we tried to inhibit their synthesis with various concentration of Caf, no decrease was noted in stimulated cells. Furthermore, a strange observation was made when AA/CaI treated cells were incubated with Zil. Zil ($5x10^{-5}$ M - 10^{-5} M) seemed to induce significantly polar lipid generation (Fig. 16c). Probably this effect was due to the purification state of Zil.

We conclude that AA and in less manner CaI stimulated non-polar lipids synthesis. By contrast, no effect was noted in polar lipids. 5-LOX inhibitors had an inhibitory effect at particular concentrations when they were administrated stimultaneously with AA/CaI. This occured only in non-polar lipids.

4.7. 5-LIPOXYGENASE IMMUNOSTAINING

The APAAP technique was applied in SZ95 sebocytes. This method was found to give good labelling in cytocentrifuged cells. The vivid red reaction color was easily identified even, when the staining reaction was relatively weak or only few positive cells were present in each sample. In our study, 5-LOX was present in cytocentrifuged preparation of sebocytes. The enzyme was present even in control and treated cells (Fig. 17). Positive stained SZ95 cells accounted for less than 10% of the total cell population. We could not find any differences in immunostaining between controls and sebocytes treated for 1 hour. Moreover, we did not observe any differences between cells treated with 100 μM AA, cells treated with 1 μM CaI and those treated with both molecules (100 μM AA and 1 μM CaI). Among all samples, 5-LOX staining was stronger in bigger well differentiated SZ95 cells than in smaller undifferentiated ones. In large sebocytes, cytosol possesses large part of the cell. The well staining of 5-LOX in sebocytes cytoplasm is evidence that the enzyme has mainly cytoplasmic allocation. This is in accord with our western blot analysis results, where 1-hour incubation with AA and/or CaI enhanced 5-LOX protein in cytosol. Probably a new translocation of the enzyme to the nucleus did not occur. However, translocation occured earlier within the first minutes of stimulation.

5. DISCUSSION

We have demonstrated the presence of LOXs in SZ95 sebocytes. The AA pathway in SZ95 sebocytes in vitro does exist and is active. The following findings support this statement:

a) 5-LOX, 15-LOX-1 and LTA_4 hydrolase are expressed in SZ95 sebocytes at protein and mRNA levels.
b) 5-LOX is translocated from the cytoplasm to the membranes, incl. the nucleal membrane, after stimulation.

c) Eicosanoids formation occurs, as we have demonstrated for LTB_4.
d) AA up-regulates LTB_4 production. In contrast, CaI does not, at least during the experimental time evaluated.
e) AA induces lipid synthesis in SZ95 sebocytes.
f) IL-1β, IL-6, IL-8 and TNF-α are released by SZ95 sebocytes. IL-6 and IL-8 release is stimulated by AA and CaI.
g) Caf and Zil have no effect on IL regulation, at least under the current experimental design.
h) Caf partially inhibits LTB_4 generation, while Zil fails to do so, at least under the current experimental design.

The discussion is divided into 5 sections. First, we discuss the problems that occurred during the procedure and some restrictions on the methods [1]. Then we briefly review basic dermatological knowledge in the field [2]. A report of eicosanoids presence in skin tissues as well the significance of our findings in sebocytes follows [3]. Furthermore, we describe typical skin disorders, where LOXs are implicated. we also analyze the magnitude of LOXs in acne with emphasis on their involvement in disease pathogenesis [4]. And finally, we update available 5-LOX inhibitors and their clinical potential in dermatology [5].

5.1. RESTRICTIONS AND TROUBLES

5.1.1. Treatment of cells

Free fatty acids (FFAs) in plasma and cells are mainly bound to membranes and proteins such as albumin and fatty acid binding proteins (FABPs) (84, 85, 86). FABPs are a family of soluble intracellular proteins. These proteins transport FFAs intracellularly, where fatty acids are utilized for oxidation. Albumin binds plasma FFAs and thus reduces their interaction with other extracellular components. FABPs reduce the utilization of FFAs for 15-LOX catalysis due to restricted availability of free substrates. When AA and LA are added exogenously to cells, 15-LOX activity is decreased in the presence of bovine serum albumin (BSA) or FABP. BSA binds FFA with greater affinity than FABPs. Furthermore, HETEs can also bind to FABPs (84). In order to minimize this effect, we have incubated SZ95 sebocytes using a high AA concentration. This concentration is not toxic, as we have shown. On the other hand, SZ95 sebocytes are sensitive in the absence of BSA for a long time and die (data not shown).

The concentration of free AA in resting cells is low. In resting platelets for instance, the concentration of esterified AA is estimated to be at 5 mM (87). A 1% estimated release of this AA reserve can give 50 μM of free substrate. In psoriatic skin lesions, AA level is high (100 μM) (87). In uninvolved skin, the concentration reaches 13 μM (87). In isolated islets of Langerhans cells, the concentration is about 15 μM (87). The amount of endogenous free AA in sebocytes is unknown. It seems that minimal concentrations of 1-10 μM are able to generate eicosanoids in cells. Generally, pH, ionic environment, cell concentration, albumin and FABPs affect AA availability (87).

Since CaI induces 5-LOX activity within the first minutes, treatment of cells with CaI lasts usually up to 15 minutes. According to other in vitro studies (15, 19, 22, 25, 26, 131, 199), the concentration of CaI can reach 5 μM. This was prohibitive in our studies, because we treated SZ95 sebocytes with CaI for a very long period. Due to our cytotoxicity assay, we decided to use 2 concentrations of CaI: 1 μM up to 1 hour and 0.1 μM for a longer incubation.

In our experiments, DMSO was used as solvent of AA, CaI and Caf. Since this molecule is cell toxic in high concentrations and induces cell differentiation in low concentrations (1%), we decided to use it in the maximal concentration of 0.1% (v/v).

Another problem was the availability of Zil. Unfortunately, Zil was not available as a pure substance for our experiment. Therefore, I have extracted the active substance from tablets available on the market. This mechanical method has many disadvantages. The Zil powder obtained also contained the vehicle, stabilizers, emulgators. Even though we have dissolved Zil in ethanol, we were not know its purity. Nor do we know the potential effects of the vehicle on SZ95 sebocytes. Moreover, we have simply assumed that Zil dissolved in ethanol remained stable and in a titrated concentration. Therefore we have our reservations about this part of the results.

5.1.2. Western blot

Many HETEs, such as 5-, 12- and 15-HETE, interact with cytosolic proteins and particularly with actin. In order to characterize these proteins, a new synthetic HETE has been tested. A biotinylated derivative, 15-HETE biotin hydrazide, has been used as a probe to detect any HETE-protein complex by western blot analysis. This compound is a 15-HETE analog that binds to cytoplasm. 15-HETE biotin hydrazide competes with other HETEs in binding to cytoplasmic proteins. When cytosolic fractions of rat basophilic leukemia cells were incubated with 15-HETE biotin hydrazide and increasing amounts of 15(S)-HETE, the band of actin was progressively decreased. 5(S)-, 15(S)-HETE and 13(S)-HODE displace 15-HETE biotin and bind to actin (88).

5-LOX also binds to α-actinin, actin and myosin (21). This association of 5-LOX with cytoskeletal structures could be a restriction for the use of β-actin as housekeeping protein in western blot analysis.

As we have discussed, the 5-LOX immunodetection in western blotting resulted in multiple unspecific bindings of the enzyme. Extra (multiple) bands could be appeared due to proteolytic degradation of the antigen. Too much loaded protein per lane may cause the same. Another reason for multiple bands, particularly in lipoxygenases, is the interaction of the enzyme with cytoskeletal proteins. In order to prove the specificity of the 79 kDa band, we did the following experiment. After blotting, PVDF membrane was blocked only with the secondary antibody. Then the membrane was incubated with ECL immunoblotting detection kit and the bands were visualized as described previously. This procedure resulted in the disappearance of the 79 kDa specific band. In contrast the unspecific bands remained visible (data not shown).

5.2. THE SKIN

The skin is the largest organ in the body regarding both weight and surface area. In adults, the weight of their skin accounts about 16% of the total body weight. It consists of 3 parts: the epidermis, the dermis and the subcutaneous tissue. The epidermis is a multilayer tissue. The lower layer, called basal cell layer, is made of epidermal stem cells. They divide giving birth to keratinocytes and further stem cells. The keratinocytes undergo terminal differentiation migrating to the upper layers. Above the basal cell layer is located the spinous cell layer (stratum spinosum). There, keratinocytes are spinoform. Next comes the granular cell layer (stratus granulosum). Keratinocytes are characterized by granules full of keratohyaline. Nucleus and other cell organelles are still present in granular keratinocytes. The cornified layer (stratum corneum) is the upper layer, which functions as a barrier between internal and external environment. The cells in stratum corneum, the corneocytes, do not contain any nuclei or other organelles. Corneocytes end by desquamating into the environment. The whole period for a cell to develop from basal to cornified layer is about 1 month. During the migration of keratinocytes from the basal layer to stratum corneum, the cellular lipid composition changes. Even though basal cells are rich in phospholipids and glycoshingolipids, cornified cells contain more ceramides, FFAs, free cholesterol and cholesterol esters. Another cell population of the skin is that of melanocytes. Melanocytes produce melanin, the substance, which is responsible for skin pigmentation. Melanin is delivered to basal keratinocytes. A third cell group is the Langerhans cells. They are restricted to the epidermis, belong to the antigen presenting cells and attract T-

lymphocytes to the skin. Because of their immunological properties, they play a significant role in infections and tumorigenesis (89, 90).

Appendages also belong to the skin. Nails and hairs are modified epidermal structures. In addition, apocrine, eccrine and sebaceous glands are present. The role of apocrine glands is not clear. The eccrine glands excrete sweat and control body temperature. The sebaceous glands will be discussed below. The connective tissue of the dermis consists of collagen fibers and elastic fibers constructed into the matrix. The matrix is made up of glycosaminoglycans and mucopolysaccharides. The main cell type of the dermis is the fibroblast. Fibroblasts produce various kinds of fibers and components of the matrix. Collagen represents the most abundant molecule of this tissue. Dermis is divided into 2 parts. The papillary dermis is the upper part consisting of a papilla-shaped structure. The papillary dermis and basal cell layer are bound together in a complex structure called epidermal-dermal junction. The rest of the dermis is the reticular dermis. There, the collagen fibers make thick bundles. Between these bundles are located fibroblasts and mast cells. The dermis has an extensive network of blood and lymph vessels, which feed the skin and remove waste products. Moreover, nerves are well developed and specific nerve end organs are present. Smooth muscles of the hairs (arrector pili) arise in the upper dermis, too. Finally, the subcutaneous tissue consists of fat. Fat acts as protective padding and energy deposit (89).

