Acta Derm Venereol (Stockh) 1996; 76: 424–428

Cyclosporin A Down-regulates the LTA_4 Hydrolase Level in Human Keratinocyte Cultures

LARS IVERSEN, MORTEN SVENDSEN and KNUD KRAGBALLE

Department of Dermatology, Marselisborg Hospital, University of Aarhus, Aarhus, Denmark

Leukotriene A_4 hydrolase is a key enzyme in the biosynthesis of leukotriene B_4, a potent pro-inflammatory compound. The purpose of this study was to determine the capacity of anti-inflammatory and anti-proliferative compounds to regulate the levels and activity of leukotriene A_4 hydrolase in cultured human keratinocytes. The content of leukotriene A_4 hydrolase was determined by Western blot analysis, and the activity of leukotriene A_4 hydrolase was expressed as the leukotriene B_4 formation after incubation of keratinocyte cultures with leukotriene A_4. Leukotriene B_4 was measured by reversed-phase high performance liquid chromatography. Preincubation for 10 min of the cultured keratinocytes with the leukotriene A_4 hydrolase inhibitor RP 64699 (0.1–10 μM) caused a significant dose-dependent inhibition of leukotriene B_4 formation (IC_{50} = 0.7 μM). Cyclosporin A (0.1 μg/ml and 1.0 μg/ml) had no direct effect on leukotriene A_4 hydrolase activity, but after incubation for 72 h there was a decrease in the mean leukotriene B_4 formation per culture dish (35% and 48%, respectively). The decreased leukotriene B_4 formation was caused mainly by a decrease in the mean leukotriene A_4 hydrolase content per mg protein (30.1% at 0.1 μg/ml cyclosporin A and 40.0% at 1.0 μg/ml cyclosporin A), although keratinocyte proliferation was also slightly decreased. Incubations with 1,25-dihydroxyvitamin D_3 (10^{-7}–10^{-10} M), all-trans retinoic acid (10^{-6}–10^{-10} M), eicosatrienoic acid (10^{-6}–10^{-8} M), dexamethasone (10^{-5}–10^{-7} M), interferon-γ (10 and 100 units/ml) or methotrexate (0.1–10 μg/ml) had no effect on either the leukotriene B_4 formation or the amount of leukotriene A_4 hydrolase in keratinocyte cultures. These results show that cyclosporin A, in contrast to other anti-inflammatory and anti-proliferative compounds, inhibits the level of leukotriene A_4 hydrolase in keratinocyte cultures. Since similar cyclosporin A concentrations are obtained during treatment of psoriasis with cyclosporin A, the effect on leukotriene A_4 hydrolase may play a role in the anti-inflammatory action of cyclosporin A. *Key words: leukotriene B_4; inflammation; psoriasis.*

(Accepted March 28, 1996.)

Acta Derm Venereol (Stockh) 1996; 76: 424–428.

L. Iversen, Department of Dermatology, Marselisborg Hospital, DK-8000 Aarhus C, Denmark.

Leukotriene B_4 (LTB_4) is present in biologically active amounts in both acute guttate lesions (1) and in chronic plaque psoriasis (2, 3). Furthermore, topical application as well as intradermal injections of LTB_4 induce skin inflammation (4, 5) and epidermal hyperproliferation (6). LTA_4 is formed from arachidonic acid (AA) by a two-step reaction of the 5-lipoxygenase enzyme (5-LO) and then further converted into LTB_4 by the LTA_4 hydrolase (7), which is the rate-limiting enzyme in LTB_4 formation (8, 9). While it is controversial whether 5-LO activity is present in the epidermis, we and others have demonstrated significant LTA_4 hydrolase activity in human epidermis (10) and cultured human keratinocytes (11, 12). Keratinocyte LTA_4 hydrolase may play an important role in LTB_4 formation, because transcellular LTB_4 formation, in which LTA_4 is formed in one cell type and converted by the LTA_4 hydrolase in another cell type, has been demonstrated between human neutrophils and cultured human keratinocytes (11, 12) and between human neutrophils and human epidermis *in vitro* (10). Furthermore, the epidermal LTA_4 hydrolase undergoes catalytic inactivation when transforming LTA_4 into LTB_4 (13). Catalytic inactivation of the LTA_4 hydrolase has also been demonstrated to occur in other cell types (14–16). Our recent results (17) have shown a decreased LTA_4 hydrolase activity in involved psoriatic skin compared to uninvolved psoriatic skin. Because of the catalytic inactivation of the enzyme, this observation is compatible with the idea of transcellular LTB_4 formation in psoriatic plaques. The epidermal LTA_4 hydrolase is, therefore, supposed to play a key role in LTB_4 formation in inflammatory skin diseases, and inhibition of this enzyme may have therapeutic implications. Previously, several compounds, including RP 64966 (18), bestatin (10, 19) and captopril (10, 20), have been shown to inhibit the activity of recombinant LTA_4 hydrolase and LTA_4 hydrolase in human neutrophils, human erythrocytes, human epidermis and porcine leukocytes.

