Biochimica et Biophysica Acta 1841 (2014) 390–400

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

The role of lipoxygenases in epidermis ☆

Peter Krieg *, Gerhard Fürstenberger

Genome Modifications and Carcinogenesis, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D69120 Heidelberg, Germany

ARTICLE INFO

Article history:
Received 4 July 2013
Received in revised form 31 July 2013
Accepted 5 August 2013
Available online 16 August 2013

Keywords:
Lipoxygenases
Epidermal barrier
Ceramides
Ichthyosis

ABSTRACT

Lipoxygenases (LOX) are key enzymes in the biosynthesis of a variety of highly active oxylipins which act as signaling molecules involved in the regulation of many biological processes. LOX are also able to oxidize complex lipids and modify membrane structures leading to structural changes that play a role in the maturation and terminal differentiation of various cell types. The mammalian skin represents a tissue with highly abundant and diverse LOX metabolism. Individual LOX isozymes are thought to play a role in the modulation of epithelial proliferation and/or differentiation as well as in inflammation, wound healing, inflammatory skin diseases and cancer. Emerging evidence indicates a structural function of a particular LOX pathway in the maintenance of skin permeability barrier. Loss-of-function mutations in the LOX genes *ALOX12B* and *ALOXE3* have been found to represent the second most common cause of autosomal recessive congenital ichthyosis and targeted disruption of the corresponding LOX genes in mice resulted in neonatal death due to a severely impaired permeability barrier function. Recent data indicate that LOX action in barrier function can be traced back to the oxygenation of linoleate-containing ceramides which constitutes an important step in the formation of the corneocyte lipid envelope. This article is part of a Special Issue entitled The Important Role of Lipids in the Epidermis and their Role in the Formation and Maintenance of the Cutaneous Barrier. Guest Editors: Kenneth R. Feingold and Peter Elias.

1. Introduction

An important role of fatty acid (FA) metabolism in skin has been suggested since the discovery that essential fatty acids (EFA) and their products are involved in the normal process of forming the water-impermeable barrier in skin. Dietary deficiency of EFA results in characteristic scaly skin disorder and excessive water loss [1]. The addition of certain FA such as linoleic acid to the diet could reverse some of the cutaneous symptoms of EFA deficiency and several lines of evidence suggested that the curative effects of EFA in the EFA deficient animal involve the lipoxygenase (LOX)-catalyzed conversion of polyunsaturated fatty acids (PUFA) to oxygenated products [2,3].

Shortly after the discovery of the first mammalian LOX [4] LOX activities and LOX products have been found in human and murine skin and epidermal cells as well [5]. Since then, a plethora of findings characterized the mammalian epidermis as a tissue with highly abundant and diverse LOX metabolism. LOX products identified in skin include a variety of oxidized free PUFA, arachidonic acid metabolites such as 12-hydroxyeicosatetraenoic acid (12-HETE), 15-hydroxyeicosatetraenoic acid (15-HETE), hepoxilins, trioxilins, and leukotrienes, and linoleic acid products such as 13-hydroxyoctadecadienoic acid (13-HODE) and 9-hydroxyoctadecadienoic acid (9-HODE) as well as docosahexaenoic acid-derived metabolites. LOX are also known to oxidize esterified structural lipids such as ceramides which in particular play an essential role in barrier function.

2. Mammalian lipoxygenases

LOX are a class of widely distributed non-heme iron containing dioxygenases that catalyze the stereo- and regio-specific incorporation of molecular oxygen into PUFA containing a (cis,cis)-1,4-pentadiene system. The primary products of LOX reactions are hydroperoxides which are rapidly transformed into their reduced hydroxyl analogs and products of subsequent enzymatic reactions (for reviews, see [6,7]). The members of the LOX multigene family exhibit highly conserved gene and protein structures. Mammalian LOX genes consist of 14 or 15 exons with exon/intron boundaries at highly conserved positions and they map close together. With the exception of the 5-LOX gene, all LOX genes are located at the human chromosome 17.p13 and the mouse chromosome 11, respectively indicating their origin from a common ancestor gene.

