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Leukotriene A₄ Hydrolase – An Evolving Therapeutic Target

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1. Introduction

Leukotriene B₄ is an important mediator of inflammation derived from successive metabolism of fatty acids by several enzymes including the terminal rate-limiting enzyme called leukotriene A₄ hydrolase. Leukotriene A₄ hydrolase is a soluble enzyme, and depending on its substrate can function as either an epoxide hydrolase or an aminopeptidase. Over the years, leukotriene B₄ has been found to be highly associated with several human diseases, and most of the reported literature has focused on the biology of the epoxide hydrolase activity of the enzyme, which generates the lipid metabolite leukotriene B₄. However, emerging data suggests that the aminopeptidase activity of the leukotriene A₄ hydrolase enzyme may also play a crucial role in the process of anti-inflammatory responses. Previous drug discovery efforts have focused on inhibition of the leukotriene B₄ metabolite by indiscriminately blocking both the epoxide hydrolase and aminopeptidase functions of the enzyme. The co-existence of a dichotomous and directly opposing biological function of this enzyme as suggested by recent studies on the aminopeptidase activity of leukotriene A₄ hydrolase is a radically paradigm-shifting and relevant concept. This manuscript will review these recent findings in the context of the classical understanding of the leukotriene A₄ hydrolase enzyme.

2. Background

The leukotrienes are important downstream effector molecules of inflammatory tissue alterations. Human diseases exhibit dysregulated inflammatory and immune responses in their pathogenesis. Therefore, 5-lipoxygenase (5-LO)-mediated lipid pathways have been investigated as possible pro-inflammatory pathways in the pathogenesis of multiple human diseases. The leukotrienes are lipid mediators of inflammation derived from the metabolism of fatty acids to arachidonic acid by phospholipase A₂ (cPLA₂), then to leukotriene A₄ (LTA₄) by 5-lipoxygenase and 5-lipoxygenase activating protein (FLAP). Further downstream metabolism yields two classes of leukotrienes: cysteinyl-leukotrienes (leukotriene C₄, D₄, and E₄) synthesized by leukotriene C₄ synthase and leukotriene B₄ synthesized by leukotriene A₄ hydrolase. Eventually, cysteinyl-leukotrienes and leukotriene B₄ bind to a cysteinyl leukotriene receptor or a leukotriene B₄ receptor, respectively, to exert final tissue effects (**Figure 1**).

Fig. 1. The 5-lipoxygenase pathway.

2.1 Properties of the leukotriene A₄ hydrolase enzyme

Leukotriene A₄ hydrolase is a monomeric soluble protein, which localizes in all different cellular compartments of several mammalian sources. The leukotriene A₄ hydrolase enzyme contains 610 amino acid residues and has a molecular weight of 69 KDa. In humans, the leukotriene A₄ gene is localized to chromosome 12q22 as a single copy gene with 19 exons. The 5' upstream region consists of several transcription consensus sequences including a phorbol ester response element and two xenobiotic response elements [1-3]. The enzyme usually resides in the cytosol, but was found to also localize to the nucleus in association with the proliferation of Type II alveolar cells [4]. Only leukotriene A₄ has been known to bind with significant affinity to the leukotriene A₄ hydrolase enzyme, whereas the isomers of leukotriene A₄, leukotrienes A₃ and A₅, are known to bind to the substrate site with much lower affinity [5, 6]. Several site-directed mutagenesis studies demonstrated that Tyr-378, Glu-271, Asp-375, Arg-563, and Lys-565 play significant roles in the epoxide hydrolase activity of leukotriene A₄ hydrolase [7-12]. High specificity of the leukotriene A₄ lipid to the catalytic site also seems to modulate the enzymatic activity by covalently binding to the catalytic site, which results in inactivation of the enzyme [5, 6].

