Drugs that Target Lipoxygenases and Leukotrienes as Emerging Therapies for Asthma and Cancer

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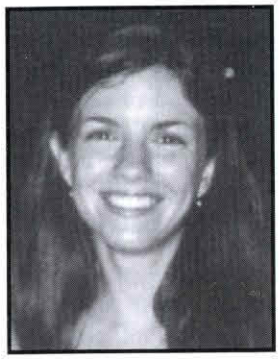

Abstract: Considerable amount of work has been done in the area of enzymatic and non-enzymatic oxidation of arachidonic acid. This effort resulted in understanding of the functions of lipid mediators – eicosanoids in various aspects of health and disease. A mechanism by which aspirin exerts therapeutic effects puzzled pharmacologists for a long time until John Vane, in 1971, discovered that aspirin and its congeners block formation of prostaglandins, a class of lipids that originate from oxidation of arachidonic acid by cyclooxygenase. Since that discovery the pharmacology of eicosanoids has substantially progressed, which resulted in new drugs available in clinics. In addition to many new inhibitors of cyclooxygenase, two isoforms of which are known, much effort has been given to find inhibitors of synthesis and function of leukotrienes, a class of lipids that are derived from 5-lipoxygenase. These lipids are generated in asthma and their uncontrolled biosynthesis aggravates the symptoms of asthma. A new class of drugs called lukasts, inhibitors of 5-LOX products, has been developed and entered clinics as the first new therapy to treat asthma in nearly 20 years. New discoveries in the field of lipoxygenase show great opportunities for drug development for cancer prevention and treatment as it has been established that lipoxygenases and their products are required for cancer growth. Intense research in this field is likely to produce new drugs in the near future.

Keywords: Asthma, Cancer, Lipoxygenases, Leukotrienes, Leukotriene antagonists, Hydroxyeicosatetraenoic acids (HETE); Lipoxins.

INTRODUCTION

Arachidonic acid is a polyunsaturated fatty acid that can be metabolized by a complex array of enzymes into important lipid mediators, eicosanoids, following activation of phospholipases by hormones and other factors, and hydrolysis of cellular phospholipids, from which it is liberated. The three major metabolic enzymatic pathways involve isoforms of cyclooxygenase (COX), lipoxygenase (LOX) and monooxygenase cytochrome P450 (CYP) and additional pathways that aim to inactivate and remove eicosanoids to terminate their function of signal mediators. Because inflammation and other pathologies increase and sustain biosynthesis of eicosanoids that have potent and detrimental effects on functions of many organs, there has been a continuous interest in finding drugs that would limit the effects of these lipids in disease. Many successes have been achieved in this area of pharmacology. Aspirin is the prototypical drug whose mechanism of action as well as side effects were first explained in terms of inhibition of COX, an enzyme that metabolizes arachidonic acid into mediators of multiple functions, prostaglandins (including thromboxane and prostacyclin) [1, 2]. Since COX is a ubiquitous enzyme, its inhibition has effects on the function of almost every organ, tissue and cell. After this important discovery, many COX inhibitors have been developed and used in a more rational way. These inhibitors are collectively known as nonsteroid, anti-inflammatory drugs or NSAIDs, to distinguish from the effects of anti-inflammatory properties of steroids such as corticosteroids, which can also inhibit prostaglandin formation by a different mechanism [3]. Dozens of NSAIDs and their formulations have been available since their discovery in the 1920s. They appear to be one of the most popular and frequently used groups of drugs. Discovery of a second, cytokine inducible form of cyclooxygenase (COX-2) stimulated research in developing new drugs having greater specificity for COX-2 and sparing the original enzyme, now dubbed COX-1, so that the organ protective effects of prostaglandins are not disturbed. This new class of drugs has been named "coxibs" with three of its members, celecoxib, rofecoxib, and valdecoxib now available for the treatment of rheumatoid arthritis and osteoarthritis [4, 5]. However, not all of the problems have been solved by the characterization of two COX enzymes originating from different genes. One such problem is the effect of another popular drug, acetaminophen (paracetamol), a component of Tylenol® and other formulations, which, unlike NSAIDs, has little effect on inflammation but produces analgesia and antipyresis. The existence of additional COX enzymes was therefore postulated [6, 7]. Recently Simmons detected a new COX isoform (named COX-3) [8] that appears to be more sensitive to acetaminophen; however, other papers question the existence of COX-3 [9, 10]. The multiple COX proteins pose a particular challenge for drug discovery because compounds of high isoform specificity and selectivity will be needed, which would not cause side effects, such as gastrointestinal bleeding and renal function depression, which may arise from inhibition of prostaglandins generated by COX-1. A second pathway of arachidonic acid metabolism involves

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lipoxygenases (LOXs) and leads to generation of important mediators of inflammation – leukotrienes, lipoxins and other products. There are five active LOXs found in humans: 5-LOX, 12*S*-LOX, 12*R*-LOX, 15-LOX-1 and 15-LOX-2. Several drugs have now been developed that either block leukotriene formation or their receptors. The group of leukotriene receptor antagonists is known as lukasts and these are the first new drugs that are available for treatment of asthma and related disorders in nearly 20 years. LOX pathways leading to numerous other lipids are complex and offer a potential for new drug development that has not yet been fully explored. Because new studies have revealed that lipoxygenase-derived lipids can mediate tumorigenesis so, there is additional interest in the development of new specific lipoxygenase inhibitors directed towards inhibition of tumor growth and potentially other effects. Finally, a third pathway of arachidonic acid metabolism involves oxidation by the inducible cytochrome P450 isozymes, and although no clinically useful drugs are yet available, promising research in this area [11] is directed toward yielding valuable drugs that block generation and symptoms of stroke [12, 13].

This article aims to review current information about lipoxygenases, their products, drugs that have been recently available, which specifically inhibit overproduction of oxidized arachidonic acids, and future prospects in this interesting area of research with emphasis on their role in asthma and cancer.

1. BIOSYNTHESIS OF HYDROPEROXYACIDS AND LEUKOTRIENES

Shortly following the identification of prostaglandins as products of COX, the significant pathway of arachidonic metabolism catalyzed by LOX enzymes was discovered (Fig. **1**). Unlike prostaglandins that have three oxygens attached to arachidonic acid from two moles of oxygen, LOX enzymes insert only one mole of oxygen into the molecular structure of arachidonic acid. Initially, the free fatty acid is oxidized to a hydroperoxy derivative (hydroperoxyeicosatrienoic acid, HpETE), which is subsequently reduced by ubiquitous glutathione peroxidase to create a more stable hydroxy acid (HETE). Like other polyunsaturated fatty acids, arachidonic acid features a *cis, cis*-1,4-pentadiene, which occurs in four locations, and is a requirement for all of the fatty acids to be substrates for mammalian, and also plant and fungal lipoxygenases [14]. These enzymes have been characterized as containing a single atom of non-heme iron at catalytic site and having a single polypeptide chain with a molecular mass of ~75-80 kDa in animals and ~94-104 kDa in plants [15, 16]. The metal is positioned among three conserved histidines and to the carboxyl group of a conserved isoleucine at the C terminus of the protein [16]. Analysis of the human and mouse genome sequences has enabled a detailed analysis of the structure and organization of the lipoxygenase genes [17]. Humans have six functional genes and at least three pseudogenes while mice have seven functional genes. The arrangement of the genes is similar between the species with most of the human lipoxygenase genes appearing on the short arm of chromosome 17 and in mice on the syntenic portion of chromosome 11 [17]. The 5-LOX gene is unique in several respects including its distinct localization in chromosome 10 and its larger size. While the knockout mice appear normal, a number of important findings have been discovered using these mice [17]. Two human 12-LOX enzyme-related genes have been characterized from 13 distinct clones isolated from three genomic bacteriophage and cosmid libraries [18]. Exon-intron boundaries for the 12-LOX genes were located in the identical corresponding positions to the previously cloned human 5-LOX and rabbit 15-LOX genes, indicating a highly related gene family. These lipoxygenases metabolize arachidonic acid to different HpETE and HETE positional isomers. Lipoxygenases can efficiently oxidize other fatty acid substrates such as eicosapentaenoic and docosahexaenoic acids [19, 20] but also other arachidonic acid derivatives such as phospholipids [21], glycerol [22, 23] and anandamide [24]. Five mammalian lipoxygenases insert oxygen into either of the 5, 12 or 15 positions of arachidonic acid (Fig. **2**). As a molecule of oxygen is inserted, a proton is removed from a *bis*- allylic methylene group with the subsequent formation of a new *trans* double bond and a hydroperoxy group. The typical observation of the *S* configuration in the HpETE products indicates a reaction that is stereospecifically controlled by lipoxygenases. An otherwise similar mechanism responsible for the spontaneous free radical mediated autoxidation of arachidonic acid differs from this mechanism in that it leads to a racemic mixture of HpETEs. However, recently Brash *et al.* described lipoxygenases that produce HETEs of opposite stereochemistry, 12*R*-HETE and 15*R*-HETE [25, 26]. It has been recognized that lipoxygenases forming the mirror image *R* configuration products are also widespread, being found in humans [16, 25]. *R*-Lipoxygenases contain the same conserved iron ligands and other sequence motifs common to *S*-lipoxygenases [16]. Although the first lipoxygenase product of arachidonic acid, 12*S*-HETE, was described in blood platelets, formation of 5*S*-HpETE in other cells appears to be of greater interest, because it is a precursor in the formation of potent biologically active leukotrienes (Fig. **3** and **4**). While COX is found in almost all mammalian cells, 5-LOX is restricted to mainly neutrophils, eosinophils, monocytes, macrophages, mast cells and dendritic cells [15]. These various cells are believed to be of clonal origin, all of them originating from a common precursor stem cell in the bone marrow. All these cells have a fundamental role in immunological defense and inflammatory response; therefore the presence of a common 5-LOX enzyme has been suggested to be of functional significance. Other lipoxygenase enzymes have been described in cells not stemming from bone marrow such as blood vessels, brain, skin and other cells [15, 27, 28]. In contrast to COX, which appears to be constantly in an active state and requires oxygen and peroxide tone for formation of prostaglandins, the 5-LOX requires a complex multi-component activation system. For example, Ca^{2+}, ATP and at least three non-dialyzable factors, one membrane bound and two cystolic, regulate the leukocyte 5-LOX [15]. The formation of leukotrienes proceeds via the removal of a water molecule from 5-HpETE by a specific dehydrase enzyme, leukotriene A_4 (LTA_4) synthase, which produces an unstable 5,6-epoxide with three conjugated double bonds (a triene molecule) (Fig. **3**). The triene, a unique structural feature, produces a characteristic ultraviolet spectrum. The first observation of these triene lipids occurred in the leukocyte

