The role of lipoxygenases in pathophysiology; new insights and future perspectives

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Abstract

Lipoxygenases (LOXs) are dioxygenases that catalyze the formation of corresponding hydroperoxides from polyunsaturated fatty acids such as linoleic acid and arachidonic acid. LOX enzymes are expressed in immune, epithelial, and tumor cells that display a variety of physiological functions, including inflammation, skin disorder, and tumorigenesis. In the humans and mice, six LOX isoforms have been known. 15-LOX, a prototypical enzyme originally found in reticulocytes shares the similarity of amino acid sequence as well as the biochemical property to plant LOX enzymes. 15-LOX-2, which is expressed in epithelial cells and leukocytes, has different substrate specificity in the humans and mice, therefore, the role of them in mammals has not been established. 12-LOX is an isomorph expressed in epithelial cells and myeloid cells including platelets. Many mutations in this isoform are found in epithelial cancers, suggesting a potential link between 12-LOX and tumorigenesis. 12R-LOX can be found in the epithelial cells of the skin. Defects in this gene result in ichthyosis, a cutaneous disorder characterized by pathophysiologically dried skin due to abnormal loss of water from its epithelial cell layer. Similarly, eLOX-3, which is also expressed in the skin epithelial cells acting downstream 12R-LOX, is another causative factor for ichthyosis. 5-LOX is a distinct isomorph playing an important role in asthma and inflammation. This isoform causes the constriction of bronchioles in response to cysteinyl leukotrienes such as LTC4, thus leading to asthma. It also induces neutrophilic inflammation by its recruitment in response to LTB4. Importantly, 5-LOX activity is strictly regulated by 5-LOX activating protein (FLAP) though the distribution of 5-LOX in the nucleus. Currently, pharmacological drugs targeting FLAP are actively developing. This review summarized these functions of LOX enzymes under pathophysiological conditions in mammals.

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1. Introduction

Lipoxygenases (LOXs) catalyze the oxygenation of polyunsaturated fatty acids such as arachidonic acid and linoleic acid [1,2]. The oxygenated lipids initiate subsequent biological reactions, activate cellular signaling mechanisms through specific cell surface receptors, or are further metabolized into potent lipid mediators. LOX can be found not only in mammals, but also in plants. Historically, biochemical characterizations have been performed mainly on soybean LOX isoforms. While the overall structure of mammalian LOX enzymes seems to be similar, each isoform has unique properties, such as substrate specificity (Table 1, reviewed in [3]). In most cases, the structure depends on the shape of the substrate cavity and the coordination of histidine residues or alternatives to a non-heme iron atom at the catalytic center [4,5]. Importantly, LOX enzymes require a lag period for the activation of enzymes from an inactive ferrous form to an active ferric form by either molecular oxygen or lipid hydroperoxides. Enzymatic activity is also regulated by the N-terminal β-barrel region of polypeptides, where this region has a similar amino acid shape of the substrate cavity and the coordination of histidine

Enzymatic activity is also regulated by the N-terminal β

NA, not available.

Aralidonic acid as a substrate except eLOX-3 where 12R-HPETE as a substrate.

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9.1. GSK2190915

9. Conclusions

Acknowledgment

References

Table 1

Properties of LOX enzymes.

| Proteins | 15-LOX | 15-LOX-2 | 12-LOX | 12R-LOX | eLOX-3 | 5-LOX | FLAP |
|----------|--------|----------|--------|---------|--------|-------|------|
| Human    |        |          |        |         |        |       |      |
| Gene     | ALOX15 | ALOX15B  | ALOX12 | ALOX12B | ALOX5  | ALOX5AP |
| Products*| 15S-HPETE | 15S-HPETE | 12S-HPETE | 12R-HPETE | 5S-HPETE | ALOX5AP |
| Expression| Leukocytes | Leukocytes | Myeloids, skin, epithelium | Skin, epithelium | Skin, epithelium | Leukocytes |
| Mouse    |        |          |        |         |        |       |      |
| Gene     | Alox15 | Alox15b  | Alox12 | Alox12b | Alox3  | Alox5  | Alox5ap |
| Products*| 12S, 15S-HPETE | 12S-HPETE | 15S-, 12S-HPETE | 12R-HPETE | 5S-HPETE | Alox5ap |
| Expression| Leukocytes | Epoxylcohols | Platelet, skin, epithelium | Skin, epithelium | Skin, epithelium | Leukocytes |

NA, not available. *Arachidonic acid as a substrate except eLOX-3 where 12R-HPETE as a substrate.

