Tissue inflammation is a major component of the acne process. Leukotriene B_4 (LTB_4) is considered to be a major player in the development of tissue inflammation. Synthesis of LTB_4 is controlled by the enzyme 5-lipoxygenase. Since Zileuton blocks the activity of 5-lipoxygenase, experimental and clinical studies have been conducted to test mode of function, as well as efficacy and safety of this compound in the treatment of acne vulgaris. Human SZ95 sebocytes and inflammatory cells in vitro express the enzymes of the leukotriene pathway at mRNA and protein levels and enzymes involved in the biosynthesis of LTB_4 are activated in sebaceous glands of acne lesions. Pre-treatment of SZ95 sebocytes with Zileuton partially prevented short-term arachidonic acid-induced effects, such as induction of LTB_4, increase of neutral lipid content and stimulation of interleukin-6 release. Long-term treatment with Zileuton directly reduced the content of neutral lipids and interleukin-6 release from SZ95 sebocytes. PPAR mRNA levels were not regulated by Zileuton. In a first pilot clinical study with 10 patients with papulopustular acne Zileuton 4 x 600 mg/d p.o. for 3 months decreased the acne severity index in a time-dependent manner being 41% of the initial score at week 12 (p < 0.05). This was mostly due to a decrease of the number of inflammatory lesions of 29% (p < 0.01). In addition, total sebum lipids significantly decreased (35%, p < 0.05) and the pro-inflammatory free fatty acids (22%) and lipoperoxides (26%) were markedly diminished in patients' sebum under treatment. The magnitude of clinical improvement strongly correlated with the reduction of total sebum lipids (p = 0.0009, r^2 = 0.81) and free fatty acids (p = 0.0003, r^2 = 0.82). In a further study, a 40-year-old female with mild disseminated sebaceous gland hyperplasia and seborrhea, responded with normalization of the casual skin surface lipids and similar reduction of facial sebum synthesis under treatment with Zileuton over 2 weeks and—after a wash-out phase—low-dose isotretinoin (10 mg/2nd d) over 5 weeks. These data are in agreement with a phase II multicenter, clinical study in 101 patients with mild to moderate inflammatory facial acne conducted in the US, which showed a significant efficacy of Zileuton in a subset of patients with moderate acne, whereas those patients treated with Zileuton showed a significant mean decrease in inflammatory lesions compared to the placebo group. In all clinical studies, Zileuton was found to be safe and well tolerated.

Introduction

Tissue inflammation is a major component in the process of the development of acne lesions. Patients with severe acne have been shown to benefit by a treatment with anti-inflammatory agents. On the other hand, enzymes involved in the biosynthesis of the proinflammatory lipids leukotriene (LT)-B_4 and prostaglandin-E_2 are activated in sebaceous glands of acne lesions. LTB_4 is a 5-lipoxygenase (LOX)/LTA_4 hydrolase product of arachidonic acid (AA) metabolism. AA induces LTB_4 and interleukin (IL)-6 release and enhances lipid synthesis in cultured human sebocytes. Interestingly, LTB_4 is also a natural ligand for peroxisome proliferator-activated receptor (PPAR)-α, PPARs, ligand-activated transcription factors belonging to the nuclear receptor superfamily, are activated by fatty-acid derivatives and can regulate lipid and lipoprotein metabolism, cell proliferation, differentiation and apoptosis of various cell types, including sebaceous gland cells. Especially, PPAR-α can modulate the inflammatory response in various cell types by inhibiting the expression of proinflammatory genes such as cytokines, metalloproteinas and acute-phase proteins.

Since 5-LOX catalyzes LTB_4 production, inhibition of 5-LOX provides an attractive target for downregulation of inflammatory processes in the sebaceous gland. Zileuton [(±)-1-(1-benzo[b]thien-2-ylethyl)-1-hydroxyurea], is an orally active and selective 5-LOX inhibitor been approved in the US for the treatment of asthma (Zyflo®). We have conducted experimental and clinical studies to test mode of function, as well as efficacy and safety of this compound in the treatment of acne vulgaris.

Experimental Study

The maximum non-cytotoxic concentration evaluated for AA was 4 x 10^{-4} M at 24 h, whereas up to 5 x 10^{-5} M Zileuton
Figure 1. Neutral (sebaceous) lipids and cell numbers (A), LTB4 generation (B) and IL-6 release from SZ95 sebocytes (C). (A) SZ95 sebocytes were treated for 14 d with AA (10^{-5} M), Zileuton (5 x 10^{-5} M) or AA (10^{-5} M) + Zileuton (5 x 10^{-5} M). Untreated cultures served as controls. (B) SZ95 sebocytes treated for 13 d as mentioned in panel A were switched for 24 h to following treatments: Control → AA; AA → no treatment; Zileuton → Zileuton + AA; AA + Zileuton → Zileuton. In (A), columns represent the amount of intracellular neutral lipids measured in nile red absolute fluorescence units (AFU) and diamonds represent cell numbers measured in fluorescein diacetate (FDA) AFU. The results are representative of three individual experiments and show the mean ± SD of 6 (A) or 3 (B and C) wells. *p ≤ 0.05, **p < 0.01, ***p ≤ 0.001 (from ref. 22).

