Biochemical and Biophysical Research Communications 396 (2010) 135–139

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Advances in eicosanoid research, novel therapeutic implications

Jesper Z. Haeggström *, Agnes Rinaldo-Matthis, Craig E. Wheelock, Anders Wetterholm

Department of Medical Biochemistry and Biophysics, Division of Chemistry 2, Karolinska Institutet, S-171 77 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 17 March 2010

Keywords:
Eicosanoids
Leukotriene
Inflammation
Asthma
Atherosclerosis
Drug design

ABSTRACT

Eicosanoids are a family of oxygenated metabolites of arachidonic acid, including the prostaglandins, thromboxanes, leukotrienes and lipoxins. These lipid mediators play essential roles in normal cellular homeostasis as well as in a number of disease states. This review will focus on recent advances in the field of eicosanoids and highlight specific discoveries and achievements. Emphasis will be placed on structure and receptor biology, which are of significant pharmacological and clinical relevance.

1. Introduction

Arachidonic acid is released from membrane phospholipids by phospholipases A_2 in response to a variety of cellular stimuli and may become oxygenated bioactive molecules called eicosanoids. Free arachidonic acid may be metabolized along the cyclooxygenase (COX) pathway, involving COX-1 and COX-2, along with terminal synthases, to generate prostaglandins (PG) and thromboxanes (TX), which act via a series of G-protein-coupled receptors. Alternatively, this fatty acid may be oxidized along the lipoxygenase pathway, with the central enzymes, 5-lipoxygenase, 12/15-lipoxygenase, leukotriene (LT) A_4 hydrolase, and LTC_4 synthase, to produce several classes of leukotrienes and lipoxins (Fig. 1). Since eicosanoids are mediators of acute inflammation, fever, and several specific diseases such as thrombosis, cancer, atherosclerosis, asthma and rhinitis, many drugs that perturb eicosanoid synthesis and actions have been developed and are widely used, e.g., Thrombyl® (low-dose aspirin; thrombosis), Cytotec® (misoprostol; gastroprotective NSAID), Celebrex® (celecoxib; gastroprotective NSAID), Singulair® (montelukast; asthma), and Xalatan® (latanoprost; glaucoma).

2. Cytosolic phospholipase A_2, the master enzyme

Phospholipase A_2 (PLA_2) hydrolyzes the ester bond in *sn*-2 position of phospholipids to yield lysophospholipids and arachidonic acid. More than 15 different PLA_2's have been characterized and grouped based on primary structure, localization and Ca^{2+} requirement [1]. Today it is widely accepted that cytosolic PLA_2 group IV ($cPLA_2$) plays a major role in arachidonic acid release that leads to prostaglandin and leukotriene production [2]. The structure of $cPLA_2$ has been solved and reveals a monomeric cytosolic protein with a molecular size of 85 kDa [3]. It has an N-terminal, lipid binding, C2 domain, and a C-terminal β-barrel catalytic unit. $cPLA_2$ exerts its activity by targeting the phospholipid membrane upon extracellular stimuli that mobilizes intracellular Ca^{2+}. The active site of $cPLA_2$ is an hydrophobic open cleft, closed by a flexible amphipathic loop that is thought to move during substrate binding [1]. Phosphorylation at Ser505 activates $cPLA_2$, probably by improving the conformation of the active site [4]. Potent and selective inhibitors of $cPLA_2$ have recently been developed [5].

3. Inhibitors of COX and the "post Vioxx" era

It is widely recognized that aspirin and several other non-steroidal anti-inflammatory drugs (NSAID) exert their action through inhibition of cyclooxygenase (COX), which leads to reduced prostaglandin synthesis. In the early 1990s, a second COX was discovered that could be specifically linked to inflammation and cancer and that exhibited low expression in the gastric mucosa [6]. Soon, specific COX-2 inhibitors were developed that exploit a unique side-pocket at the active site, with less gastric side effects and these drugs, referred to as coxibs, became widely prescribed for inflammatory pain associated with rheumatoid arthritis and osteoarthritis. Coxibs were also candidate drugs for the treatment and prevention of colon cancer via inhibition of COX-2 and subsequent effects on PGE_2 synthesis.