The skin separates the internal environment from the external. It maintains the form of the body and protects internal organs from mechanical or chemical damage. The epidermis is resistant to xenobiotics. It prevents water loss and reduction of electrolytes and other macromolecules. The skin barrier defends the organism from infectious agents such as bacteria, fungi and viruses. On the other hand, natural skin flora competes with pathogenic flora. Keratinocytes, Langerhans cells, melanocytes can synthesize a wide range of anti-inflammatory agents. Pigmented skin shields against ultraviolet (UV) light exposure and antioxidants. Sebaceous glands produce sebum, which lubricate the skin surface and the hair. The sebum has bacteriostatic and fungistatic properties. Salts contained in sweat are antimicrobial. Moreover, sweat carries off heat. Generally, skin regulates temperature with the cooling system of sweat glands and dermal vasodilatation and on the other hand it prevents heat loss together with the subcutaneous fat and hair coat. Furthermore, skin has various apparatuses that serve as sensors for touch, pressure, pain, and temperature (89, 91).

One of the most amazing properties of the skin is its ability to produce hormones and vitamins. The skin is not only a target of circulating hormones but can also synthesize and release hormones. Moreover it can act as a main endocrine organ. Human skin is also able to

metabolize hormones and to activate or inactivate them. Skin is capable of producing cholesterol and it contains all requisite enzymes to convert cholesterol to testosterone and the potent dihydro-testosterone. It can convert androgen to estrogens. Epidermal cells catabolize corticosteroids to less potent ones. Androgens control sexual hair growth and stimulate sebocytes proliferation and differentiation. On the other hand, estrogens prolong the anagen and postpone the catagen phase of hairs and suppress sebaceous gland function. Furthermore, a large part of testosterone in blood circulating testosterone, dihydro-testosterone and estrogen are produced by the skin, especially in postmenopausal women (92, 93, 97). Skin cells produce the corticotrophin-releasing hormone (CRH), and pro-opiomelanocortin derivatives (POMC) such as adrenocorticotropic hormone (ACTH), melanocyte-stimulating hormone (α- and β-MSH), β-endorphin and express their receptors. These hormones are important for cutaneous responses to stress. CRH induces lipid synthesis in sebocytes. CRH also enhances the production and secretion of POMC products. POMC peptides regulate skin pigmentation and exert immunosuppressive effects on skin (92, 94). Another anabolic hormone that is locally formed in the skin is the insulin-like growth factor (IGF-1). IGF-1 regulates sebocyte proliferation and differentiation. Moreover, IGF is involved in the hair growth cycle (92, 97).

Not only hormones, but vitamins are also present in skin. Keratinocytes can produce vitamin A derivative, all-trans retinoic acid. Retinoids inhibit sebaceous gland proliferation and lipogenesis (92). Vitamin D_3, a calcitriol precursor, is a unique product of the skin. Calcitriol exerts its anti-proliferative and anti-inflammatory properties in the skin (92). Vitamin E (α-tocopherol) is delivered to the skin surface via the sebum. Vitamin E has strong anti-oxidative action. In areas rich in sebaceous glands, high level of vitamin E protects against ultra-violet (UV) irradiation. UV-rays induce sebum lipids peroxidation and cause DNA damage, immunosuppression and carcinogenesis (95, 96).

5.3. THE SEBACEOUS GLAND AND THE SEBUM

We are describing the sebaceous gland separately, as it is particularly interesting for our study. Sebaceous glands are almost always associated with hair follicles. This complex is called pilosebaceous unit. The sebaceous duct connects the sebaceous gland with the follicular infudibulum. Sebaceous glands are present everywhere in the skin except in palms and soles. They vary significantly in number and size throughout the body. The highest gland density is on the face and scalp. These glands are well developed in the newborn but undergo atrophy during childhood. At puberty, the gland grows up under the stimulus of androgens. On the other hand, estrogens suppress sebaceous gland function. The gland consists of one or more lobules leading

into a common duct. Each sebaceous lobule possesses a peripheral layer of cuboital cells, which cells do not contain lipid droplets. In contrast, the centrally located cells are rich in fat vesicles. Finally, full of lipids, sebocytes form sebum as they burst and die.

The sebum, the secretion product of sebocytes is excreted into hair infundibulum. The sebum is a unique mixture of lipids. The lipid composition is generally species specific. In humans, the following classes of lipids are detected: glycerides (50%), wax esters (25%), squalene (10%), cholesterol and cholesterol esters (5%), FFAs (5%) and other lipids. Wax esters and squalene are synthesized only by sebocytes. Wax ester production is a marker of sebaceous cell function and differentiation. The ratio of wax esters to cholesterol and cholesterol esters is known to increase with increasing sebaceous gland activity. The most preferred fatty acids that are incorporated in wax esters are palmitic (16:0), stearic (18:0) and oleic acid (18:1). Generally, saturated fatty acids are incorporated in esters at higher rates than unsaturated ones. In mammalian cells other than sebocytes, all these fatty acids are uncommon, since they have very long carbon chain. In fact, human sebocytes contain elongases, enzymes, which synthesize very long chain fatty acids and desaturases, which add double bonds to saturated fatty acids. Linoleic acid (18:2) is also present in cytosol and membranes of sebocytes. Desaturase prefers to catalyze palmitic acid to sapienic acid (16:1) rather than utilize linoleic acid. Instead of desaturation of linoleic to γ-linolenic acid, linoleic acid undergoes β-oxidation by lipoxygenases. On the other hand, AA is present only incorporated in sebocyte phospholipids (98, 99).

5.4. SZ95 SEBOCYTES

Due to the fact that acne is a human disease and that the secretory activity of the sebaceous gland is species specific, we requested the use of a human skin model for performing our study. A major role of sebocytes in the skin is the production of sebum. When sebocytes undergo differentiation, they accumulate neutral fat droplets until they burst and die. Primary human sebocytes can only be cultured for few passages. After some passages, the proliferation rate was low and sebocytes died after final differentiation. To solve this problem, we used the immortalized human sebaceous gland cell line SZ95. Sebocytes isolated from human facial skin were transfected with a plasmid containing the Simian Virus-40 (SV-40)-Large-T-Antigen. The infected cells have been cloned and investigated over a period of 10 years. The cells did not loose their vitality for over 50 passages. SZ95 sebocytes are highly hyperdiploid (mean chromosome number 64.5) and aneuploid with chromosomal abnormalities. The morphology of SZ95 cells is like that of primary sebocytes. They vary in size. The cytoplasm is large with abundant organelles, well developed endoplasmic reticulum and Golgi apparatus. Vacuoles and

myelin figures are present indicating lipid synthesis. Many droplets containing components similar to sebum are also found. SZ95 sebocytes synthesize several fractions of neutral lipids such as squalene, wax esters, cholesterol and cholesterol esters, triglycerides and free fatty acids. SZ95 sebocytes express molecules typically associated with human sebocytes. Antigens for keratin-7, -13, -19, 5α-reductase-1 and proteins of the epithelial mucin family were present. Furthermore, retinoids (drugs against acne) significantly inhibit the proliferation of certain SZ95 sebocyte clones (100).

5.5. ESSENTIAL FATTY ACIDS

The most abundant fatty acid in human skin is linoleic acid (LA). Its main role is in the construction and maintenance of the epidermal water barrier. Ceramides of the barrier are rich in LA. On the other hand, AA is the second most frequent fatty acid in the skin. AA constitutes 10% of the total fatty acids of the epidermal phospholipids. AA in the skin is implicated in the genesis of eicosanoids (101). Depletion of essential fatty acids from the nutrition causes an essential fatty acid deficiency syndrome. This occurs as a scaly skin with associated skin barrier defect and increase of transdermal water loss (102). In other studies, SZ95 sebocytes treated with AA produce lipids marked with oil red staining. Control cells mostly exhibit negative staining. Furthermore, fragmented nuclei, which are characteristic of apoptotic cells, are noted in AA-treated sebocytes. Thus, finally differentiated sebocytes induced by AA undergo apoptosis and die (103). This is in accord with an observation that addition of exogenous AA or high cellular accumulation of AA in HL60 cells can prevent cell proliferation and initiate apoptosis (87). Whereas in our data, 100 μM AA induces the lipid synthesis, no toxicity is noted as a mean of LDH release. On the other hand, CaI is not implicated in sebum lipids formation.

5.6. 5-LIPOXYGENASE IN THE SKIN

Very few articles are available about the expression of 5-LOX in the skin. In vitro studies show that undifferentiated keratinocytes express very low 5-LOX if any, whether it is determined by protein, mRNA, leukotriene production or cell free enzyme activity. However, when normal human keratinocytes and HaCaT cells are cultured in conditions that promote more differentiation (FBS, 1.5 mM Ca^{+2}), 5-LOX expression is stronger. An up-regulation of 5-LOX is demonstrated at protein and mRNA levels. The enzyme activity is also elevated as a means of 5-HETE and LTB_4 production. The 5-LOX induction is less vigorous in normal human keratinocytes than in HaCaT cells. Moreover, when cells are treated with CaI, both LTB_4 and

LTC_4 are detected with HPLC. Then the addition of the FLAP inhibitor, MK886 blocks leukotrienes formation (104).

5-LOX is involved in lysophosphatidic acid (LPA) stimulated hydrogen peroxide release in HaCaT cells. LPA is a mitogen for keratinocytes that regulates production of reactive oxygen species (ROS). Zileuton or nordihydroguaiaretic acid (NDGA) pre-incubated cells generated much less H_2O_2 than cells without inhibitors (105).

In contrast, other authors report no presence of 5-LOX in keratinocytes (106, 107). In these studies, cultured keratinocytes stimulated with AA or phorbol myristyl acetate (PMA) failed to form LTB_4. Neither 5-LOX nor FLAP proteins, nor mRNA were detected.

In situ hybridization of 5-LOX in normal human skin shows a positive signal in basal and spinous layers, outer hair root sheath and sebaceous glands. The percentage of detected 5-LOX-positive epidermal cells is about 2%. Similar results were revealed with indirect immunofluorescence. By this method, Langerhans cells were detected as the dominant 5-LOX expressing population. 5-LOX in Langerhans cells was located in the nucleus and in the cytoplasm close to nuclear envelope. These cells expressed FLAP as well LTA_4 hydrolase protein (108).