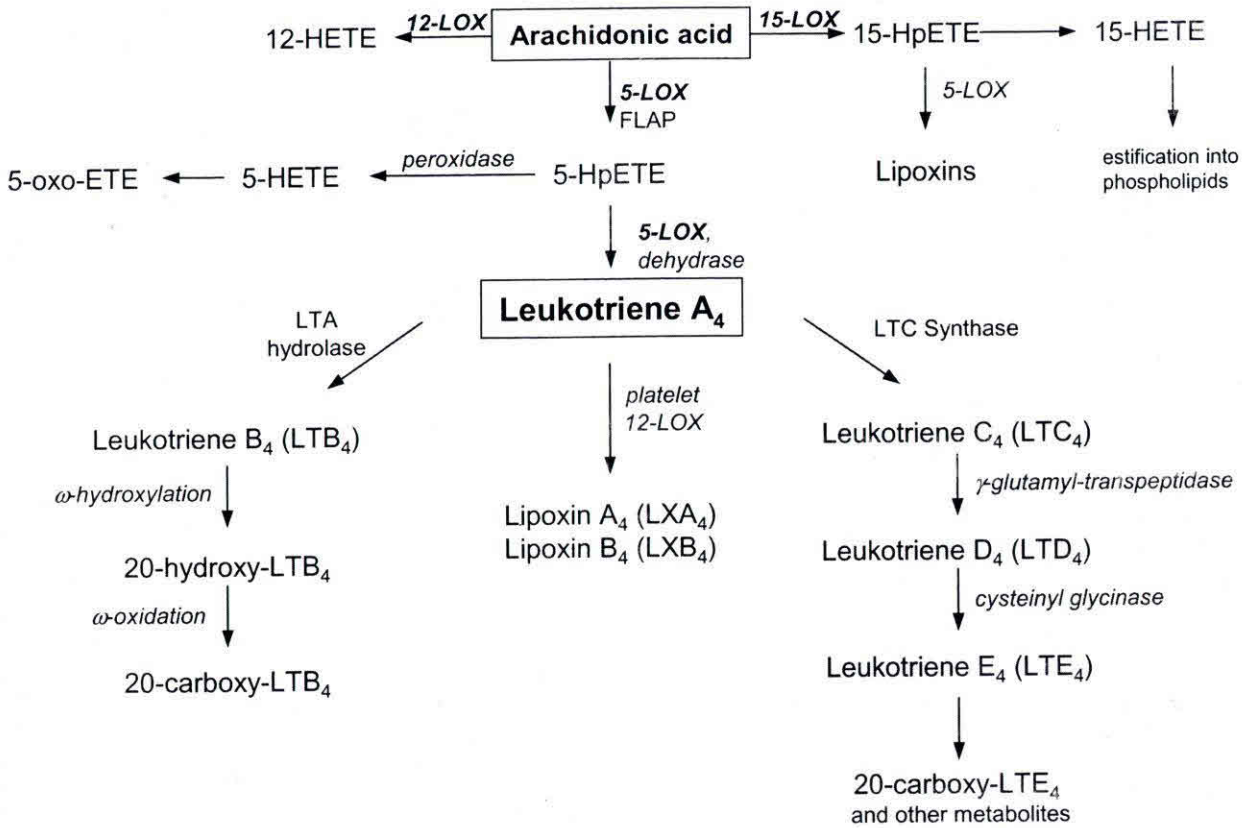

Fig. (1). Major biochemical steps involved in arachidonic acid metabolism by lipoxygenase pathways.

leading Bengt Samuelsson to coin the name leukotrienes to describe this type of lipids in 1979 [29, 30]. The role of endoperoxides in the prostaglandin cascade is analogous to the role LTA_4 that plays as a crucial intermediate in the biosynthesis of two groups of leukotrienes: leukotriene B_4 (LTB_4) and leukotriene C_4 (LTC_4). Enzymatic hydrolysis of LTA_4 by a soluble hydrolase leads to LTB_4 (Fig. **3**). Non-enzymatic hydrolysis of LTA_4 results in a mixture of 5,6- and 5,12-dihydroxy acids. LTA_4 can also be conjugated with glutathione to form LTC_4 (Fig. **4**) by a distinct form of glutathione S-transferase, which is known as LTC synthase (LTC4S). At least two forms of the enzymes have been described, and in humans the LTC4S gene promoter shows significant polymorphism [31, 32]. Furthermore, LTC_4 can be hydrolyzed by a γ-glutamyl transpeptidase to LTD_4; and from LTD_4 to LTE_4 by cysteinyl glycinase. The products that form a group of sulfidopeptide leukotrienes, LTC_4, D_4, E_4, (Fig. **4**), are now known as the components of the "slow reacting substance of anaphylaxis" (SRS-A), an active principal released by pulmonary tissue in response to antigen challenge, first described by Brocklehurst in 1953 [33]. The mixture of sulfidopeptide leukotrienes accounts for the biological activity of SRS-A, which had been originally described as having a potent "bronchoconstrictor activity" and further noted for contracting smooth muscle of the respiratory tract by Kellaway and Trethewie in the late 1930's [33].

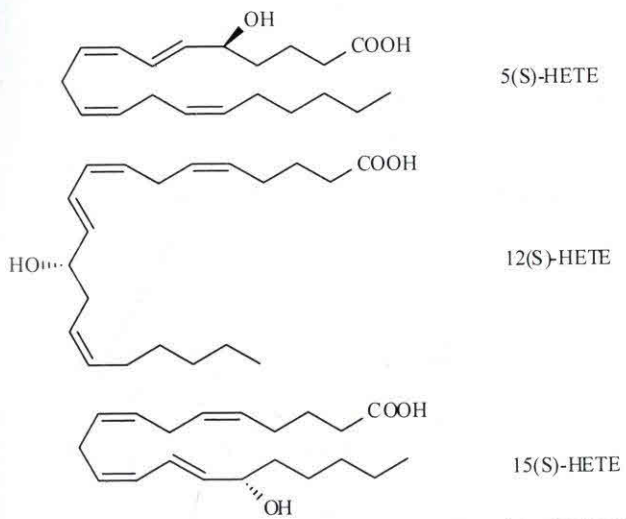

Fig. (2). Structures of hydroxyeicosatetraenoic acids (HETEs), products of 5-, 12-, 15-lipoxygenases having the *S* stereoconfiguration of the hydroxyl group.

2. METABOLISM OF LEUKOTRIENES

The metabolic pathways of leukotrienes are not understood as well as the metabolism of prostaglandins; thus a gap exists in our knowledge of their metabolic fate. As previously discussed, specific peptidases rapidly convert LTC_4 to LTD_4 and then, LTD_4 to LTE_4. All three compounds, LTC_4, LTD_4 and LTE_4, are biologically active. LTE_4 is excreted in urine and thus the measurement of the fluctuation of LTE_4 levels in urine has provided a useful index of systemic leukotriene synthesis. For example,

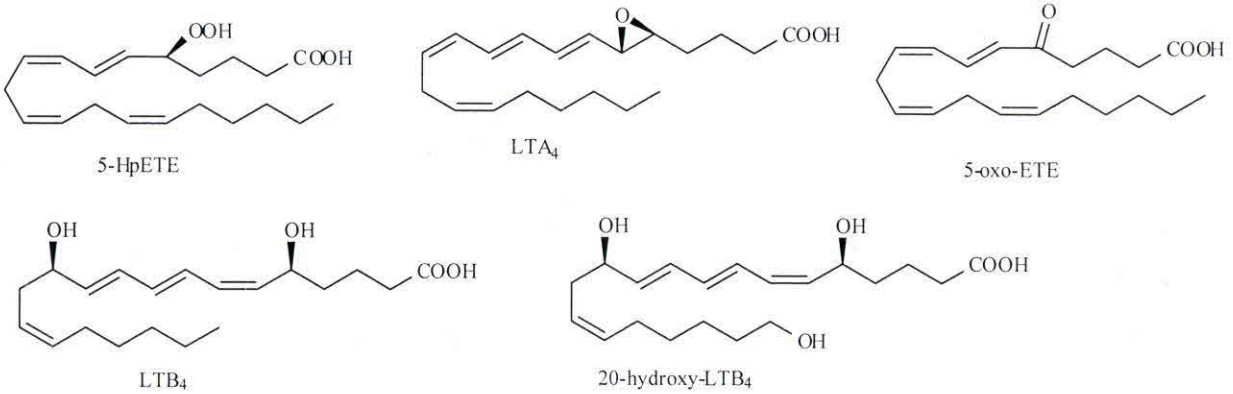

Fig. (3). Structures of 5-hydroxyeicosatetraenoic acid (5-HpETE), 5-oxo-eicosatetraenoic acid (5-oxo-ETE), leukotrienes A_4 (LTA_4), B_4 (LTB_4) and a major metabolite of LTB_4, 20-hydroxy-LTB_4.