The leukotriene A₄ hydrolase enzyme processes hydrolysis of leukotriene A₄ to afford leukotriene B₄. The biological activity of leukotriene B₄ is dependent on a specific stereochemical configuration at carbon-12 and a specific geometric configuration of the olefin between carbon-6 and carbon-7. The leukotriene A₄ hydrolase enzyme promotes stereoselective hydrolysis of leukotriene A₄ by addition of water at carbon-12 to give the 12R adduct. The intermediate carbocation that is formed prior to hydrolysis is oriented by the enzyme to afford exclusively the 6Z olefin product. This catalytic hydrolysis performed by leukotriene A₄ hydrolase is significant, because the leukotriene A₄ lipid metabolite contains an unstable allylic epoxide that can undergo uncatalyzed hydrolysis. In this scenario, non-enzymatic hydrolysis of the leukotriene A₄ lipid results in the formation of 6E-leukotriene B₄ as a mixture of diastereomers at carbon-12 [13]. As shown in **Figure 2**, the olefin at carbon-6 has the Z configuration and the carbon-12 stereocenter is defined as R for leukotriene B₄. Under non-catalytic conditions, the olefin at carbon-6 is formed to give the E olefin, and the stereocenter at carbon-12 is formed to give a mixture of the R or S epimer.

As compared to native leukotriene B₄, both 6E-leukotriene B₄ and the 12S epimer of 6E-leukotriene B₄ have demonstrated significantly reduced affinity for the leukotriene B₄ receptors in human leukocytes and guinea pig lungs [14, 15]. Sala and colleagues also demonstrated that a substantial amount of the leukotriene A₄ metabolite can be released out

of the human polymorphonuclear leukocytes for transcellular biosynthetic processing [16]. In combination, this suggests that the conversion of leukotriene A₄ to leukotriene B₄ may play crucially important biological roles, and simple analysis of the total amount of leukotriene B₄ produced at the local tissues cannot fully explain the observed phenotypes associated with these pathways.

Fig. 2. Enzyme-catalyzed and uncatalyzed hydrolysis of LTA₄.

2.2 The dual catalytic activities of the leukotriene A₄ hydrolase enzyme

The leukotriene A₄ hydrolase enzyme functions as either an epoxide hydrolase or aminopeptidase. As an aminopeptidase the enzyme efficiently catalyzes the hydrolysis of small peptides of three-amino acid length [17]. Leukotriene B₄ is a potent neutrophil and monocyte chemo-attractant and activator, and therefore has been the subject of most discussions concerning leukotriene A₄ hydrolase [18-21]. Leukotriene B₄ is known to associate with two G protein-coupled seven transmembrane domain receptors, whose genes are located in very close proximity to each other in the human and mouse genomes [22]. This metabolite maintains important immune functions in the areas of defense and inflammatory diseases. It is an important intracellular messenger with numerous effector functions to stimulate immune responses. Leukotriene B₄ promotes chemotaxis of several types of leukocytes (monocytes [21, 23-25], neutrophils [26-29], macrophages [20, 30-32], dendritic cells [18, 33]) which lead to the initiation of inflammatory responses at the site of local tissues. Leukotriene B₄ subsequently promotes endothelial adhesion [34-37] and degranulation of toxic intracellular materials from the leukocytes [38-41]. Eventually, leukotriene B₄ facilitates phagocytosis and clearing of the inciting foreign agents that initiated the inflammatory cascade [42-46]. Consistent with these biological observations, a variety of inflammatory diseases have been associated with the over-production of leukotriene B₄. Some of these diseases are sepsis [47-50], shock [51, 52], cystic fibrosis [53-57], coronary artery disease [58-60] connective tissue disease [19, 61-65], and COPD [66-68].