Abbreviations: ARCI, autosomal recessive congenital ichthyoses; CE, corneocyte envelope; CLE, corneocyte lipid envelope; CoA, Coenzyme A; E, embryonic day; e12-LOX, epidermis-type 12-lipoxygenase; EFA, essential fatty acids; eLOX-3, epidermis-type lipoxygenase-3; EOS, esterified omega-hydroxyacyl-sphingosine; FA, fatty acid; FATP 4, fatty acid transport protein 4; GlcCer, Glucosylceramide; H(P)ETE, hydro(pero) xyeicosatetraenoic acid; H(P)ODE, hydro(pero)xy-octadecadienoic acid; HxA, HxB, hepoxilin A or B; l12-LOX, leukocyte-type 12-lipoxygenase; LT, leukotriene; LOX, lipoxygenase; LX, lipoxin; OS, omega-hydroxyacyl-sphingosine; 5-Oxo-ETE, 5-Oxo-eicosatetraenoic acid; p12-LOX, platelet-type 12-lipoxygenase; PUFA, polyunsaturated fatty acids; TEWL, transepidermal water loss; TPA, 12-O-tetradecanoylphorbol-13-acetate; VLC-FA, very long chain fatty acid

☆ This article is part of a Special Issue entitled The Important Role of Lipids in the Epidermis and their Role in the Formation and Maintenance of the Cutaneous Barrier. Guest Editors: Kenneth R. Feingold and Peter Elias.

* Corresponding author. Tel.: +49 6221 42 4506; fax: +49 6221 42 4932.
E-mail address: p.krieg@dkfz.de (P. Krieg).

1388-1981/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbalip.2013.08.005

LOX proteins constitute single polypeptide chains (molecular mass of mammalian LOX: 75–81 kDa, 662–711 amino acids) containing highly conserved domains and sequence motifs which are important for the distinct structure and the binding of the catalytic iron. The tertiary structure which is the same in plant and mammalian LOX reveals two domains, an N-terminal β-barrel and a C-terminal part containing the catalytically active non-heme iron and the substrate binding cavity. The volume of the substrate binding cavity and the orientation of the substrate determine the positional specificity of the distinct isoforms.

Six different functional LOX isoforms have been identified in humans, and seven in mice. Historically mammalian LOX are classified based on the positional specificity of arachidonic acid oxygenation (e.g., 5-, 8-, 12- and 15-LOX) and after the prototypical tissue of their occurrence [i.e. platelet-type 12-LOX (p12-LOX), leukocyte-type 12-LOX (l12-LOX) and epidermis-type 12-LOX (e12-LOX). Based on the phylogenetic relatedness mammalian LOX are divided into four subfamilies: 5-LOX, 12-LOX, 12/15-LOX (reticulocyte-type 15-LOX-1 and leukocyte-type 12-LOX, both exhibiting a dual positional specificity) and epidermis-type LOX (12R-LOX, 15-LOX-2, 8-LOX, epidermis-type LOX-3 (eLOX-3)).

3. Lipoxygenases in human and mouse skin: cloning, expression, activity and function

All LOX are expressed in skin of both humans and mice as detected by activity, immunohistochemistry and/or polymerase chain reaction (PCR) of the corresponding mRNA.

As key enzymes in the production of a variety of signaling lipid metabolites individual LOX isozymes are thought to play a role in the modulation of epithelial proliferation and/or differentiation as well as in inflammation, wound healing, inflammatory skin diseases and cancer. Emerging evidence furthermore indicates a structural function of LOX metabolism in the development and maintenance of skin permeability barrier (Table 1).

3.1. 5-LOX

5-LOX catalyzes the rate-limiting step of leukotriene (LT) and lipoxin (LX) synthesis which requires the activity of various other enzymes including 5-lipoxygenase-activating protein, LTA_4 hydrolase, and LTC_4 synthase. 5-LOX has been purified from leukocytes of humans and other species and 5-LOX cDNAs have been cloned from human, rat, mouse and hamster (for recent review see [6]). Recombinant 5-LOX converts arachidonic acid to the unstable intermediate products 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and LTA_4. 5-HPETE can be reduced to 5-HETE and further metabolized to 5-oxo-eicosatetraenoic acid (5-oxo-ETE) while LTA_4 serves either as an intracellular intermediate in the synthesis of the leukotrienes LTB_4 and LTC_4, or may be released extracellularly and subsequently taken up by adjacent cells devoid of 5-LOX activity but expressing LTA_4-hydrolase and/or LTC_4 synthase yielding LTB_4 and LTC_4 [8]. As a substrate for 12-LOX LTA_4 can be converted to the trihydroxy products lipoxin A_4 and B_4. The 5-LOX products exert a wide spectrum of biological activities. Leukotrienes are proinflammatory mediators while lipoxins are involved in resolution of acute inflammation.