The purpose of the present study was to investigate some anti-inflammatory and anti-proliferative compounds for their capacity to regulate the activity and/or the content of LTA_4 hydrolase in cultured human keratinocytes. The novel compound RP 64699 (18) inhibited the LTA_4 hydrolase activity, and cyclosporin A (CsA) down-regulated the amount of the keratinocyte LTA_4 hydrolase at concentrations similar to those found in the epidermis during CsA treatment of psoriasis.

MATERIAL AND METHODS

Materials

The LTA_4-methyl ester and the authentic LTB_4 were obtained from Cascade Biochem Limited, Reading, UK. The organic solvents were all HPLC-grade from Merck, Darmstadt, Germany. Octadecylsilyl C18-SEP-PAK catridges were from Waters (USA). Human serum albumin was obtained from Nordisk Gentofte, Bagsvaerd, Denmark. Culture dishes, RPMI 1640 medium and the low-calcium, fatty acid and serum free keratinocyte growth medium were from Life Technologies, European Division, Paisley, UK. Fetal calf serum was from AH Diagnostics, Aarhus, Denmark. The protein assay kit was purchased from Bio-Rad, Munich, Germany. Hybond enhanced chemiluminescence (ECL) nitrocellulose-membrane, ECL detection system and hyperfilm-ECL were all obtained from Amersham, Denmark. Trypsin, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) methotrexate, all-trans retinoic acid and dexamethasone were purchased from Sigma Chemical, St. Louis, USA. 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2$ D_3) was a kind gift from LEO, Ballerup, Denmark. CsA was obtained from Sandoz AS, Denmark. Eicosatrienoic acid was from Cayman Chemical, European Division, Paris, France. Human IFN-γ was obtained from R&D Systems Europe

 ISSN 0001-5555

Ltd., Abingdon, UK. Recombinant LTA_4 hydrolase and affinity purified rabbit anti LTA_4 hydrolase IgG antibody was a kind gift from Professor Takao Shimizu, Tokyo University, Japan. The LTA_4 hydrolase inhibitor RP 64966 was a kind gift from Dr. Norbert Dereu, Rhone-Poulenc Rorer, France.

Cultures of human keratinocytes

Human keratinocytes were cultured in serum-free keratinocyte growth medium as previously described (12). At subconfluence first passage cell cultures were incubated with 1,25-$(OH)_2$ D_3 (10^{-7}–10^{-10} M), all-trans retinoic acid (10^{-6}–10^{-10} M), eicosatrienoic acid (10^{-6}–10^{-8} M), dexamethasone (10^{-5}–10^{-7} M), IFN-γ (10 and 100 units/ml), RP 64966 (10^{-5}–10^{-7} M), methotrexate (0.1–10 μg/ml) or CsA (0.1 and 1.0 μg/ml) for 24 or 72 h. Then, incubation with LTA_4 for determination of the LTA_4 hydrolase activity was carried out (see below) or the keratinocytes were scraped off in Tris-HCl buffer (pH 7.4) for quantitation of LTA_4 hydrolase.

Detection and quantitation of LTA_4 hydrolase by Western blot analysis

The released keratinocytes were sonicated 3×10 s on ice and then centrifuged $8,000 \times g$ for 15 min. The protein content in the supernatant was determined according to Bradford (21). The method used for quantitation of LTA_4 hydrolase was Western blot analysis followed by immunological detection using an affinity purified antibody. This method has previously been described in detail (17).