7. 5-Lipoxygenase (5-LOX)

7.1. ALOX5

7.2. Alox5

7.2.1. Inflammation

7.2.2. Atherosclerosis

7.2.3. Neuronal disorder

7.2.4. Tumor

8. 5-Lipoxygenase activating protein (FLAP)

8.1. ALOX5AP

8.2. Alox5ap

9. Clinical Trials

9.1. GSK2190915

10. Conclusions

Acknowledgment

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| Gene     | ALOX15 | ALOX15B  | ALOX12 | ALOX12B | ALOX5  | ALOX5AP |
| Products*| 15S-HPETE | 15S-HPETE | 12S-HPETE | 12R-HPETE | 5S-HPETE | ALOX5AP |
| Expression| Leukocytes | Leukocytes | Myeloids, skin, epithelium | Skin, epithelium | Skin, epithelium | Leukocytes |
| Mouse    |        |          |        |         |        |       |      |
| Gene     | Alox15 | Alox15b  | Alox12 | Alox12b | Alox3  | Alox5  | Alox5ap |
| Products*| 12S, 15S-HPETE | 12S-HPETE | 15S-, 12S-HPETE | 12R-HPETE | 5S-HPETE | Alox5ap |
| Expression| Leukocytes | Epoxylcohols | Platelet, skin, epithelium | Skin, epithelium | Skin, epithelium | Leukocytes |

NA, not available. *Arachidonic acid as a substrate except eLOX-3 where 12R-HPETE as a substrate.
potential link between atherosclerosis and its inhibition with antioxidants was explored, 15-LOX has been hypothesized to initiate and/or promote atherosclerosis through low density lipoprotein (LDL) oxidation. This is based on the “oxidative LDL theory” that oxidation of lipids induces atherosclerosis and its inhibition by antioxidants prevents atherogenesis. To initiate oxidation in vivo, there must be some initiators for lipid peroxidation. One potential candidate includes carbon-centered free radicals, which react with molecular oxygen to give rise to peroxyl radicals. Since these radicals trigger oxidation of lipids continuously, therefore, generation of such free radical-generating initiators must be tightly regulated in vivo. Lipid hydroperoxides generated from polyunsaturated fatty acids by LOX enzyme can induce this reaction since these oxidation products further decompose into other free radicals in the presence of metal ions. 15-LOX can be found in macrophages and other immune cells as well as epithelial cells. Human and mouse enzymes are known to be induced by Th2 cytokines such as IL-4 and IL-13 via STAT6-dependent manner [9,10].

2.1. ALOX15

It is widely accepted that cyclooxygenase (COX) inhibitor non-steroidal anti-inflammatory drugs (NSAIDs) induce colon cancer in humans [11]. One suggested reason is that the balance between COX and LOX determines tumorigenesis critically. Under low COX activity, arachidonic acid released from cell membranes in response to external stimuli is preferentially metabolized by LOX

Table 2
Human diseases that potentially links to lipoxygenase genes.

| Genes | Atherosclerosis/heart disease | Immune response | Neurological disorder | Cancer | Skin disease | Others |
|-------|-------------------------------|-----------------|----------------------|--------|-------------|--------|
| ALOX15 | A near null mutant (T560M) in coronary artery disease [140] | Rectal cancer [100] | | Colon cancer [141] | Adenoma recurrence [142] | Breast cancer [39] | Prostate cancer [12] | Bone mineral density [56,143–144] |
| ALOX15B | † in carotid plaque with thrombosis [43] † in carotid lesion [32] Variants in coronary artery disease [145] | Esophageal cancer [35] | | Adrenocortical tumor [36] | Breast cancer [39] | Epithelial tumors [37] | Prostate cancer cells [38] | Head and neck carcinoma [40] |
| ALOX12 | Variants in subclinical atherosclerosis [146] | Variants in Toxoplasmosis [147] | Bipolar disorder [49] Schizophrenia [50] | Methylation in AML [61] | Esophageal squamous cell carcinoma [64] | Rectal cancer [100] | Adenoma recurrence [142] | Colorectal cancer [148] | E261R mutation | Breast cancer [48] | Colon cancer [47] | Colorectal cancer [65] | Colorectal adenoma [149] | Bone mineral density [54–57,150] | Fat mass [53] |
| ALOX12B | | | | | | | | |
| ALOXE3 | | | | | | | | |
| ALOX5 | Variants in atherosclerosis [152] | Variants in asthma [97,154,155] | Inverse correlation | Rectal cancer [100] | | | | |
| | † in atherosclerosis [153] | Variants in AHR [98,99] | | | | | | |
| | Variants in subclinical atherosclerosis [146] | | | | | | | |
| ALOX5AP | Variants in subclinical atherosclerosis [146] | Variants in asthma [126,156] | | Rectal cancer [100] | | | | Adenoma [100] |