The biosynthesis of leukotriene B₄ is initiated by the conversion of arachidonic acid to leukotriene A₄, which requires sequential actions by 5-lipoxygenase and 5-lipoxygenase activating protein. The 5-lipoxygenase enzyme and 5-lipoxygenase activating protein catalyze sequential reactions to produce the unstable metabolite leukotriene A₄. The fate of leukotriene A₄ is determined by either leukotriene C₄ synthase, which conjugates glutathione to leukotriene A₄ to form leukotriene C₄ [69, 70], or leukotriene A₄ hydrolase, which generates leukotriene B₄ by its epoxide hydrolase activity [10, 29]. The 5-lipoxygenase enzyme has been mostly found in the leukocytes, and therefore, leukotriene B₄ has been primarily found to be produced by leukocytes. However, the biosynthesis of leukotriene B₄ was also found to occur in the absence of 5-lipoxygenase. For example, in the cases of alveolar epithelial cells, due to the lack of 5-lipoxygenase, these cells cannot produce leukotriene A₄, a mandatory precursor to leukotriene B₄. However, when co-incubated with neutrophils, the alveolar epithelial cells were found to produce a measurable amount of

leukotriene B₄. This was found to occur by transferring pre-made leukotriene A₄ from neutrophils to the alveolar epithelial cells [71]. Therefore, the only enzyme that these alveolar epithelial cells require was the presence of intracellular leukotriene A₄ hydrolase. This demonstrated that cells lacking 5-lipoxygenase could influence the total amount of leukotriene B₄ in local tissues populated by the recruited leukocytes. As described above, this very mechanism can potentially alter the effects of this pathway by how the leukotriene A₄ lipid is metabolized (i.e. enzymatic vs. non-enzymatic mechanisms).

For the past several years, leukotriene A₄ hydrolase has been known to carry two catalytic functions. One function is a well-characterized epoxide hydrolase activity described above and a poorly characterized aminopeptidase activity. The second catalytic site of the enzyme can bind short peptide sequences such as PGP, dynorphins, enkephalins, bestatin, and captopril [72-79]. The mammalian leukotriene A₄ hydrolase enzyme is homologous to *C. Elegans* aminopeptidase-1, but the *C. Elegans* aminopeptidase-1 enzyme does not possess epoxide hydrolase activity [80]. A subsequent study has demonstrated that the mammalian aminopeptidase B enzyme shares significant homology with the leukotriene A₄ hydrolase enzyme, but lacks epoxide hydrolase activity [81]. However, a clear understanding on the role of the aminopeptidase activity of leukotriene A₄ has yet to be clarified.

2.3 The possible significance of leukotriene A₄ hydrolase aminopeptidase activity

To date, studies addressing the aminopeptidase activity of the leukotriene A₄ hydrolase enzyme remain sparse. This reflects the presumption that the leukotriene B₄ metabolite alone is biologically relevant in human diseases associated with this enzyme. Naturally, all known pharmacological investigations to date have targeted only the epoxide hydrolase activity of the leukotriene A₄ hydrolase enzyme rather than the aminopeptidase activity. Numerous *in vitro* and *in vivo* animal studies have demonstrated significant pathologies induced by the exaggerated activity of the epoxide hydrolase activity of the leukotriene A₄ hydrolase enzyme (Table 1).

Pre-clinical animal modeling demonstrated that these findings are associated with cystic fibrosis, inflammatory bowel disease, chronic obstructive pulmonary disease, sepsis, asthma, adult respiratory distress syndrome, and atherosclerotic coronary artery disease [4, 64, 89, 160-164]. Exaggerated levels of leukotriene B₄ have also been found in patients with rheumatoid arthritis [65, 147], cystic fibrosis [57, 165], obstructive pulmonary diseases [68, 166], sepsis [47, 107], adult respiratory distress syndrome [56, 132, 138-141], inflammatory bowel diseases [167, 168], and atherosclerosis [58]. These observations led to FDA clinical trials to target the epoxide hydrolase activity of the leukotriene A₄ hydrolase enzyme and the leukotriene B₄ metabolite with several pharmaceutical agents. Interestingly, these clinical trials in humans mostly failed to show similar beneficial effects in several diseases such as rheumatoid arthritis, cystic fibrosis, inflammatory bowel diseases, sepsis, and atherosclerosis [59, 84, 88, 99-102, 113, 114, 126, 128, 130, 148, 150, 151, 159, 169, 170].

