1. INTRODUCTION

1.1. THE LIPOXYGENASE FAMILY

The first report on lipoxygenases (LOXs) was published in 1976 (1). Since then, our knowledge about LOXs has been well established. The LOXs are widely distributed in nature. They are found in many plants and animals. In contrast, they are absent in bacteria and yeasts. The substrates for LOXs are poly-unsaturated fatty acids (PUFA), which are essential in mammals. Bacteria and yeasts lack the desaturase enzyme. Thus, they cannot convert saturated fatty acids to unsaturated ones (2, 3). The nomenclature of LOXs is defined by the location of the oxidation reaction that they catalyze. For example, 12-LOX oxidizes the 12^{th} fatty acid carbon (C12). In some LOXs, it is also necessary to mention the stereochemistry {e.g. 12(R)-LOX, 12(S)-LOX} or the tissue, where they are found (e.g. platelet, leukocyte, epidermal 12-LOX) (2, 3).

All LOXs contain a non-heme iron ion. The role of iron is to act as electron acceptor and donor during hydrogen abstraction and peroxide formation. Comparisons of the amino-acid sequences of various LOXs have shown several similarities. Six conserved histidine residues are present in all LOXs. The carboxyl termini are similar, too. Two of the conserved residues and the carboxyl-terminus make up a pocket where the iron is anchored. The enzymatic specificity of each LOX depends on the size of the pocket binding the fatty acid substrate (4, 5).

1.2. METABOLISM OF ARACHIDONIC ACID

Free fatty acids (FFA) such as arachidonic acid (AA) and linoleic acid (LA) can be substrates for 5-LOX. AA is the major substrate for lipoxygenase catalyzation. We term all the products of LOX metabolism “eicosanoids” (eicosi: twenty means in *Greek*) according to the number of carbon backbone of the product.

AA and LA are incorporated in the phospholipids of the cellular membranes. Phospholipase A_2 (PLA_2) catalyzes the hydrolysis of phospholipids and liberates AA. There are 4 types of PLA_2: a secretory, a cytosolic, a Ca^{2+} independent PLA_2 and the platelet activating factor acetyl-hydrolase, PAF-AH (6). Among them, cytosolic PLA_2 ($cPLA_2$) shows significant selectivity for AA. Cytosolic PLA_2 is expressed in most cells including keratinocytes and fibroblasts. Calcium is required for binding the enzyme to its substrate in cellular phospholipids. After various stimulation (interferons, lipopolysaccharides, cytokines, growth factors, Ca^{2+} ionophore A23187), $cPLA_2$ is translocated from the cytosol to intracellular membrane compartments, such as the Golgi apparatus, endoplasmic reticulum and nuclear envelope. Translocation to nuclear

envelope is very important for the metabolism of AA, because the eicosanoid synthesis-related enzymes are located there, too (6, 7). The activation of $cPLA_2$ is regulated by phosphorylation (PKC, MAP kinase)(6, 8).

Free AA can also be generated by phospholipase C (PLC). PLC catalyzes the phospholipid phosphatidylinositol-biphosphate (PIP_2) to diacylglycerol (DAG) and inositol-triphosphate (IP_3). Both of them are intracellular mediators. DAG-lipase converts DAG to free AA.

AA can be metabolized in three different ways: by cytochrom P450 AA mono-oxygenase, cyclo-oxygenase (COX) and lipoxygenase (LOX).

1.3. P450 AA MONO-OXYGENASE

Microsomal P450s are a family of membrane bound hemoproteins, which are located in the endoplasmic reticulum of the most eucaryotic cells. These enzymes participate in the oxidative metabolism of various lipophilic molecules, xenobiotics, cholesterol, steroids, vitamins and fatty acids. P450 AA mono-oxygenase has been found in the skin, liver, kidney, heart and pituitary of humans. The catalytic properties of P450 involve the NADH-dependent cleavage of molecular oxygen, the insertion of one atom of oxygen into the substrate and the release of the remaining oxygen atom as water. AA can be oxygenated by P450 in four ways:

a) hydroxylation of carbon at the ω-side of the carbon backbone (16-, 17-, 18-, 19-, 20-HETEs),
b) hydroxylation of carbons located between double bonds (7-, 10-, 13-HETEs),
c) hydroxylation with double bond migration (5-, 8-, 9-, 11-, 12R-, 15R-HETEs),
d) epoxidation of double bonds, produced epoxy-eicosatrienoic acids (EET): 5,6-EET; 8,9-EET; 11,12-EET and 14,15-EET. The latter products play an important role in the membrane homeostasis, because they can be esterified and thus, incorporated into phospholipids.

The type of products generated from AA depends on the tissue origin, animal species, sex, age, hormonal status, diet and exposure to xenobiotics (9, 10).

1.4. CYCLO-OXYGENASE

Cyclo-oxygenase (COX) or prostaglandin endoperoxide synthase catalyzes the initial steps of prostaglandin and thromboxane anabolism. Although COX prefers AA as substrate, many other polyunsaturated fatty acids may be used. The enzyme catalyzes the double oxygenation of AA producing the hydroperoxy endoperoxide prostaglandin G2 (PGG_2). PGG_2 is further converted by COX to hydroxy endoperoxide prostaglandin H_2 (PGH_2). COX is a heme-containing protein.