AHR, airway hyperresponsiveness; AML, acute myeloid lymphoma; ARCI, autosomal recessive congenital ichthyosis; NCIE, nonbullous congenital ichthyosiform erythroderma; TAM, tumor-associated macrophages.
enzymes. There is evidence that a 15-LOX metabolite 13S-HPODE (13S-hydroperoxyoctadecenoic acid) generated from linoleic acid induces apoptosis in colon cancer cells; thus, defective expression of ALOX15 in colon cancers could promote tumorigenesis [11]. ALOX15 expression itself is controlled, at least in part, by the epigenetic process, as an alteration of methylation in the ALOX15 promoter has been observed in prostate cancer patients [12]. Furthermore, the expression of 15-LOX in epithelial cancer cells is tightly regulated by additional mechanisms. As mentioned, STAT6 is a critical regulator of ALOX15 expression regulated by its phosphorylation and acetylation, as well as histone modification [13,14]. Recent studies have also shown the importance of ALOX15 expression can be modulated by the chromatin-dependent STAT6-independent mechanism [15,16]. Biochemically, the produced 13 S-HPETE interacts with PPAR-δ, followed by the induction of apoptosis prior to carcinogenic conditions [17]. The importance of 15-LOX-derived metabolites has also been defined by its aberrant failure in conditional transgenic mice, expressing it in the mouse prostate, inducing prostatic intraepithelial neoplasia once the apoptotic function is dysregulated [18].

2.2. Alox15

Atherosclerosis is an inflammatory disease characterized by an accumulation of lipid-loaded macrophages in blood vessels. Evidence has suggested a close link between Alox15 expression and atherosclerosis in the mouse, characterized mostly on the atherosclerosis-prone genetic background of mice lacking the Alox15 gene or LDL receptor [19–23]. In both cases, the initiation and/or development of atherosclerosis depends on 15-LOX enzymatic
activity. Recent studies have suggested that atherosclerosis is involved, at least in part, in sterile inflammation characterized by augmented IL-1β and the activation of caspases. The accumulation of lipids in blood vessels causes a failure of vascular function; therefore, disruption of Alox15 shows impaired migration of vascular smooth muscle cells [24]. In addition, ischemia-induced cardioprotection plays an important role in maintaining proper heart function. A previous study showed that Alox15 deficiency caused its impairment after reperfusion-mediated preconditioning by PKC activation in the heart [25]. These results suggest that this enzyme might also contribute to the pathogenesis of cardiovascular disease.

Lipid hydroperoxides, the primary reaction products of Alox15 from polyunsaturated fatty acids, readily induce oxidative stress through their decomposition to free radicals. Thus, Alox15-deficient mice might produce less oxidative stress under physiological conditions. However, Alox15-deficient mice have shown an increase in oxidative stress markers such as isoprostane 8-epi-prostaglandin F2α in a zymosan-induced peritonitis model [26]. These apparently paradoxical results might be explained by the concomitantly enhanced 5-LOX-, but not 15-LOX-, dependent mechanism, which facilitates the accumulation of neutrophils by 5-HPETE and leukotrienes, thereby leading to enhanced oxidative stress at the site of inflammation in Alox15-deficient mice.