However, several studies emerged which suggested that COX-2 inhibitors may confer a cardiovascular risk and, eventually, the

* Corresponding author. Fax: +46 8 736 0439.
E-mail address: jesper.haeggstrom@ki.se (J.Z. Haeggström).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.03.140

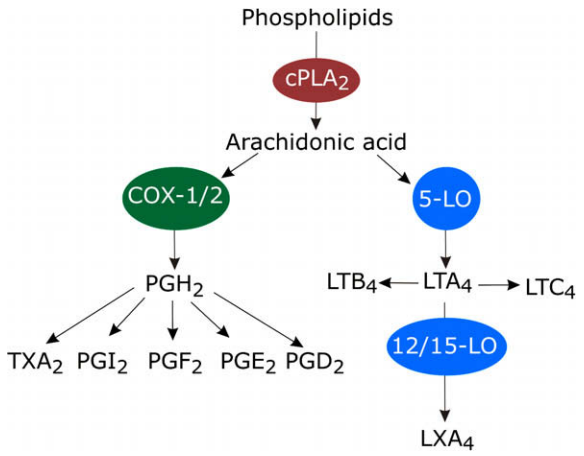

Fig. 1. Schematic overview of the cyclooxygenase and lipoxygenase pathways.

results of a clinical trial of rofecoxib for treatment of colon cancer, the so called APPROVe (Adenomatous Polyp Prevention on Vioxx) study, were so alarming that Vioxx was immediately withdrawn from the market [7]. The mechanism(s) behind these serious side effects are not fully understood. However, the current most widely accepted hypothesis is that coxibs block COX-2 dependent PGI_2 synthesis, particularly in the endothelium, which in turn can both augment the response to thrombotic and hypertensive stimuli and initiate and accelerate atherogenesis [8]. More recent studies have revealed that not only COX-2 inhibitors, but also a range of traditional NSAIDs are associated with a cardiovascular risk, perhaps with a somewhat lower risk for naproxen [9]. It should be noted that COX-2 inhibitors, e.g., Celebrex® (celecoxib) are still widely prescribed drugs.

After the COX-2 turmoil, researchers began the development of new generations of coxibs with an improved gastro-intestinal and cardiovascular safety profile. Several strategies have been proposed, e.g., linking NSAIDs to a nitric oxide releasing moiety, resulting in NO-NSAIDs, or targeting microsomal prostaglandin E synthase type 1 (mPGES-1) [10,11]. This enzyme is an integral membrane protein, which is induced by pro-inflammatory cytokines and mitogens in tandem with COX-2. The joint action of COX-2 and this down-stream membrane PGE synthase appears to be responsible for biosynthesis of pro-inflammatory PGE_2, a potent mediator of fever and pain. Hence, the overall strategy is to inhibit mPGES-1, rather than COX-2, thereby selectively blocking PGE_2 formation while sparing other prostanoids including the vasodilatory PGI_2 [11]. Since several reports have suggested that mPGES-1 is over expressed in cancer, efforts are also aimed at developing mPGES-1 inhibitors as anti-cancer drugs.

4. 5-Lipoxygenase and FLAP

5-LO is a non-heme iron dioxygenase and a soluble monomeric enzyme (Mr = 78 kDa) which catalyses the oxidation of arachidonic acid to yield 5-HPETE and its subsequent dehydration to form LTA_4 [12]. There is no crystal structure of 5-LO available, but sequence identities of 40–57% to rabbit 15-LO [13] and coral 8R-LO [14], both of which are structurally characterized, suggest that 5-LO has two domains, with one regulatory β-barrel C2 domain and one α-helical catalytic domain.

5-LO is regulated at multiple levels. Concentrations of intracellular Ca^{2+} regulate the translocation of 5-LO from the cytosol to the nuclear membrane, a process which is also regulated by interactions with the actin binding Coactosin Like Protein (CLP). The 5-LO activity is further regulated by ATP, phosphocholine, FLAP (*vide infra*) and by phosphorylation at three Ser residues in positions 271, 523 and 663 residing on the catalytic domain [12]. Due to the pivotal role of 5-LO in the production of leukotrienes, 5-LO inhibitors have been developed such as Zileuton (Zyflo™) [15].

In intact cells, 5-LO interacts with an 18 kDa integral membrane protein called 5-lipoxygenase activating protein (FLAP), which is located at the nuclear membrane. It promotes the synthesis of LTA_4 and in this process FLAP is believed to "present" arachidonic acid to 5-LO. Together with $cPLA_2$, 5-LO and FLAP form a biosynthetic complex assembled at the nuclear membrane [16]. Several FLAP inhibitors are currently in clinical development for treatment of respiratory and atherosclerotic diseases [17,18].