Two isozymes are described, COX-1 and COX-2. Both amino acid sequences are 60% identical in humans. They are expressed in many tissues such as prostate, brain, colon, stomach, skin, synovial fluid. The properties of the isozymes differ. Even though COX-1 is widely distributed, COX-2 is absent or expressed at low levels in most tissues. COX-1 vs COX-2 is located in chromosome 9 vs. 1, contains 11 vs. 10 exons and 576 vs 604 amino acids. They are both found in endoplasmic reticulum and nuclear envelope, but COX-2 is more concentrated in the envelope. Moreover, there is a functional interaction between $cPLA_2$ and the enzyme. COX-1 gene acts as housekeeping gene. In contrast, COX-2 is induced by growth factors, hormones, cytokines, lipopolysaccharides, tumor promoting agents, UVB and mechanical stress. COX-1 cannot utilize endogenous AA. Low concentrations of AA can be catalyzed exclusively by COX-2. Because the AA is liberated by phospholipase in small amounts, it is metabolized by COX-2. On the other hand, COX-1 catalyzes preferably high concentrations (>10 μM) of AA (11, 12, 13).

1.5. 5-LIPOXYGENASE

1.5.1. Purification and amino sequence

5-LOX has been purified from leukocytes and has a molecular weight between 72 and 80 kDa. Mammalian 5-LOX cDNAs have been cloned from human, rat, mouse and hamster. It is mostly expressed in neutrophils, eosinophils, macrophages, lymphocytes, mast cells and related tissue culture lines. There are also some reports of 5-LOX presence in placenta and brain. The human protein contains 673 amino acids, 14 exons and 13 introns. A major transcription initiation site is found 65 bases upstream of the ATG initiation code. The promoter contains several GC boxes, but lacks TATA and CCAAT sequences. The human 5-LOX gene is located in chromosome 10 (10q11). The six conserved histidines of 5-LOX are His-372, -550, -367, -362, -390 and –399. The first two together with the carbon terminal Ile-673 are permanent ligands for 5-LOX. When such a ligand is removed, iron is lost and the enzymatic activity, too. The His-367 is a replaceable ligand, which means that iron is present, but not the oxygenase activity. His-367 is replaced by AA during the catalyzation. The residues His-362, -390, -399 are non-iron ligands and they are important for the maintenance of the stability of 5-LOX (3, 4, 5).

1.5.2. Enzyme location

In resting cells, 5-LOX is located in the cytoplasm or in the nucleus. Hence in some cells such as neutrophils, eosinophils, monocytes and peritoneal macrophages, the enzyme is present in the cytoplasm. On the other hand, in mast cells, alveolar macrophages and rat basophilic leukemia cells, 5-LOX is located in both pools. 5-LOX is bound to cellular membranes after cell stimulation. 5-LOX does not translocate to plasma membranes, but only to the nuclear envelope (5, 7, 14, 15). Moreover, this subcellular distribution of 5-LOX is not necessarily fixed. For example, differentiation of monocytes to alveolar macrophages is associated with nuclear localization of the enzyme. In contrast, removal of macrophages from the alveolar environment results in a new localization of 5-LOX in the cytosol (16, 17, 18).

Within the 5-LOX protein is a sequence (Arg 638 - Lys 655) that resembles a bipartite nuclear localization signal (NLS). NLSs are sequences that are necessary for nuclear import of their respective proteins. Molecules larger in size than 50 kDa require NLS for their transport from cytoplasm into nucleoplasm through the nuclear pore complex. Substitution of residues including NLS-sequences completely blocks the nuclear import of 5-LOX (18, 19, 20).

5-LOX may bind with cytoskeletal proteins such as α-actinin, actin and myosin. Because cytoskeletal proteins are responsible for cell shape and mobility, an association between 5-LOX mobility and these proteins is possible (21).

1.5.3. Catalytic reactions

5-LOX catalyzes free AA in 2 steps. The first step is the oxidation of AA to the hydroperoxide intermediate 5(S)-hydroxy-6-trans-8,11,14-cis-eicosatetraenoic acid (5-HPETE). The second step is the dehydration of 5-HPETE to form leukotriene A_4. Thus, the enzyme has 2 activities, acting as oxygenase as well as dehydrogenase (leukotriene A_4 synthase). The first reaction of 5-LOX is the abstraction of hydrogen from the C-7 carbon of AA. At the same time the ferric (Fe^{3+}) non-heme iron is reduced to ferrous (Fe^{2+}). Molecular oxygen is added to the C-5 carbon and 5-HPETE is formed. This reaction is followed by the reoxidation of iron to ferric state. Then, 5-LOX is ready to catalyze the intermediate unstable product 5-HPETE. In the second step, a hydrogen is abstracted from the C-10 carbon and a hydroxyl group from the C-5 carbon of HPETE, generating water and the epoxide leukotriene A_4 (LTA_4). The reaction is followed by a new reduction of ferric iron to ferrous. The enzyme undergoes inactivation so long as the iron remains in ferrous state. 5-LOX can react with exogenous 5-HPETE, but endogenous hydroperoxide is preferred (3, 5).