LTA_4 hydrolase activity

LTA_4-free acid was prepared from LTA_4-methyl ester, as previously described (12, 22), and the concentration was estimated by UV-absorption at 280 nm. Subconfluent keratinocytes grown with the various compounds (see above) were equilibrated in the culture dishes in 3 ml of keratinocyte growth medium supplemented with 1 mg/ml human serum albumin for 10 min at 37°C before incubation with LTA_4 (60 nmol) for 5 min at 37°C. In separate experiments preincubation with RP 64699 A (0.1, 1.0, 10 μM) or CsA (0.1, 1.0 μg/ml) was carried out for 10 min before incubation with LTA_4. All incubations were terminated by the addition of 2 vol of ice-cold MeOH. Because LTA_4 was dissolved in a mixture of EtOH/NaOH, a pH of 7.8 was obtained in the incubation medium while LTA_4 was added.

Lipid extraction and reversed-phase high performance liquid chromatography (RP-HPLC)

The reaction mixture was kept at −20°C for 20 min. Cells were released from the culture dishes by scraping and then centrifuged ($1,500 \times g$) for 10 min at 4°C to precipitate cellular debris and denatured protein. Lipids were then extracted from the collected supernatant as previously described (23). Identification of LTB_4 was by chromatographic comparison to authentic LTB_4 and by characteristic UV absorption (12).

Authentic LTB_4 was used to construct a calibration curve expressing the absorption as a function of the amount of standard LTB_4. Quantification of the LTB_4 was by integrated optical density and comparison to the calibration curve. Because incubations were carried out in the culture dishes, LTB_4 formation was expressed as ng LTB_4/culture dish/5 min.

Cell viability and proliferation

MTT (tetrazolium) assay. The tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) is used in a colorimetric assay for determination of living cells. MTT is cleaved in active mitochondria and can therefore be used to measure cytotoxicity and proliferation (24). Briefly, keratinocytes were grown in 96-well plates and then incubated for either 24 or 72 h with the various inhibitors investigated. Then 25 μl MTT stock solution (5 mg/ml in phosphate-buffered saline) per well was added to culture medium. The plates were shaken for 5 min at room temperature and then incubated for 4 h at 37°C before the medium was removed. Then the plates were frozen at −20°C for 30 min and thawed for 30 min at room temperature. Finally, 0.2 ml fixative (ethanol/acetone, 60/40, v/v) per well was added and the plate was shaken for 30 min at 4°C before the optical density was measured at 570 nm using an ELISA reader. A reference wavelength of 630 nm was also measured.

Trypan blue exclusion. Keratinocytes were grown in culture dishes and then incubated for 24 or 72 h with CsA (0.1, 1.0 μg/ml) or RP 64699 (10^{-5}–10^{-7} M). To assess cell viability incubations with trypan blue were carried out for 12 min. Then cells were counted under the microscope in three different fields in each culture dish. The total number of cells counted per field were 100.

Statistical methods

Results were expressed as mean ± standard error of the mean (SEM). Statistical significance was assessed by Wilcoxon's rank sum test.

RESULTS

The LTA_4 hydrolase activity in keratinocyte cultures, expressed as ng LTB_4 formed/culture dish/5 min, was 63.7 ± 7.6 (mean ± SEM, $n=6$). Preincubation of cultured keratinocytes for 10 min with RP 64966, a known inhibitor of LTA_4 hydrolase activity, resulted in a significant and dose-dependent inhibition of the conversion of LTA_4 into LTB_4 (Fig. 1). CsA had no direct effect on the LTA_4 hydrolase activity (Fig. 1). However, incubation for 72 h resulted in a decrease in the mean LTB_4 formation of 35% at 0.1 μg/ml CsA ($p<0.05$ vs. control) and 48% at 1.0 μg/ml CsA ($p<0.05$ vs. control) (Fig. 2). Also, keratinocyte proliferation was inhibited by CsA, although to a smaller degree than LTB_4 formation (Fig. 2).

To determine whether the reduced conversion of LTA_4 into LTB_4 was due to an inhibition of the activity or the amount of LTA_4 hydrolase, Western analysis was performed. Analysis of protein extracts from keratinocyte cultures with an affinity