The central intermediate in the 5-LO pathway, *viz.* LTA_4, may also be converted by 12- or 15-LO into lipoxins, often via transcellular routes involving neutrophils and platelets (Fig. 2). In the presence of aspirin, COX-2 is acetylated and capable of converting arachidonic acid into 15R-HETE, which in turn is the substrate for production of 15-epi-LXA_4, also termed aspirin-triggered lipoxin (ATL) [19]. Lipoxins and ATL have anti-inflammatory and

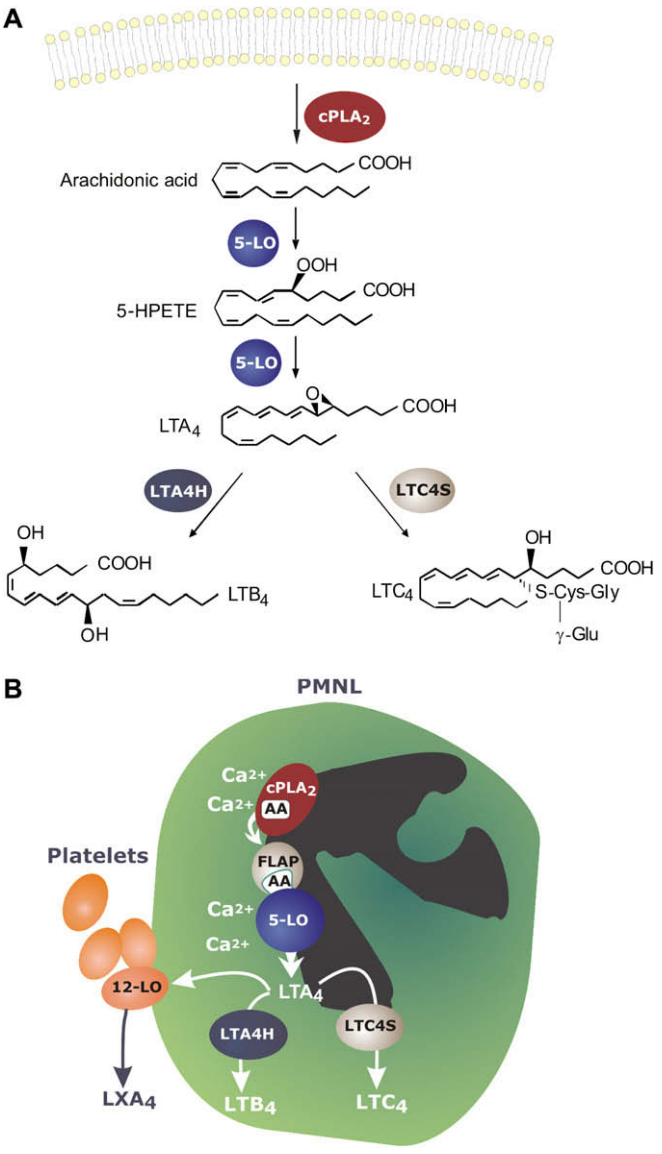

Fig. 2. (A) The 5-lipoxygenase/leukotriene pathway. (B) The leukotriene biosynthetic pathway and transcellular metabolism of lipoxins.

proresolving properties and may be regarded as novel lead structures for development of anti-phlogistic drugs. Several other classes of molecules derived from eicosapentaenoic and docosahexaenoic acid have been described, which possess potent bioactivity promoting the resolution of inflammation [20]. In this context it is interesting to note that inhibitors of LTA4H block LTB_4 synthesis, while sparing LTA_4 synthesis to allow shunting into lipoxin formation (Fig. 1). Thus, inhibitors of LTA4H may alleviate inflammation by two mechanisms, reduced LTB_4 synthesis and enhanced formation of lipoxin [21–22].

5. Structure-based design of novel anti-leukotrienes

There are two classes of leukotrienes, *viz.* the dihydroxy acid LTB_4 and the so called cysteinyl-leukotrienes (cys-LT), LTC_4, LTD_4 and LTE_4, previously referred to as slow reacting substance of anaphylaxis. Leukotrienes signal via two sets of GPCR, denoted BLT1/2 and CysLT1/2. Leukotrienes are established mediators of asthma and therapeutics that interfere with the synthesis or action of cys-LT are regularly used in treating asthma [23]. Thus far, an inhibitor of 5-lipoxygenase, Zileuton, and several classes of selective CysLT1 antagonists, typified by montelukast, are available on the market. More recently, an increasing body of evidence indicates that leukotrienes, in particular LTB_4, are involved in atherosclerosis, myocardial infarction, and stroke [24–25], raising the possibility that anti-leukotrienes may also be effective drugs in the treatment and/or prevention of these diseases.