Accumulating evidence suggests that Alox15 is critically involved in the regulation of cell differentiation. For example, the development of hematopoietic stem cells into the myeloid lineage is impaired in Alox15-deficient mice [27]. Alternatively, the formation of osteoclasts in Alox15-deficient mice and mice treated with enzymatic inhibitors has indicated its attenuation [28]. This impaired bone-desorbing osteoclastogenesis is negatively regulated by osteoblast formation, which derives from mesenchymal stem cells. Given that this bone-absorption is impaired in Alox15-deficient mice, bone-generating osteoblast formation is likely to be enhanced. In this case, adipocytes, which also stem from mesenchymal stem cells, would be impaired. Consistent with this speculation, Alox15-deficient mice have displayed impaired obesity [29], indicating a critical role of Alox15 in cell differentiation.

3. 15-Lipoxygenase, type B (15-LOX-2)

15-LOX-2 shows higher similarity to 15-LOX, initially discovered in the skin in humans [30]. Human 15-LOX-2 generates 12R-HPETE from arachidonic acid specifically, showing unusual specificity for the regioisomeric lipid mediators in contrast to most isozymes, which produce S-regioisomers. Furthermore, mouse ortholog of 15-LOX-2 generates 8S-HETE and 8S-, 15S-diHETE from arachidonic acid, thus enzymologically murine Alox15b is considered to be similar to human ALOX12 [31]. Due to this, there are almost no overlapping diseases/phenotypes in the human and mouse in this isoform.

3.1. ALOX15B

Although a link between atherogenesis and Alox15 expression has long been hypothesized, it is known that the expression of ALOX15B in human carotid plaque macrophages is higher compared to Alox15 [32,33]. An in vitro experiment of ALOX15B silencing reported an attenuated lipid accumulation in human macrophages, indicating that it is functional for lipid uptake into the cells [34]. Thus, it is suggested that ALOX15B plays an important role in the initiation and development of atherosclerosis in humans.

Several studies have shown the downregulation of ALOX15B in epithelial tumors, suggesting that ALOX15B has an antiproliferative role [35–40]. This reduction of 15-LOX-2 in tumor cells was restored by an inhibitor for COX enzyme, demonstrating that its expression is negatively regulated by prostaglandins, at least in part [35]. PPAR-γ, a nuclear receptor regulated by endogenous LOX products, was upregulated in some epithelial tumors, suggesting that the downregulation of ALOX15B is autonomously controlled by PPAR-γ in epithelial cancer cells [37]. In prostate epithelial cells, Alox15 expression is positively regulated by transcription factor Sp1, whereas transcription factor Sp3, which is closely related to Sp1, negatively regulates its expression, suggesting that ALOX15B expression is critically regulated by multiple regulators [41]. Apart from epithelial cells, a separate study demonstrated increased ALOX15B expression in tumor-associated macrophages from renal cell carcinoma, suggesting that ALOX15B expression is distinctly regulated in epithelial cancer cells and macrophages [42]. Similar to tumor-associated macrophages, the upregulation of ALOX15B in carotid plaque macrophages has also been described [32,43].

3.2. Alox15b

There are a limited number of studies involving Alox15b. In chimeric mice transplanted with Alox15b-silenced bone marrow cells in mice lacking LDL receptor showed defective atherogenesis, suggesting that 15-LOX-2 is required in the murine atherosclerotic model [34]. As mentioned previously, the enzymatic action of murine 15-LOX-2 preferentially generates 12S-HPETE rather than 15S-HPETE from arachidonic acid, suggesting that mouse Alox15b and human ALOX12 have similar roles in vivo. The skin of Alox15b-deficient mice has been shown to display an ichthyosiform appearance, as in Alox12b– and Alox13e-deficient mice [44]. This example clearly shows that the skin phenotype found in Alox1b-deficient mice seems to require specific oxidation products from polyunsaturated fatty acids. Furthermore, this result suggests that Alox15b could be a functional alternative for Alox12b and Alox3.

4. 12-Lipoxygenase (12-LOX)

12-LOX enzymes derived from ALOX12 in humans and its murine ortholog Alox12 (also known as platelet-type 12S-LOX) produce 12S-HPETE from arachidonic acid. These enzymes are expressed in leukocytes in humans and in platelets, megakaryocytes, and skin in mice [45,46]. Genetic studies have suggested that ALOX12 gene polymorphisms are associated with cancers [47,48], neurological disorders [49,50], hypertension [51,52], fat mass [53], and bone mineral density [54–57]. The mechanism of regulation of 12-LOX expression has been studied in several models. The ALOX12 promoter region contains at least four binding sites for RUNX1, leading to its suppressive effect in human erythroleukemic cells [58]. Alox12 expression is also regulated by transcription factor p63 in the skin, which plays a key role in the development and terminal differentiation of the epidermis [59]. Similar to other isozymes, human 12-LOX has a catalytic domain in the C-terminal. Truncation of the N-terminal domain (which needs to be explored in terms of function) decreases its enzymatic activity at approximately 20% without altering substrate specificity [60].