during an anaphylactic attack, urinary LTE_4 levels are increased [34]. However, Szczeklik *et al.* noted that only 10-20% of total cysteinyl-leukotriene body production is excreted by the kidney, which may limit usefulness of urinary LTE_4 levels to predict clinical outcomes [35]. An active transport system collects a concentration of sulfidopeptide leukotrienes in the hepatocyte, which can further oxidize LTE_4 at the C20 terminus. Several rounds of β-oxidation convert ω-hydroxy-LTE_4 to several ω-carboxy LTE_4 metabolites [36]. Omega-oxidation and subsequent β-oxidation from the methyl terminus of the LTE_4 appear to be the major metabolic route for sulfidopeptide leukotrienes, also known as cysteinyl leukotrienes (cysLTs) in humans. Such metabolites of LTE_4 could be useful in assessing *in vivo* production of these leukotrienes; yet further studies are needed to reveal the potential of these metabolites as biological markers of LT formation. In addition to the known presence of leukotrienes in urine and hepatocytes, sensitive methodologies have also been developed to directly measure levels of sulfidopeptide leukotrienes in lung homogenates, sputum, exhaled breath condensates and in the lung lavage of infants suffering from bronchopulmonary dysplasia [37]. Much like LTE_4, LTB_4 is also inactivated within cells in which LTB_4 is formed as well as by hepatocytes. LTB_4 metabolism was studied in liver cells, keratinocytes, human PMN, cultured HepG2 cells and primary cultures of human hepatocytes [38]. The oxidation pathway for LTB_4 involves ω-hydroxylation by a unique membrane-bound NADH dependent cytochrome P450 monooxygenase system into 20-hydroxy-LTB_4, which can be further oxidized by a separate dehydrogenase to 20-carboxy-LTB_4. These oxidations substantially reduce LTB_4 activity, and were observed in human polymorphonuclear leukocytes and liver. In neutrophils, a specific $CYP450_{LTB\omega}$ enzyme oxidizes LTB_4 into ω-hydroxy-LTB_4 [39-41] (Fig. **3**) whereas in hepatocytes, CYP4F proteins act as specific LTB_4 ω-hydroxylases [42, 43]. Recent work suggests that the leukocyte-derived ω-hydroxylase is also a CYP4F type protein [41]. Novel pathways involving glucuronidation, β-oxidation from the carboxyl terminus and cysteinyl LTB_4 formation have been also identified in human hepatocytes [38].

3. PHARMACOLOGICAL PROPERTIES OF LEUKOTRIENES

Leukotrienes are very potent molecules acting through receptors at subnanomolar concentrations (Table 1). LTC_4 and LTD_4 cause hypotension in humans by a significant reduction in coronary blood flow. LTC_4 and LTD_4 constrict coronary arteries and distal segments of the pulmonary artery at nanomolar concentrations. The sulfidopeptide leukotrienes show prominent and potent effects on microvasculature. LTC_4 and LTD_4 appear to act on the endothelium of post capillary venules, cause plasma exudation and are more than 1000-times more potent than histamine in this activity. In addition to their effects on coronary blood flow, LTC_4 and LTD_4 are potent constrictors of bronchial smooth muscles. The constriction of bronchial smooth muscle is achieved by the activation of receptors located on smooth muscle in peripheral airways. Leukotrienes also stimulate bronchial mucus secretion and cause mucosal edema. Immunohistochemical studies of mucosal biopsies from the bronchi of aspirin-intolerant asthmatics demonstrate that LTC4S is amplified in individuals with this phenotype, and this finding correlates with an overproduction of cysteinyl leukotrienes and lysine-aspirin bronchial hyperreactivity [44].

Table 1. Characteristics of Leukotriene Receptors

Receptor	Agonist	Antagonist	K_m (nM)	References
$CysLT_1$	$LTD_4 > LTC_4 \geq LTE_4$	Lukasts (see Fig. **5**), BAYu9773	0.25	[51, 52]
$CysLT_2$	$LTC_4 = LTD_4 > LTE_4$	BAYu9773	0.015	[50, 63]
BLT1	LTB_4 (high affinity)	CP-105696, U-75302, CP-195543, ZK-158252	0.15	[61, 62]
BLT2	LTB_4 (low affinity) 12(*S*)-HETE, 15(*S*)-HETE	ZK-158252, LY-255283, CP-195543	23	[62, 131]

Though LTC_4 and LTD_4 show considerable activity in blood vessels and the bronchi, they do not activate most leukocytes. LTB_4 is unique with respect to other leukotrienes in that it acts as a potent chemotactic and chemokinetic lipid for PMN leukocytes, eosinophils, and monocytes [45]. The potency of LTB_4 is comparable to that of platelet-activating factors and chemotactic peptides. LTB_4 is biologically important for the removal of pathogens by activating and recruiting granulocytes to the inflamed lesions as well as stimulating phagocytosis and the killing of microbes [46]. Higher concentrations of LTB_4 are responsible for the causation of PMN aggregation, degranulation and generation of superoxide, adhesion of neutrophils to vascular endothelium and trans-endothelial migration. An overproduction of LTB_4, however, can lead to various inflammatory diseases including psoriasis, bronchial asthma, rheumatoid arthritis, ulcerative colitis, and ischemic reperfusion injury in various tissues [45, 47, 48].

4. LEUKOTRIENE RECEPTORS

4.1 CysLT receptors

Pharmacological probes and radiological binding techniques have been utilized to identify the distinct receptors that bind LTB_4, LTC_4, and LTD_4/LTE_4 in various preparations. Many leukotriene antagonists of different structural types have been obtained and studies support the existence of four leukotriene receptors in two major categories, CysLT receptors and BLT receptors. The experimental data also support existence of not yet fully characterized additional receptors [49]. The biological actions of CysLTs such as bronchoconstriction, microvascular edema, mucus hypersecretion and eosinophil chemotaxis are mediated by CysLT type 1 ($CysLT_1$) and type 2 ($CysLT_2$) receptors distinct from those for LTB_4 receptors [50] (Table 2). Both receptors, which have been cloned and characterized at the molecular level [51-53] show interesting differences in distribution. Whereas expression of $CysLT_1$ mRNA has been found mainly in leukocytes, airway smooth muscle and spleen, large amounts of $CysLT_2$ mRNA have been found in the heart, brain and central nervous system, placenta, spleen and leukocytes, but not in the lung [50]. Differences in function between the two receptors also arise when considering the different molecules that target each type of receptor. The $CysLT_1$ receptor is the target for a recently introduced group of drugs named "lukasts" [54-56] (montelukast, zafirlukast, pranlukast, pobilukast and others) (Fig. **5**). These drugs do not antagonize the $CysLT_2$ receptor and the activities of cysteinyl leukotrienes mediated by this receptor are not yet well characterized. For the two CysLT receptors, one dual antagonist has been identified, BAYu9773 [50]. The CysLTs relax many vessels at very low concentrations (10^{-8} – 10^{-7} M) by the production of nitric oxide and prostanoids and supposedly via the $CysLT_2$ receptor on the vascular endothelium [50]. At higher concentrations vascular constriction occurs apparently via $CysLT_1$ receptor. The activation of $CysLT_1$ receptor appears to lead to a cascade of reactions resulting in the mobilization of intracellular Ca^{2+}, reduction of cyclic AMP, and activation of phospholipase C. The possibility that these two receptors may mediate opposing effects of leukotrienes in other tissues is under current investigation. Particularly puzzling is high distribution of the $CysLT_2$ receptors and lack of $CysLT_1$ receptors in the brain [57]. While neuroendocrine function of LTC_4 has been reported [58], studies in this area suggest a role for $CysLT_2$ in neurological inflammation and other pathologies. Further studies directed towards cloning CysLT receptor-deficient animals [51, 59] are likely to yield more leads concerning the pathophysiological roles of these receptor subtypes.

Table 2. Summary of Cysteinyl Leukotriene Receptor-Mediated Effects

Effect/action	$CysLT_1$	$CysLT_2$
Airway actions and asthma	+	
bronchoconstriction, bronchial hyperesponsiveness	+	
microvascular permeability	+	
retraction of endothelial cells	+	
pulmonary edema	+	
mucus secretion	+	
eosinophil infiltration	+	
airway obstruction and inflammation in asthma	+	
eosinophil receptor distribution	+	
Cardiovascular functions	+	+
coronary constriction	+	+
cardiac anaphylaxis induced by PAF	+	
arterial relaxation		+
cardiac failure	–	+
pulmonary vein relaxation		+
Neuroendocrine modulation		+
LH and LHRH release	–	+
neuroendocrine functions		+
neurological inflammation	+	+
brain, spinal cord receptor distribution	–	+

4.2 LTB receptors

Two G-protein-coupled receptor types for LTB_4 have been cloned and characterized, BLT1 and BLT2 [60-62]. The second LTB_4 receptor identified, BLT2, shows a high homology with BLT1 of 36-45% amino acid identity [63], yet differences arise in their affinity for the ligand and the locations at which they are expressed. BLT1 is a high affinity receptor exclusively expressed in leukocytes [64], and BLT2 is a low affinity receptor expressed more ubiquitously and found in the spleen, liver, ovary and leukocytes [60]. Whereas numerous BLT receptor antagonists have been developed to block LTB_4 actions in vitro, no BLT antagonist is available for clinical use. Molecular characterization of these receptors is likely to accelerate research into clinically useful BLT blockers.