The committed steps in the biosynthesis of LTB_4 and cys-LT are catalyzed by the two enzymes LTA_4 hydrolase (LTA4H) and LTC_4 synthase (LTC4S), respectively. LTA4H is a soluble 70 kDa enzyme that is abundantly expressed in polymorphonuclear neutrophils, monocytes and macrophages. Interestingly, the enzyme contains one atom of zinc, bound to a catalytic zinc site, and exhibits an aminopeptidase activity against Arg tripeptides, the physiological function of which is not yet established. The crystal structure of LTA4H was solved by Thunnissen et al. [26] revealing three domains, an N-terminal, a central catalytic, and a C-terminal domain, packed in a flat triangular arrangement with a deep cleft in between, harbouring the active center [26]. The active site pocket is divided in a wide section, containing the catalytic zinc, and a narrow, L-shaped, hydrophobic channel that is believed to bind the lipid substrate, LTA_4. Using site-directed mutagenesis, biochemical characterization and X-ray crystallography, the molecular details of the catalytic mechanisms for the epoxide hydrolase and aminopeptidase activities have been elucidated [27–28]. Interestingly, the active site of LTA4H offers a chemical landscape that is ideal for structure-based drug design (Fig. 3) and successful efforts in this direction have already been carried out by several pharmaceutical companies [29–30]. Based on recent biochemical data, molecular genetic analysis, and results from experiments in animal disease models, potent and selective LTA4H inhibitors are potential anti-inflammatory agents for treatment and prevention of atherosclerosis and asthma [22].

LTC4S belongs to the MAPEG (membrane associated proteins in eicosanoid and glutathione metabolism) family of integral membrane proteins and catalyzes a GSH *S*-transferase reaction, which is specific for LTA_4 and generates LTC_4. The enzyme is mainly expressed in eosinophils, mast cells, monocytes, and platelets and is regulated by PKC dependent phosphorylation. In 2007, two independent groups solved the crystal structure of human LTC4S [31–32], a major achievement not only in the eicosanoid field but also in X-ray crystallography since, at that time, only one other human integral membrane protein (aquaporin) had been structurally characterized at high resolution. The LTC4S structure revealed a homotrimer with a GSH binding site composed of residues from two adjacent monomers, which in turn demonstrated that a monomer is not sufficient to support enzyme activity. GSH was bound in a unique "horseshoe shaped" conformation. In between two monomers, the aliphatic side chain of one detergent molecule (dodecyl maltoside) was bound in a hydrophobic cleft, possibly mimicking the binding of LTA_4. Most of this cleft is open to the surface of the trimer except at its deepest end where a putative lipid anchoring lid is formed by Trp116. Several candidate catalytic and substrate binding residues were identified in the structure, in particular Arg104 as a thiol activator, which are now being subjected to functional analysis.

Recent work has uncovered new receptors for cys-LTs (*vide infra*), which in turn has made the biosynthetic upstream enzyme, LTC4S, an attractive target for pharmacological inhibition of cys-LT signaling. Since high-quality crystals (2 Å) of human LTC4S can be generated and are stable, it is now possible to carry out structure-based design of LTC4S inhibitors. This is most likely the first time a human integral membrane protein has become amenable to this sophisticated technique.

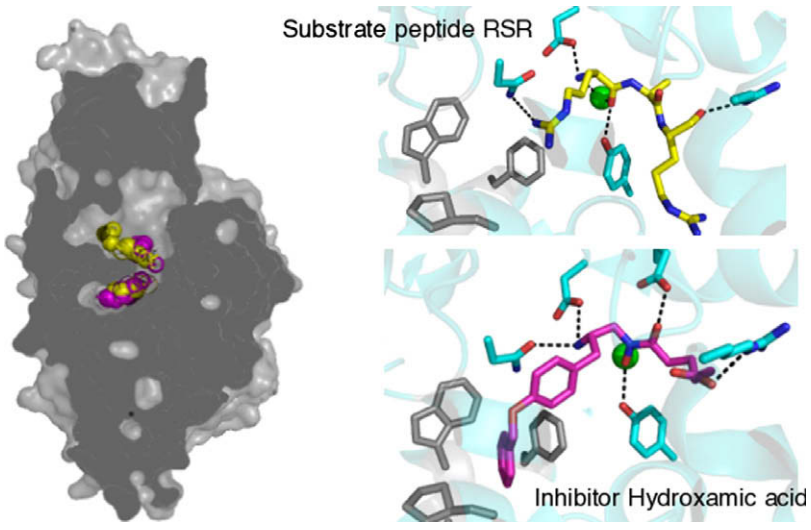

Fig. 3. Left, cross-section through the active center of LTA4H. The wide section of the active site is filled with a tripeptide substrate (yellow) whereas a hydroxamic acid inhibitor (cyan), designed to mimic LTA_4, occupies a narrow hydrophobic channel. Right, comparison between the binding of the tripeptide substrate, Arg-Ser-Arg (top panel), and the hydroxamic acid inhibitor (bottom panel) to the active site of LTA4H.