5-LOX is present in SZ95 sebocytes. The enzyme is present at the mRNA level irrespective of whether or not cells are treated with AA/CaI. Western blotting shows that the protein is expressed and upregulated by AA and CaI at 30 minutes of incubation. The enzyme has the classic properties: in resting cells is located in cytosol and after stimulation is translocated to nucleus. The concentrations of CaI look more effective than that of AA in 5-LOX translocation. Although 5-LOX induction by AA and CaI after 1 hour of treatment is not significant, it indicates that the enzyme is up-regulated and probably capable of generating eicosanoids in the cytoplasm. 5-LOX in SZ95 sebocytes is possibly able to reproduce leukotrienes after a reasonable period of time. A second cycle of intracellular migration may occur later (12 hours). 5-LOX immunocytochemical staining showed that sebocytes are positively stained for the enzyme. However, only a small proportion of SZ95 sebocytes are stained. After 1 hour's stimulation with AA and/or CaI, the enzyme is located in the cytosol. The staining is more pronounced in well differentiated cells. In conclusion, we assume that 5-LOX is active in SZ95 sebocytes and this activation is significant in the first minutes. It is not certain whether the enzyme is constitutively expressed in sebocytes, since our control cells were treated with high concentrations of Ca^{2+}, a factor that activates 5-LOX.

Our results are in accord with similar experimental data from keratinocytes. The simultaneous treatment of keratinocytes with AA and CaI has a synergistic effect on LTB_4 production (109).

Optimal stimulation occurs within the first 10 minutes and occasionally lasts for up to 30 minutes. However, the cells are further able to generate LTB_4 after 18 hours of incubation (110).

5.7. LEUKOTRIENE A_4 HYDROLASE IN THE SKIN

LTA_4 hydrolase is detected in human cultured keratinocytes and in human epidermis. Immunohistochemical staining in epidermis detects this enzyme in the basal and spinous layers (111). Normal human epidermis shows low, but significant enzyme activity. In contrast, human whole skin or dermis does not exhibit significant activity. By immunohistochemistry, fibroblasts, neutrophils and dermal endothelial cells are positively stained (35). The enzyme has the classic properties. It is located in the cytoplasm and undergoes suicide inactivation. In normal skin, peptidase activity of the enzyme seems to be its main function. If no substrate is available, the hydrolase is inactive. Under various stimuli of the skin such as mechanical trauma, ultraviolet irradiation, histamine, calcium and tumor promoters, the hydrolase undergoes activation. Inflammatory conditions activate the hydrolase, too. Since production of LTB_4 is problematic in normal keratinocytes, an interaction between infiltrating neutrophils and keratinocytes may be required. Co-incubation of human neutrophils with keratinocytes results in a significant increase in LTB_4 in comparison with neutrophil monoculture. When neutrophils are incubated with supernatant taken from CaI-stimulated keratinocytes, they fail to increase LTB_4 formation. In this sense, LTA_4 is released by leukocytes into the extracellular space and utilized by keratinocytes (106). LTA_4 hydrolase activity is inhibited by bestatin and captopril in human epidermis (35).

Another skin cell type, in which the enzyme is active are the melanocytes. LTA_4 hydrolase protein is found in melanocytes in the basal layer of healthy skin as well in nevus pigmentosus lesions and malignant melanoma cells. Its location is the cytoplasm (112).

SZ95 sebocytes express LTA_4 protein. The protein is up-regulated after 1 hour of treatment with AA and CaI, however no statistical significance could be obtained. In other points in time, no great differences were observed. Thus, there is no evidence of AA- or CaI-induced regulation of LTA_4 protein levels. Moreover, translocation of the enzyme does not occur. This finding together with other studies indicates that LTA_4 hydrolase does not need translocation to become active. It can generate LTB_4 in cytosol. The enzyme is constitutionally expressed at the mRNA level. CaI and AA do not up-regulate the expression of the enzyme. The intensity of the signals shows that LTA_4 hydrolase is strongly expressed in sebocytes. Moreover LTA_4 hydrolase is widely distributed in various cells and organs.

The presence of LTA_4 hydrolase in sebocytes may play a particular role in the development of sebaceous gland diseases, since this enzyme converts precursor eicosanoids to LTB_4, a very potent inflammatory mediator. Since sebocytes express LTA_4 hydrolase, sebocyte LTB_4 formation in inflammatory lesions is independent of the activity of other cell populations. Therefore, sebocytes can attract neutrophils and macrophages without any further mediation.

5.8. 15-LIPOXYGENASE IN THE SKIN

15-LOX is expressed in human keratinocytes. It has been demonstrated that both isoforms of the enzyme are present in the skin. Moreover, the amount of 15-HETE produced by keratinocytes is higher than that of leukocytes. However, normal cultured keratinocytes seem to possess 15-LOX activity only when the cellular membrane is damaged or after treatment with exogenous AA (113). Human keratinocyte sonicates contain much higher quantities of 15-LOX than any other lipoxygenase. When exogenous AA is provided to keratinocytes, 15-HETE is produced. Keratinocyte 15-LOX does not generate detectable amounts of other mono HETEs (114). 15-LOX in keratinocytes can convert 5-HETE to 5,15 di-HETE. Furthermore, keratinocytes are able to metabolize 5,15 di-HETE to a lipoxin-like product. The reported biological effects of lipoxins are related with immune regulation (113). This interaction could be efficient for the synthesis of lipoxins by the keratinocytes. This means that eicosanoids derived from other cells could be utilized by keratinocytes in order to produce several lipid mediators. Suspensions of 10000 X g pellets convert less than 4% of AA to 15-HETE. The greater quantities of 15-HETE are found in 400000 X g supernatant (93%) and a small one in 400000 X g pellet (7%). This indicate us that the localization of 15-LOX in human keratinocytes is mainly cytoplasmic. The enzyme function requires Ca^{2+}. Minimal activity is observed when Ca^{2+} concentration is up to 0.5 mM. The activity reaches the maximum when the ion concentration is over 2 mM. According to 15-LOX-1 properties, the enzyme can also metabolize LA and yield the corresponding hydroxy-acid, 13-hydroxy octadecadienoic acid (13-HODE). LA is incorporated in epidermal sphingolipids. The addition of the non-specific LOX inhibitors, nordihydroguaiaretic acid (NDGA) and 5,8,11,14-eicosatetraynoic acid (ETYA), to AA-stimulated keratinocytes causes the reduction of 15-LOX activity (114, 115). When ETYA is applied on the skin or given orally to rats, the animals present an LA deficiency-like syndrome (102). The epidermal elevated concentration of 13-HODE, derived from dietary LA, down-regulates keratinocyte hyperproliferation. In vivo, 13-HODE has an anti-proliferative effect as demonstrated by the reduction of scaly lesions. In addition, 13-HODE acts as an anti-inflammatory molecule in vitro (101, 116).

The presence of 15-LOX-1 was also demonstrated at the mRNA level. Keratinocytes express mRNA, which is identical to the reticulocyte 15-LOX. Furthermore, keratinocytes express mRNA of platelet 12-LOX (117). In ex-vivo tissue experiments, incubation of freshly plucked human hair roots with AA leaded to generation of 15-HETE. When LA was also added, 13- and 9-HODE were detected. 15-HETE production was greater in anagen hair roots than in telogen but the difference was not significant (118). Another interesting discovery comes from the observation that different epidermal layers produce diverse eicosanoids. Skin biopsies were used and basal cells were separated from suprabasal cells. The two populations have been cultured in vitro and were treated with AA. The basal cells predominantly formed 15-HETE and the suprabasal 12-HETE. Depletion of spinous cells in an epidermal mixture caused undetectable amounts of 12-HETE. Likewise 15-HETE is not detected if basal cells are absent in the mixture. Therefore, differential production of eicosanoids depends on the status of keratinocyte differentiation (119).

Eventhough 15-LOX-2 presence is limited in tissues, it is detected in the skin. Immunohistochemical results show that the strongest expression of 15-LOX-2 is noted in sebaceous glands. 15-LOX-2 immunostaining in sebaceous glands is found in normal and hyperplastic tissues. The cytosol of differentiated sebocytes, which have abundant cytoplasm, is strongly stained. Tissues from sebaceous adenoma and sebaceous carcinoma are stained positively, but with a variable pattern. On the other hand, keratinocytes, undifferentiated basal cells of the gland and cells from the outer hair root sheath are generally not stained. The follicular epithelium is also negatively stained for 15-LOX. Eccrine and apocrine glands are also positively stained. Other skin adnexa do not exhibit 15-LOX expression. Tissue samples from dermis, erector pili smooth muscle, blood vessels and nevus are negative. This is in accord with in situ hybridization studies. 15-LOX-2 is expressed in sebaceous glands of neonatal or adult skin in situ. Tissues from foreskin, breast or abdominal area are positive. In contrast, hair and hair follicles, dermal papillae, follicular epidermis and dermis are negative for 15-LOX-2 hybridization (120).

Our results showed presence of 15-LOX-1 in SZ95 sebocytes. The classical isoform is expressed at protein level and mRNA. In western blot analysis, untreated cells express 15-LOX-1 protein. Although studies in keratinocytes report that intact cells do not express 15-LOX, we detected such expression in intact sebocytes. High concentration of Ca^{2+} in the culture medium (1.5 mM) may have played a role. In further intact cells, low 15-LOX-1 expression could be detected. When AA or CaI are added, the protein in the soluble pool is reduced. This evidence supports the assumption that the enzyme in resting sebocytes is cytoplasmic and after activation

translocates to membranes. The observation that the stimulus of CaI is stronger than that of AA is in accord with the results from 5-LOX blotting. The translocation of 15-LOX occurs during the first minutes of the addition of AA and/or CaI (30 min). In latter points in time, the addition of AA and / or CaI does not play a significant role in enzyme kinetics. Studies have shown that the enzyme is active within the first minutes and later undergoes suicide inactivation by its product. Another important finding is that 15-LOX-1 in SZ95 sebocytes is expressed in a less vigorous manner than 5-LOX. Densitometry analysis shows a weaker band for reticulocyte 15-LOX than for 5-LOX. Moreover, much protein of 15-LOX-1 (50 μg per well) was loaded to electrophoresis gel than this of 5-LOX (30 μg per well).

The expression of 15-LOX-1 in SZ95 sebocytes at the mRNA level was also demonstrated. No transcriptional differences are found here between treated and untreated sebocytes. According to the western blot analysis, we have to expect that the expression of 15-LOX should also be up-regulated after treatment with AA and/or CaI. It is possible that other transcriptional factors or genes regulate the expression of the enzyme. Although the weak 15-LOX-1 expression might not allow a quintitative evaluation of the AA/CaI effects.

As mentioned previously differences are detected in the size of the active site of lipoxygenases. The cause of the modification of the binding site for AA in the same lipoxygenase molecule is unknown. Moreover, in psoriatic skin, a stimulation of skin samples with CaI A23187 and AA increases the amount of leukotriene B_4 4-fold. The 12-LOX product, 12-HETE, and the 15-LOX product, 15-HETE, are both increased 2.7-fold (121). Thus, CaI and AA stimulate the activity of 12- and 15-LOX, too, but in a less vigorous manner than in the case of 5-LOX. The profile of eicosanoid products generated by sebocytes may depend on how 5-LOX and 15-LOX are differentially regulated to use AA. The balance between 5-LOX and 15-LOX in sebocytes can be seen under the prism of inflammation. 5-LOX exerts pro-inflammatory activities, whereas 15-LOX has mainly anti-inflammatory effects. The inhibition of one LOX pathway can result in an increased activity of another LOX pathway. It will be interesting in future studies to find out what kind of eicosanoids apart from LTB_4 are produced by sebocytes. It would be also useful to study eicosanoid diversity after switching one LOX activity to another. Moreover, the balance between 15-LOX-1 and 15-LOX-2 in SZ95 sebocytes has to be investigated. Perhaps the second isoform is the dominant enzyme in sebaceous glands and responsible for HETEs generation.