Except AA, other eicosanoids can also be substrates for 5-LOX. Fatty acids with 5,8-cis-double bonds such as 5,8,11,14,17-eicosapentanoic acid, 5,8,11-eicosatrienoic and 5,8-eicosadienoic acid are good substrates. Moreover, 12- and 15-hydroxy-eicosatetraenoic acid (HETE) are also used as substrates, too (5). It is interesting that in human neutrophils, cytosolic 5-LOX cannot metabolize 12- or 15-HETE to the corresponding 5,12- and 5,15-diHETE, respectively. However, membrane-associated enzyme can (22).

Ca^{2+} stimulates both the oxygenase and the LTA_4 synthase activities of 5-LOX. Ca^{2+} promotes the membrane association of 5-LOX. In alveolar macrophages and rat basophilic leukemia cells, the addition of EGTA (Ca^{2+} chelator) causes 5-LOX to return from the nuclear envelope to its resting site. However, 5-LOX can act without additional Ca^{2+}. Stimulation of cells with Ca^{2+} ionophore A23187 (CaI) binds 5-LOX to membrane (5, 16, 23).

1.5.4. Phosphorylation

For many proteins, nuclear import is modulated by phosphorylation. ATP may be required for this process. 5-LOX can be phosphorylated at the NLS site and thus, can affect the 5-LOX transportation to nuclear envelope. Kinases can influence cellular 5-LOX activity. Protein kinase C upregulates the enzyme. Other kinases, which are implicated in 5-LOX pathway are tyrosine kinases and mitogen-activated protein kinase-associated protein kinases (MAPKAP-kinases). MAPKAP-kinases are phosphorylated and activated by p38 mitogen-activated protein kinase, which is activated by cell stress or treatment of cells with inflammatory cytokines (3, 5, 7, 18).

1.5.5. 5-Lipoxygenase inactivation

5-LOX is an unstable enzyme. It undergoes suicide inactivation. The product of 5-LOX catalysis, LTA_4, inactivates irreversibly 5-LOX. The recovery of the enzymatic activity after inactivation remains very low. LTA_4 inactivates 5-LOX without inhibiting its translocation to the envelope (24). Calcium ionophore-induced translocation of the enzyme to nuclear envelope and production of leukotrienes cause a loss of the enzymatic activity, but also loss of protein mass from the cytosol (25). This means that, Ca^{2+} chelators such as EDTA and BAPTA, prevent 5-LOX inactivation (25, 26). The role of HPETEs is bifunctional. Initially, they stimulate lipoxygenase catalysis and later block it. 12(S)-HETE and 15(S)-HETE inhibit 5-LOX activity. H_2O_2 inactivates lipoxygenase, too. Glutathione peroxidase-1, which is H_2O_2 scavenger, inhibits the product formation and thus, enzyme inactivation (5). In addition, nitrotyrosination of 5-LOX, induced by peroxinitrite ($ONOO^-$), reduces enzymatic activity. Peroxynitrite is generated by nitric oxides (NO) and reactive oxygen intermediates (ROS). It is an oxidant that acts as

cytotoxic agent. Nitrotyrosination of 5-LOX is blocked by the 5-LOX inhibitor, zileuton. This effect of zileuton is independent of its lipoxygenase inhibitory property (27). The suicide inactivation of 5-LOX can be prevented by zileuton. Significant enzymatic activity can be found in the membranes fraction of CaI-stimulated neutrophils, when inactivation of the enzyme is prevented by zileuton. In resting neutrophils, some enzymatic activity is observed in cytosol. After incubation with zileuton and CaI, most of the activity is removed to the membranes fraction. 5-LOX activity is lower when cells are stimulated only with CaI. The incubation with zileuton does not affect the translocation and the association of the enzyme with the nuclear membrane (22). Thus, membrane binding does not always lead to inactivation. Very long incubation time with CaI causes such inactivation. On the other hand, leukotriene formation in the cells can occur in the absence of membrane association when exogenous AA is provided (5).

1.6. 5-LIPOXYGENASE ACTIVATING PROTEIN

An additional protein, called 5-lipoxygenase activating protein (FLAP), is required for 5-LOX activity. Most of FLAP is located on the nuclear envelope with some quantities in endoplasmic reticulum. The protein is 18 kDa in size and the gene comprises 5 exons and 4 introns. FLAP functions as a substrate transfer protein. Thus, it presents AA to 5-LOX, without affecting the binding of 5-LOX to the nuclear envelope. FLAP may also be bound with 12- and 15-hydroxy-eicosatetraenoic acid (HETE). Moreover, FLAP stimulates both the conversion of AA to 5-HPETE and the LTA_4 synthase activity of 5-LOX (5, 16, 28).