5. LEUKOTRIENE ANTAGONISTS

Both newly developed drugs that are available for clinical application and other drugs still in early experimental phases act to treat asthma and other related conditions either by 5-LOX inhibition, acting as a specific leukotriene antagonist, or binding to 5-LOX activating protein (FLAP). The development of drugs targeting FLAP has yet to proceed beyond experimental use but they function by binding to the

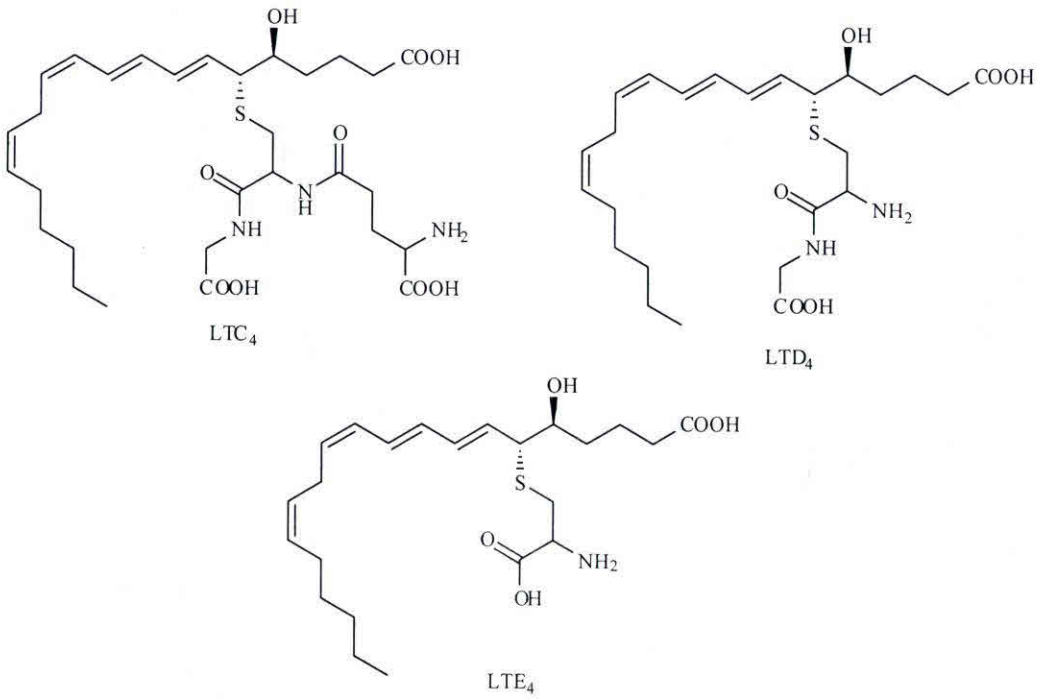

Fig. (4). Structures of cysteinyl leukotrienes.

FLAP and blocking it from trafficking 5-LOX from the cytosol to the membrane compartment for the formation of leukotrienes [65]. Other studies suggest that FLAP functions as an arachidonic acid presenting protein in which case drugs that target FLAP in this manner will need to be developed. Both 5-LOX inhibitors and leukotriene receptor antagonists have advanced to clinical stages and are most commonly associated with the treatment of asthma. This resulting new class of asthma therapy drugs includes montelukast (Singulair^{TM}), zafirlukast (Accolate^{TM}), pranlukast (Onon^{TM}), and zileuton (Zyflo^{TM}) (Fig. **5**). A naturally occurring flavonoid also known to inhibit 12-LOX, baicalein inhibits LTB_4 and LTC_4 biosynthesis at micromolar concentrations [66]. Zileuton is both potent and selective as a 5-LOX inhibitor but short acting (Zileuton needs to be administered four times a day) due to a half-life of about 2.5 hours. Zileuton inhibits all 5-LOX –derived lipids (Fig. **6**). It may seem that a therapy that uses 5-LOX inhibitor should be more effective because all products that originate from 5-HpETE will be inhibited; however, clinical studies suggest that zileuton is not more efficacious than $CysLT_1$ receptor blockers in asthma therapy probably, in part, because other LT receptors are also involved that are not inhibited by the drug [35]. Montelukast is a potent, specific leukotriene receptor antagonist. Administered once daily in a tablet form, montelukast reduces the signs and symptoms of chronic asthma in children as young as two years of age with toxicity profiles similar to that of the placebo [67]. In addition to the relief for chronic asthma, montelukast has been shown to inhibit exercise-induced broncho constrictors in asthmatic patients. Interestingly, montelukast has demonstrated effectiveness both in patients with aspirin-induced asthma and patients with aspirin-tolerant asthma in a similar manner suggesting tachyphylaxia and/or the involvement of downregulation of LTC receptors. There is some recent data that suggest that aspirin-induced asthma may be associated with increased $CysLT_1$ expression [68]. In some countries, but not in the USA, pranlukast is also used as a $CysLT_1$-receptor antagonist in the treatment of asthma.

Receptor antagonists that have been recently developed are: cinalukast and ablukast (Hoffmann-La Roche, Nutley, NJ, USA), pobilukast (SmithKline and Beecham, King of Prussia, PA, USA), tomelukast (Eli Lilly, Indianapolis, IN, USA), iralukast (Ciba-Geigy, Basel, Switzerland), and verlukast (Merck, Montreal, Canada). Recent studies of cinalukast found the drug to be effective in providing 8 hours of relief from the signs and symptoms of asthma for those experiencing exercise-induced bronchoconstriction. Cinalukast has been withdrawn from development due to the overall ineffectiveness of the drug for asthmatics [69]. After one week of regular administration, the drug lost its relieving effects. Studies examining pobilukast also identify it as a bronchoconstricting drug similar in effects to histamine. Unlike histamine, however, it has been found that pobilukast only elicits a small change in the intracellular concentration of calcium ions and it inhibits the translocation of PKC-α [70]. Only recently has a study shown that Singulair may be of some benefit as an acute bronchodilator in the emergency room for the treatment of asthma exaberations [71], previously it was believed that Singulair and other CysLT receptor antagonists were not designed for the fast relief of acute asthma attacks or the prevention or treatment of asthma induced by exercise. These drugs can help to control children's asthma for up to 24 hours; other drugs continue to be used for treatment of acute asthma symptoms [63]. Tomelukast has been withdrawn from clinical trials but has been used in recent experiments on animals as a smooth muscle contractor. Like tomelukast, verlukast and ablukast have also been withdrawn from development due to the inability to develop pharmaceuticals from these drugs that are both safe and effective [72].

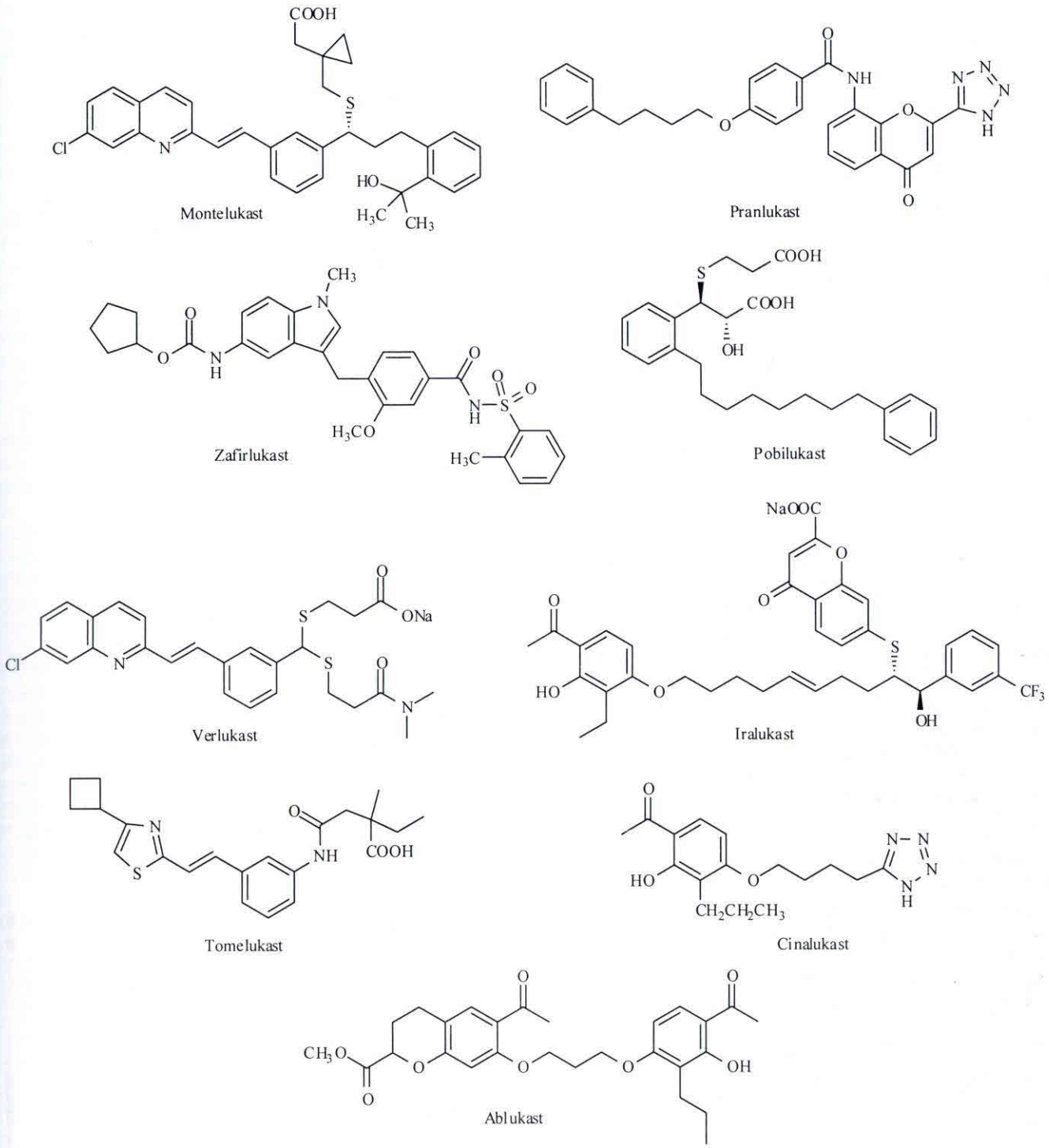

Fig. (5). Selected cysteinyl leukotriene receptor antagonists (lukasts).