5.9. LEUKOTRIENE B_4 AND THE SKIN

LTB_4 is the most potent leukocytes chemotactic agent. Other such agents, although lower in potency, include: 5-HETE, 20-OH-LTB_4, 12-HETE, 15-HETE and 20-COOH-LTB_4 in decreasing order (109).

LTB_4 is detected in many skin disorders such as bullous pemphigoid, pressure and pigmented urticaria, psoriasis and atopic dermatitis (109). Intradermal injection of LTB_4 leads to transient erythema and wheal formation during the first 10 minutes. The wheal remains for 2 hours and an indurated papule appears more than 6 hours after exposure. Synchronized combination of LTB_4 and PGD_2 causes a more prolonged erythema (122, 123). Application of LTB_4 topically to healthy skin results in a dose-dependent wheal and burning sensation which persists for several days. Biopsies of the provoked lesions reveal epidermal neutrophil microabscesses and neutrophil and monocyte infiltrates. In vitro, prior exposure of polymorphonuclears to LTB_4 leads to a specific loss of responsiveness. In fact, repeated topical application of LTB_4 on skin causes diminished inflammatory response (124). Similar effects are also observed in rabbits when intravascular LTB_4 is continuously injected. A dose-dependent inhibition of neutrophils accumulation in inflammatory sites occurs. Moreover, intradermal injection of various chemoattractants such as C5 and formyl-methionyl-leucyl-phenylalanine (FMLP) induces a rapid increase of LTB_4 levels in the skin. LTB_4 returns to baseline after 30 minutes (125).

Co-incubation of a fixed number of keratinocytes with an increasing number of neutrophils does not alter the percentage increase of LTB_4 formation when compared to a neutrophil monoculture. Keratinocytes have the ability to generate LTB_4 by the enzyme LTA_4 hydrolase (106). LTB_4 and peptido-LTs stimulate the DNA synthesis of cultured human epidermal keratinocytes as determined by experiments utilizing the incorporation of thymidine in the DNA (126).

Melanocytes, too, generate LTB_4 by catalysing LTA_4 (112). LTB_4, unlike peptido-LTs, does not induce the development of melanocytes, but has been shown to induce their pigmentation. This finding agrees with the post-pigmentation observed in many inflammatory dermatoses as a result of LTB_4 overproduction (127).

Keratinocytes are able to further metabolize LTB_4. One mechanism is by the formation of a glutathione conjugate of LTB_4. The conjugate is similar to LTC_4 and can be converted to LTD_4 and LTE_4 analogs. Another mechanism is by LTB_4 reduction to dihydro metabolites (128). However, LTB_4 is mainly catabolized by P450 enzymatic cascade. In human skin, ω-oxidation occurs in the cytochrome resulting in the conversion of LTB_4 to 20-OH-LTB_4 and 20-COOH-LTB_4. The catabolism of LTB_4 in the skin can be considered as a protective mechanism against

LTB_4 pro-inflammatory effects (129), not only because degradation is associated with loss or decrease in biological activity, but also because degradative enzymes are increased by PPARα (61). As it will be reported later, PPARα is implicated in eicosanoids metabolism and may control the duration and extent of an inflammatory response (130).

LTB_4 production is CaI-dependent. Studies have shown that there is an almost linear rate of LTB_4 synthesis correlating with increasing concentrations of CaI (up to 5 μM). When a fixed concentration of CaI is used, the LTB_4 synthesis is also time-dependent. In human cultured leukocytes, LTB_4 formation reaches its maximum after 5 minutes (131). However, zymosan-induced LTB_4 from whole blood increases to a maximum from 30 to 60 minutes and remained constant for many minutes (132).

In our studies, SZ95 sebocytes are able to produce LTB_4 themselves. Induction of sebocytes with AA for an hour resulted in a significant up-regulation of LTB_4 production. In contrast, application of CaI under the same conditions failed to stimulate LTB_4 production. We believe that administration of CaI for a long time inactivates 5-LOX and causes translocation of the enzyme to the nuclear envelope. Even though part of 5-LOX is now located in the nuclear envelope, another part is still located in the cytosol. The cytoplasmic 5-LOX can generate LTB_4 in the presence of exogenous AA for a long time. Our experiments suggest that thereafter only AA is responsible for the significant elevation of LTB_4.

Leukocytes incubated with AA or CaI produce various eicosanoids, including 5-HETE and 15-HETE, as a result of 5- and 15-LOX activity. When 15-HETE is added for a second time, 5-HETE and LTB_4 formation is inhibited (131, 133). Similar effects occur when CaI- and AA-stimulated leukocytes are treated with 5-LOX inhibitors, where the cells show an elevated 15-HETE formation (134). Moreover, LTB_4 production in human monocytes is down-regulated by IL-4-induced 15-LOX (135). 5-LOX inhibition and 15-LOX activation are associated phenomena. The inhibitory effect of 15-HETE on LTB_4 generation is due to competition of 15-HETE with AA for the substrate binding site on 5-LOX. 13-HODE and 12-HETE are weaker 5-LOX inhibitors (131).

Two cell surface LTB_4 receptors are known in humans: a high affinity receptor BLT-1 and a low affinity receptor BLT-2. BLT-1 seems to mediate chemotaxis and Ca^{2+} increase and BLT-2 degranulation. Apart from LTB_4, 20-OH-LTB_4 and 12(R)-HETE are also ligands for BLT-1 and 12(S)-HETE and 15-HETE for BLT-2 respectively (136). The distribution of BLT receptors varies in cells. Skin tissues possess LTB_4 receptors. In in vitro cell preparations of keratinocytes, leukotrienes bind to cellular membranes with high affinity receptors (137). The development of BLTs antagonists can be useful for modulating the LTB_4 inflammatory signal.

5.10. PEPTIDO-LEUKOTRIENES AND THE SKIN

While human epidermis cannot form detectable amounts of peptide leukotrienes by 5-LOX, it can transform exogenous leukotriene A_4 into peptide leukotrienes. Furthermore, coincubation of human epidermis and neutrophils results in a marked increase (90%) in peptide leukotriene formation when compared with neutrophils alone, indicating that human epidermis can transform neutrophil-derived leukotriene A_4 into peptide leukotrienes (138). Moreover, LTs increase DNA synthesis in cultured human keratinocytes (124). Cultured human melanocytes and melanoma cell line are able to form LTC_4 when LTA_4 is given exogenously. LTC_4 from microsomal fraction of the cells, but not LTD_4 is detected by HPLC (112). These results indicate that human skin contains LTC_4 synthase activity capable of producing significant amounts of LTC_4 from LTA_4. LTC_4 and LTD_4 stimulate melanocytes growth. These LTs are potent mitogens for cultured neonatal melanocytes. At the same time, LTC_4 decreases the melanin content of this cell population (127). Intracutaneous injection of peptido-LTs elicits erythema and wheal on the skin (122, 123). In addition, LTs cause increased vascular permeability and vasodilation in vivo (34).

5.11. 12-LIPOXYGENASE IN THE SKIN

Keratinocytes express isoforms of 12-LOX. The epidermal type is present in both the cytosol and membranes of culture keratinocytes. The enzyme metabolizes only AA to 12(S)-HETE. In normal skin, 12-HETEs are both of the S and R enantiomers at a ratio 97:3 (139). Provided that 12(R)-HETE is not a cytochrome P450 product, 12(R)-LOX must be present. In fact, 12(R)-LOX is found in human keratinocytes and hair follicles (140). 12-HETE is found in cultured human hair roots. NDGA inhibits 12-LOX activity in hairs (118). The enzyme is present not only in healthy skin, but is also found in much higher levels in psoriatic lesions. 12(R)-HETE is highly produced in psoriasis and other squamous diseases. Moreover, in mice, 12(R)-LOX is implicated in embryonic skin development (57). 12(R)-HETE is more potent than 12(S)-HETE in inflammatory skin. The properties of 12-HETE in skin are similar to LTB_4, however much weaker. 12-HETE attracts neutrophils and stimulates DNA synthesis in human keratinocytes. Its topical application provokes erythema and infiltration of leukocytes (124). 12-LOX is also involved in papillomas and squamous cell carcinomas in mice. Tumors induced by phorbol esters contain much higher amounts of 12-HETE than normal skin (141).

5.12. CYCLO-OXYGENASE IN THE SKIN

In normal human skin, COX-1 immunostaining is observed in the epidermis, whereas in the more differentiated, suprabasal keratinocytes, COX-2 immunostaining is increased.

Immunostaining of COX-1 or COX-2 in basal cell carcinomas was very weak, whereas both isozymes were strongly expressed in squamous cell carcinomas derived from a more differentiated layer of the epidermis. The expression of COX-2 in human epidermis and in human cultured keratinocytes may occur as part of normal keratinocyte differentiation (142). Moreover, COX-2 expression is induced by UVB irradiation. Exposure of human keratinocytes to UVB results in an increased PGE_2 production and up-regulation of COX-2 protein.When sun protected skin is irradiated, similar results are obtained. An up-regulation of COX-2 protein expression is observed (143). Transgenic mice overexpressing COX-2 produce high levels of epidermal PGE_2, $PGF_{2\alpha}$, and 15d-$\Delta^{12,14}$-PGJ_2, a metabolite of PGJ_2. These molecules promote tumorigenesis. Squamous cell carcinomas and sebaceous gland adenomas are appeared. The phenotype depends on the level of COX-2 expression and COX-2-derived prostaglandins (144). It has been recently demonstrated that SZ95 sebocytes express both isozymes at protein and mRNA level. They produce PGE_2 and 15d-$\Delta^{12,14}$-PGJ_2 (unpublished data by Muller-Decker K, DKFZ, Heidelberg, Germany).

5.13. P450 MONO-OXYGENASE IN THE SKIN

Although cytochrome P450 arachidonic acid mono-oxygenase has been known to be present in other tissues for many years, a novel P450 enzyme, CYP2B19, was recently discovered in fetal murine skin. CYP2B19 was shown to be exclusively expressed in differentiated keratinocytes of the epidermis, hair follicles and sebaceous glands. This mono-oxygenase can serve as keratinocyte differentiation marker. Recombinant CYP2B19 metabolizes AA and generates epoxy-eicosatrienoic acids (EETs) and HETEs. In endothelial cells, EETs activate ion channels and increase the cytoplasmatic Ca^{2+} concentration. It could be possible that skin CYP2B19 participates in Ca^{2+} dependent intracellular signalling pathways and in the formation of eicosanoids (145).