1.7. FORMATION OF HYDROXY-EICOSATETRAENOIC ACIDS (HETEs)

The intermediate products of lipoxygenase catalyzation, HPETEs, are unstable. HPETEs, apart from LTA_4 formation, can be converted to their corresponding monohydroxy acids. HPETEs are reduced to HETEs, either spontaneously or by the action of peroxidases. HETEs are more stable products. Further dihydro acids (diHETEs) can be produced via double oxygenation. (29, 30, 31).

1.8. LEUKOTRIENES

LTA_4 can follow two different ways of catalyzation. LTA_4 hydrolase converts LTA_4 to leukotriene B_4 (LTB_4) and leukotriene C_4 synthetase to leukotrienes C_4, D_4 and E_4.

1.8.1. Peptido (cysteinyl)-leukotrienes

Leukotriene C_4 synthetase (glutathione S transferase) catalyzes the synthesis of leukotriene C_4 (LTC_4). Its genomic structure is similar to FLAP and PGE synthase (32). Leukotriene C_4 synthetase is found in the nuclear envelope. LTC_4 is transferred out of the cell. There, γ-glutamyl transpeptidase converts LTC_4 to leukotriene D_4 (LTD_4). Finally, leukotriene E_4 (LTE_4) is formed by LTE_4 dipeptidase. These LTs are often referred to as peptido-leukotrienes since all possess a cysteinyl residue at carbon 6. Peptido-LTs formation is induced by CaI (23, 33). The inflammatory potency of LTs is diverse. LTE_4 is less potent than the other two cysteinyl-leukotrienes. The dipeptidase action, which leads to LTE_4 generation, is rather an inactivation step (31). Cysteinyl-LTs are found in mast cells, basophils, eosinophils and dendritic cells (34).

1.9. LEUKOTRIENE A_4 HYDROLASE

Leukotriene A_4 hydrolase was purified from several mammals. It is found in blood cells, such as leukocytes, erythrocytes, neutrophils, in lung and liver (35). The protein molecule has a mass of 69 kDa. Human, mouse, rat and guinea pig cDNAs have been cloned. The human gene is localized in chromosome 12. The coding sequence is shared into 19 exons.

The enzyme has two activities, an epoxide hydrolase and an aminopeptidase. LTA_4 hydrolase was found to catalyze a varying numbers of amino acids. This activity is involved in the processing of peptides related to inflammation and host defense. The hydrolase activity is the main one (36). The molecule contains zinc, which plays role for both activities (37). Its presence is essential for catalysis. The metal is bound to three residues (His 295, His 299 and Glu 318). Furthermore, other amino acids (Glu-296, Tyr-383 and Glu-271) are associated with the reaction mechanism. During catalysis, Glu-271 stereospecifically opens the 5, 6 epoxide ring. The next step is the insertion of a water molecule at carbon 12. LTA_4 hydrolase is suicide-inactivated and modified by its substrate, LTA_4. This occurs during catalysis (38). LTA_4 hydrolase is located in the cytoplasm and potentially in the nucleus. Thus, LTB_4 can be formed in both cellular compartments (33).

1.10. 15-LIPOXYGENASE

As mentioned before, 15-lipoxygenase (15-LOX) catalyzes the stereoselective insertion of molecular oxygen at the carbon 15 of the AA. The enzyme converts AA to 15-HPETE (39, 40). This is unstable and 15-HETE is formed. 15-LOX has dual positional specificity. Oxygenation of AA produces not only 15-HETE, but also 12-HETE in a ratio 10:1 (40, 41). In eosinophils, 15-HPETE can be further metabolized to 14,15-epoxy-leukotriene (39, 42). 15-LOX is found in

plants, bacteria and animals. Two isoforms of the enzyme are discovered, the 15-LOX-1 and the 15-LOX-2.

1.10.1. 15-Lipoxygenase-1

The classical 15-LOX-1 was first described in rabbit reticulocytes (40). In humans, the enzyme is strongly expressed in blood cells, such as reticulocytes, eosinophils, alveolar macrophages, in colon, prostate, lung epithelial cells and skin. 5-LOX and 15-LOX-1 have 61% identical amino acids (43). 15-LOX-1 is very similar to leukocyte 12-LOX. They share the same enzymatic properties and mechanism of cytokine-dependent regulation of gene expression (44). Our knowledge about 15-LOX is relatively poor in comparison with 5-LOX. Its protein has a molecular mass of 75 kDa. As a typical lipoxygenase, it contains one non-heme iron. Three dimensional structure shows 2 domains. The amino-terminal domain seems to be responsible for the localization of the enzyme closed to the specific substrates, biomembranes and lipoproteins. The second domain, the carbon terminal, includes the iron, which is the catalytic area. The iron together with 5 ligands makes the catalytic pocket. Four histidine ligands belong to the amino-terminal domain and an isoleucine residue to the carbon-terminal (44). Arachidonic acid, as a substrate is placed with its methyl end at the bottom of the pocket and its acid end is bound tethered by a conserved basic residue on the surface of the enzyme. Modelling studies show that the binding site for AA is smaller in 15-LOX-1 than in 5-LOX. That makes the difference in the introduction of molecular oxygen at the AA carbon backbone (45). Mutagenesis studies in 15-LOX-1 reveal that amino acid 353 plays a significant role in the positional specifity of 12/15-LOX. Mutation in position 353 switchs 15-LOX-1 to 12-LOX. The mutant enzyme has 12-LOX properties and converts AA to 12-HETE instead of 15-HETE (44). Similar effects are shown with Ile-417 and Met-418 aminoacids. Moreover, Arg-402 and Phe-414 seem to be important for the enzymatic activity and the positioning of fatty acids in the correct catalytic area (41). 15-LOX-1 accepts many FFAs as substrates. Among them are arachidonic, linoleic and linolenic acid. It is interesting that 15-LOX-1 can catalyze a variety of ester lipids such as phospholipids, cholesterol esters, triglycerols, but also more complicated structures such as biomembranes and lipoproteins.