There are two plausible explanations for the failure to translate the significant pathobiology of the leukotriene A₄ hydrolase enzyme and leukotriene B₄ found in less complex *in vitro* or *in vivo* animal models to more complex human systems. First, the entire leukotriene A₄ hydrolase enzyme pathway may not be suitable as a therapeutic target. Second, the non-specific targeting to completely inhibit all activities of the leukotriene A₄ hydrolase enzyme may not be appropriate because of the unknown but potentially important biological contribution by the aminopeptidase activity of the enzyme. Taken together, it becomes apparent that significant confusion and knowledge gaps exist on this matter as a result of

incomplete understanding of the biology of the aminopeptidase activity of the leukotriene A₄ hydrolase enzyme.

Human Disease	Pre-Clinical Animal Models	Observational or <i>in vitro</i> Human Studies	Pharmaceutical Trials
Cystic Fibrosis	De Lisle[116] Tetaert[117] van Heeckeren[100, 118]	Daryadel[119] Lawrence[54, 55, 120] O'Driscoll[56] Cromwell[57]	Schmitt-Grohe(BLTR)[99] Panchaud(LTA ₄ H) [100]
Inflammatory Bowel Diseases	Habib[83] Stenson[121] Bailon[122] Nancey[123] Murthy[124]	Ikehata[125] Kjeldsen[126] Cole[127] Casellas[128] Pavlenko[129]	Roberts(5-LO)[101] Hawkey(5-LO)[102] Rask-Madsen(5-LO)[103] McCall(LTA ₄ H)[104]
Sepsis	Hartiala[50] Mack[130] Doi[49] Rasmussen[131] Marshall[132] Rios-Santos[133]	Tavares-Murta[47] Alves-Filho[91] Arraes[134] Ball[135] Nakae[136] Takakuwa[137, 138]	Winning(BLTR)[105]
Obstructive Lung Disease	Freisch[139] Taki[140] Johnson[141] Turner[142] Henderson[143] Fretland[144]	O'Driscoll[56] Payan[145] Tanno[146] Wardlaw[147] Radeau[148] Koh[149]	Arm(LTA ₄ H)[107]
ARDS	Thomsen[150] Sprague[93, 151] Goldman[152] Czarnetzki[153] Hicks[154] Furue[155]	O'Driscoll[56] Davis[92] Sprague[93] Czarnetzki[94] Schonfeld[95] Loick[96]	
Rheumatoid Arthritis	Suarez[156] Fretland[144, 157] Grespan[61]	Sperling[19, 65, 158] Nielsen[159] Elmgreen[160] Smith[88, 112]	Diaz-Gonzalez(BLTR)[113] Alten(BLTR)[114]
Atherosclerosis	Hagihara[161] Amsterdam[162] Senoh[163]	Qiu[164] Dwyer[165] Elgebaly[166] Hakonarson[59] Maznyczka[58] Back[167]	Tardif(5-LO)[115] Hakonarson(FLAP)[59]

Table 1. Representative review of literature on pre-clinical and clinical studies targeting LTB₄. References in the “Pharmaceutical Trial” column are matched with the LTB₄ associated pharmaceutical targets. LTA₄H = LTA₄ hydrolase. BLTR = LTB₄ Receptor. 5-LO = 5-Lipoxygenase. FLAP = 5-Lipoxygenase Activating Protein.

2.4 Emerging data on the leukotriene A₄ hydrolase aminopeptidase activity

New findings from the murine model of influenza pneumonia have demonstrated that the aminopeptidase activity of the leukotriene A₄ hydrolase enzyme was necessary and crucial in the resolution phase of neutrophilic inflammation induced by intranasal influenza exposure [73]. An investigation was undertaken to explain how murine lungs clear neutrophilic inflammation induced by intra-nasal influenza exposure in association with the previously discovered tri-amino acid chemotactic peptide called PGP [72]. These studies demonstrated that timely resolution of neutrophilic infiltration into the lungs occur in parallel with degradation of a simple tri-peptide sequence, PGP. Further analysis of this murine model demonstrated that the leukotriene A₄ hydrolase enzyme was the major aminopeptidase enzyme that degraded PGP, and this degradation of PGP by the aminopeptidase activity of the leukotriene A₄ hydrolase enzyme was crucial to resolve acute neutrophilic infiltration into the lungs post influenza exposure. These findings were recapitulated in an *in vivo* murine model by confirming paradoxically increased neutrophil infiltration into the lungs of the leukotriene A₄ hydrolase -/- mice post influenza exposure. This was presumed to occur in the setting of decreased PGP degradation and clearance as compared to wild-type mice.