5.14. PPARS AND SKIN

The function of eicosanoids plays an important role in their nuclear receptors, the PPARs, since they are involved in lipid homeostasis, apoptosis, proliferation and differentiation. We are concentrated on their presence and significance in the skin. All three types of PPARs are expressed in human keratinocytes (149) and sebocytes (154). The epidermis serves as a border between inner tissues and the outer environment. A barrier protects the organism from dehydration and from microbial, chemical and mechanical attack. This barrier is made up of hydrophobic ceramides and cholesterol derivatives, which are produced by keratinocytes and

secreted extracellularly. The PPARs ligands clofibric and linoleic acid stimulate the building of the barrier. PPARα increases the expression of genes, which are responsible for the synthesis of ceramides and cholesterol derivatives (90). Moreover, clofibric acid-activated PPARα stimulates the formation of cornified keratinocytes and thus inhibits their growth and promotes their differentiation. Elevation of PPARα protein level is associated with the differentiation of murine keratinocytes. The 8-LOX derivative 8(S)-HETE activates PPARα. 8(S)-HETE produced by murine keratinocytes increases the expression of keratin-1, a differentiation marker. Hence PPARα regulates keratin-1 and keratinocyte differentiation (146). Similar results show that the PPARα activators Wy-14643 and clofibric acid induce differentiation and inhibit proliferation. When these were applied topically in mice, they resulted in an elevated expression of the proteins loricrin, fillagrin and involucrin. These proteins are structurally important for the building of the terminal differentiative keratinocytes, the cornified keratinocytes. Moreover, this topical application blocks the keratinocyte proliferation as shown by the epidermal thickening. Another effect is the acceleration of the recovery of the epidermis after mechanical damage (147). In skin carcinomas and papillomas, the protein level of PPARα is increased in comparison with its level in normal human skin. Similar results are obtained from primary cultured keratinocytes and tumorigenic cell lines. Likewise PPARβ and -γ protein levels are elevated in tumors although their expression is low in healthy skin. According to their properties PPARα activators Wy-14643 and conjugated LA have a protective effect on skin tumors, while PPARβ and -γ activators have not (67). PPARα activators partially inhibit mouse skin tumor promotion (33). In general, it is not clear whether PPARs activators generally inhibit or promote tumors. Wy-14643 and clofibric acid downregulate the production of IL-6 and IL-8. These pro-inflammatory cytokines are produced by UVB irradiation of murine keratinocytes. On the other hand, UVB exposure decreases all forms of PPARs at the level of mRNA (151).

PPARβ is particularly involved in the skin wound healing process. During healing, the neo-formation of skin is developed via two mechanisms: 1) migration of keratinocytes from the healthy edge of the trauma, and 2) their stratification in order to build a new barrier. Thus, both proliferation and differentiation participate in the neo-epithelialization process. PPARβ seems to be up-regulated during this phenomenon in murine tissues (148).

PPARγ inhibits the proliferation of cultured normal and psoriatic human keratinocytes. In psoriatic skin lesions, mRNA of PPARα and -γ is downregulated (149). On the other hand, PPARβ is overexpressed in hyperplastic and psoriatic lesions (150).

In sebocytes, PPARα and -γ activators stimulate lipid droplet accumulation. PPARγ activation induces the differentiation of cultured sebocytes (152) and PPARα increases the differentiative

response of sebocytes to androgens (153). PPARγ induces the transformation of early sebocytes, characterized by perinuclear lipid accumulation, to mid-differentiated sebocytes characterized by fused lipid droplets. PPARγ mRNA is found in lower quantities in cultured sebocytes than in newly dispersed ones. Primary cultured sebocytes are predominantly immature in comparison with well differentiated freshly dispersed sebocytes (152). The differentiation effect of PPARs is accumulative after synchronised stimulation of PPARα and -γ activators (152).

Since LOX products act mostly as pro-inflammatory mediators and induce cancer, the use of LOX inhibitors could also affect PPARs. In fact, there is a relation between LOX inhibitors and PPARs. LOX inhibitors directly block PPARs. As well as MK886 and nordihydroguaiaretic acid (NDGA), quercetin and morin also inhibit ketatinocyte differentiation (155).

Since LTB_4 is highly expressed in inflammatory diseases such as atopic dermatitis and psoriasis, its receptor PPARα may play a significant role in LTB_4 regulation. The PPARα agonist, clofibrate, induces the catabolism of LTB_4 in rats. Catabolism of LTB_4 takes place in cells of the immune system at the inflammation site as well as in the liver. The liver is the major organ that clears LTB_4 from the blood circulation. In culture hepatocells, LTB_4 activates PPARα to induce transcription of enzymes, which catabolize LTB_4. Degradation products of LTB_4 are less potent inflammatory mediators than LTB_4 itself. Therefore PPARα agonists could control the duration of inflammatory response through a feedback mechanism (156). However, activation of PPARα in endothelial cells prior to treatment with LTB_4 has no effect on the adhesion of neutrophils to endothelial cells (157). In contrast, a PPARα antagonist could prolong the duration of the inflammation. In fact, transgenic mice, which lack the expression of PPARα extend the inflammation response (156). On the other hand, PPARs are involved in the differentiation of sebocytes participating in the accumulation of fat droplets (152). In our study we have demonstrated that LTB_4 is present in SZ95 sebocytes and is upregulated under certain conditions, leading to inflammation and differentiation. It is reasonable to assume that regulation of LTB_4 at PPAR level may work.

Another important point is the relationship between PPARs and RXRs. As reported before, PPAR and RXR form an active dimer. Different to RX-receptor is the retinoic acid receptor (RAR). The first has as natural ligand 9-cis retinoic acid and the second all-trans retinoid acid. RXR activator stimulates sebocyte differentiation and proliferation according to PPAR-RXR dimer formation. All-trans retinoic acid has a reverse anti-proliferative effect (150). Many precursors of all-trans retinoic acid are used in the treatment of acne.

Finally, dehydroepiandrosterone-sulfate (DHEA-S) is a PPARα ligand. DHEA-S is a precursor molecule of testosterone and of its active metabolite 5α-dihydrotestosterone (5α-DHT).

Sebocytes can convert circulating DHEA to 5α-DHT (92). Taking into account that overproduction of androgens may cause acne, this finding could be a new field of research in acne therapy.

5.15. CYTOKINES AND LIPOXYGENASES

Cytokines are present in skin tissues. Skin cells such as keratinocytes, Langerhans cells and melanocytes produce many cytokines. These mediators have various properties: they attract inflammatory cells, may affect the immune system, influence keratinocyte proliferation and differentiation and stimulate the production of other cytokines. Cytokines can be divided into two groups: Th-1 and Th-2 pattern. Th-1 secreted cytokines (IL-2, TNF-β, IFN-γ, etc) are generally implicated in cellular immunological reactions and Th-2 type (IL-4, -5, -6, etc) in humoral immunological mechanisms. An example of Th-1 disease pattern is psoriasis and of Th-2 is atopic dermatitis (200). We will concentrate on keratinocytes. Cytokines are generated by keratinocytes constitutionally, but also after stimulation. Keratinocytes produce a plethora of cytokines including interleukine (IL) –1, -6, -7, -8, -10, -12, -15, -20, interferons (IFN) –α, -β, -γ and tumor necrosis factor α (TNF-α).

Of these, IL-1, -6, -8 and TNF-α are pro-inflammatory mediators. Keratinocytes, Langerhans cells and many melanoma cell lines produce IL-1. There are two functionally almost equivalent forms of IL-1, IL-1α and IL-1β. IL-1β is the predominant form in humans. IL-1 acts locally and also systemically. The synthesis of IL-1 can be induced by other cytokines and also by bacterial endotoxins, viruses, mitogens and antigens. The main biological activity of IL-1 is the stimulation of CD4+ T-lymphocytes, which are induced to secrete IL-2. IL-1 acts directly on B-lymphocytes promoting their proliferation and synthesis of immunoglobulins (158). IL-1 enhances the metabolism of AA, particularly of prostacyclin and PGE_2 in inflammatory cells. IL-1 also influences the functional activities of Langerhans cells of the skin. IL-1 induces keratin-6 expression in keratinocytes and decreases adherence of certain bacteria to keratinocytes (159). IL-1α is decreased in lesional psoriatic and eczematic lesions (160). IL-1 induces the secretion of other cytokines by the keratinocytes and enhances melanin production by melanocytes (161).

IL-6 influences antigen-specific immune responses and inflammatory reactions. It is one of the major physiological mediators of acute phase reaction. In the presence of IL-2, IL-6 induces the differentiation of mature and immature T-cells into CD+8 T-lymphocytes. IL-6 may function also as an autocrine growth modulator for tumors. IL-1, bacterial endotoxins and TNFs are the physiological stimuli for the synthesis of IL-6 (158). IL-6 is also present in keratinocytes. It stimulates keratinocytes proliferation. IL-6 is overproduced in diseases associated with

epidermal hyperplasia and wound healing and is increased in many dermatoses and after UVB exposure (159).

IL-8 is a strong neutrophilic attractant. It is produced by keratinocytes after stimulation of contact sensitizers and irritants. It is present in auto-immune diseases, such as pemphigus herpetiformis and bullous pemphigoid.

IL-10 and IL-12 belong to anti-inflammatory cytokines. UVB irradiation activates IL-10 gene promoter in mice keratinocytes. The IL-10 receptor is downregulated in psoriasis and acute atopic dermatitis, but up-regulated after UVA exposure. The main source of this cytokine in human skin is the melanocyte population. It is not clear if human keratinocytes form IL-10. IL-12 is important in the induction of Th-1 response. IL-12 is generated after UVA exposure in keratinocytes. Allergens, but not irritants, evoke this cytokine production. IL-12 is present in allergic contact dermatitis. IL-10 and IL-12 have antagonistic properties in the same cells (159).

TNF-α and TNF-β display a similar spectrum of biological activities in vitro systems, although TNF-β is often less potent. The synthesis of TNF-α is induced by many different stimuli including INF, IL-2, GM-CSF, substance P, bradykinin, immune complexes, inhibitors of cyclo-oxygenase and PAF. IL-6, TGF-β, vitamin D, PGE_2, dexamethasone and antagonists of PAF inhibit the production of TNF. TNF-α causes cytolysis and cytostasis of many tumor cell lines. The factor also enhances phagocytosis and cytotoxicity in neutrophilis and also modulates the expression of many other proteins. In vivo, TNF-α in combination with IL-1, is responsible for many alterations of the endothelium. TNF-α is a potent chemoattractant for neutrophils. TNF-α induces the synthesis of a number of chemoattractant cytokines. TNF stimulates the expression of class I and II HLA and the production of IL-1, colony stimulating factors, IFN-γ and AA metabolism. IL-6 suppresses the synthesis of TNF induced by endotoxins (158). The functional capacities of Langerhans cells are also influenced by TNF-α. The enhanced ability of maturated Langerhans cells to process antigens is significantly reduced by TNF-α. TNF is produced by keratinocytes after various kinds of stimulation. High TNF expression has been found in cutaneous T-cell lymphoma and psoriasis. TNF is also involved in keratinocytes apoptosis. UV irradiation induces the release of this factor (159). TNF-α as well IL-1α and -β mRNA are present in hair follicular epithelium, in sebaceous glands, in sweat gland ductals and in arrector pili muscles (161).

