidently indicate the roles of leukotrienes in IBD and motivates the researchers to find possibilities for curing IBD by targeting lipoxygenase.

Cancer
The leucotrienes and prostaglandins are allied with some carcinogenic processes such as tumor cell proliferation, differentiation, and apoptosis. Overexpression of platelet 12- lipoxygenase (p12-LOX) has been pragmatic in human prostate cancer cells. The role of 5-LOX metabolites have been reported in the development of breast cancer by promoting the invasion of tumor cells into the lymphatic vessels and the formation of lymph node metastasis. Increased expression of the 5-LOX enzyme and the LTB4 receptors were observed in pancreatic cancer. In addition, 5-LOX expression levels were suggested as indicator for early neoplastic lesions. Albeit, the cyclooxygenases are reported to aggravate mammary cancer due to eminent PGE2 tumour yields isolated from the breast tissues. The prostaglandins such as PGE1, PGF2, PGI2 and TXA2 are formed by human mammary cancers. These studies visibly indicate that the increased lipoxygenase and cyclooxygenase activity is associated with the development of cancer and this enzyme could be the probable target for cancer treatment as well.

Therapeutic Perspectives for Cyclooxygenase enzyme
Non-Steroidal Anti-inflammatory drugs (NSAIDs). It is an important class of drugs that have been used as effective analgesics, antipyretics and as anti-inflammatory agents. NSAIDs inhibit the activity of cyclooxygenases, thereby affecting the production of prostaglandins and thromboxanes. These inhibit the activities of COX-1 and COX-2, hence also lead to gastrointestinal side effects associated with the inhibition of COX-1. Based on their difference in the mechanism of action, NSAIDs have been broadly classified into various categories including; Salicylates. The drugs are used for the treatment of arthritis pain and inflammation and have been divided into two categories including the acetylated and non-acetylated salicylates. One of the prominent drug, aspirin (acetyl salicylic acid, 1) which was first discovered from the bark of willow tree in 1763 and was first synthesized in the year 1897 relates to the acetylated subset of the salicylates. Quite lately after its consistent use as a common analgesic and anti-inflammatory agent, its mechanism of action came into limelight. It was later found that aspirin inhibits the COX-1 variant (50% inhibitory concentration, IC50 1.67 µM) more than the COX-2 variant (IC50 278 µM). Aspirin suppresses the production of prostaglandins and thromboxanes by irreversibly inhibiting cyclooxygenases. Other
drugs [42] including trilisate (2), salsalate (3) and trolamine salicylate (4) also came into use as anti-inflammatory agents (Chart 4).

**Chart 4: Acetic acid derivatives**

*Acetic acid derivatives*
This category incorporates the most common anti-inflammatory drugs including indomethacin (5) which was discovered in 1963 and was approved in the U.S. by Food and Drug Administration (FDA) in 1965. Indomethacin inhibits COX-2 with an IC50 970 nM [43]. Since, it also inhibits COX-1, therefore it is associated with gastrointestinal side-effects like causing peptic ulcers. Another important drug belonging to this class includes diclofenac (6) which was first discovered in 1973 [44] and is one of the most widely used drugs for pain, fever and swelling. Diclofenac sodium exhibits an IC50 60 and 200 nM for ovine COX-1 and COX-2 respectively [45] while it shows IC50 0.9-2.7 μM for human COX-1 and 1.5-20 μM for human COX-2 [46] and hence, a non-selective inhibitor of cyclooxygenases. This category also includes other drugs (Chart 5) like sulindac (7), ketorolac (8), etodolac (9), aceclofenac (10) and tolmetin (11). A number of reports on pyridizinone derivatives are available [47,48]. Abouzid et al. have also reported pyridazinone derivatives (12) as anti-inflammatory agents in 2008 [49] followed by a recent report in 2012 [50].

**Propionic acid derivatives**
One of the core medicines declared by the WHO incorporates the drug Ibuprofen (13) which was derived from propionic acid in 1960s [51]. Along with analgesic, antipyretic and anti-inflammatory effects, it also show antiplatelet and vasodilation effects as well and works by inhibiting both COX-1 and COX-2 isoforms of cyclooxygenases.
It exhibits an IC50 223 µM against COX-2 [52]. Other clinically used members of the family include naproxen (14), ketoprofen (15), oxaprazin (16) and flurbiprofen (17) are shown in Chart 6. Various reports have been published based upon the derivatization of clinically used drugs and their bioevaluation as anti-inflammatory agents [53,54].

**Chart 6**

*Enolic acid derivatives*

It incorporates the oxicam class of anti-inflammatory drugs [55,56]. Piroxicam [57] (18) is one of the representative members of this category. It shows both analgesic and anti-pyretic effects and is a non-selective NSAID exhibiting an IC50 0.6 µM for COX-2. Meloxicam [58,59] (19), another member of this class has been found to show lesser side-effects than piroxicam with an IC50 1.9 nM for COX-2. The sister drugs include tenoxicam (20) and lornoxicam (21) shown in **Chart 7**. Isoxicam [60] (22) was also used clinically but its marketing was banned because of its fatal skin reactions. Several piroxicam derivatives [61,62] were developed in order to reduce their gastrointestinal side-effects and prodrugs like ampiroxicam, droxicam and cinnoxam have been marketed. The prodrugs were found to be stable under gastric conditions.