15-LOX-1 is a cytoplasmic enzyme. In contrast with 5-LOX, 15-LOX does not translocate to the nuclear envelope after activation. In rat macrophages, activation of the cells with calcium ionophore A23187 does not prove binding of 15-LOX to the nuclear envelope. Moreover, 15-LOX-1 lacks NLS domains (43). This is evidence of absence of this kind of tranlocation. Studies also in IL-4 treated monocytes demonstrate neither translocation of the enzyme to the envelope

nor any kind of docking protein (46). The ability of 15-LOX-1 to oxidize membranes is associated with the binding of the enzyme to membranes. In rabbit reticulocytes and in IL-4-treated human monocytes, the enzyme is localized in cytosol and in cytoplasmic side of intracellular membranes, such as mitochondria. The binding to membranes demands calcium. In the presence of Ca^{2+}, the majority of 15-LOX-1 was found on cellular membranes. The absence of exogenous Ca^{2+} or the addition of Ca^{2+} chelators causes mainly cytoplasmic localization of the enzyme. The membrane binding of the enzyme is reversible. It depends on the Ca^{2+} concentration. In concentrations close to the normal intracellular one, the main pool of 15-LOX-1 is in cytosol. The translocation to membranes means enhanced activity of the enzyme. The addition of Ca^{2+} ionophore A23187 increases the production of HETEs. Thus, Ca^{2+} may act as a regulator of 15-LOX-1 activity (46). Normally, the expression of 15-LOX-1 in resting cells is low. Artificial anemia causes the reticulocytes to express high amounts of 15-LOX protein (44). Interleukines (IL) 4 and 13 upregulate the 15-LOX-1 expression, too (47, 48, 49). In dendritic cells, IL-4 switches 5-LOX expression to 15-LOX. Differentiating dendritic cells express predominantly 5-LOX. After IL-4 treatment, 5-LOX is eliminated from its nuclear pool and 15-LOX is retained in cytosol. When these cells are treated with AA and Ca^{+2+} ionophore, they produce mainly 5-HETE. In the presence of IL-4, 5-HETE is reduced and 15-HETE is significantly increased (47). In addition, IL-4 is able to modulate the incorporation of 15-HETE into cellular lipids. In lung epithelial cells, IL-4 induces the esterification of 15-HETE into inositol containing phospholipids. This incorporation leads to a reduction of LTB_4 produced by neutrophils. Thus, a potent anti-inflammatory effect could be the resulting function (48).

15-LOX undergoes suicide inactivation, like other LOXs. 15-HETE inactivates the enzyme. The inactivation of lipoxygenase by 15-HETE is associated with covalent modification of the peptides in the active site of 15-LOX (42, 50). However, small amounts of 15-HETE can initially activate the enzyme. The activation of the lipoxygenase requires the oxidation of ferrous iron to ferric status (51).

1.10.2. 15-Lipoxygenase-2

Recently, a new isoform of 15-LOX has been discovered. It is found in human lung, prostate, cornea and hair root tissues. The second type is localized in cytosol of resting cells like 15-LOX-1. After stimulation with Ca^{2+} or Ca^{2+} ionophore A23187, the enzyme is bound to membranes. The novel enzyme is 40% identical to the classical 15-LOX. The molecule weighs of 76 kDa. Some differences are known between the two isotypes. The maximal membrane association of 15-LOX-2 occurs 20 minutes after Ca^{2+} stimulation, whereas 15-LOX-1 occurs within 5 minutes.

15-LOX-1 also exhibits 12-LOX catalytic properties and can form small amounts of 12-HETE. No 12-HETE products are found in 15-LOX-2 catalysis. 15-LOX-1 accepts AA and LA as favorite substrates. 15-LOX-2 can metabolize LA, but prefers AA. 15-LOX-1 is widely distributed in tissues. On the other hand, the presence of the second isoform is restricted to few tissues (52, 53).

1.11. 12-LIPOXYGENASE

12-Lipoxygenase (12-LOX) inserts a molecular oxygen at the carbon 12 of AA. Thus, a 12-hydroperoxy acid (12-HPETE) is produced, which has a S- or R-configuration. The stereoselective HPETE derivatives are converted to the corresponding HETEs. Two classical isozymes are present in mammals: the platelet and the leucocyte 12(S)-LOX. Recently, 3 novel isoforms have been discovered: the epidermal 12(S)-LOX, the epidermal LOX-3 (only in mice) and a 12(R)-LOX. The nomenclature for the isoforms is taken from the source where the enzymes were first discovered.