4.1. ALOX12

Since the discovery of the high expression of LOX in platelets, the mechanism of its expression has been studied in detail. A previous study reported that ALOX12 expression was attenuated in platelets by the haplodeficiency of RUNX1, a hematopoietic transcription factor associated with familial thrombocytopenia, platelet dysfunction, and a predisposition to acute leukemia in patients.
with thrombocytopenia [58]. ALOX12 expression is also regulated epigenetically, as indicated by the increase in DNA methylation of ALOX12 genes in myelodysplastic syndrome and acute myeloid leukemia patients with megakaryocytic dysplasia [61,62]. It has been suggested that ALOX12 is associated with diminished bone mineral density as well [54–57]. Given that 12-LOX produces endogenous lipid ligands for nuclear receptors, such as PPAR-γ, which facilitate adipocyte differentiation from mesenchymal stem cells, the number of osteoblasts decreases, followed by impairment of bone mineral density [55].

An earlier study suggested that the 12-LOX-mediated pathway is associated with the risk of colorectal cancer [63]. The best-studied example includes mutation of E261R (835A > G), which causes an increase in 12-LOX activity, with a potential link to esophageal squamous cell carcinoma [64]. This mutation has also been associated with colorectal cancer [47,65] and breast cancer [48]. An in vitro and in vivo study has shown that 12-LOX plays a role in the proliferation and antiapoptosis of hepatocellular cells, suggesting that this carcinogenic function of ALOX12 requires endogenously generated lipid mediators [66].
4.2. Alox12

Consistent with the expression of \textit{ALOX12} in the platelets of humans, mice lacking \textit{Alox12} have shown increased platelet sensitivity and mortality due to thrombosis in response to the administration of adenosine diphosphate, whereas aggregation and secretion in response to most agonists seemed normal [67]. Alox12 deficiency has led to a reduced incidence of carcinoma in a C57BL6/129 genetic background and of papilloma in a tumor-sensitive SENCAR genetic background, showing that Alox12 is involved in tumorigenesis in the skin in a context-dependent manner [68]. Most notably, Alox12 deficiency has caused basal trans-epidermal water loss in the skin with unaltered inflammatory responses in Alox12b- and Alox3-deficient mice [69]. This finding suggests that Alox12 has a critical role in the maintenance of the skin barrier in association with other isoforms, as explained in detail later.

5. 12-Lipoxygenase, 12R type (12R-LOX)

In humans, 12R-LOX has been detected in keratinocytes, tonsil squamous epithelial cells, bronchial epithelial cells, and psoriasis scales, as well as in B cells [8,70–74]. In mice, its expression has been induced at embryonic day (E) 15.5 in the epidermis, nasal epithelium, and surface of the tongue, suggesting that 12R-LOX is required for the proper development of neonates [71].

The physiological role of 12R-LOX is rather specific, in conjunction with its limited expression profile in epithelial cells. The best-characterized example is the skin. The important physiological role in the skin is to maintain appropriate moisture by preventing unnecessary water evaporation through epithelial cells, as well as in B cells [8,70–74]. In mice, its expression has been induced at embryonic day (E) 15.5 in the epidermis, nasal epithelium, and surface of the tongue, suggesting that 12R-LOX is required for the proper development of neonates [71].

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6. Epidermal lipoxygenase 3 (eLOX-3)

As mentioned previously, the conversion of linoleoyl ceramide in CLE into CE plays a crucial role in the proper maintenance of epidermal barrier formation. The eLOX-3 enzyme plays a major role in the second step, which involves the conversion of 9R-HPODE esterified with ω-hydroxylacyl-sphingosine into its related epoxylated derivative (Fig. 1B) [91–93]. Biochemical reactions catalyzed by eLOX-3 are essential, and their failure leads to ARCI.