6. LIPOXYGENASES

Lipoxygenases are abundant enzymes that have been identified in numerous cells including platelets, leukocytes, and vascular and neural tissue. Typically, the same HETE isomer can be produced by several distinct lipoxygenase isoforms. For example, two types of 12-LOX have been referred to as "platelet" type and "leukocyte" type both of which synthesize 12(*S*)-HETE [73]. Since their identification, many studies have explored the 12-LOX and 15-LOX pathways and their implications for asthma, inflammation, cancer and other disease processes; these studies are described in greater detail below. A more recently identified lipoxygenase that is just beginning to be researched is 8(*S*)-lipoxygenase, which has been detected in rats and mice but not human tissues [74]. Nevertheless, studies of 8-LOX have provided valuable information on the role of LOX in rodent cancer models.

6.1 12-LOX

12(*S*)-HETE, a product of 12-LOX, was first observed in human platelets. It is an abundant product that has been

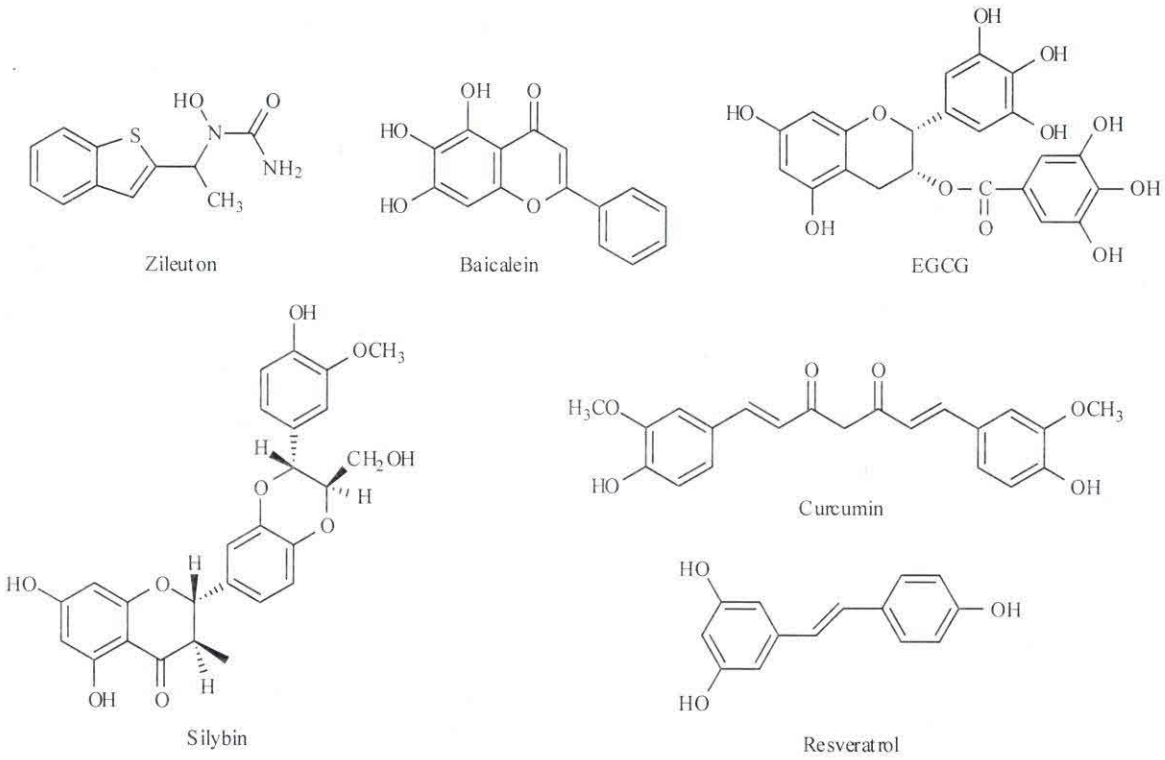

Fig. (6). Structures of 5-LOX inhibitor (zileuton), 12-LOX inhibitor (baicalein) and several natural anticancer compounds.

known to reach microgram levels per sample though it should be noted that no enzyme activity has been observed in the prostate [75]. Although 12(*S*)-HETE is a major arachidonic acid metabolite in platelets, the acid has also been produced by macrophages, erythrocytes, and lymphocytes [76, 77]. Various activities of 12(*S*)-HETE have been studied in addition to the modulation of platelet activation such as its postsynaptic responses to histamine.

Recent studies have focused on characterizing the roles of 12(*S*)-HETE and 12-LOX in cancer growth and angiogenesis [73, 78, 79]. The overexpression of 12-LOX in human prostate cells correlates with prostate tumor growth and stimulates angiogenesis. In contrast, baicalein, a flavonoid and a potent 12-LOX inhibitor, slows down metastatic prostate tumor growth and tumor angiogenesis. These experiments propose that 12-LOX and possibly other lipoxygenases could be important regulators of tumor angiogenesis via generation of proangiogenic lipids, such as 12(*S*)-HETE, and that inhibition of 12-LOX could be a novel therapeutic approach for the treatment of prostate cancers and tumor angiogenesis [80].

Detailed descriptions of the chemical structure and molecular activity of binding proteins and receptors for HETEs have become available only recently. For example, a 12(*S*)-HETE high affinity-binding protein (dissociation constant < 1 nM) has been found in lung carcinoma cells. This binding protein activates a signaling mechanism involving nuclear receptor co-activator protein suggesting a role of 12(*S*)-HETE in gene transcription [81]. Another possible function of HETEs involves the interaction of HETEs with the biological membrane to affect permeability. Unlike prostaglandins, HETEs are easily esterified into membrane phospholipids making such an interaction likely. Thus, HETE molecules may influence membrane properties such as permeability and also release secondary signaling molecules in a more delayed and hormone- dependent way e.g. following stimulation by phospholipases. In addition, 12-LOX interacts with low-density lipoproteins (LDL) and demonstrates the ability to directly oxygenate unsaturated fatty acids contained in cell-mediated LDL by a mechanism that does not involve the secretion or leakage of the enzyme. The assumption was made that a membrane receptor provided the means by which the oxidation of LDL occurred, but experiments revealed that neither the LDL receptor nor scavenger receptor type BI were required for the oxidation of LDL by 12-LOX to occur, however, the presence of the LDR receptor was required. The 15-LOX functioned similarly to 12-LOX in atherogenesis, therefore the 12/15-LOX –LRP receptor system is a possible target of research for atherosclerosis [77]. 12-LOX may have further significance for inflammatory processes. Animal studies show that rats injured by a balloon catheter display a marked upregulation of 12-LOX mRNA. In addition to these findings, patients with hypertension and patients with sickle cell anemia both showed higher levels of platelet 12-LOX protein suggesting that 12-LOX-derived arachidonic acid metabolites may play a critical role in inflammation processes as well as blood vessel diseases [77].

6.2 15-LOX

Two forms of 15-LOX metabolize arachidonic acid to 15(*S*)-HETE [82, 83]. 15-LOX-1, known as reticulocyte type, has been implicated in erythrocyte maturation, cell differentiation, inflammation, asthma and bronchial, epithelial functions, carcinogenesis, atherogenesis and other [82]. Since the leukocyte-type 12-LOX that occurs in mouse

and some other mammals but not in humans is functionally very similar to the 15-LOX-1, these enzymes are frequently referred to as 12/15-LOX. One interesting property of 15(*S*)-HETE is its rapid esterification into phosphatidylinositol of PMNs, which can be mobilized and transformed upon exposure of the cells to a secondary signal [84]. Similar to arachidonic acids, HETEs can be stored by cells, released by signal transduction mechanisms, and oxygenated to generate alternative profiles of eicosanoids [84]. According to additional studies, 15-LOX may also be involved in processes that modify native LDL into an oxidized form that is readily taken up by tissue macrophages. The identification of 15-LOX-1 located about macrophage-rich arterial lesions and epitopes of modified LDL supports a role of 15-LOX in atherogenesis. Investigations using transgenic animals also suggest that the locus of 15-LOX expression may be an important factor in the development of this disease.

Because 15-HpETE has been shown to be a potent inhibitor of PGI_2 biosynthesis, it has been proposed that overproduction of lipid hydroperoxides in the vascular wall by lipoxygenases may be an important contributing factor in the development of atherosclerosis [85]. Studies also established that lipoxygenase products and enzymes play a role in renin release, aldosterone secretion, and vasopressor effects of angiotensin II in arterial smooth muscle cells and hypertension [86]. Recently, Brash *et al.* have discovered a second new form of 15-LOX (15-LOX-2), which is expressed in human hair roots, lung, cornea, prostate [83, 87] and skin [88]. 15-LOX-2 converts arachidonic acid exclusively to 15S-HpETE, while linoleic acid is not metabolized as well. These and other features contrast with the previously reported 15S-lipoxygenase (15-LOX-1), which oxygenates arachidonic acid mainly at C-15, but also partly at C-12, and for which linoleic acid is an excellent substrate. The different catalytic activities and tissue distribution suggest a distinct function for the new enzyme compared with the previously reported human 15-LOX [83].