Zileuton (Fig. 2). This was already significant by two weeks compared to baseline. The mean reduction in inflammatory lesions was 71% at 12 weeks (95% CI 54–89%; median reduction 75%; p = 0.007). Likewise, the acne severity index was suppressed at 12 weeks (mean suppression 59%, 95% CI 28–75%; median 63%;
Zileuton in acne treatment

There was a trend in reduction of non-inflammatory lesions (mean reduction 36%, 95% CI 6–66% at 12 weeks; median 14%; p = 0.076). Total sebum lipids were also significantly suppressed, showing a mean reduction of 65% at 12 weeks (95% CI 29–100%, median 47%; p = 0.038). Free fatty acids [mean reduction 78% (95% CI 65–90%), median 58%] and hydroperoxides [mean 74% (95% CI 53–95%), median 72%] in sebum were markedly, but not significantly decreased at week 12. LTB₄ levels in blood were not affected by the treatment. The reduction of inflammatory lesions strongly correlated with the reduction of total sebum lipids (p = 0.0009, r² = 0.81) and free fatty acids in sebum (p = 0.0009, r² = 0.81).

In the patient with the disseminated sebaceous gland hyperplasia and seborrhea CSSL were normalized under treatment with Zileuton and NSS decreased (Fig. 3). Six weeks after discontinuation of treatment CSSL were found increased again and average NSS had returned to baseline. Subsequently, low-dose oral Isotretinoin 10 mg/2nd day was
administered over five weeks leading to normalization of NSSL and to decreased NSS after two and five weeks.

Patients, Materials and Methods

Experimental study.22 Immortalized human facial SZ95 sebocytes, been shown to conserve the major characteristics of normal sebocytes25 were cultured in Sebomed® medium with 10% heat-inactivated fetal calf serum, 5 ng/ml human recombinant epidermal growth factor, 1 mM CaCl2 and 50 μg/ml gentamicin at 37°C in a 5% CO2 atmosphere. After leaving the cells to attach for 24 h, the culture medium (and compounds to be tested) was replaced thrice, namely every 2nd day. Cells were subcultured once weekly.

A first panel of SZ95 sebocytes were treated for 14 d with AA (10-9 M), Zileuton (5 x 10-5 M) or AA (10-9 M) + Zileuton (5 x 10-5 M) dissolved in dimethyl sulfoxide (DMSO) with a final DMSO concentration of 0.2% in medium. Untreated cultures in medium supplemented with 0.2% DMSO served as controls. A second panel of identical SZ95 sebocyte cultures were washed twice with phosphate-buffered saline without Ca2+ and Mg2+ (PBS) after 13 d and were subsequently further treated with or without AA and/or Zileuton for 24 h.

For detection of cytotoxicity, SZ95 sebocytes were cultured for 24 h, washed with PBS and treated with AA (10-9 to 10-4 M) or zileuton (10-7 to 5 x 10-5 M). Lactate dehydrogenase (LDH) release was measured in supernatants at 4 and 24 h with a LDH assay kit.13

For cell quantification, SZ95 sebocytes were treated with the compounds mentioned above for 24 h and assessed by the fluorescein diacetate assay (FDA).25 The detection of intracellular lipids was performed in parallel wells with SZ95 sebocytes incubated with 100 μl of a 1 μg/ml nile red solution at 37°C for 20 min.25 For LTB4 detection, SZ95 sebocytes were treated with the compounds mentioned above for 24 h. The culture supernatants were assayed for LTB4 by a colorimetric enzyme immunoassay.22 IL (interleukin)-6 and IL-8 levels were determined in the same supernatants using ELISA kits.

PPAR mRNA levels were assessed by quantitative RT-PCR. SZ95 sebocytes were treated with the compounds mentioned above for 24 h. mRNA was extracted and reverse transcribed to cDNA. The following primers and probes were used: PPARα sense 5'-GTA GCG TAT GGA AAT GGG TTT ATA ACT-3', antisense 5'-CCT TAG GCT TTT TAG GAA TTC ACG A-3'; PPARδ sense 5'-ACC AGG TGA CCC TCA AGT A-3', antisense 5'-GCA TGG CAA AGA TGG CCT-3'; PPARγ1 sense 5'-TCG AGA ACA CCC GAG AGG-3', antisense 5'-TGG TAT ATT GGT GGT TTA GTG TCG G-3'; PPARγ2 sense 5'-GGT TGA ATT ACA GCA AAC CCC TAT T-3', antisense 5'-TCC CAG AGT TTT ACC CAT AAC A-3'. Primers for the L27 ribosomal protein gene and probe serving as internal control were added in the thermal cycling procedure as reference controls.