These studies by Blalock and co-workers were the first to demonstrate an important biological function performed by the aminopeptidase activity of the leukotriene A₄ hydrolase enzyme in association with neutrophilic inflammation. These studies were also the first to demonstrate PGP as a natural biological substrate to the aminopeptidase catalytic site of the leukotriene A₄ hydrolase enzyme. Subsequently, Barber and colleagues reported that the enzyme producing PGP, prolyl endopeptidase, made important contribution to cigarette smoke-induced pulmonary emphysema in a murine model [174]. Xu and colleagues reported that the prolonged presence of PGP may also contribute to lung tissue destruction in cystic fibrosis patients by PGP secretion leading to CXCR1 and CXCR2, receptor activation, exaggerated influx of neutrophils and chemotaxis into the lungs [171].

3. Structural biology

The leukotriene A₄ hydrolase enzyme is a fairly large (69 KDa) cytosolic protein. Its solubility likely facilitated crystallization of the enzyme, which allowed for high resolution X-ray crystallographic structure elucidation [176]. The endogenous ligand for leukotriene A₄ hydrolase is leukotriene A₄, and unstable epoxide-containing lipid derived from arachidonic acid. As mentioned previously, the leukotriene A₄ lipid is known to undergo two possible transformations as follows. First, stereoselective hydrolysis at C-12 is mediated by the leukotriene A₄ hydrolase enzyme to give leukotriene B₄. Second, leukotriene C₄ synthase catalyzes the conjugation of glutathione to give leukotriene C₄. Although leukotriene A₄ hydrolase and leukotriene C₄ synthase both recognize leukotriene A₄ as an endogenous substrate, they share very little similarity. The 3-dimensional crystal structure of leukotriene C₄ synthase resembles glutathione transferase enzymes as was expected on the basis of its primary structure and catalytic activity [177, 178]. On the other hand, leukotriene A₄ hydrolase is a cytosolic protein and catalyzes the hydrolysis of leukotriene A₄ to leukotriene B₄. Leukotriene A₄ hydrolase was found to be homologous to enzymes that exhibit aminopeptidase activity, and indeed leukotriene A₄ was also found to catalyze the hydrolysis of short peptides [17]. Both leukotrienes B₄ and C₄ are responsible for inflammatory responses, and therefore previous pharmacological efforts have targeted these metabolites. However, recent literature suggests that the mostly uncharacterized

aminopeptidase activity of the leukotriene A₄ hydrolase enzyme might also be a key player in inflammatory responses. In this section, we will review the structural elements that contribute to substrate binding and enzymatic processing by leukotriene A₄ hydrolase.

3.1 The structure of the leukotriene A₄ hydrolase enzyme

In 2001, Haeggström and co-workers published a high-resolution X-ray crystal structure of the leukotriene A₄ hydrolase enzyme in complex with bestatin, a competitive inhibitor [176]. Over the past 10 years, X-ray crystallography of the leukotriene A₄ hydrolase enzyme has become commonplace, and over 40 subsequent structures of the enzyme in complex with a variety of ligands have been published [10-12, 78, 179-182]. The abundance of crystal structures with high resolution (< 3.0 Å) of the leukotriene A₄ hydrolase enzyme has enabled detailed understanding of its molecular mechanisms in substrate binding and processing. Nevertheless, a very intriguing feature of the dual activity of the enzyme can be realized by a simple 2-dimensional comparison of the conversion of leukotriene A₄ to leukotriene B₄ and the hydrolysis of short peptides such as proline-glycine-proline (PGP).

Fig. 3. A 2-dimensional analysis of leukotriene A₄ hydrolase-mediated hydrolysis.