Eicosanoids and cytokines are involved in intracellular communication processes such as inflammation, host defence and vascular events. Cytokines affect eicosanoids on multiple levels. They stimulate, de novo, biosynthesis of eicosanoids; they stimulate enzymes, which regulate eicosanoids synthesis or degradation; they act synergistically with lipid mediators. Vice-versa,

lipid mediators regulate cytokines metabolism and actions. Thus, various interactions between cytokines and lipid mediators lead to diverse effects, some pro-inflammatory and other anti-inflammatory. IL-1, -6 and TNF-α increase the expression of PLA_2. Induction of PLA_2 activates the AA metabolic pathway. IL-8 stimulates LTB_4 release by the neutrophils. IL-4 and -13 induce 15-LOX expression (162). IL-4, –13 increase also the production of LTB_4, upregulating the activity of LTA_4 hydrolase (163). IL-1, -2 stimulate 5- and 15-HETE formation by T-lymphocytes cell lines. Conversely 5-LOX stimulates IL-1-induced IL-2 release as well IL-2-mediated regulation of proliferation and IFN-γ production. LTB_4, LTC_4, LTD_4 and LTE_4 are able to stimulate IFN-γ secretion, too. Moreover, LOX inhibitor ETYA has an inhibitory effect on IL-1 induced IL-2 production. Similar effects are shown on IL-2 induced IFN-γ release in the presence of ETYA (164).

Our results show that IL-1β and TNF-α are produced by SZ95 sebocytes, which are in agreement with other reports (161, 165). However, the treatment of the sebocytes with AA and CaI has no effect on IL-1β and TNF-α release. On the other hand, IL-6 and IL-8 not only are produced by SZ95 sebocytes, but they are increased by AA and CaI stimulation. 1μM CaI is more potent than 100 μM AA. Neither Caf nor Zil cause any down-regulation of these cytokines. It is hypothesized that cytokine production is independent of 5-LOX inhibition. If not, the concentration of the administrated inhibitors is too low. SZ95 sebocytes do not release IL-10 and IL-12(p70). We conclude that SZ95 sebocytes have some properties of pro-inflammatory cells. LOXs may play a significant role in inflammatory signaling. Sebocytes not only produce lipid mediators such as LTB_4, but they also act as modulators of the generation of other pro-inflammatory molecules.

5.16. INVOLVEMENT OF EICOSANOIDS IN SKIN DISEASES

5.16.1. Psoriasis

Psoriasis is the best studied dermatological disorder, in which eicosanoids are implicated. It is a hereditary disease of the skin and occurs as chronic, reccuring, scaling papules and plaques. The principal abnormality in psoriasis is an alteration of the cell kinetics of the keratinocytes. A shortening of the cell cycle causes an acceleration of the production of new cells. Psoriatic lesions are characterized by hyperplasia and incomplete differentiation of the keratinocytes. Lesional areas are infiltrated by leukocytes. At the same time, capillaries of the papillary dermis are dilated. Although immunological phenomena are implicated in the pathogenesis of the disorder, eicosanoids might play a significant role in the etiology.

Lipoxygenase is activated in the presence of AA. AA as substrate can be liberated in keratinocytes by phospholipase A_2 (PLA_2). Cytosolic PLA_2 is increased in psoriatic lesions. Actually, in psoriatic lesions, free AA is elevated (166). The high expression of $cPLA_2$ in psoriasis can be associated with the absence of the anti-inflammatory protein annexin-I. Annexin is induced by corticoids and inhibits PLA_2. Annexin is present in human skin and the level of $cPLA_2$ remains low. Additionally, in $cPLA_2$ deficient mice, the production of eicosanoids is decreased (167). Glucocorticoids inhibit PLA_2 and therefore suppress COX and LOX production. In clinical experience, topical application of corticoids improves psoriatic lesions.

As previously mentioned, keratinocytes expres 5-LOX in low quantities or not at all. The enzyme is present in psoriatic patients. Furthermore, 5-LOX is more elevated in lesional skin than in uninvolved areas of psoriatic patients (167). The presence of LTA_4 hydrolase is demonstrated in involved and uninvolved psoriatic skin. The levels of LTA_4 hydrolase in lesional and non-lesional skin are the same; however the activity of the enzyme is decreased in lesional areas. This could be explained by the suicide inactivation of the enzyme (111). In contrast, other studies (168) show that in non-lesional skin, no significant LTB_4 activity is found. It is not known whether LTA_4 is produced by the keratinocytes themselves or is derived from other cells. Taking into account that keratinocytes lack FLAP and 5-LOX activity is limited, it is possible that leukocytes or macrophages produce LTA_4 in the site of lesions (138, 169). However, non-lesional skin shows a small 5-LOX activity in the absence of leukocytes (170). It is a matter of discussion, whether LTB_4 can initiate the inflammatory response or the formation of leukotrienes is presented as a secondary phenomenon in the inflammatory process. In other words, do LTB_4 or molecules such as cytokines and complement primarily attract neutrophils to the lesions?

The expression of LTB_4 is high expressed in psoriatic biopsies and blister fluids. LTB_4 is detectable in acute gutate lesions supporting an early involvement of this mediator in psoriasis (124). Other leukotrienes, such as LTC_4 and LTD_4, are also elevated in psoriatic plaques and serum (167). LTs have been shown to induce DNA synthesis in human cultured keratinocytes (126). Elevated LTE_4 is also found in the urine of psoriatic patients (124). At least 9 monohydroxy acids are detected in psoriatic lesions, among them 5-, 12- and 15-HETE as well the LA derivatives 9- and 13-HODE. 12-HETE is the most abundant metabolite followed by 15- and 5-HETE (170, 171). 12-HETE levels are significantly higher in psoriatic lesions than in uninvolved skin. It is interesting that this molecule has an R-configuration. 12(R)-HETE is a product of 12(R)-LOX catalysis and not of platelet 12(S)-LOX. Platelet 12-LOX is only slightly elevated when compared to 12-HETE, which is produced in large quantities. However, the platelet type is present in psoriasis (172). 15-HETE is the main hydroxy fatty acid in normal

skin. In contrast, its quantity is decreased in non-lesional skin of psoriatic patients (167). It might be possible that 15-HETE regulates the inflammation. In many chronic psoriatic plaques, 15-HETE is present in concentrations capable of inhibiting both the formation and the chemotactic effects of LTB_4. 15-HETE and 13-HODE are also found incorporated in membrane phospholipids of lesional psoriatic skin. The quantities of fatty acids are lower in skin lesions than in uninvolved skin (124).

Finally, COX is present in psoriasis, too. The major COX derivative in psoriatic skin is PGE_2 (160, 166, 167, 173).

When keratomed psoriatic skin specimens were incubated in the presence of AA and CaI A23187, the amount of LTB_4 increased 4-fold. 12- and 15-HETE both increased 2.7-fold. The PGE_2 increased 8-fold. Similar incubations using psoriatic scales did not result in the formation of eicosanoids. Incubations with the 5-LOX inhibitor RS-43179 inhibited the formation of LTB_4 and PGE_2 without significantly affecting the formation of 12- and 15-HETE (121).

Not only are eicosanoids produced in human skin, but they act as pro-inflammatory mediators. They regulate the immune response by cytokines, regulate growth and differentiation of the keratinocytes and cause vasodilatation. All these phenomena are present in psoriasis. Hence, the disease could be partially explained under the prisma of the eicosanoids presence.

5.16.2. Atopic dermatitis

Atopic dermatitis (AD) is an acute, subacute or chronic relapsing disease that usually begins in infancy. It is characterized by erythematous plaques, papules and pruritus, which with rubbing and scratching leads to lichenification (hyperplasia of the skin). AD is often associated with a personal or family history of AD, allergic rhinitis and asthma. Type I (IgE mediated) hypersensitivity reaction occurs as a result of the release of vasoactive substances from both mast cells and basophils that have been sensitized by the interaction of the antigen with IgE. The serum IgE is usually elevated.

Eicosanoids, as pro-inflammatory mediators, are also implicated in the pathogenesis of atopic dermatitis (AD). Healthy patients' skin and uninvolved skin from atopic patients do not form LTB_4. On the other hand, LTB_4 is significantly increased in skin lesions. Association between LTB_4 amounts and disease severity was not noted (173). However, other studies show correlation between the LTA_4 hydrolase activity and the disease (174). Furthermore, there is no correlation between LTB_4 and serum IgE levels. PGE_2 shows no significant differences between lesional, non-lesional atopic skin and healthy skin (173). Compared with uninvolved skin, the levels of 12- and 15-HETE are elevated 7-fold and 11-fold, respectively, in lesional skin, but do

not reach biologically active concentrations (175). The platelet secreted LTB_4 and 12-HETE in atopic patients is increased, too (176). Studies with animal skin and rat peritoneal mast cells suggest that substance P induces the release of histamine and LTB_4 from skin mast cells. Substance P is located in cutaneous nerve fibres and induces wheal and flare responses after intradermal injection, accompanied by granulocyte infiltration. The amount of LTB_4 is correlated with the amount of tissue histamine (177). Moreover, it was demonstrated that intradermal injected LTB_4 causes itching in mice (178). In AD, an accumulation of monocytes-macrophages is present. It might be possible that LTB_4 attracts this population in lesions (173). The source of LTB_4 in AD is not clear either. Neutrophils, macrophages, eosinophils or keratinocytes are possible candidates. Cultured mixed leukocytes (neutrophils, monocytes, eosinophils) stimulated with AA and CaI, generate much higher quantities of LTB_4 and LTC_4 than leukocytes from healthy persons (179). In addition, stimulation of leukocytes with IL-3, IL-8 and complement 5a (C5a) leads to higher release of leukotrienes (180). The LTA_4 hydrolase activity of neutrophils and monocytes is elevated in patients with severe AD compared to patients with moderately-mild disease or healthy people. This elevation does not appear to be due to an increase of the number of eosinophils (174).