6.1. ALOXE3

Genetically, many mutations in \textit{ALOXE3}, together with \textit{Alox12B}, have been found in ichthyosis [81–84,86,94]. The outcome of disease in \textit{ALOXE3} variants seems to be similar to that found in \textit{Alox12B} variants, showing clearly that this sequential oxidation by \textit{ALOXE3} and \textit{Alox12B} are equally important. As a substrate, it is known that eLOX-3 favors oxygenated lipids like 12R-HPETE rather than unoxidized compound such as arachidonic acid (Fig. 1B). Other than ARCI, other diseases associated with \textit{ALOXE3} variants...
have not been reported.

6.2. Aloxe3

A previous study showed that Aloxe3-deficient mice also exhibit a similarly severe ichthyosis phenotype with the loss of covalently bound ceramides and impaired CLE development [86].

As expected from its hepoxilin synthase activity of eLOX-3, in vivo results also showed a marked reduction of its metabolites in the skin. Similar to Alox12b-deficient mice, Aloxe3-deficient mice displayed a postnatal lethal phenotype [95].
7. 5-Lipoxigenase (5-LOX)

5-LOX plays an important role in the control of asthma. Under asthmatic conditions, activated immune cells first produce arachidonic acid by an enzymatic action of phospholipase A₂ from the plasma membrane, followed by 5-HPETE production through the 5-LOX enzyme. The produced 5-HPETE then converts into leukotrienes that have a potent biological effect on the constriction of bronchioles through cysteinyl leukotriene receptor 1, which is expressed exclusively onto bronchiolar smooth muscle cells, but not epithelial cells [96]. Conversely, cysteinyl leukotriene receptor 2 is expressed strongly in pulmonary interstitial macrophages and weakly in smooth muscle cells.

7.1. ALOX5

The expression of the ALOX5 gene is transcriptionally regulated at a basal level and regulated by external stimuli such as Ca²⁺. Numerous agents have been developed as antiasthmatics. A potent LOX-5 inhibitor zileuton introduced in the United States blocks a considerable amount of cysteinyl leukotriene production for a short period of time. Although the prevalence is low, a genetic increase in the production of lipid products from 5-LOX means that have a potent biological effect on the constriction of bronchioles through cysteinyl leukotriene receptor 1, which is expressed exclusively onto bronchiolar smooth muscle cells, but not epithelial cells [96]. Conversely, cysteinyl leukotriene receptor 2 is expressed strongly in pulmonary interstitial macrophages and weakly in smooth muscle cells.

7.2. Alox5

7.2.1. Inflammation

As mentioned previously, immune cells express leukotriene receptor 1 and 2 as well as cysteinyl leukotriene receptor 2; as such, the role of Alox5 could account for the migration of these immune cells. As expected, neutrophilic inflammation is one of the apparent phenotypes in Alox5-deficient mice. These mice have been shown to be resistant to anaphylaxis induced by platelet-activating factor (PAF), showing that Alox5 seems to be closely involved in this process [102,103]. Similarly, Alox5-deficient mice exhibit a suppressed response to chemically induced local inflammation [104,105]. These mice are also susceptible to Borrelia burgdorferi-induced arthritis [106]. In this study, the authors showed that enzymatic activity of 5-LOX is not required for the initiation of infection, but it is required for earlier joint swelling and retarded arthritis recovery, suggesting a potential increase in the accumulation of neutrophils. Similarly, in a Toxoplasma gondii infection model, Alox5-deficient mice exhibited suppressed leukotriene A₄ production and increased interleukin-12 and interferon-γ production, followed by an increase in mortality rate due to marked encephalitis [107]. Such altered cytokine production seems to be explained, at least in part, by impaired neutrophilic inflammation.

In an ovalbumin-induced asthma model, Alox5-deficient mice exhibited a suppressed methacholine-induced response to airway hyperresponsiveness with impaired eosinophilic inflammation in the lung [108]. Thus, the production of lipid products from 5-LOX plays an important role under physiological conditions, and its level is tightly regulated by the balance between LOX and other enzymes, such as COX, as shown in an earlier study. Another study reported an increased mortality rate in Alox5-deficient male mice with an autoimmune-prone MRL-lpr/lpr genetic background, raising the possibility that an enhanced renal autoimmune inflammation could be involved in this process [109].