Two other medically important MAPEG members have also been structurally characterized, albeit to a relatively low resolution. Thus, crystal structures of human FLAP in complex with inhibitors, at 4 Å resolution, have been reported [33]. FLAP forms a homotrimer and each monomer is composed of four transmembrane helices connected by extended loops. An inhibitor binding site was observed in the crystal structure of FLAP in complex with MK591. Unexpectedly, this site was located within the plane of the nuclear membrane, distant from the GSH binding site in the LTC4S crystal structure. Based on mutagenesis studies, the binding site for arachidonic acid and MK886 was seen to overlap within the membrane ideally positioned for FLAP to capture laterally diffusing arachidonic acid molecules to enable presentation to 5-LO. A distinct feature of the FLAP structure is an internal pocket at the bottom of the trimer open to the ER lumen with an internal diameter of 6 Å. Although LTC4S and FLAP are very similar in structure, this opening does not exist in LTC4S and there is so far no evidence for a function of this aperture.

Shortly after the structure determinations of LTC4S and FLAP, Jegerschöld et al. reported a structure of mPGES-1 at 3.5 Å resolution, obtained by electron crystallography [34]. Just as in LTC4S, GSH was bound in a U-shaped conformation, and, interestingly, the authors could not find an access path for PGH_2 to the bound GSH molecule, indicating that the protein was in a closed conformation and that dynamic changes, involving helices 1 and 4, occur at the active site during binding and turnover of the lipid substrate. A putative structure of an open conformation of the active site, with a critical Arg residue (Arg126), was obtained through modeling against the crystal structure of human LTC4S. In our hands, mutants of Arg126 were catalytically compromised and displayed an altered product profile [35]. Together with the structural data one may conclude that further mechanistic studies must take into account the consequences of protein dynamics. Although the structures of FLAP and mPGES-1 are helpful in inhibitor design, their resolutions have to be improved in order to allow a structure-based drug design program.

6. Novel GPCRs for leukotrienes, development of receptor antagonists

Groundbreaking work by Shimizu and coworkers led to the identification of two types of surface receptors for LTB_4, denoted BLT1 and BLT2, respectively. The two receptors differ with respect to their affinity for LTB_4 and cellular expression patterns. The BLT_1 receptor has been cloned and characterized as a 43 kDa, G-protein-coupled receptor with seven-transmembrane-spanning domains (7TM) [36]. The BLT1 receptor is only expressed in inflammatory cells and shows a high degree of specificity for LTB_4 with a K_d of 0.15–1 nM [37]. Computer assisted sequence comparisons revealed that the receptor is distantly related to certain somatostatin receptors as well as some of the chemokine receptors, e.g., those which bind fMLP, LXA_4, and C5a. Based on the crystal structure of rhodopsin, models of BLT1 structures have been generated and residues implicated in catalysis and signaling have been identified by site-directed mutagenesis [38–39]. However, reliable molecular insights to the structure and function of leukotriene GPCR can only be achieved by crystallography.

A second GPCR for LTB_4, BLT2, has recently been identified [37]. This receptor is homologous to the BLT1 receptor, but has a higher K_d value for LTB_4 (23 nM) and a different ligand-specificity and binding profile for various BLT antagonists. Recently, it was discovered that 12-HHT, a side product during thromboxane synthesis, is an endogenous high-affinity ligand for BLT2, thus connecting the lipoxygenase pathway with the cyclooxygenase pathway [37]. In contrast to the BLT1 receptor, which is predominantly found in various inflammatory, bone marrow derived, cells, BLT2 is ubiquitously expressed in various cells and tissues. The genes encoding the LTB_4 receptors are located on chromosome 14 and, interestingly, the open reading frame of the gene encoding BLT2 is located within the promoter region of the BLT1 gene, an unusual gene structure previously not described among mammals [37]. This unique arrangement also means that it is very difficult, or even impossible, to delete one BLT receptor without affecting the other.