The 5-LOX pathway is largely restricted to cells of myeloid origin including Langerhans cells of the skin [6]. In human skin, Langerhans cells have been identified indeed as the sole source of 5-LOX mRNA and protein within this tissue [9]. While 5-LOX metabolism may not play a prominent role in normal epidermal homeostasis as indicated by the lack of an obvious skin phenotype of 5-LOX-deficient mice [10], leukotrienes have been ascribed an important role in inflammatory skin diseases like psoriasis and atopic dermatitis [11,12]. Leukotriene formation in epidermis apparently occurs through transcellular leukotriene synthesis [13]. In fact, human keratinocytes and human epidermis have been shown to transform neutrophil-derived LTA_4 into LTB_4 by means of LTA_4 hydrolyse which has been identified in keratinocytes [14,15]. Transcellular leukotriene synthesis may therefore be an important mechanism by which human epidermis can contribute to LTB_4 formation in inflammatory skin diseases. In addition, LTB_4 has been shown to stimulate keratinocyte proliferation most probably mediated via specific LTB_4 receptors in these cells. Thus, LTB_4 may also be at least in part responsible for epidermal hyperproliferation in inflammatory skin diseases [16].

Treatment of mouse skin in vivo with the irritant and hyperplasiogenic tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) increased the epidermal content of LTC_4, LTD_4 and LTE_4 but not of LTB_4. Suppression of leukotriene synthesis by the 5-LOX inhibitor MK-886 prevented TPA-induced edema but did not show a significant reduction of epidermal hyperproliferation and tumor development induced by chronic TPA administration [17]. A threefold increased dose of MK-886 used in an independent experiment was reported to inhibit tumor formation in this skin carcinogenesis model [18]. Data from other animal models and clinical observations also indicate a role of 5-LOX pathway in carcinogenesis. Expression of 5-LOX and enzymes of the 5-LOX pathway was found to be upregulated with a corresponding increase in product formation in oral carcinogenesis in hamsters and humans and in tumors of colon, esophagus, lung, breast, prostate and pancreas. Furthermore, inhibition of 5-LOX pathway delayed the onset and development of tumors in various animal models (for review see [19]).

3.2. 12/15-LOX

The LOX isoforms now referred to as 12/15-LOX include the 15-LOX first discovered in rabbit reticulocytes and in human airway epithelial cells and eosinophils (with regard to the second 15-LOX isoform in humans this enzyme is also referred to as human 15-LOX-1) as well as the leukocyte-type 12-LOX (l12-LOX) identified in porcine, murine and bovine leukocytes. The human 15-LOX-1 cDNA was cloned from both a reticulocyte and a bronchus cDNA library [20,21] and the mouse l12-LOX cDNA from murine macrophages, spleen and from skin papillomas [22–24]. 12/15-LOX are characterized by a dual positional specificity of oxygenation, i.e. they simultaneously convert arachidonic acid to both 12- and 15-HPETE. Depending on the species there is a polarity towards one regioisomer. While the human and rabbit enzymes convert arachidonic acid predominantly to 15-HPETE, the other orthologs mainly produce 12-HPETE. The 12/15-LOX also efficiently metabolize linoleic acid to 13-HPODE. Unlike most other mammalian LOX, 12/15-LOX oxygenate esterified PUFAs such as arachidonyl-phosphatidylcholine and -ethanolamine as well as cholesterol esters (for review see [25]).

The human12/15-LOX (15-LOX-1) is predominantly expressed in reticulocytes, eosinophils, in colon, and respiratory epithelium. In skin, increased levels of 15-HETE were first found in psoriatic scales [26]. Thereafter 15-LOX activity was identified in rat epidermis [2], in cultivated human epidermal cells [27] and in psoriatic scales [28]. Moreover, 15-LOX-1 was found to be expressed in cultured human epidermal and oral keratinocytes [29] and exposure of human keratinocytes to UV irradiation was found to induce the expression of 15-LOX-1 mRNA [30]. At the time, however, the epidermal 15-LOX-2 (see below) was not detected. Thus, 15-HETE production could not exclusively be assigned to the activity of 15-LOX-1.

In mice, the highest expression of 12/15-LOX (l12-LOX) is found in peritoneal macrophages and lower expression in adipose tissue. In normal mouse skin 12/15-LOX was the only isoform that was not found to be expressed [31]. On the other hand, epicutaneous application of 12-O-tetradecanoylphorbol-13-acetate (TPA) led to a transient induction of l12-LOX. It cannot be excluded, however, that 12/15-LOX originates from invading inflammatory cells. A characteristic feature of 12/15-LOX activity in skin is the abundant production of 13-HODE upon oxygenation of linoleic acid. In mouse keratinocytes 13-HODE has been shown to counteract the effects of 12-HETE on epidermal