1.11.1. Platelet 12(S)-lipoxygenase

The platelet 12-LOX is found in humans, mouse, rat, rabbit and cattle. In humans, the enzyme is isolated from platelets and the epidermis. The expressed protein of platelet and leukocyte types has a molecular weight of 75 kDa. The murine platelet isoform is 58% identical to the leucocyte one and both of them are only 40% identical to 5-LOX. Their gene contains 14 exons and 13 introns and it is located on human chromosome 17. The lipoxygenase is composed of an amino-terminal β-barrel domain and a carboxy-terminal domain, containing the active site. The non-heme iron is located between 3 histidine residues and the carboxy-terminal isoleucine. The histidines at 361, 366 and 541 are essential for the catalysis. Platelet 12-LOX is located in cytoplasm, but also in microsomal fractions of keratinocytes. The latter is detected during psoriatic inflammation. Thrombin stimulation activates the enzyme and causes translocation from the cytosol to membranes. The enzyme accepts as substrate AA. LA has a low specificity for this isozyme. Platelet 12-LOX is active over 30 minutes upon initial activation. HETEs inactivate the enzyme. Albumin regulates the availability of substrates for 12-LOX and inhibits the metabolism of 12-HETE (54, 55).

1.11.2. Leukocyte 12(S)-lipoxygenase

The leucocyte 12(S)-LOX is present in mouse, rat, rabbit, pig and calf. It has a very wide distribution in tissues, including blood cells, lung, heart, intestine, liver. This isoform, as

previously discussed, is highly identical with 15-LOX-1. Humans do not express this LOX, but the 15-LOX-1. However, in some cases, such as in rabbit reticulocytes, both enzymes are present. It has been demonstrated that the 12(S)-, 12(R)- and 15-LOXs form a unique gene cluster on human chromosome 17. The enzymatic properties of leucocyte 12(S)-LOX are identical with those of 15-LOX-1 (54, 55).

1.11.3. Epidermal 12(S)-lipoxygenase

The enzyme is expressed in murine skin. Humans seem to lack a functional gene for this isotype, but have a pseudogene on chromosome 17. The encoding protein has a mass of 81 kDa. AA can be metabolized to 12(S)-HETE and LA to 13(S)-HODE. This reaction is very poor (less than 7%) in comparison with platelet 12-LOX. The real substrates for this enzyme are the corresponding AA and LA methyl esters (56).

1.11.4. 12(R)-lipoxygenase

Although lipoxygenases with R-configuration properties are widely distributed in lower species, their presence in mammals is very limited. It is demonstrated that humans and mice express this "R"-LOX. It is found in healthy and psoriatic skin and in tonsils. 12(R)-HETE is the major eicosanoid in psoriatic lesions. Lung, testis, adrenal gland, ovary and prostate express also the enzyme at mRNA level. The only known substrate for 12(R)-LOX is AA methyl ester. The catalytic capacity of the enzyme, including that of the epidermal type, is depended on the pH and the Ca^{2+} concentration of the culture medium. In addition, dimethyl sulfoxide (DMSO) increases the enzymatic activity and the ability of the enzyme to catalyze more substrates (56, 57).

1.12. HEPOXILINS AND LIPOXINS

We will briefly discuss these 2 families of eicosanoids. The 12-LOX metabolite, 12(S)-HPETE can be reduced to 12(S)-HETE. Alternatively, 12-HPETE undergoes isomerization. The intramolecular rearrangement leads to the formation of hepoxilins. Two isoforms are discovered, 11,12-hepoxilin A_3 (8-hydroxy-11,12-epoxy-eicosatrienoic acid) and 11,12-hepoxilin B_3 (10-hydroxy-11,12-epoxy-eicosatrienoic acid). 15-HPETE is transformed to 14,15-hepoxilin A_3 (11-hydroxy-14,15-epoxy-eicosatrienoic acid) and 11,12-hepoxilin B_3 (13-hydroxy-14,15-epoxy-eicosatrienoic acid). The molecules are found in rat pancreatic islets of Langerhans, the pineal gland and brain (58, 59).

Lipoxins can be generated by human cells under infection, injury or inflammation stimuli. These lipid mediators have a trihydroxy-tetraene structure and are products of cellular

interaction. They can be formed in three different ways: a) 15-LOX produced 15-HPETE can be catalyzed by 5-LOX. The final product is lipoxin. The diverse catalytic steps occur in different cells; for instance, 15-LOX catalysis in monocytes and 5-LOX in neutrophils, b) conversely, the 5-LOX product LTA_4 can be converted by 15-LOX to lipoxins, c) COX-2, acetylated by aspirin, converts AA to 15(R)-HETE. 5-LOX oxidation of 15(R)-HETE forms lipoxin. Lipoxins, which are 2 isoforms (LXA_4 and LXB_4), are made by the blood cells (60).