The cys-LTs are recognized by at least two GPCR denoted CysLT1 and CysLT2 [40–41]. CysLT1 contains 336 amino acid residues and mRNA is found in, e.g., the spleen, peripheral blood leukocytes, lung tissue, smooth muscle cells, and tissue macrophages. The preferred ligands for CysLT1 are LTD_4 followed by LTC_4 and LTE_4 in decreasing order of potency. The gene encoding the receptor is located on the X chromosome. CysLT2 contains 345 amino acids with approximately 40% sequence identity to CysLT1 [41]. This receptor binds LTC_4 and LTD_4 equally well, whereas LTE_4 shows low affinity to the receptor. Studies on the tissue distribution of CysLT2 show high levels of mRNA in, e.g., heart, brain, peripheral blood leukocytes, spleen, placenta, and lymph nodes, whereas only small amounts are found in the lung. The functional role(s) of CysLT2 is presently unclear, but high expression levels in vascular endothelial cells and a functional profile similar to CysLT1 in the vasculature *in vivo* have suggested a role for this receptor in heart and vessel regulation [42–43]. Further work using transgenic mice over expressing CysLT2 in the endothelium have suggested that this receptor may play a role in ischemia–reperfusion injury [44]. Clearly, the wide tissue and cellular distribution of CysLT2 opens many possibilities, including yet unidentified endogenous ligands and involvement in the regulation of brain and/or cardiac functions.

The existence of a third CysLT receptor has been discussed for a long time. In 2006, Abrachio et al. reported that gpr17 had been deorphaned as a receptor for nucleotides and cys-LTs [45]. Further studies have indicated that gpr17 is not a receptor for cys-LT but rather plays a role in regulating the signaling from CysLT1 [46]. In yet other studies, two additional receptors specific for LTE_4 have been described and in one case it was demonstrated that montelukast did not block the putative CysLT3 [47–48]. Apparently, there is a whole keyboard of cross-talking receptors for cys-LTs, allowing cells to play a complex signaling pattern. Given the fact that montelukast, the prototype for a selective CysLT1 antagonist, is ineffective in a substantial number of patients, novel receptor antagonists with different or broader selectivity is of considerable pharmacological interest. As discussed above, complete inhibition of cys-LT synthesis, by blocking LTC4S, is also a feasible strategy to circumvent the high number of isoreceptors. Accordingly there are ample opportunities to further expand investigations into LT biology and their putative roles in multiple diseases.

Acknowledgments

This work was supported by the Swedish Research Council (10350, 20854, Linneus Grant CERIC), the EU integrated projects EICOSANOX (005033) and Atheroremo (201668), the CIDaT consortium (Vinnova), the Torsten & Ragnar Söderbergs Foundation and the Jeanssons Foundation. C.E.W. was supported by a Center for Allergy Research (Cfa) Fellowship.

References

[1] J.E. Burke, E.A. Dennis, Phospholipase A_2 structure/function, mechanism, and signaling, J. Lipid Res. 50 (Suppl.) (2009) S237–242.
[2] N. Uozumi, K. Kume, T. Nagase, et al., Role of cytosolic phospholipase A_2 in allergic response and parturition, Nature 390 (1997) 618–622.
[3] A. Dessen, J. Tang, H. Schmidt, et al., Crystal structure of human cytosolic phospholipase A_2 reveals a novel topology and catalytic mechanism, Cell 97 (1999) 349–360.