5.16.3. Systemic sclerosis

Systemic sclerosis (SS) is a chronic disease characterized by progressive fibrosis of the skin and internal organs. The etiology of this disease is unknown. Genetic, autoimmune, environmental factors, abnormally reactive vessels and inappropriate regulation of collagen synthesis are possible causes. Thinned skin, loss of appendages, inflammation and vascular injury occur in SS, followed finally by extracellular matrix deposition, called fibrosis. Fibrosis is characterized by enhanced collagen production. Lesional skin is infiltrated by blood cells. This infiltration is mainly perivascular. Vascular involvement is associated with increased permeability, microangiopathy and blood vessel hyperactivity. Apart from leukocytes, vascular endothelial cells, macrophages and fibroblasts are also implicated in the tissue damage. Fibroblasts are cells, which produce collagen, the main component of the extracellular matrix. They are involved in most connective tissues disorders such as SS. Fibroblasts are able to release pro-inflammatory mediators. Thus, fibroblasts may participate in the initiation of acute and chronic inflammatory diseases.

In situ hybridization and immunostaining reveal 5-LOX presence in healthy individuals and SS patients. The expression is significantly higher in sclerotic lesions than in healthy skin. The enzyme is located in perivascular infiltrations. Fibroblasts and monocytes-macrophages are

marked positive for 5-LOX. RT-PCR and FACS analysis also shows 5-LOX expression in cultured fibroblasts, taken from SS patients and healthy individuals. Moreover, LTB_4 and LTE_4 are demonstrated in cultured fibroblasts. Enzyme immunoassay shows that fibroblasts stimulated with CaI A23187 form leukotrienes, but untreated cells do not. Cells from both patients and normal controls produce them. The level of LTB_4 is higher in SS samples than in control samples. In addition, production of LTB_4 is higher than LTE_4. LTE_4 production remains low and without differences between SS and control fibroblasts (181).

5.16.4. Acne vulgaris

Acne vulgaris is a very common disease. It appears mainly in puberty. More or less all individuals are affected once in their life. The typical lesions of acne are comedones, papules, pustules, nodules and scars. Acne is a disorder of the pilosebaceous unit. Four major factors are resposible for its etiology: comedogenesis, seborrhoea, colonization of the duct with Propionibacterium acnes and inflammation (182).

The earliest morphological change in a pilosebaceous unit is an abnormal follicular epithelial differentiation. The upper part of the follicle duct is plugged with desquamated cornified cells. These cells, instead of shedding, remain abnormally attached. The accumulation of corneocytes is also due to hyperproliferation of ductal keratinocytes. This process is known as comedogenesis. Alteration in sebum-containing lipids induces hypercornification. Increased FFAs, squalene, oxidative squalene and decreased LA lead to comedogenesis. Moreover, androgens stimulate the proliferation of ductal corneocytes, whereas retinoids suppress comedogenesis (97, 183).

Overproduction of sebum (seborrhoea) is the second factor. Acne patients produce high quantities of sebum and their sebaceous glands are enlarged. The growth of glands is androgen dependent. However, there is no correlation between free testosterone level in the plasma and severity of acne. The intensity of the androgens effect in sebaceous gland proliferation depends on the body area. Face, neck, chest, upper back and arms are more sensitive. In fact, these areas are affected by acne (184).

Furthermore, sebum serves as a growth medium for Propionibacterium acnes. P. acnes is an anaerobic bacterium, naturally occurring in the skin. The follicular duct flora in normal and acne individuals also includes staphylococcus and malassezia species. In puberty, there is a significantly higher density of P. acnes in acne patients than in healthy individuals (184). However, there is no association between the number of bacteria and the severity of acne (185). An infection of static sebum takes place in comedones. P. acnes hydrolyzes triglycerides to

glycerol for its nutritional demands. However, FFAs are also liberated from triglycerides causing irritation. P. acnes releases chemotactic factors and activates complement (C5α) providing infiltration of neutrophils. Along with neutrophil migration, the early inflammatory event is the infiltration of monocytes in comedones. CD+4 T-lymphocytes and Langerhans cells accumulate and infiltrate the lesions. These inflammatory reactions cause further damage and rupture of the follicular wall (97, 182, 185).

The presence of inflammation is the critical link between acne and eicosanoids. Sebaceous gland is the key tissue in this relationship. Sebum contains FFAs, which are ideal substrates for LOXs. We have demonstrated that 5- and 15-LOX-1 are expressed in sebocytes. Moreover, LTA_4 hydrolase is present, too. It is important that stimulation of sebocytes with AA and /or CaI causes the activation of the enzymes and their translocation to membranes. The formation of elevated quantities of LTB_4 under various stimuli is evidence that LOX undergoes activation. The fact that SZ95 sebocytes produce LTB_4 in the absence of other cells suggests that skin cells can themselves produce leukotrienes. Probably, sebocytes have particular properties which are absent in other skin cells populations. One of them might be the presence of FFAs in sebum.

Another point is the coexistence of 5- and 15-LOX-1 in sebocytes. In contrast to 5-LOX derivatives, 15-LOX-1 products have anti-inflammatory properties. Pro-inflammatory mediators such as prostaglandins and leukotrienes are in balance with anti-inflammatory mediators such as 15-HETE and lipoxins (101). The role of lipoxins in the control and resolution of inflammation is established. Generally, the major lipoxygenase pathway in the epidermis is 15-LOX. As we reported previously, 5-LOX expression is limited in human keratinocytes. On the other hand, we have demonstrated that 5-LOX is the main lipoxygenase in cultured sebocytes. 15-LOX-1 expression is much lower. Of course the presence of epidermal 15-LOX-2 in SZ95 sebocytes is a matter of further investigation.

Lipid analysis of the sebum shows neither the presence of free nor esterified AA. The only compartment that AA can be found in is incorporated in the cellular membranes. Thus, we have to find out which AA pool 5-LOX utilizes to form LTB_4 in SZ95 sebocytes. In our study, 5-LOX is expressed in untreated SZ95 cells, too. It is certainly present at both protein and mRNA levels. A possible explanation could be the high concentration of Ca^{2+} in culture medium (1.5 mM). We remind that Ca^{2+} activates 5-LOX. In addition, small amounts of endogenous free AA could be utilized. On the other hand, cultured sebocytes might use exogenous AA liberated from neighbouring cells. AA incorporated in phospholipids of burst sebocytes could be this source. In contrast, LA is a sebum component both in free and esterified forms. It is likely that LA serves

as lipoxygenase substrate in sebaceous glands and its derivatives act as pro-inflammatory mediators.

Another expression of inflammation in sebaceous glands is the presence of cytokines. Cytokines are not only present in sebaceous glands, but they are also formed locally. Both interleukines IL-1α and IL-1β can be detected by immunohistochemistry in normal human sebaceous glands (161, 165). In acne patients, IL-1α production is increased in uninvolved follicles in comparison with normal control skin. In early inflamed lesions, IL-1α and IL-1β receptors are further elevated. Furthermore, IL-1α causes comedonal appearance in vitro as demonstrated by hyperproliferation and abnormal differentiation of keratinocytes (186). The early presence of cytokines in microcomedones can be seen as the initial inflammatory event in acne, without the participation of bacteria.

Bacteria found typically in comedones seem to fail to upregulate IL-1α release by keratinocytes, in vitro (185, 187). On the other hand, P. acnes induces the production of monocyte derived cytokines. Monocytes isolated from acne patients, when are treated with heat killed or supernatants of P. acnes, produce higher amounts of IL-1β, IL-8 and TNF-α than controls (187).

Whether or not bacteria are important in triggering cytokine release, lipoxygenases they are. IL-6 and IL-8 produced by SZ95 sebocytes are upregulated in the presence of 5-LOX activators, AA and CaI. It seems that cytokine induction occurs as a result of 5-LOX activation. On the other hand, the generation of LTB_4 is increased by the stimulation of C5α, IL-1, TNF-α, PAF, granulocyte-macrophage colony-stimulating factor (GM-CSF), as a means of enhanced AA release from the cells. LTB_4 is upregulated not only by other inflammatory mediators and itself, but can also multiply the potential of their actions (61). Thus, our results in the stimulatory effects of LTB_4 on cytokine production are in agreement with these reviews.

Upregulation of cytokine production by AA and/or CaI suggests an involvement of LOXs in inflammation. We do not know if there is a correlation between LOX activation and upregulation of cytokines by AA and CaI. It is possible that in sebaceous glands locally produced eicosanoids could stimulate in a later time the production of pro-inflammatory cytokines. Eicosanoids are formed within minutes, whereas cytokines in some hours. It is certain that both phenomena are expressions of inflammation in SZ95 sebocytes.

One step further, LTB_4 can attract macrophages and leukocytes to the comedones. In addition, Unlike in the case of healthy individuals skin, CD4+ T-lymphocytes are increased in uninvolved acne skin and in early lesions. Elevation is also noted in the number of macrophages (186). As a result, these cells release cytokines. It is known that LTB_4 induces the production of IL-1, IL-6,

IL-8 and TNF-α in macrophages as well IL-2, IL-4, IL-5, IL-10 and INF-γ in lymphocytes. Moreover, leukotrienes stimulate the proliferation of CD+8 T-lymphocytes and decrease that of CD+4 cells (34, 61). Thus, an endless inflammatory cycle takes place, in which LTB_4 triggers the generation of inflammatory mediators and these induce more leukotriene production. Even though LTB_4 is not released by sebocytes in acne, but by other cells, the outcome is the same. Topical inflammation is present.

LTB_4 production can be reduced in three ways:

a) Gene deletion through 5-LOX deficient organisms.
b) Substitution of AA with other dietary fatty acids. This idea is not realistic since AA is an essential fatty acid. However, dietary supplementation with EPA causes a clinical improvement in many LTB_4-implicated disorders (61). Nutrition rich in ω-3 fatty acids such as EPA and DHA can modify the fatty acids profile of epidermal cellular phospholipids. Release of these lipids from the plasma membrane generates eicosanoids by the action of 15-LOX. These eicosanoids reduce LTB_4 synthesis (101).
c) LTB_4 inhibition. This will be discussed extensively here.

5.17. LIPOXYGENASES INHIBITORS

Since 5-LOX catalyzes the first steps in leukotriene anabolism, application of 5-LOX inhibitors provides a definitive target in order to modulate the effect of leukotrienes. LOX blockers can be applied in two fields. Inflammatory and allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, psoriasis, inflammatory bowel diseases, and rheumatoid arthritis belong to the first entity. The second one involves cancer of the breast, colon, prostate, lung and skin tumors.

Caffeic acid (Caf) is a natural extract of many plants. Caf inhibits 5-LOX in a non-competitive manner. In human platelets, Caf inhibits completely the generation of LTB_4 at a concentration of 10^{-4} M. On the other hand, Caf increases COX activity. At 10^{-5} M doses, it does not show any inhibitory effect on COX. The inhibitor can inhibit 12-LOX, too, but at higher concentrations. For Caf inhibitory effect on LTB_4, no preincubation is required. The methyl-esters of Caf are stronger inhibitors than Caf (188).

