

$\mu\text{M}$  was observed to inhibit  $\text{LTB}_4$  formation in AA- and CaI-induced sebocytes. The reduction was significant. In contrast, higher Caf concentration had no inhibitory effect on  $\text{LTB}_4$  release from AA/CaI stimulated cells. Even though Caf ( $10 \mu\text{M}$ ) 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.  $\text{LTB}_4$  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  $\text{LTB}_4$  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

well established. Furthermore, eicosanoids are present in atopic dermatitis, an inflammatory skin disorder. Human keratinocytes are capable of producing LTB<sub>4</sub>, 12- and 15-HETE. In vivo, LTB<sub>4</sub> has been demonstrated to induce skin inflammation, and its topical application causes epidermal hyperproliferation. Furthermore, leukotrienes have chemoattractant properties in the skin and they provoke pigmentation. In the field of malignancies, 12-HETE and COX-2 are implicated in skin cancer such as squamous cell carcinoma and sebaceous gland adenoma. As a result eicosanoids are applied in dermatological practice. Hence leukotriene antagonists or 5-LOX inhibitors are used in atopic dermatitis with encouraging results.

A very common disorder of the skin, which affects the majority of human population, is acne. The main characteristic of acne is the overproduction of sebum, the formation of comedones, the bacterial attack and colonization. However the primary cause of acne must be inflammation. Cytokines, and particularly IL-1 $\alpha$ , cause comedonal feature in vivo. Cytokines may initiate an inflammatory process in acne. It has been demonstrated that IL-1 $\alpha$  is increased not only in comedones, but also in uninvolved pilosebaceous follicles of acne patients. In these follicles, a CD+4 T-lymphocytes infiltration as well macrophages are noted. In addition, there is a notable absence of neutrophils and a low number of CD+8 T-lymphocytes (186). These findings suggest that an inflammatory event occurs in early stages of acne. Moreover, the mere presence of microorganisms in acne lesions can not explain the inflammation since bacteria are found in follicles of healthy individuals, too. On the other hand, there is no correlation between levels of any cytokine and quantity of bacteria (197). According to the inflammatory origin of acne, some factors should be responsible for the initiation of the process. Here, the presence of eicosanoids is reasonable.

Eicosanoids are present in skin, but little is known in the field of sebaceous gland disorders. In order to investigate the presence of eicosanoids in sebaceous glands, we have used human cultured immortalized SZ95 sebocytes. SZ95 cell line is a unique tool for investigating the molecular biology in sebaceous gland diseases, since they maintain all the main characteristics of primary sebocytes. The use of a cell line has many advantages, since the culture of primary sebocytes is problematic. As a cell line, a large number of identical cells, is provided and no interference with other cellular populations occurs. In vitro cultured cells simplify used methods. Therefore, protein and mRNA expressions can be better studied through isolation of large amounts of pure protein and mRNA. Cell supernatants can be easily used for ELISA and enzyme immuno-assay. The subcellular localization of the enzymes in cell culture can be better identified. Moreover, sebocytes produce sebum, a natural source of fatty acids, which are LOXs substrates.

We have demonstrated the presence of 5-LOX, LTA<sub>4</sub> hydrolase and 15-LOX-1 in human cultured sebocytes. The enzymes are expressed at protein and mRNA level. It is interesting that 5- and 15-LOX are translocated to the membranes fraction after stimulation with CaI and AA. This occurs within the first 30 minutes of the stimulation. Besides, 5-LOX has been detected by immunocytochemistry. The enzyme is stained at least in the cytosol of SZ95 cells. Well differentiated SZ95 cells are mostly stained. The presence of 5-LOX and LTA<sub>4</sub> hydrolase in sebocytes is evidence that sebaceous glands can utilize fatty acids and convert them into eicosanoids. Fatty acids in sebocytes can be served either as free lipids from the sebum (e.g. linoleic acid), or esterified in cellular membranes in the case of 15-LOX (e.g. arachidonic acid). As a proof of eicosanoid formation in sebocytes, we have determined the 5-LOX activity as LTB<sub>4</sub> generation. SZ95 cells are not only capable of generating LTB<sub>4</sub>, but this formation is upregulated by AA. Thus, sebocytes in specific circumstances may trigger an inflammatory cascade. LTB<sub>4</sub> attracts neutrophils into comedones, independently of the presence of bacteria. It seems that bacterial infection is an epiphenomenon in comedones. Furthermore, LOX stimulators (CaI and AA) induce cytokine production such as IL-6 and IL-8 in sebocytes. SZ95 sebocytes produce IL-1 and TNF- $\alpha$ , too. It might be an evidence of eicosanoids capability to amplify the potential of sebocytes to attract T-cells and macrophages. The existence of two isozymes in human sebocytes is another important point. 5- and 15-LOX have opposing properties. 5-LOX-derived eicosanoids are potent pro-inflammatory mediators. In contrast, 15-HETE and lipoxins derivatives of 15-LOX act as anti-inflammatory molecules. In addition, 15-HETE inhibits 5-LOX activity. This antagonism between the two isoforms has an effect also within sebocytes. 5-LOX expression seems to be stronger in SZ95 cells. Is this further evidence that inflammation does begin from sebocytes in acne? What is actually the role of 15-LOX-2 in sebocytes? Surely, there are critical questions in LOXs implication in sebaceous glands. In our study, we have investigated the arachidonate LOXs in sebocytes. We found that AA induces lipid production in SZ95 sebocytes. We remind the overproduction of sebum in acne.

If eicosanoids in general and LTB<sub>4</sub> in particular, play a significant role in acne, their inhibition can be an alternative therapy for acne patients. We have tested in vitro two different 5-LOX inhibitors in SZ95 sebocytes, caffeic acid and zileuton. Caf has succeeded in significantly blocking LTB<sub>4</sub> production after 5-LOX activation. LTB<sub>4</sub> is reduced 20% by 10  $\mu$ M Caf. On the other hand, extract of zileuton tablets has failed to inhibit LTB<sub>4</sub>. We have tested also the ability of 5-LOX inhibitors to downregulate the lipid formation of SZ95 sebocytes. Here the tested molecules have reduced non-polar lipid accumulation.

Hence, specific 5-LOX inhibitors can reduce the enzyme activity in sebocytes. Therefore, a decrease in LTB<sub>4</sub> is effective in the treatment of acne skin lesions. Moreover, the inhibitors have a directly antilipidic action on sebocytes. If 5-LOX activity is limited in vivo, then the administration of 5-LOX inhibitors would have no effect. In this case the use of LTB<sub>4</sub> antagonists or LTA<sub>4</sub> inhibitors in skin disorders would be more effective. Beyond the classic inhibitors that we have already discussed, an alternative therapy could be the administration of PPAR $\alpha$  agonists. LTB<sub>4</sub> acts as PPAR $\alpha$  ligand. PPAR $\alpha$  induces the transcription of enzymes involved in lipid oxidation. As a result, PPAR $\alpha$  induces the catabolism of LTB<sub>4</sub> to its less potent metabolites (156). In conclusion, the presence of LOXs activity in sebocytes opens a new amazing chapter in the treatment of acne as well as in the theory of inflammatory origin of acne.