[4] M.A. Gijon, D.M. Spencer, A.L. Kaiser, et al., Role of phosphorylation sites and the C2 domain in regulation of cytosolic phospholipase A_2, J. Cell Biol. 145 (1999) 1219–1232.
[5] S. Marusic, P. Thakker, J.W. Pelker, et al., Blockade of cytosolic phospholipase A_2 alpha prevents experimental autoimmune encephalomyelitis and diminishes development of Th1 and Th17 responses, J. Neuroimmunol. 204 (2008) 29–37.
[6] W.L. Smith, D.L. Dewitt, R.M. Garavito, Cyclooxygenases: structural, cellular, and molecular biology, Annu. Rev. Biochem. 69 (2000) 145–182.
[7] R.S. Bresalier, R.S. Sandler, H. Quan, et al., Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial, N. Engl. J. Med. 352 (2005) 1092–1102.
[8] S. Fries, T. Grosser, T.S. Price, et al., Marked interindividual variability in the response to selective inhibitors of cyclooxygenase-2, Gastroenterology 130 (2006) 55–64.
[9] P.M. Kearney, C. Baigent, J. Godwin, et al., Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials, BMJ 332 (2006) 1302–1308.
[10] P. Wallace, J.L. Del Soldato, The therapeutic potential of NO-NSAIDs, Fundam. Clin. Pharmacol. 17 (2003) 11–20.
[11] B. Samuelsson, R. Morgenstern, P.J. Jakobsson, Membrane prostaglandin E synthase-1: a novel therapeutic target, Pharmacol. Rev. 59 (2007) 207–224.
[12] O. Radmark, O. Werz, D. Steinhilber, et al., 5-Lipoxygenase: regulation of expression and enzyme activity, Trends Biochem. Sci. 32 (2007) 332–341.
[13] S.A. Gillmor, A. Villasenor, R. Fletterick, et al., The structure of mammalian 15-lipoxygenase reveals similarity to the lipases and the determinants of substrate specificity, Nat. Struct. Biol. 4 (1997) 1003–1009.
[14] D.B. Neau, N.C. Gilbert, S.G. Bartlett, et al., The 1.85 A structure of an 8R-lipoxygenase suggests a general model for lipoxygenase product specificity, Biochemistry 48 (2009) 7906–7915.
[15] W. Berger, M.T.M. De Chandt, C.B. Cairns, Zileuton: clinical implications of 5-lipoxygenase inhibition in severe airway disease, Int. J. Clin. Pract. 61 (2007) 663–676.
[16] M. Peters-Golden, T.G. Brock, Intracellular compartmentalization of leukotriene synthesis: unexpected nuclear secrets, FEBS Lett. 487 (2001) 323–326.
[17] G. Bain, C.D. King, M. Rewolinski, et al., Pharmacodynamics and pharmacokinetics of AM103, a novel inhibitor of 5-lipoxygenase-activating protein (FLAP), Clin. Pharmacol. Ther. 87 (2010) 437–444.
[18] N. Stock, C. Baccei, G. Bain, et al., 5-Lipoxygenase-activating protein inhibitors. Part 2: 3-{5-((*S*)-1-acetyl-2,3-dihydro-1H-indol-2-ylmethoxy)-3-tert-butylsulfanyl-1-[4-(5-methoxy-pyrimidin-2-yl)-benzyl]-1H-indol-2-yl}-2,2-dimethyl-propionic acid (AM679) – a potent FLAP inhibitor, Bioorg. Med. Chem. Lett. 20 (2010) 213–217.
[19] J. Claria, C.N. Serhan, Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell–leukocyte interactions, Proc. Natl. Acad. Sci. USA 92 (1995) 9475–9479.
[20] C.N. Serhan, N. Chiang, T.E. Van Dyke, Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators, Nat. Rev. Immunol. 8 (2008) 349–361.
[21] N.L. Rao, P.J. Dunford, X. Xue, et al., Anti-inflammatory activity of a potent, selective leukotriene A_4 hydrolase inhibitor in comparison with the 5-lipoxygenase inhibitor zileuton, J. Pharmacol. Exp. Ther. 321 (2007) 1154–1160.
[22] N.L. Rao, J.P. Riley, H. Banie, et al., Leukotriene A_4 hydrolase inhibition attenuates allergic airway inflammation and hyperresponsiveness, Am. J. Respir. Crit. Care Med., in press. Epub ahead of print.
[23] J.M. Drazen, E. Israel, P. O'byrne, Treatment of asthma with drugs modifying the leukotriene pathway, N. Engl. J. Med. 340 (1999) 197–206.
[24] C.D. Funk, Lipoxygenase pathways as mediators of early inflammatory events in atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 1204–1206.
[25] H. Qiu, A. Gabrielsen, H.E. Agardh, et al., Expression of 5-lipoxygenase and leukotriene A4 hydrolase in human atherosclerotic lesions correlates with symptoms of plaque instability, Proc. Natl. Acad. Sci. USA 103 (2006) 8161–8166.
[26] M.G.M. Thunnissen, P. Nordlund, J.Z. Haeggström, Crystal structure of human leukotriene A_4 hydrolase, a bifunctional enzyme in inflammation, Nat. Str. Biol. 8 (2001) 131–135.
[27] J.Z. Haeggstrom, Leukotriene A_4 hydrolase/aminopeptidase, the gatekeeper of chemotactic leukotriene B_4 biosynthesis, J. Biol. Chem. 279 (2004) 50639–50642.