Recently, the roles of oxidation products derived from eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6) have been studied extensively (Fig. 2). As seen in their chemical structures, 20:5 and 22:6 have four and five bisallylic carbon atoms, respectively, in their molecules, providing a variety of oxidation products through free radical-mediated mechanisms. For example, 20:5 is a primary substrate for conventional LOX enzymes such as 15-LOX, 12-LOX, and 5-LOX. In addition, 18-hydroperoxy-5,8,11,14,16-eicosapentaenoic acid (18-HPETE), a free radical-mediated oxidation product of 20:5, can be further metabolized by 5-LOX to produce a novel class of oxidation product collectively called resolvins (Fig. 2) [110,111]. Emerging evidence has shown that resolvins assist in terminating inflammation through specific GPCR ChemR23 at nM concentrations in vitro [112]. The formation of resolvins seems to be critically regulated by local O₂ concentration, as well as the expression and activity of multiple LOX enzymes, both of which influence the final yield of resolvins from its initial substrate 20:5. Due to its anti-inflammatory function, whether these oxidized lipids might modulate the functions of microRNAs is under investigation [113].

7.2.2. Atherosclerosis

Apart from reactions in asthma and neutrophilic inflammation, there is some research showing that 5-LOX plays a key role in the initiation and/or development of atherosclerosis. Impaired expression of functional 5-LOX in LDL receptor-deficient mice has revealed suppressed atherogenesis, suggesting that 5-LOX plays a causative role in this disease [114]. Consistently, another atherosclerotic model induced by COX-2 disruption attenuated disease formation in Alox5-deficient mice [115]. Given that LOX-15 is involved in atherosclerosis, these studies provide examples that atherosclerosis can be induced by lipid peroxidation products formed from any isoforms. This finding is entirely consistent with observations indicating that antioxidants generally exhibit protective effects in experimental models.

7.2.3. Neuronal disorder

Alox5 is known to be highly expressed in neuronal tissue, particularly in Alzheimer’s disease; thus, its role in neuronal disorders has been actively characterized [116]. A recent study reported that aged female Alox5-deficient mice exhibited protective effects against anxiety-like behavior on a C57BL/6 genetic background, raising the possibility that Alox5 could modulate neuronal function [117]. A subsequent study using a transgenic mouse model of Alzheimer’s disease demonstrated the efficacy of a 5-LOX inhibitor zileuton and hypothesized that Alox5 could facilitate the initiation or progression of this disease [118]. Using such a disease model, Alox5 deficiency consistently improved disease phenotypes [119].

7.2.4. Tumor

Colorectal cancer is often caused by mutation of the tumor suppressor Adenomatous polyposis coli (APC) gene. Among many mouse models generated by Apc mutations, ApcΔ468 mice specifically bear a truncated Apc gene that develops severe polyposis by four months [120]. Interestingly, immunohistochemistry showed an increase in Alox5 expression in ApcΔ468 mice, suggesting that LOX-5 might contribute to tumorigenesis in colorectal cancer [121–123]. Mast cells play an important role in the development of colorectal cancer in this animal model, as they induce epithelial proliferation [122]. Consistently, the number of mast cells increased in ApcΔ468 mice compared to WT controls. In this model, a deficiency in Alox5 led to impairment, implying that LOX5 acts as an important role in colorectal tumorigenesis [122].
8. 5-Lipoxygenase activating protein (FLAP)

FLAP is a small protein that activates 5-LOX through protein interaction. The formed complex of 5-LOX and FLAP in the nucleus efficiently generates 5-HEPE from arachidonic acid, which is subsequently converted into various leukotrienes. FLAP protein stays on the nuclear membrane and acts as a transporter for 5-LOX. FLAP expression is limited in myeloid cells.

8.1. ALOXSAP

Drugs targeting FLAP protein have been actively developed in humans. DG-031 (velipalon, BAY x 1005), first licensed by DeCode Genetics and then developed by Bayer, is one example. This compound also reduces the incidence of ischemic myocardial infarction by reducing LTB4 production [124]. A recently developed AM-103/GSK2190914 was designed based on the three-dimensional structure of FLAP protein [125]. In asthma, two intronic single-nucleotide polymorphisms have been associated with ALOXSAP, suggesting that these mutations can be used for diagnostic markers [126].