15-HETE is another inhibitor the effects of which have already been discussed.

Nordihydroguaiaretic acid (NDGA) and 5,8,1,14-eicosatetraynoic acid (ETYA) are non-specific LOX inhibitors. NDGA (10^{-7} M) is a more potent 5-LOX inhibitor than ETYA or 15-HETE. This molecule also blocks 12-LOX and COX at higher concentrations. NDGA acts as a free radical scavenger. ETYA, an AA analogue, inhibits all LOXs and COX, but prefers 12- and

15-LOX. At a concentration of 10^{-7} M, it stimulates the synthesis of 5-HETE, probably because of the concomitant inhibition of 12-LOX and of the resulting increase of available AA (189).

Most of the known LOX inhibitors are non-selective, act as anti-oxidants, provoke toxicity and other side-effects, lack oral bioavailability or interfere with other biological processes (1). New agents with greater potency and enzyme specificity have been developed. Generally, these agents can be classified into four groups: 5-LOX inhibitors, peptido-leukotrienes receptor antagonists, LTB_4 receptor antagonists and FLAP inhibitors. We include LTs antagonists in this chapter due to their relation with LOX. Several potential inhibitory mechanisms are considered. Inhibitors may prevent neutrophil and lymphocyte activation and accumulation. They modulate transduction signals such as down-regulation of phosphate kinase C, tyrosine kinase, cAMP. Moreover, they block lipid peroxidation and generation of free radicals and induce apoptosis. LOX inhibitors prevent tumor growth by inhibition of DNA synthesis, oncogenes, growth factors and neoangiogenesis; they are involved in cancer metastasis regulating tumor cell motility, adherence to endothelial matrix and extravasation (190). Next, we will describe some inhibitors of various categories.

MK-886 belongs to FLAP inhibitors. MK-886 is believed to work by binding to the AA binding side of FLAP. Although in vitro inhibition of leukotrienes is excellent, the molecule has failed in vivo (1). MK-0591, another FLAP inhibitor, improves the pulmonary function of moderate asthmatics (1). MK-0591 intravenously administration in rabbits reduces neutrophil accumulation in skin after LTB_4 induction (125). However, the degree of clinical improvement was not as good as expected (1).

LTB_4 antagonists belong to a group of promising agents. However, only preliminary reports have appeared on their clinical evaluation (1).

In contrast, peptido-LTs antagonists are well developed and clinically evaluated. Many drugs of this category have been released on the market. These drugs are used against asthma. To date, montelukast (Singulair^{TM}) provides the most potent and longest lasting blockade of LTD_4 inducing bronchoconstriction in humans. Montelukast has high efficacy and is devoid of liver toxicity. In moderate asthmatics, Singulair^{TM} (100 mg, single dose) evokes bronchodilation and blocked exercise induced broncho-constriction 24 hours after dosage. In a dosage of 10 mg daily, it produces a significant improvent in lung function and in the quality of life. Zafirlukast (Accolate^{TM}) provides an improvement in lung function of asthmatic patients, too. This antagonist is applied also in acute seasonal allergic rhinitis. Significant improvement in nasal congestion, symptoms, sneezing and rhinorrea formation is noted. Another 2 peptido-LTs

antagonists, verlukast (Venzair^{TM}) and pranlukast (Onon^{TM}), which have similar efficacy, are available on the market.

5.17.1. Zileuton

Lipoxygenases possesses an iron atom in the active site. An ideal LOX inhibitor shall be designed to interact with the catalytically required iron. Zileuton [N-(1-benzothien-2-ylethyl)-N-hydroxyurea] is binding strongly to Fe^{+3}, thus inhibiting the enzyme activity. Zileuton (Zil), in vitro, causes a concentration-dependent inhibition of 5-LOX (IC_{50}=0.5 μM). Zil is less active than NDGA. LTB_4 synthesis by human neutrophils is blocked when Zil is added. Zil is a reversible inhibitor. When Zil is removed from the suspensions, inhibition ceases. Zil is a specific human 5-LOX blocker; it fails to inhibit or has limited effect on other LOXs. Partial inhibition of COX is succeeded at 100-fold concentrations. The enzyme is effective in preventing 5-HETE and LTB_4 generation of Ca^{+2} ionophore A23187-induced cells. Moreover, Zil restricts the accumulation of neutrophils (191).

Zileuton (Zyflo^{TM}) is the first selective orally administered 5-LOX inhibitor in humans. Zil reduces IgE-associated airway inflammation. In chronic asthmatics, a dosage of 600 mg qid, improves the lung function and overall asthma symptoms. It improves the symptoms in aspirin sensitive asthmatics as well the tolerance in cold dry air (1).

Zil is also used in patients suffering of ulcerative colitis. Although the treatment is effective, there are no therapeutic advantages in comparison with classic therapeutic schemata (1).

5.17.2. Lipoxygenase inhibitors and skin diseases

LOX inhibitors may have therapeutic application in inflammatory skin disorders and cancer. We will report the results of clinical trials of the 5-LOX inhibitors used in dermatology.

Lonapalene (RS 43179) is a 5-LOX inhibitor. Topical application of 2% lonapalene ointment in psoriatics causes significant clinical improvement. In addition, there is a reduction of LTB_4 in skin chamber fluid samples (192).

R-68151, another 5-LOX inhibitor, has a moderately-mild therapeutic effect on psoriatic plaques. Topical application reduces scaling and erythema. The drug is effective in the treatment of hyperkeratotic skin diseases such as lamellar ichtyosis, bullous congenital ichtyosiform erythroderma and vulvar lichen sclerosus et atrophicus (192).

Zafirlukast™ has been used in psoriatic patients. The drug has been applied as monotherapy in patients, who did not tolerate the classical therapy. After this treatment, a clinical improvement was noted (193).

Zileuton has been used in various diseases. Atopic patients, who were put on zileuton monotherapy, showed significant improvement. On the disease dissatisfaction scale, the score was reduced. The pruritus score was also reduced after Zil administration (194). Zil was also found to improve the clinical symptoms of patients suffering from systemic lupus erythematosus (SLE). LTE_4 level in urine, which is correlated with SLE disease activity, was reduced (192).

Particularly interesting is the administration of LOX inhibitors in acne. The first use of eicosanoids inhibitor in acne patients was performed in the ˋ60s. Then, patients were treated with ETYA. Orally administrered ETYA reduced the sebum excretion rate. However, this drug was unsuitable for long treatment. Topical application of 2% ETYA caused a slight but significant sebum decrease. This reduction was not significant, when ETYA was applied in the presence of 20% propylene glycol (195, 196).

At our clinical department, we have also investigated the effects of Zil on acne patients. 10 young patients suffering from moderate acne were treated with Zil, 600 mg, qid for 3 months. All other topical or oral medicines, cosmetics and cleansers were banned. For the evaluation of the trial, the acne severity index, the number of lesions, the total lipids and FFAs in sebum and LTB_4 in serum were measured. During Zil treatment, a gradual and time-dependent reduction of inflammatory lesions was observed. The acne severity index also showed an improvement of the disorder. Total sebum lipids were reduced significantly. A non-significant reduction of FFAs was observed, too. Nevertheless, LTB_4 in serum was not affected. The decrease in inflammatory lesions was correlated with the reduction of total sebum lipids and fatty acids in sebum (99, 201).

On the other hand, our in vitro data show limited effects of 5-LOX inhibitors Caf and Zil on lipid synthesis of sebum. Treatment of resting cells with various concentrations of Caf or Zil does not significantly alter lipid matabolism. However, AA and CaI induced lipid production in SZ95 sebocytes is significantly decreased, when the cells are treated with 20 μM Caf or 5 μM Zil, particularly. In contrast, in some concentrations of Zil there is a stimulatory effect on lipid production. The last finding might be explained by the fact that the Zil administrated was not purified. Some vehicles of the drug could affect the outcome.

When we investigated the effect of Zil on LTB_4 production in vitro, the results were disappointing. Zil failed to block LTB_4 production. 5 μM Zil failed to reduce LTB_4 synthesis in resting sebocytes and in AA/CaI induced sebocytes. It is possible that the concentration of Zil was inappropriate. Furthermore, two concentrations of Caf were examined (10, 20 μM). Only 10

μM was observed to inhibit LTB_4 formation in AA- and CaI-induced sebocytes. The reduction was significant. In contrast, higher Caf concentration had no inhibitory effect on LTB_4 release from AA/CaI stimulated cells. Even though Caf (10 μM) was used as mono-treatment, no difference was observed compared to the control.

6. CONCLUSION

LOXs form a family of lipid peroxidising enzymes. The enzymes are widely distributed in plants and animals. Although LOXs were discovered as plant enzymes, soon the investigation was concentrated on their presence in mammals. The fact that arachidonate 5-LOX is involved in leukotriene metabolism has created new aspects in inflammatory process. Studies on LOXs have lasted over 30 years. However, our knowledge of their structural biology and biological significance is limited. The multiplicity of the isozymes and the interactions of various eicosanoids raise many difficulties. Amongst LOXs, the functions of 5-LOX are better understood thanks to implication of leukotrienes. The enzymes are involved in the generation of lipid mediators such as HETEs, leukotrienes and lipoxins. Some of them have anti-inflammatory and others pro-inflammatory properties. Lipoxins and 15-HETE belong to the former group. On the other hand, leukotrienes act as potent pro-inflammatory mediators. LTB_4 is the best "known" potent mediator. Hence, 5-LOX is strongly associated with inflammatory phenomena. In fact, LOXs are mainly expressed in cells, which are involved in inflammation or anaphylactic reactions. Neutrophils, eosinophils, macrophages, mast cells are some of them. The presence of LTB_4 in diseases such as asthma, rheumatoid arthritis, atopic dermatitis, inflammatory bowel diseases support this. The enzymes are also expressed in epithelia, a location that is of great importance for an organism's immunological defence. At the cellular level, LOXs have many implications. 5-LOX is up-regulated in well differentiated HL60 cells and HaCaT cells. 15-LOX is involved in differentiation and maturation of blood cells. Moreover, 15-LOX can peroxidize membrane lipids and modify cellular structures. The same enzyme can oxidize low-density lipoproteins, a phenomenon associated with atherosclerosis. On the other hand, 5-LOX can bind to cytoskeletal proteins. Moreover, LOXs are associated with tumorigenesis and apoptosis. It is found that LOXs are implicated in prostate malignancy, colon, and breast and lung cancers.

The investigation of eicosanoids in dermatology has seen going on for many years. Because keratinocytes are the predominant cells in the skin, most of the research is focused to this population. Fewer studies have been performed on sebocytes, Langerhans cells, melanocytes. The involvement of LOXs in diseases with keratinocyte hyperproliferation, such as psoriasis is