1.13. BIOLOGICAL ROLE OF EICOSANOIDS

Eicosanoids are generated intracellularly, but they are not stored. They act locally and regulate autocrine and paracrine functions. Different cell types have a characteristic range of enzymes for eicosanoid synthesis and thus, different eicosanoid profile.

LTB_4 is a major product of leukocytes, which has the ability to activate a wide range of immune effector cells. Neutrophils and monocytes/macrophages are the main blood cells that form predominantly LTB_4. The formation of LTs seems to be limited in these cells in normal situations. Other blood cells such as lymphocytes, eosinophils and other tissues cells can produce LTB_4 under inflammatory conditions (32). Enhanced LTB_4 is found in many respiratory and skin diseases, cystic fibrosis, rheumatoid arthritis and inflammatory bowel diseases. Leukocyte is the main target of LTB_4. The molecule at nanomolar concentrations stimulates neutrophils chemotaxis. It increases neutrophil adherence to venule walls, as well lysosomal release and generation of superoxide radicals. In addition, it activates complement and induces IL-8 and LTB_4 production by neutrophils (61). It regulates the function of ion channels, too (32). Similar properties characterize HETEs, too. However, their potency is weaker than that of LTB_4 (29).

The major importance of 15-LOX is its capability to modify membranes and lipoproteins. The incorporation of 15-HETE into phospholipids leads to an alteration in membrane properties and function (46). 15-LOX is involved in blood cells differentiation and maturation. 15-LOX is strongly expressed in reticulocytes. In contrast to mature erythrocytes, reticulocytes contain mitochondria. The disappearance of mitochondria due to cell evolution is associated with 15-LOX activity. The damage of mitochondrial membrane could be explained by the activity of lipoxygenase, which causes peroxydation of phospholipids (40). 15-LOX inhibitors can delay this transformation. In addition, 15-HETE upregulates the proliferation of erythroleukemia cells.

15-LOX also regulates the differentiation of macrophages (44) and it is thus involved in the atherogenesis process. Macrophages from atherosclerotic lesions highly express this enzyme. 15-LOX has atherogenetic effects and is able to oxidize low density lipoproteins (LDL) in vitro. 15-LOX inhibitors prevent the formation of atheroma (44).

There is much evidence showing implication of 15-LOX in inflammation. 15-HETE is often increased during inflammation. 15-HETE inhibits: a) superoxide production in neutrophils induced by platelet activating factor, b) synthesis, and c) activity of LTB_4 (44). The incorporation of 15-HETE in cellular membrane may decrease the extracellular and intracellular concentration of 15-HETE and thus reduce the inflammatory response (48). In asthma, 15-HETE is overproduced. 15-HETE may modulate the mucus production and induce bronchocontraction (44).

15-LOX is also involved in carcinogenesis. 15-LOX is downregulated in colon cancer. 15-LOX oxidative products of LA suppress cell proliferation and induce apoptosis. In cancer of the oral cavity, the free levels of AA and 15-HETE are used as markers of the accompanying inflammatory process (44). 15-LOX is expressed in benign hyperplasia of prostate and 15-HETE is also present. In contrast, 15-LOX expression and 15-HETE formation are reduced in prostate carcinoma (62).

The physiological role of the 12-LOX family is not fully understood. Certainly, its enzymes are implicated in proliferative skin dermatoses. They are also expressed in parts of mammalian brain such as cerebrum, hypothalamus, hippocampus and ganglia. Moreover, it is involved in myeloproliferative disorders. Patients with deficient 12-LOX tend to have hemorrhagic episodes and bleeding complications. Some studies report the implication of the enzyme in hypertension, diabetes melittus, rheumatoid arthritis, malignant prostatic hypertrophy and uterine cervical cancer (54).

COX-1 is involved in platelet aggregation. The enzyme produces thromboxanes and prostaglandins, which play an important role in thrombosis. COX-1-derived prostaglandins in kidney act as vasodilators. In stomach, COX-1-produced PGs protect the mucosa from ulceration and ischemia. COX-2 is implicated in defective ovulation, fertilization, implantation and decidualisation. COX-2 is involved in inflammation such as in rheumatism. Pain and fever are associated with COX-2. Tumorigenesis and tumor growth in colon and skin are COX-2-dependent, too. COX-2 expression is also critical in Alzheimer's disease (11, 12, 13).

The hepoxilins enhance the release of insulin, facilitate the transport of Ca^{2+} in membranes and raise its cytosolic concentration. They modulate synaptic neurotransmission, potentiate vascular contraction, regulate cell volume and provoke skin vascular permeability (58, 59).

The lipoxins have anti-inflammatory properties. They play a role in the resolution of the inflammatory response; they promote repair and wound healing. They also act as immunomodulators. They inhibit IL-6 and IL-8 production of fibroblasts. Lipoxins inhibit leukocytes chemotaxis and leukocytes-epithelial cell interaction. They block superoxide

formation. Moreover, they induce vasodilatation. In skin, they regulate delayed hypersensitivity reactions and inhibit neutrophil infiltration and vascular permeability (60).