[28] F. Tholander, A. Muroya, B.P. Roques, M.C. Fournié-Zaluski, M.M. Thunnissen, J.Z. Haeggström, Structure-based dissection of the active site chemistry of leukotriene A_4 hydrolase: implications for M1 aminopeptidases and inhibitor design, Chem. Biol. 15 (2008) 920–929.
[29] T.A. Kirkland, M. Adler, J.G. Bauman, et al., Synthesis of glutamic acid analogs as potent inhibitors of leukotriene A_4 hydrolase, Bioorg. Med. Chem. 16 (2008) 4963–4983.
[30] D.R. Davies, B. Mamat, O.T. Magnusson, J. Christensen, M.H. Haraldsson, R. Mishra, B. Pease, E. Hansen, J. Singh, D. Zembower, H. Kim, A.S. Kiselyov, A.B. Burgin, M.E. Gurney, L.J. Stewart, Discovery of leukotriene A_4 hydrolase inhibitors using metabolomics biased fragment crystallography, J. Med. Chem. 52 (2009) 4694–4715.
[31] H. Ago, Y. Kanaoka, D. Irikura, et al., Crystal structure of a human membrane protein involved in cysteinyl leukotriene biosynthesis, Nature 448 (2007) 609–612.
[32] D. Martinez Molina, A. Wetterholm, A. Kohl, et al., Structural basis for synthesis of inflammatory mediators by human leukotriene C_4 synthase, Nature 448 (2007) 613–616.
[33] A.D. Ferguson, B.M. Mckeever, S. Xu, et al., Crystal structure of inhibitor-bound human 5-lipoxygenase-activating protein, Science 317 (2007) 510–512.
[34] C. Jegerschold, S.C. Pawelzik, P. Purhonen, et al., Structural basis for induced formation of the inflammatory mediator prostaglandin E_2, Proc. Natl. Acad. Sci. USA 105 (2008) 11110–11115.
[35] T. Hammarberg, M. Hamberg, A. Wetterholm, et al., Mutation of a critical arginine in microsomal prostaglandin E synthase-1 shifts the isomerase activity to a reductase activity that converts prostaglandin H_2 into prostaglandin $F_{2\alpha}$, J. Biol. Chem. 284 (2009) 301–305.
[36] T. Yokomizo, T. Izumi, K. Chang, et al., A G-protein-coupled receptor for leukotriene B_4 that mediates chemotaxis, Nature 387 (1997) 620–624.
[37] T. Shimizu, Lipid mediators in health and disease: enzymes and receptors as therapeutic targets for the regulation of immunity and inflammation, Annu. Rev. Pharmacol. Toxicol. 49 (2009) 123–150.
[38] K. Kuniyeda, T. Okuno, K. Terawaki, et al., Identification of the intracellular region of the leukotriene B_4 receptor type 1 that is specifically involved in Gi activation, J. Biol. Chem. 282 (2007) 3998–4006.
[39] A. Sabirsh, R.P. Bywater, J. Bristulf, et al., Residues from transmembrane helices 3 and 5 participate in leukotriene B_4 binding to BLT1, Biochemistry 45 (2006) 5733–5744.
[40] K.R. Lynch, G.P. O'neill, Q. Liu, et al., Characterization of the human cysteinyl leukotriene CysLT1 receptor, Nature 399 (1999) 789–793.
[41] C.E. Heise, B.F. O'dowd, D.J. Figueroa, et al., Characterization of the human cysteinyl leukotriene 2 (CysLT2) receptor, J. Biol. Chem. 275 (2000) 30531–30536.
[42] M. Sjostrom, A.S. Johansson, O. Schroder, et al., Dominant expression of the CysLT2 receptor accounts for calcium signaling by cysteinyl leukotrienes in human umbilical vein endothelial cells, Arterioscler. Thromb. Vasc. Biol. 23 (2003) e37–41.
[43] Y. Hui, Y. Cheng, I. Smalera, et al., Directed vascular expression of human cysteinyl leukotriene 2 receptor modulates endothelial permeability and systemic blood pressure, Circulation 110 (2004) 3360–3366.
[44] W. Jiang, S.R. Hall, M.P. Moos, et al., Endothelial cysteinyl leukotriene 2 receptor expression mediates myocardial ischemia–reperfusion injury, Am. J. Pathol. 172 (2008) 592–602.
[45] P. Ciana, M. Fumagalli, M.L. Trincavelli, et al., The orphan receptor GPR17 identified as a new dual uracil nucleotides/cysteinyl-leukotrienes receptor, EMBO J. 25 (2006) 4615–4627.
[46] A. Maekawa, B. Balestrieri, K.F. Austen, et al., GPR17 is a negative regulator of the cysteinyl leukotriene 1 receptor response to leukotriene D_4, Proc. Natl. Acad. Sci. USA 106 (2009) 11685–11690.
[47] A. Maekawa, Y. Kanaoka, W. Xing, et al., Functional recognition of a distinct receptor preferential for leukotriene E_4 in mice lacking the cysteinyl leukotriene 1 and 2 receptors, Proc. Natl. Acad. Sci. USA 105 (2008) 16695–16700.
[48] S. Paruchuri, H. Tashimo, C. Feng, et al., Leukotriene E_4-induced pulmonary inflammation is mediated by the P2Y12 receptor, J. Exp. Med. 206 (2009) 2543–2555.