7. LIPOXINS

Lipoxins (LX) is a group of lipid mediators discovered in the 1980s that contain three hydroxyl groups and four double bonds within the arachidonic acid structure (trihydroxyeicosatetraenes). They originate from interactions of lipoxygenases via sequential coordinated metabolism of arachidonic acid by two different LOX enzymes; one being 5-LOX, the other 15-LOX or 12-LOX, typically from different populations of cells. Original work has discovered these compounds in experiments that studied metabolism of 15(*S*)-HETE in human leukocytes stimulated with calcium ionophore [89]. These studies produced two prominent new lipids characterized as LXA_4 and LXB_4 [90] as metabolites of 15(*S*)-HETE by 5-LOX (Fig. 7). LX molecules can be generated at mucosal surfaces via leukocyte-epithelial cell interactions (15-LOX-initiated route) and platelet-leukocyte interactions (the vascular route). It is quite interesting that platelets, which contain 12-LOX but no 15-LOX, can initiate formation of lipoxins from leukocyte-derived LTA_4 [91] much like they can form LTC_4 from LTA_4 by platelet-leukocyte interaction [92]. Isolated platelets do not make LX and LTs but it will be of interest to know factors that determine the LX /LTC_4 ratio from external LTA_4 taken up by platelets. This is of particular significance because LX molecules have potent anti-inflammatory properties; therefore, understanding conditions that favor LX over LTC_4 might have therapeutic implications. The lipoxygenase and cell-cell interactions that generate LX appear to be particularly important in inflammation, reperfusion injury, vascular damage and asthma [93]. But unlike leukotrienes, which are proinflammatory, LX block leukocyte formation in inflammation processes and protect organs from leukocyte-mediated injury. Recent concepts have focused around the idea that LX and their congeners might have impact on the resolution of acute inflammation. Leukocyte-cell interactions use transcellular biosynthetic pathways to produce specific lipids (LX) as "stop signals", which function as key steps in leukocyte trafficking and prevent neutrophil-mediated acute tissue injury. A very interesting implication of this concept is that aspirin could potentiate such signals. Serhan *et al.* have found that another group of lipoxins named 15-epi-LX can be formed from 15(*R*)- HETE [94]. It appears that a major source of 15(*R*)-HETE is COX-2 acetylated with aspirin. Although prostaglandin formation is blocked, acylated COX-2 can form 15(*R*)-HETE [95]. Metabolism of 15*R*-HETE by 5-LOX produces 15-epi-LX molecules also known as aspirin-triggered lipoxins (ATL). The rapid synthesis of LX in response to a variety of stimuli, in addition to their rapid enzymatic inactivation, makes LX and analogs great candidates for small molecule therapeutics and useful as a tool for revealing anti-inflammatory pathways. Lipoxins inhibit several key events in inflammation including PMN chemotaxis, transmigration across endothelial and epithelial cells and neutrophil adhesion [96]. Recent studies suggest that in addition to the downregulation of PMN movement, LX further demonstrates anti-inflammatory activity by the downregulation of the PMN secretion of antibacterial superoxide and peroxynitrite among other mediators [97]. PMN secretions act as a line of defense from infection but have also been known to cause damage to the host tissue. When defense processes become unregulated, PMN secretions can lead to the over-promotion of the inflammatory state and PMN-induced injury. The accumulation of PMN is a key factor in the pathogenesis of inflammatory states such as rheumatoid arthritis, atherosclerosis, and psoriasis [98]. The anti-inflammatory actions of LX are potentiated by aspirin-triggered lipoxins, 15-epi-LX, which have shown greater inhibitory activities.

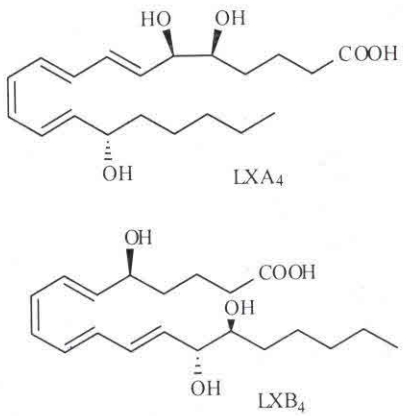

Fig. (7). Structures of lipoxin A_4 (LXA_4) and lipoxin B_4 (LXB_4).

LX could function as anti-inflammatory mediators of asthma [99] as further described in a study by Levy *et al.* in which the administration of an analog of LXA_4 blocked airway hyper-responsiveness [100]. In this study the expression of human LXA_4 receptors led to the inhibition of pulmonary inflammation showing that the LXA_4 system may have a significant role regulating airway responses. More stable analogs of these lipoxins are being developed and studied as potential drugs for anti-leukocyte therapy.

8. ASPIRIN, NSAIDS AND LEUKOTRIENES

Following the discovery of leukotrienes, the investigation of the inhibition of COX by aspirin and other NSAIDs revealed that one of the side effects of COX inhibition could be the potentiation of leukotriene production particularly in the development of gastrointestinal and other effects. While the mechanism has yet to be fully elucidated, it ultimately results in the excess production of vasoconstrictor sulfidopeptide leukotrienes (LTC_4, D_4, E_4), chemoattractive LTB_4, and various HpETEs and HETEs having numerous biological effects. The mechanism involved in this over-production of LTs appears to proceed by the diversion of arachidonic acid metabolism through lipoxygenase pathways. Such a diversion of arachidonic acid metabolism from COX to LOX can be demonstrated *in vitro* e.g. in platelets. Such a diversion could have additional importance because the effects of leukotrienes are potentiated in conditions of NSAIDs-induced COX inhibition and removal of prostaglandins, some of which have protective properties. While there may be beneficial health aspects of aspirin therapy through the inhibition of thromboxane in platelets, for asthmatics the use of aspirin and other NSAIDs may prove hazardous due to the ability of the NSAIDs to induce severe disorders. While the specific mechanism by which NSAIDs induce the syndrome, aspirin-induced asthma (AIA), is not fully understood, it appears that aspirin increases leukotriene levels in these patients [101]. In these patients, inhibition of COX-1 by aspirin and other NSAIDs causes elevation of LTs in all body fluids [101]. Studies have proposed that platelets may play a critical role in the development of AIA. Platelets do not synthesize leukotrienes but they are capable of converting LTA_4, originating from leukocytes, into LTC_4 via a trans-cellular mechanism [92]. Blockade of COX in platelets by aspirin increases LTC_4 formation from exogenous LTA_4. Additional important interaction of NSAIDs with arachidonic metabolism occurs when aspirin triggers biosynthesis of new lipid mediators related to lipoxins (LX) – lipoxygenase interaction products of arachidonic acid metabolism. Aspirin inhibits the constitutive form of cyclooxygenase, COX-1, better than the inducible form, COX-2. The inhibition of COX-1 proceeds via acetylation of critical serine while COX-2 remains active following acetylation by aspirin and forms 15(*R*)-HETE. While 15(*S*)-HETE, a product of 15-LOX found in endothelial and lung epithelial cells, can be further metabolized by 5-LOX to lipoxin molecules, recent work by Serhan *et al.* has shown that 15(*R*)-HETE could also be a precursor for a new class of lipids, ATL by cell-cell interactions and transcellular biosynthesis [94]. Aspirin may also contribute to enhanced formation of lipoxins by platelets from PMN-derived LTA_4. Although much additional work is needed in this area to understand the potential beneficial aspects of these molecules, it is believed that ATL derived drugs may potentially inhibit inflammation and protect in reperfusion injury [93].

9. LOX INHIBITION AS POTENTIAL ANTICANCER STRATEGY

In recent years, eicosanoids have emerged as important regulators of cancer biology [65, 102, 103]. Studies on the polyunsaturated fatty acid metabolism and carcinogenesis have identified potential novel molecular targets for cancer chemoprevention. One such target is COX-2, which is upregulated in colon, prostate, breast, pancreas and other cancers [104]. COX-2-derived prostaglandins are required for tumor proliferation and angiogenesis [103, 104]. Thus, COX-2 inhibitors (coxibs) have the potential to slow or prevent tumor growth. Interestingly, LOX products have also been identified as procarcinogenic and these include 5- and 12-LOX, and anticarcinogenic LOX, including 15-LOX-1 and possibly 15-LOX-2. The studies confirm the role of LOX enzymes and inhibitors as well as leukotriene receptor antagonists in cancer [65, 105]. The investigation of LOX inhibitors for their potential as cancer-fighting drugs began with experimental evidence that the LOX enzymes and their products are essential to cancer metastasis and growth, and tumor angiogenesis [65, 106, 107]. The levels of LOX metabolites in cancer cells of various sites such as lung, prostate, colon, breast, skin and other are significantly higher than the levels found in normal tissue. Lipid products of the LOX pathways, possibly HpETEs or HETEs, regulate and/or stimulate activities associated with neoplasm including cell proliferation, growth factor and transcription factor activation, tumor cell adhesion, and apoptosis [65]. Inhibitors of 5-LOX have anticarcinogenic activity and are being developed for clinical chemoprevention study. An emerging concept has suggested that LOX pathways exist in a dynamic balance that can shift during carcinogenesis toward 5- and 12-LOX (and COX-2) and potentially away from 15-LOX [108]. A novel strategy for cancer chemoprevention may thus involve LOX modulators, i.e., compounds that can induce the anticarcinogenic and/or inhibit the procarcinogenic LOXs and their products, thereby shifting the balance of LOX activities from procarcinogenic to anticarcinogenic metabolism of polyunsaturated fatty acids.