Peptido-LTs were previously described as slow reacting substances of anaphylaxis (SRS-A). They are considered to be mediators of allergy and immediate hypersensitivity. Generally, peptido-LTs affect mainly smooth muscles and other cells with contractile capacity (63). Cysteinyl LTs are very potent bronchoconstrictors and arteriolar vasoconstrictors. Their potency is 100 to 1000 times stronger than histamine. In addition, they can increase mucus secretion and stimulate eosinophils migration (34, 64). They induce microvascular permeability and leakage of plasma and decrease blood pressure (63).

The fact that leukotrienes are secreted, it is remarkable that they are produced in the nuclear envelope. This fact strongly indicates that they must be involved in important functions within the nucleus. Moreover, in resting human alveolar macrophages, 5-LOX is located in the euchromatin of the nucleus and may be associated with chromatin (65). This discovery suggests that the enzyme itself may interact with intranuclear proteins or genes. In fact, a variety of eicosanoids can be ligands for peroxisomal proliferator-activated receptors (PPARs). Peroxisomes are small cellular organelles. They play an important role in regulating cellular proliferation and differentiation as well as in the modulation of inflammation. In addition, peroxisomes have broad effects on the metabolism of lipids, hormones and xenobiotics. Despite these effects, peroxisomes affect cellular membranes, adipocyte formation and insulin sensitivity. Their effects on oxidative stress affect aging and tumorigenesis (66). Their receptors, the PPARs, are nuclear transcription factors, like steroids hormones, vitamin D and retinoids. PPARs bind to DNA by recognizing target sequences, termed peroxisome proliferator response elements (PPREs). Upon activation by a ligand, PPAR forms a heterodimer with retinoid X receptor (RXR). Then this complex interacts with a PPRE. There are 3 subtypes of human PPARs: PPARα, PPARβ/δ and PPARγ. The expression of each form is tissue specific: PPARα is the predominant form in liver, heart and kidney, whereas PPARγ is the most expressed in adipocytes and epithelial cells. PPARβ is highly expressed in almost all tissues and it might function as a “housekeeping” molecule (67). PPARα regulates the catabolism of fatty acids mobilized during energy demanding physiological situations such as fasting or stress. Its regulatory action is distributed over the entire oxidative pathway including uptake, activation and peroxisomal and mitochondrial β-oxidation of fatty acids and ketogenesis. It modulates the inflammatory response, too. PPARα is also involved in cell proliferation and apoptosis. PPARγ favors fatty acid storage by stimulating triglyceride accumulation in adipocytes. It is involved in the adipocyte differentiation process (68, 69, 70). In some carcinoma cell lines, PPARγ agonists

inhibit proliferation and induce differentiation (62). PPARγ activity abrogates the induction of macrophage inflammatory mediators (198). As mentioned before, various eicosanoids, but also fatty acids bind to PPARs. Arachidonic, linolenic and linoleic acids are all PPARs ligands. The synthetic AA analogue and LOX inhibitor 5,8,11,14-eicosatetraynoic acid (ETYA) is another PPAR ligand. Of the eicosanoids, 8(S)-HETE and 15-HETE can also serve as PPAR ligands (62). The prostaglandin J_2 metabolites 15d-$\Delta^{12,\ 14}$-PGJ_2 and LTB_4 are specific PPARγ and PPARα ligands respectively (68, 69, 70, 71). It is interesting that MK886, a FLAP inhibitor, inhibits PPARs. MK886 induces apoptosis and blocks PPARα-induced differentiation (72). Fatty acids such AA, LA, docosahexaenoic (DHA) and eicosapentanoic acid (EPA), which activate PPARs, may have anticancer effects (68).

2. AIM OF THE STUDY

Since all LOX isoforms are expressed in the skin, a wide spectrum of associated skin disorders have been investigated. The involvement of eicosanoids in dermatology has been concentrated to the fields of inflammatory diseases and skin cancers. Psoriasis and atopic dermatitis are major chronic skin diseases, in which LTs may be involved. Many studies investigated the presence of LOXs in keratinocytes, since psoriasis is characterized by keratinocyte hyperproliferation.

In contrast, the involvement of LOXs in acne, another chronic inflammatory skin disease, and consequently in the sebaceous gland has not been evaluated. In acne, neutrophil infiltration plays a significant role and LTB_4-mediated inflammation is believed to participate in this process. Few studies are available about 5-LOX or other LOXs expression in the pilosebaceous unit. In our study we tried to determine:

1) the expression of 5-LOX, 15-LOX and LTA_4 hydrolase in human SZ95 sebocytes in vitro, at protein and mRNA levels, as well as by immunocytochemistry,
2) the activity of 5-LOX, defined as LTB_4 formation and associated release of pro-inflammatory cytokines,
3) the effect of 5-LOX on sebaceous lipid synthesis,
4) the potential effects of 5-LOX specific inhibitors, in vitro. This was particularly interesting since a current in vivo study performed in our department detected an anti-acne effect of zileuton, an oral 5-LOX inhibitor.