As shown in **Figure 3**, the common group in both substrates is the carboxylic acid moiety, which are highlighted in red. Extension of the molecule beginning from the carboxylic acid moiety shows that the cleavage site of the peptide aligns with the site for epoxide ring opening, which suggests that enzyme activation occurs in this region. However, hydrolysis of the peptide occurs directly at the site of cleavage, whereas hydrolysis of leukotriene A₄ occurs 6 atoms away at carbon-12 to give leukotriene B₄. The binding pocket of the enzyme can be labeled using the nomenclature devised by Schechter and Berger to describe protease subsites [183]. Thus, the N-terminal proline residue that is proximal to the site of hydrolysis is labeled P1' (P for peptide) and resides in the S1' subsite of the enzyme. The 2-dimensional analysis in **Figure 3** places the epoxide group of leukotriene A₄ slightly toward the C-terminus side of the scission site, which is labeled the S1' subsite of the enzyme. Therefore, peptide cleavage and epoxide ring opening appear to occur in the same region in the S1' subsite of the enzyme. However, whereas hydrolysis of the peptide occurs directly at the scission site in the S1' subsite, hydrolysis of leukotriene A₄ occurs ~6 atoms away at carbon-12 in what would be the S2 subsite for extended peptide substrates.

A more sophisticated analysis of the binding pocket of the leukotriene A₄ hydrolase enzyme can be delineated from structures elucidated by X-ray crystallography. As mentioned above, X-ray crystallography of the leukotriene A₄ hydrolase enzyme has become commonplace and gives intricate details about substrate binding. Currently, there are 44 solved crystal structures of the enzyme deposited in the Protein Data Bank (PDB) [184]. Structural studies

of the leukotriene A_4 hydrolase enzyme continue to be the subject of active research with 9 crystal structures released to the PDB in the past year. Most of the published structures contain a co-crystallized substrate. Drug discovery efforts by deCODE Genetics, an Iceland-based pharmaceutical company, utilized the facility in which the leukotriene A_4 hydrolase enzyme could be co-crystallized with small molecule substrates to identify molecular fragments that could be pieced together to design a new drug [181, 182].

Fig. 4. The binding pocket of LTA_4H .

A 3-dimensional analysis of the binding pocket of leukotriene A_4 hydrolase was rendered using the Visual Molecular Dynamics (VMD) software package (**Figure 4A**) [184]. Several crystal structures of the leukotriene A_4 hydrolase enzyme containing small molecular fragments were aligned, and then the ligands were displayed. The ligands shown in blue occupy an L-shaped binding pocket with the zinc atom centered between the two regions. A 2-dimensional schematic of the putative binding mode for leukotriene A_4 is presented in **Figure 4B**. For comparison, a schematic of a transition-state analog of a tripeptide, which was co-crystallized with leukotriene A_4 hydrolase, is shown in **Figure 4C** [180].

The X-ray crystal structure of the leukotriene A_4 hydrolase enzyme demonstrates three distinct binding regions. The $S1'$ subsite is located within the C-terminal domain binding region and the $S2$ subsite resides within the N-terminal domain binding region. The catalytic domain contains the zinc atom, which anchors the two flanking C-terminal and N-terminal domains. The binding pocket is made up of a narrow hydrophobic cavity that is $\sim 6\text{--}7\text{ \AA}$ wide by $\sim 15\text{ \AA}$ deep [176]. The depth of the pocket is an important aspect with regards to binding leukotriene A_4 , which must extend into the $S2$ pocket with a long aliphatic chain.

The reaction mechanism for hydrolysis by the leukotriene A_4 hydrolase enzyme involves activation of the epoxide of leukotriene A_4 , or the amide carbonyl group of a small peptide, by the weakly Lewis acidic zinc atom. The oxidation state of the zinc atom is +2 and complexes with bestatin with a trigonal bipyramidal geometry [176]. Removal of the zinc atom by treatment with 1,10-phenanthroline results in loss of enzymatic activity. The catalytic activity of the enzyme is restored when treated with a stoichiometric amount of Zn^{2+} . The Zn^{2+} cation can be exchanged for a Co^{2+} cation, also a weakly Lewis acidic transition metal ion, to give a functional leukotriene A_4 hydrolase enzyme [185]. Presumably, coordination of the epoxide of leukotriene A_4 results in formation of a resonance-stabilized carbocation between carbons 6 and 12. The shape of the cavity apparently drives hydrolysis at the C-12 position mediated by Asp375 to give exclusively the *R* configuration and an *E* olefin between carbons 6 and 7 (**Figure 5A**).