9.1 5-LOX

The production of 5(*S*)-HETE is stimulated by several autocrine growth factors, GRP and IGF, which also stimulate 5-LOX activity. In turn, studies show that 5-LOX products stimulate lung cancer cell growth, which may involve a specific receptor for 5(*S*)-HETE and its metabolite, 5-oxo-ETE [109, 110]. The study of 5-LOX inhibitors has received much less attention than the study of COX-2 inhibitors with regards to the treatment of cancer tumors, yet evidence demonstrates that 5-LOX inhibitors such as A-79175 and A-79715 are effective in the reduction of lung tumor growth [65, 111]. *In vivo* studies have shown that 5-LOX inhibitors significantly lower tumor incidence and multiplicity in strain A/J mice. The mice were subjected to similar doses over time of 4-(methylnitrosamino)-1-(3-

pyridyl)-1-butanone (NNK) as received by smokers. NNK, a carcinogen activated by lipoxygenase, was specifically inhibited by A-79175, which was found to subsequently reduce the incidence and multiplicity of lung cancer tumors [65, 111]. A-79715 also acts synergistically with acetylsalicylic acid (ASA) to lower multiplicity and incidence, the former by 87% and the latter by 24% [111]. A-79715 has potential as an effective chemopreventive agent because it shows a dual function of decreasing cellular proliferation and inhibiting NNK activation. MK-886, a 5-LOX inhibitor, which acts by inactivating the 5-LOX-activating protein (FLAP), reduced tumor multiplicity in rats by 52% [111]. Both the inhibitors described above reduced cell proliferation in lung tumors in a concentration-dependent manner. MK-886 functions to inhibit 5-LOX movement to membrane domain by binding to FLAP; therefore preventing the activating protein from catalyzing the production of 5-LOX, for example in differentiated HL60 cells [112, 113]. Although A-79715 was a more effective agent than MK-886, both 5-LOX inhibitors were ~10 times more effective than ASA as *in vivo* anti-proliferate agents. This suggests that 5-LOX-derived lipids play a significant role in events leading to cancer. Further implications for MK-886 as an anticancer agent arise from biochemical evidence that the inhibitor programs cell-death in an atypical form [114] because an *in vitro* study of the response of bronchiolar carcinoma cells to MK-866 revealed that cells exposed to MK-886 undergo apoptosis without detectable DNA laddering, as seen in necrosis. The results of this study suggest that MK-886 is less effective as a chemopreventive agent than treatments involving radiation due to the causation of oxidative stress, formation of free radicals, and altered redox potential. Evidence suggests that MK-886 causes metabolic stress as the cells exposed upregulate DNA repair mechanisms and alter apoptosis components to compensate for the inactivation of the 5-LOX pathway resulting in incomplete cell death and a resistance to anticancer therapy as seen with ionizing radiation treatment [115]. The atypical apoptosis stimulated by MK-886 when applied to bronchiolar carcinoma cells (H-358) varies depending on the cell involved. Similar effects were seen in Panc-1 cells but in U937 cells type 1 "normal" apoptosis has been observed [114]. The overall effects of these 5-LOX inhibitors, which are all responses characteristic of apoptosis, could be reversed by 5-oxo-ETE, a metabolite produced following the conversion of arachidonic acid to 5-HETE and the subsequent oxidation of 5-HETE to 5-oxo-ETE [116].

Two 5-LOX inhibitors, SC41661A and MK886, block prostate cancer (PC-3) cell proliferation and induce apoptosis [117]. Interestingly, these drugs exhibit different profiles of apoptosis whereby SC41661A induces type 1 "apoptotic" programmed cell death whereas MK886, type 2 "autophagic" cell death [117]. The occurrence and function of the 5-LOX system in the prostate tumor requires additional studies because the identification both of 5-LOX and 5-HETE in malignant tissue of prostate cancer is inconclusive [102]. A recent study did not detect 5-LOX activity in prostate tumor tissues as indicated by absence of 5-HETE [75]. Current findings with 5-LOX and PCA have revealed that the inhibition of 5-LOX by MK886 prevented the production of 5-HETE and induced apoptosis both in hormone-responsive and non-responsive human prostate cancer cells and that exogenous 5-HETE protected these cancer cells from apoptotic death induced by MK886 indicating that dietary AA might promote the progression of prostate cancer by acting as an anti-apoptotic factor. Another study has reported RT-PCR measurements of 5-LOX mRNA in patients with prostate cancer; however more data of that sort is needed for establishing the specific role of 5-LOX in prostate cancer [102]. More studies are also needed to confirm the role of 5-LOX inhibitors in prostate cancer. While the induction of apoptosis by MK886 appears to be a confirmed observation as evidenced above, some concerns have been raised as to whether or not the MK886-dependent apoptosis is due to the inhibition of 5-LOX activity or some other effects [102]. In addition to evidence of the role of 5-LOX in prostate cancer, several studies implicate 5-LOX in the progression of pancreatic cancer as well. The expression of 5-LOX was observed in pancreatic cancer cells but not in normal human pancreatic ductal cells. Also, blocking 5-LOX activity with LOX inhibitors decreased in vitro pancreatic cancer cell proliferation and was associated with the induction of apoptosis and differentiation.

9.2 12-LOX

Similar to 5-LOX, studies have also linked 12-LOX to pancreatic cancer due to the expression of 12-LOX found in pancreatic cancer cells, which is uncommon to normal pancreatic cells. The effectiveness of LOX inhibitors blocking pancreatic cancer cell proliferation and the possible role of 12-LOX in the regulation of pancreatic cancer cell growth and apoptosis strengthens this association. 12-LOX is also expressed in prostate, breast, lung and other cancers [104]. The biochemical evidence for the involvement of 12-LOX in prostate tumor growth suggests a dose dependent relationship between the levels of 12(*S*)-HETE in cancer cells and the advanced stage, marked lack of differentiation and invasiveness of tumor cells [78]. Studies also suggest a role in tumor metastasis via the promotion of prostate tumor cell motility and invasion by stimulating proteolytic enzyme release [78, 79], such as the secretion of cathespin B. The level of expression of 12-LOX mRNA is correlated with the clinical stage of the cancer; sections of prostate cancer tumors and breast cancer tumors show higher levels of 12-LOX mRNA than benign cells [77].

The two main 12-LOX inhibitors examined for the regulation of prostate tumor growth in prostate are baicalein and N-benzyl-N-hydroxy-5-phenylpentamide (BHPP). These 12-LOX inhibitors achieve regulation of neoplasm via growth arrest in the G_0/G_1 phase [78]. This cell growth arrest results in a time-dependent decrease in levels of cyclin-D1; otherwise ample levels of cyclin-D1 act to block the actions of pRB by phosphorylation leading to the release of transcription factors and cellular proliferation. In addition to the inactivation of pRB, studies suggest the potential existence of other inhibitory pathways secondary to the ability of baicalein and BHPP to reduce levels in p107 and p130 thereby blocking cell cycle progression. Baicalein is a natural flavonoid that has been identified in many plants and is also an active component of a herbal preparation [118]. The application of baicalein also results in a decrease of survivin, a protein responsible for halting caspase-mediated

apoptosis. Treatment with baicalein resulted in undetectable levels of survivin within 14 hours. Baicalein was also effective in the treatment of 12(S)-HETE induced apoptosis as demonstrated by the reduced apoptotic fraction of 6.6% from 18.6% upon application of the inhibitor [119].

While an understanding of the role of the 12-LOX pathway in prostate cancer is well underway due to recent experimental advances, much less is known about the involvement in the 12-LOX pathway in cancers of the breast or skin. An association between 12-LOX mRNA and breast cancer tissue has been established[65]. In one study that examined the 12-LOX mRNA expression in matched, normal and cancerous tissues it was found that in cancerous tissue 12-LOX mRNA expression increased 3-30 fold compared to normal tissue [65]. Other studies also document greater amounts of 12-LOX mRNA in breast cancer cells and suggest that 12-LOX cDNA grew without the presence of estrogen [65]. In addition to the finding that the 12-LOX enzyme is estrogen-independent the role of 12-LOX was further defined by a study that suggests the promotion of angiogenesis by the enzyme. The role of 12-LOX in breast cancer was further examined using two specific 12-LOX inhibitors, which led to the inhibition of serum-induced growth of MCF-7 cells implying that 12-LOX may be responsible for the activation of oncogenes of breast cancer, protein kinase C (PKC) and mitogen-activated protein kinases. Although 12-LOX metabolites are probably involved in breast cancer cells, there is insufficient evidence concerning the possible regulation of tumor growth by 12-LOX inhibitors [77]. Similar to breast cancer, elevated levels of 12(*S*)-HETE were found in papillomas and carcinomas when compared to normal skin cells. In addition to the 50-60 fold greater levels of 12(S)-HETE found, higher levels of 12-LOX enzyme activity were observed as well. The corresponding metabolites of 8-LOX, which is found only in animals, and 12-LOX namely 8- and 12- HpETE were reported to cause chromosomal damage in the epidermis leading to skin tumors. In addition, LOX inhibitors were found to suppress skin carcinogenesis in rats. Despite the association between breast cancer cells, skin cancer cells and 12-LOX, no studies provide sufficient evidence that 12-LOX inhibitors would be effective anticancer agents for these two cancer types [65]. Progress in this area will benefit from new inhibitors of high specificity for 12-LOX.