8.2. Alox5ap

Alox5ap-deficient mice exhibited unique phenotypes similarly observed in Alox5-deficient mice, such as an impaired response to PAF-induced anaphylaxis and zymosan-induced peritonitis [127]. In a collagen-induced arthritis model, Alox5ap-deficient mice displayed impaired arthritis, whereas the accumulation of antibody against collagen remained unchanged, suggesting that 5-LOX positively regulated inflammation without affecting the immune response. In a cerebral artery occlusion model, disruption of the Alox5ap gene caused impaired median infarct size and a better functional score, demonstrating that FLAP protein positively regulates cerebral inflammation [128]. The expression of Alox5ap is independent of the expression of 5-LOX. Alox5ap induces 12S-HETE production in the 12-LOX-induced signaling pathway, suggesting that 5-HPETE or downstream leukotriene metabolites might be involved in this process [129].

In atherosclerosis, FLAP inhibitor MK-886 and BAY x 1005 effectively attenuated disease formation in mice lacking ApoE and LDL receptors [130,131]. Similarly, another atherosclerotic model developed by transgenic mice expressing a dominant negative form of TGFβ receptor II in ApoE-deficient mice was suppressed by MK-886 [132]. These examples strongly suggested that FLAP protein is required for atherogenesis in these mouse models. Consistently, Alox5ap-deficient mice exhibited attenuated disease formation in an experimental model generated by Cox-2-deficient mice [115].

Alox5ap-deficient mice displayed an improved Alzheimer’s disease-like phenotype [133] and an apparent increase in anxiety-like behavior in aged mice [134]. These results supported the phenotype of Alox5-deficient mice, showing that both 5-LOX and FLAP mutually and collaboratively play critical roles in the leukotriene pathway in neurological disorders.

9. Clinical Trials

There are many studies reporting synthesis and characterization of lipoxygenase inhibitors (reviewed in [135,136]). Generally, substances sharing similarity to either lipids or phenolic anti-oxidants have lower inhibitory activity for LOX enzyme. Furthermore, due to the presence of multiple isoforms, the development of selective inhibitor seems to be challenging. Therefore, there is a limited number of successful drugs that can be used for therapeutic purposes. One clinically applicable example includes zileuton that acts as anti-asthmatic agent by inhibiting 5-LOX. Apart from direct regulation of enzymes, there are some studies targeting FLAP for modulating 5-LOX under pathophysiological conditions in clinical trials.

9.1. GSK2190915

This is a FLAP inhibitor that inhibits the production of LTB4 and other cysteinyl leukotrienes. The results of Phase I study showed that there was no clear difference in adverse events between placebo and drug-treated subjects in Western Europe (EUR-DACT2007-00484872) and Japan (NCT00955383) [137]. Plasma concentration of GSK2190915 reaches maximal at two hours after oral administration. Consistently, LTB4 production in drug-treated subjects was significantly impaired (EC50 in plasma is approximately 85 nM). Its beneficial effect is proven in adults and adolescents with persistent asthma (NCT01147744) [138]. Comparison between GSK2190915 and established asthma treatment such as montelukast and the inhaled corticosteroid fluticasone propionate revealed that GSK2190915 30 mg once daily has similar effect compared to montelukast 10 mg once daily as assessed by forced expiratory volume in 1 s (FEV1), a widely used measure for asthma evaluation. A subsequent study reported that GSK2190915 50 mg daily showed clear attenuation in early asthmatic response induced by inhaled allergens in a placebo-controlled double-blind randomized study in UK (NCT00812773) [139].

10. Conclusions

The physiological roles of LOX enzymes have been studied extensively, due to the close link between them and various diseases. Apparently, the best characterized example includes the relation between 5-LOX and asthma, because cysteinyl leukotriene receptor 1, a GPCR activated by leukotrienes produced by 5-LOX, is widely expressed on bronchiolar smooth muscle cells. Genetically, both ALOX12B and ALOX13 play a critical role in the development of ichthyosis through TEWL. In humans, some mutations in ALOX12 are found in tumor cells, suggesting this isoform might have anti-tumor effect. An increased expression of LOX enzymes in response to Th2 cytokines has been well established; how their expression is controlled and its consequences need to be investigated in the future.

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