Clinical studies.23,24 An open-label drug, ethic committee-approved, industry-independent study in a small cohort of 10 consecutive patients (4 female, 6 male; median age 17 years) moderate inflammatory acne was conducted to evaluate efficacy and safety of Zileuton (4 x 600 mg/d p.o. over 3 months).25 All patients provided written consent, had been off all topical and oral acne therapy for at least 1 month, and none had received oral isotretinoin or anabolic steroids. The use of cleansers and personal care products were prohibited during the study. Patients who had atypical disease such as Gram-negative folliculitis were not included, nor were patients with any other disease, pregnant or breast feeding females, any patients taking any other medications or females using any form of hormonal contraception.

The acne was assessed by counting inflammatory lesions, being the primary outcome measure, and non-inflammatory lesions on the entire face and upper chest and back. Acne severity was determined by the method of Allen and Smith.26 Patients were photographed before and at the end of treatment. To obtain sebum samples patients were always invited at 4 p.m., a defined central area of the forehead was wiped twice with ethanol and sebum samples (500 μl) were obtained one hour later, were suspended in 600 μl CHCl/CH3OH 2:1 and were split into aliquots. Total lipids and free fatty acids in sebum were measured by gas chromatography-mass spectrometry, hydroperoxides in sebum by a photometrical method based on the use of N,N-diethyl-1,4-phenylene-diamine after comparison to the reaction of the reference standard compound cumene hydroperoxide, and LTB4 in blood by a commercial LTB4 enzyme immunoassay system. Clinical assessments performed at the same time of the day were made at baseline, 2, 4, 8 and 12 weeks. Laboratory assessments were made at baseline and 12 weeks. Although the study was not double-blind the assessor did not have access to the previous or other visit data until the study was completed.

In another case study, a 40-year-old female with histologically confirmed, mild, disseminated sebaceous gland hyperplasia and seborrhoea on face, scalp and neck since puberty was treated with Zileuton (4 x 600 mg/d o.o. over 3 weeks) after receiving written consent.24 Casual skin surface lipids (CSSL) of the central forehead and the left temple were wiped twice with ethanol after measuring CSSL. Total lipids were measured 1 h later using the device as described above.

Conclusion

The involvement of products of the LT pathway in inflammatory skin diseases is well established.12 5-LOX and the other enzymes which are involved in the LT pathway form a family of lipid peroxidation enzymes and generate lipid mediators.10 On the other hand, LT are leukocyte chemotactic mediators with LTB4 being the most potent among them.4 It increases neutrophil adherence, as well as lysosomal release and generation of superoxide radicals. In addition, it activates complement and induces interleukin production by neutrophils. Intradermal injection of LTB4 provokes the local attraction of neutrophils and monocytes.

There is increasing evidence that acne is a genuine inflammatory disorder.4-6 Since 5-LOX catalyzes the first step in AA metabolism towards LTB4, we have considered the application of 5-LOX
inhibitors as a definitive step for modulation of LT effects in acne patients. Our pilot clinical study with systemic administration of Zileuton in 10 patients with inflammatory acne has detected anti-inflammatory and lipid reducing effects of the compound.23 Searching for the primary effect, we have observed that Zileuton inhibited the synthesis of sebaceous lipids in a patient with no inflammatory signs.24 Inhibition of lipid synthesis, especially of pro-inflammatory lipids, is likely to be the major effect of Zileuton on sebaceous glands followed by reduction of inflammatory lesions in acne.25 The direct effect of Zileuton on the synthesis of sebaceous lipids was confirmed in the in vitro study. This effect was associated with the ability of Zileuton to reduce IL-6 release from SZ95 sebocytes. In addition, pre-treatment with Zileuton partially antagonised the short-term AA-induced induction of LTB4 release, increase of sebaceous lipids, and stimulation of IL-6 release. Zileuton ability to inhibit LTB4 formation in the present study is in agreement with published data on inflammatory cells.21 On the other hand, 5-LOX inhibitors may also down-regulate the inflammatory activity of locally involved lymphocytes and macrophages leading to the anti-inflammatory activity which is clinically observed.

Finally, a phase II multicenter, clinical study in 101 patients with mild to moderate inflammatory facial acne conducted in the US showed a significant efficacy of Zileuton in a subset of patients with moderate acne (baseline inflammatory lesions ≥30), whereas those patients treated with Zileuton (n = 26) showed a mean decrease in inflammatory lesions of 41.6% compared to 26.2% in the placebo group (p = 0.025).28 In all clinical studies, Zileuton was found to be safe and well-tolerated.

In summary, Zileuton directly inhibits lipogenesis in human sebocytes and prevents the activation of the leukotriene pathway by AA. Zileuton inhibited sebum synthesis to a similar level with that of low-dose Isotretinoin.24 This is probably the most intriguing aspect of our work and may seem difficult to explain. However, an effect on PPARs can be implicated.28 LTB4 is a natural ligand for PPAR-α. The latter is shown to regulate lipid and lipoprotein metabolism, inflammatory response, cell proliferation, differentiation and apoptosis of various cell types, including sebaceous gland cells,29 providing a comprehensive link towards the introduction of Zileuton in the treatment of sebaceous gland diseases, and especially of acne.

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