In addition to the potential of 12-LOX inhibitors such as baicalein as an anticancer drug in epithelial cancer cell lines, recent findings showed that baicalein induced massive cell death when applied to block the 12-LOX pathway in gastric cancer cells. Apoptosis occurred in a concentration-dependent manner and appeared to be independent of p53, based on levels of bcl-2 which were observed but did not change following baicalein treatment; however baicalein treatment did result in a change in the levels of *bcl-2*, an anti-apoptotic gene. The anti-apoptotic gene was decreased while the pro-apoptotic gene, *bax*, remained unaltered [73].

9.3 15-LOX

15-LOX appears to be involved in human prostate cancer, breast cancer and gastric cancer [82, 120] but conflicting reports are found as to whether 15-LOX is induced in colon cancer. 15(*S*)-HETE can be generated by two isoforms of 15-LOX, which only share about 40% homology and both have been identified in prostate and other cancers [75, 82]. Recent studies indicate that 15-LOX-1 is downregulated in colorectal cancer cells and that the ability of NSAIDs, a class of clinically active cancer chemopreventive agents, to induce apoptosis and growth inhibition in these cells was dependent on the induction of 15-LOX-1 and its metabolic product 13-S-hydroxyoctadecadienoic acid [108]. Another study reported increased activity of 15-LOX-1 in colorectal cancer as well as a greater presence of 15-LOX-1 in tumor samples than in comparable adjacent normal tissues [121]. The examination of human colorectal carcinoma Caco-2 cells revealed that the expression of 15-LOX-1 is specific to apoptosis and cell differentiation and metabolites of 15-LOX-1 may be responsible for the suppression of apoptosis due to the observation that the inhibition of LOX was followed by an increase in apoptosis. The cause of the increased expression of 15-LOX-1 in colorectal tumors is unclear although the conflicting claims of 15-LOX-1 as being both anticarcinogenic as well as procarcinogenic can be best explained by the dual functions of its primary metabolite, 15-HETE, which can promote cell growth, play a role in signal transduction and alter cell migration. In contrast to the colorectal studies, 15-LOX very recently has shown exclusively anti-carcinogenic activity in esophageal and prostatic carcinogenesis. Furthermore, the anti-carcinogenic activity of 15-LOX-1 has also been observed in gastric cancer cell lines. Applying the COX-2 specific inhibitor to four human gastric cancer cell lines resulted in an increase in the expression of 15-LOX-1 and subsequently the induction of apoptosis[120]. The finding that 15-LOX-1 induces apoptosis suggests that for tumorigenesis to occur, 15-LOX-1 must be downregulated. When 15-LOX-1 was inhibited, apoptosis was blocked due to the inactivity of the lipoxygenase. These findings also further established 13-*S*-HODE, a product of linoleic acid by 15-LOX, as a mediator of apoptosis[120]. Despite the current strides in research surrounding the role of 15-LOX in cancer biology, underlying mechanisms of such interactions remain unknown. Unlike the gastric cancer data, studies of human prostate cancer cell lines show elevated levels of 12/15-LOX products suggesting that such products may be pro-carcinogenic rather than anti-carcinogenic. Likewise the results from a recent study using the BT-20 cell line suggested that 15-LOX products may be pro-carcinogenic in breast cancer cells also. The increased production of 13-*S*-HODE from 15-LOX obtained as a consequence of high dietary intake of linoleic acid could increase breast cancer cell proliferation by setting off the EGF receptor-signaling pathway. However, much more needs to be learned about 15-LOX and the integrated function of all LOX isoforms in cancer and the distribution of arachidonic acid metabolism between COX-2 and LOX.

9.4 Natural Anti-LOX Compounds

In addition to the LOX inhibitors previously described, there are also several naturally occurring compounds that possess anti-inflammatory and antioxidant properties that enable them to behave as LOX inhibitors [122]. One such compound is curcumin, a common component of curries and mustards that has shown potential in cancer therapy both in a topical and dietary form. The topical application of

curcumin has inhibited tumor development in mouse skin and edema of mouse ears. Studies have shown that a dietary application of curcumin inhibited tumor progression also in mouse skin as well as the forestomach, large intestine and small intestine of mice and reduced the multiplicity and incidence of adenomas and adenocarcinomas [122]. Another compound, this one derived from the herb, *silybum marianum* (milk thistle), that has proven useful in the treatment of skin tumors in mice is silymarin, which is composed mainly of silybin. By protecting against lipid peroxidation, several studies have shown that silymarin is useful for the treatment of cancer due to its ability to inhibit ornithine decarboxylase (ODC) activity and its effectiveness in protecting mouse skin from UVB radiation-induced tumor development [123]. A major component of green tea, epigallocatechin gallate (EGCG) is known to decrease the mutagenicity of several different types of carcinogens and is also effective as a free radical scavenger. Current studies are examining the chemopreventive effects of EGCG in skin, neck, head and colon cancers [122]. EGCG could function as inhibitor of protein nitration [124], which can be induced by nitrated products of lipoxygenases (lipid peroxynitrates/ peroxynitrites) [125, 126].

Another common compound found to inhibit LOX pathways is resveratrol, which occurs naturally in grapes and other medicinal plants and is known to protect against infections. Resveratrol is one of a group of compounds called phytoalexins that are produced in plants during times of environmental stress such as adverse weather or insect, animal or pathogenic attack. Resveratrol has been identified in more than 70 species of plants, including mulberries and peanuts and in the skin (not flesh) of grapes. Fresh grape skin contains about 50 to 100 micrograms of resveratrol per gram, while red wine concentrations range from 1.5 to 3 milligrams per liter. Resveratrol inhibits the development of precancerous lesions in mouse mammary glands and blocks tumorigenesis in another study using a two-stage model of skin cancer in mice [127]. Pezzuto and colleagues were able to show that resveratrol was effective during all three phases of the cancer process: initiation, promotion and progression [128]. Resveratrol was found to have antioxidant and antimutagenic activity and also increased levels of enzyme quinone reductase, an enzyme capable of metabolically detoxifying carcinogens. All three of these physiological effects are indicative of resveratrol preventing cancer initiation. Resveratrol also demonstrated anti-inflammatory effects and inhibited the activity of the cyclooxygenase and hydroperoxidase enzymes in addition to causing the differentiation of human promyelocytic leukemia cells, indicating that this compound may also depress the progression phase of cancer. Finally, resveratrol inhibited the development of preneoplastic lesions in mouse mammary glands treated with a carcinogen in culture and inhibited tumor formation in mice. Much more work will be required particularly in the area of clinical studies before these compounds or their analogs will be available for cancer treatment in clinics.

10. CONCLUSIONS

Discovery of first mammalian lipoxygenase in the human platelet triggered intensive research resulting in the discovery of leukotrienes, lipoxins and many other lipoxygenase-derived mediators. These lipids appear to play an important role in host defense, inflammation, asthma, cancer and other disorders acting with specific receptor proteins and potentially through other mechanisms. Much effort has been given to find new drugs that could function as leukotriene receptor antagonists and 5-LOX inhibitors, which resulted in the development of drugs such as montelukast and zileuton. Much needs to be learned about the function of 5-LOX, other lipoxygenase enzymes, and receptors and more research is required in the area of lipoxygenase-arachidonic metabolism as well. This area of research continuously undergoes rapid advances and new drugs are expected to emerge from this research. Recent findings of 5-LOX in the nucleus has raised many hypotheses of potentially novel roles for this enzyme in the nucleus that may or may not be leukotriene dependent [129]. Furthermore, the discovery that LTB_4 could act through a nuclear hormone receptor (PPAR-α) [130] has intensified the research surrounding novel aspects of lipoxygenase pathway. Recent work by Shimizu *et al.* also suggests that 12(*S*)-HETE and 15(*S*)-HETE can bind and activate BLT2 receptor suggesting a broader role for these molecules [131]. A recently discovered lipoxygenase that can produce HETE molecules of *R* configuration, [132] as well as novel forms of lipoxygenases [87] and their novel functions[133] contributes to the complexity of lipid mediators that can be generated by this pathway and make drug discovery particularly challenging. The continuation of research working towards new drug developments has yielded recent studies, which are focused on dual inhibitors of cyclooxygenase and 5-lipoxygenase. One such inhibitor is ML3000, which is now in Phase III clinical trials [134]. Knowledge of arachidonic acid metabolism by lipoxygenase is extensive at the biochemical level yet insufficient in that there lacks a full understanding of their role in health and disease. While new drugs which block 5-LOX and CysLT receptors have been available for treatment of asthma, one can anticipate that new anticancer therapies based on modulation of lipoxygenase pathways will be developed. Judging by the intensity of research in this area it is likely that new drugs whose main function is to modulate lipoxygenase pathway will be available in the foreseeable future.

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ABBREVIATIONS

COX	=	Cycloxygenase
LOX	=	Lipoxygenase
CYP	=	Cytochrome P450
NSAIDS	=	Nonsteroidal anti-inflammatory drugs
HpETE	=	Hydroperoxyeicosatetraenoic acid
HETE	=	Hydroxyeicosatetraenoic acid
LT	=	Leukotriene
LX	=	Lipoxin

PMN = Polymorphonuclear

BHPP = N-benzyl-N-hydroxy-5-phenylpentamide

A-79175 = *R*(+)-*N*-[3-[5-(4-fluorophenyl)-2-furanyl]-1-methyl-2-propynyl]-*N*-hydroxyurea

NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

MK-886 = 3-[1-(4-chloro-benzyl)-3-t-butyl-thio-t-isopropyl-indol-2-yl]-2,2-dimethylpropanoic acid

ASA = Acetylsalicylic acid

NDGA = Nordihydroguaiaretic acid.

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