On the basis of the structure of RB3041 (see **Figure 4C**) co-crystallized with the leukotriene A_4 hydrolase enzyme, peptide hydrolysis appears to occur by activation of the carbonyl

group of the P1 amide bond (Schechter and Berger nomenclature) followed by addition of water by Glu296 to give the tetrahedral intermediate. Subsequent decomposition of the tetrahedral intermediate releases the free carboxylic acid and free amine (**Figure 5B**) [186].

Fig. 5. Reaction mechanism for hydrolysis by the leukotriene A₄ hydrolase enzyme.

3.2 The history of leukotriene A₄ hydrolase inhibitors

As noted in these reaction mechanisms, inhibition of leukotriene A₄ hydrolysis by chelation with the zinc atom will inevitably lead to inhibition of aminopeptidase function. Inhibition of the leukotriene A₄ hydrolase enzyme was motivated by the potential clinical utility that could be realized by reducing the biosynthesis of the leukotriene B₄ metabolite. Therefore, initial efforts to design leukotriene A₄ hydrolase inhibitors focused on analogs of the lipid substrate (**Figure 6**). Prescott first reported that eicosapentaenoic acid, an omega-3 fatty acid, inhibited leukotriene B₄ biosynthesis in a dose-responsive manner [187]. Leukotriene A₃, derived from metabolism of exogenously added 5,8,11-eicosatrienoate, also known as mead acid, was then found to also inhibit leukotriene A₄ hydrolase [5, 188]. Shimizu and co-workers then reported the effect of a series of leukotriene A₄ analogs on the leukotriene A₄ hydrolase enzyme. Their work demonstrated that an appropriately positioned allylic epoxide is sufficient for inhibition of the enzyme. However, their study suggested that the free carboxylic acid moiety, the 5,6-epoxide, and the (7*E*,9*E*,11*Z*,14*Z*)-tetraene structure of the leukotriene A₄ substrate were all required components for binding to the enzyme [6].

Fig. 6. First discovered inhibitors of the leukotriene A₄ hydrolase enzyme.

On the basis of the aminopeptidase activity of the leukotriene A₄ hydrolase enzyme, Orning and co-workers realized that the general metallohydrolase inhibitor bestatin was also a potent inhibitor of the leukotriene A₄ hydrolase enzyme [79]. Subsequent studies revealed

that the enzyme is also sensitive to captopril (**Figure 7**), an inhibitor of the angiotensin-converting enzyme (ACE) [79].

Fig. 7. Examples of zinc-chelating inhibitors of the leukotriene A₄ hydrolase enzyme.

The mechanism of captopril inhibition of the leukotriene A₄ hydrolase enzyme likely involves chelation to the zinc atom via its sulfhydryl group. Strategies to target the leukotriene A₄ hydrolase enzyme on the basis of zinc chelation has led to some of the most potent inhibitors known for the enzyme [189]. As expected on the basis of a shared binding mode in the enzyme, the aminopeptidase inhibitors also inhibit the epoxide hydrolase activity of the leukotriene A₄ hydrolase enzyme.