**Chart 7**

*Fenamic acid derivatives*

Fenamates are a class of NSAIDs family having a common structure of Narylanthranilic acid [63] in their molecules. The fenamates are differentiated by their aryl substituents as shown in **Chart 8**. One of the members, meclofenamic acid (23) was approved by the FDA in 1980. It is a non-selective NSAID and is used for the treatment of arthritis and pain. It works by inhibiting prostaglandin production. Other members [64] include flufenamic acid (24), tolfenamic acid (25) and mefenamic acid (26). Fenamates are variously derivatized [65-67] with benzofurans, triazoles, etc. in order to improve their efficiency and to lower the risk of side-effects.
Selective COX-2 inhibitors (COXIBs)

Unlike non-selective NSAIDs, the COXIB class [68] of COX-2 inhibitors slow down the activity of COX-2 only. Very first examples of this category include DUP697 [69] (Chart 9) and NS398 [70] (28). They acted as the building blocks for the discovery of selective COX-2 inhibitors. Celecoxib [71,72] (29) and Rofecoxib [73] (30) were launched as selective COX-2 inhibitors in 1998 and 1999 respectively. Both the drugs did not produce the ulcerogenic effects like the other NSAIDs. Later, valdecoxib [74] (31) was also introduced to be used as a selective COX-2 inhibitor but because of the increased side effects associated with rofecoxib and valdecoxib, causing heart attack and strokes (as a result of activation of 5-LOX pathway), they were withdrawn from the market [75,76]. After the exclusion of rofecoxib and valdecoxib, celecoxib is the only drug which is available in the market as a selective inhibitor of COX-2. Celecoxib exhibits an IC50 40 nM for COX-2 as compared to an IC50 15 μM for COX-1 thus having a 375-fold selectivity for COX-2 recombinant enzyme assays [77].
Etoricoxib (32) and Parecoxib (33) are the other selective COX-2 inhibitors used in around 80 countries all over the world but have not still been approved by FDA. Tilmacoxib (JTE-522) [78] (34) has also been reported as COX-2 selective inhibitor and is used against osteoarthritis and rheumatoid arthritis.

Therapeutic perspectives for lipoxygenase enzyme

FLAP inhibition

MK-886 is an effective anti-inflammatory agent [79] which reduces the leucotrienes biosynthesis by binding to a 1800 KDa protein [80], better known as Five Lox Activating Protein [81]. For the optimum catalytic activity of lipoxygenase enzyme the FLAP should be bound to the substrate in the membranes but in the presence of MK-886, this becomes limiting [82]. BAY-X-1005 (Chart 10) is a compound of this class with inhibitory concentration in µM range [83]. However the FLAP inhibition therapy is of less significance as there is a competitive binding in the presence of the natural substrate.

Iron chelation

The activity of lipoxygenase is closely related to the oxidation states of the cofactor [84,85]. Molecules which can form the chelate complexes with iron could be the prospective inhibitors of 5-LOX enzyme. Hydroxamic acid and N-hydroxyurea functionalities are identified with effective inhibitory properties against 5-LOX enzyme [86]. Zileuton (Chart 11) which is commercially available for the treatment of asthma effectively inhibits the activity of 5-LOX enzyme by forming a chelate complex with the iron cofactor [87]. Due to its distinctive affects on the liver, the use of zileuton is still questionable [88]. Furthur, atreleuton with a higher effectiveness over zileuton was developed [89] which mainly focuses on the inhibition of cysteinyll leukotrienes. The capability of atreleuton to cure atherosclerosis is under clinical tests.
Redox inhibition
Redox inhibitors have a limiting effect on the expression of lipoxygenase enzyme by acting as antioxidants. Phenidone and BW755C (Chart 12) are well known drugs in this category [90]. The caffeoyl clusters have been identified for their redox inhibition for 5-LOX enzyme with an IC50 0.79 μM and 0.66 μM [91]. However, the redox inhibitors have only an average selectivity for 5LOX [92]. Apart from their appreciable potential to inhibit biosynthesis of leukotrienes, the major drawback is that these encumber with the biological redox processes. The formation of methaemoglobin is a setback that was reported upon application of redox inhibitors. [93,94].

Non-redox inhibitors
Non-redox inhibitors have a mode of action different from redox inhibitors, FLAP inhibitors and iron chelation inhibitors [95]. These inhibitors act by competitively binding to the active site of lipoxygenase enzyme [96]. The non-redox inhibitors like methoxyalkyl thiazoles (ICI211965) (Chart 13) are reported to selectively inhibit 5-LOX activity and cut down the LTB4 and LTC4 synthesis in animal as well as human blood samples [97]. The compound ICI211965 has been acknowledged with a good 5-LOX inhibitory activity. It however, has a below average oral effectiveness [98]. To cope with this problem, methoxytetrahydropyrans (ZD-2138) [99] are developed for the treatment of arthritis and asthma [100] with an improved oral potency compared to ICI211965. Despite all the positive outcomes of this class of compounds, their use as an anti-arthritis agent has been discouraged due to the poor results obtained from their clinical trials.

Leukotriene antagonists
Another class of compounds, known as leukotriene receptor antagonists, has been developed which have quite applaudable properties against the leukotriene biosynthesis [101]. Pranlukast, Zafirlukast and Montelukast (Chart 14) are the most popular leukotriene receptor antagonists known for their effectiveness in the treatment of asthma [102,103].
Chart 14

These therapeutics perform by blocking the cysteinyl leukotrienes C4, D4 and E4 in the bronchi [104] which lessens the constriction in the bronchial airways and mucus accumulation in the lungs [105]. It has also been reported that montelukast inhibits the cysteinyl leukotrienes, LTC4, LTD4 and LTE4 [106] but despite its efficacy against asthma, the safe use of montelukast is still under question as it causes liver related problems [107,108].

CONCLUSION

The cyclooxygenase-2 isozyme is associated with several medical complications which are expressed in the form of acute/ chronic inflammation or even cancers. This has led to the development of several medications. The research is on rise to find the methods to curb the menace caused by the overexpressed metabolites of arachidonic acid metabolism. Yet only a limited success has yet been ensured.

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