Despite the impressive potencies achieved with zinc-chelating agents, selective inhibition of the leukotriene A₄ hydrolase enzyme over other zinc-containing aminopeptidases remained a challenge [189]. Therefore, most of the current inhibitors of the leukotriene A₄ hydrolase enzyme are derived from the scaffold provided by Penning and co-workers. Optimization of SC-22716, a lead compound identified through the Monsanto Structure-Activity Screening Program, identified important structural motifs for inhibition of leukotriene A₄ hydrolase without a zinc-chelating component. These inhibitors contain a bis-aryl substituent, a two-carbon linker, and an amine (or nitrogen atom-containing heterocycle) substituent [190]. The large number of inhibitors derived from Penning's work has recently afforded an efficient *in silico* pharmacophore model for identification of new inhibitors of the leukotriene A₄ hydrolase enzyme by virtual screening (**Figure 8**). In this model, the chemical features that make up the best pharmacophore includes a hydrogen bond acceptor (HBA), a hydrophobic region (HYP), and two ring-aromatic regions [191].

Fig. 8. A. Non-chelating inhibitors [189]. B. In silico pharmacophore model [191].

3.3 Contemporary approach in targeting the leukotriene A₄ hydrolase enzyme

The most advanced leukotriene A₄ hydrolase inhibitor in clinical trials is a small molecule developed by deCODE Genetics and named DG-051 (**Figure 9**). As described earlier, deCODE Genetics underwent an impressive fragment-based screening program using X-ray crystallography to design new inhibitors of the leukotriene A₄ hydrolase enzyme. Their efforts afforded molecules that fit the pharmacophore model derived from Penning's work. However, the use of X-ray crystallography unveiled a potential interaction with the zinc

atom that could be achieved with an extended carboxylic acid substituent. Addition of a carboxylic acid moiety allowed for optimization of other important physicochemical properties such as solubility for oral administration [181, 182]. Building upon the pre-clinical findings, which demonstrated the pathogenic roles of the leukotriene A₄ hydrolase epoxide hydrolase activity, DG-051 is now at an advanced stage of FDA clinical trial as a therapy for preventing atherosclerosis.

Fig. 9. Structure of DG-051.

Exploitation of the newly found biology of the aminopeptidase activity of the leukotriene A₄ hydrolase enzyme is the subject of current research efforts. A reported strategy targets the biological role of PGP as a CXCR1/CXCR2 activating ligand in the process of neutrophil chemotaxis [73]. One approach is to inhibit the prolyl endopeptidase enzyme or metalloproteinase-8 in order to reduce endogenous bio-production of PGP [171, 174]. A second approach is to target neutrophil chemotaxis by creating a synthetic PGP analog, which would emulate the N-terminus of interleukin-8 but antagonize the CXCR1/CXCR2 receptors [173]. These two pharmaceutical strategies would mitigate deleterious neutrophil chemotaxis in various states of human diseases.

4. Conclusion

The leukotriene A₄ hydrolase enzyme has been a center of intense biological investigations for several decades. The relevance of the leukotriene A₄ hydrolase enzyme in human diseases has proved to be substantial, but pharmaceutical attempts to exploit this pathway have been disappointing. The aminopeptidase activity of the leukotriene A₄ hydrolase enzyme was recently found to be an important factor in human diseases. Attempts to exploit the biology of the leukotriene A₄ hydrolase enzyme is actively ongoing and is certainly expected to continue in the foreseeable future. This review has considered both the classical and new understandings of the biology of the leukotriene A₄ hydrolase enzyme, and it is reasonable to conclude that both catalytic functions of the enzyme (i.e. epoxide hydrolase and aminopeptidase activities) need to be carefully considered for pharmaceutical investigations targeting this enzyme. Taken together, the leukotriene A₄ hydrolase enzyme and the leukotriene B₄ associated pathways are sure to remain an important topic of discussion in the arenas of drug discovery for years to come.

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This book is a collection of comprehensive reviews contributed by experts in the diverse fields of acute and chronic inflammatory diseases, with emphasis on current pharmacological and diagnostic options. Interested professionals are also encouraged to review the contributions made by experts in a second related book entitled "Inflammation, Chronic Diseases and Cancer"; it deals with immunobiology, clinical reviews, and perspectives of the mechanisms of immune inflammatory responses that are involved in alterations of immune dynamics during the genesis, progression and manifestation of a number of inflammatory diseases and cancers, as well as perspectives for diagnosis, and treatment or prevention of these disabling and potentially preventable diseases, particularly for the growing population of older adults around the globe.

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