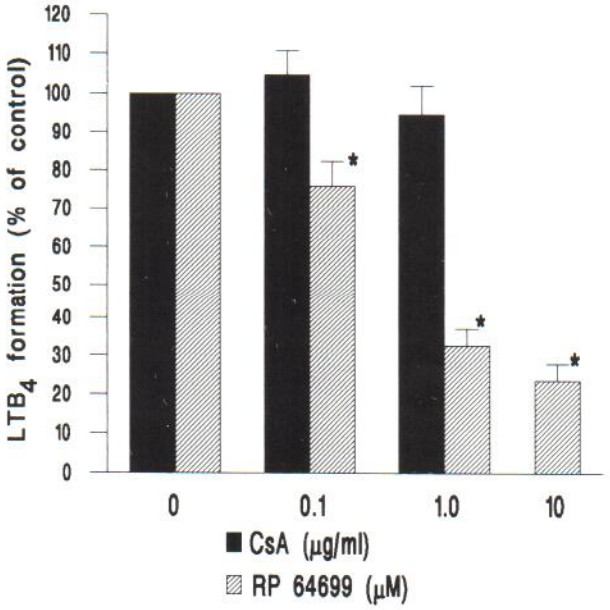

Fig. 1. Formation of LTB_4 by subconfluent keratinocytes preincubated for 10 min with cyclosporin A (CsA) (0.1, 1.0 μg/ml) or RP 64699 (0.1, 1.0, 10 μM) in keratinocyte growth medium supplemented with human serum albumin (1 mg/ml) at 37°C. Controls contained vehicle (ethanol) of CsA or RP 64699. Incubations were carried out with LTA_4 (60 nmol) for 5 min at 37°C. A LTA_4 hydrolase activity of 100% corresponds to 63.7 ± 7.6 ng LTB_4/culture dish/5 min. Results are expressed as the percentage of control (mean ± SEM, $n=6$). * = $p<0.05$.

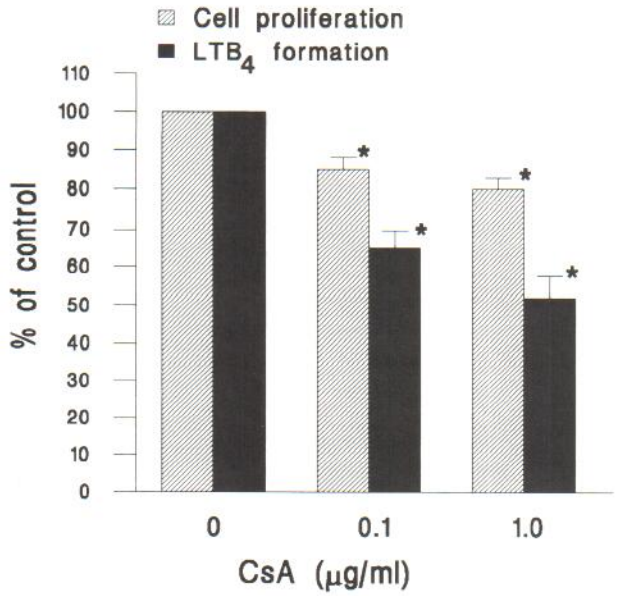

Fig. 2. Effect of cyclosporin A (CsA) on cell proliferation and LTB_4 formation after incubation of subconfluent keratinocyte cultures with CsA (0.1, 1.0 µg/ml) or vehicle (ethanol) of CsA for 72 h. LTB_4 formation was determined by incubating keratinocyte cultures with LTA_4 (60 nmol) for 5 min at 37°C. A LTB_4 formation of 100% corresponds to 63.7 ± 7.6 ng LTB_4/culture dish/5 min. Cell proliferation was assessed by the MTT (tetrazolium) assay. Results are expressed as the percentage of controls (mean ± SEM, $n=6$). * = $p < 0.05$.

purified anti-LTA_4 hydrolase antibody showed a single band (Fig. 3). Incubation of keratinocyte cultures with CsA for 24 h did not change the amount of LTA_4 hydrolase (Fig. 3), but after 72 h there was a dose-dependent decrease in the amount

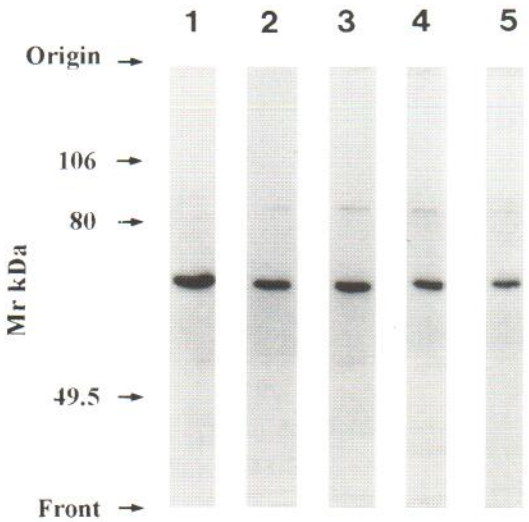

Fig. 3. Representative immunoblots of keratinocyte cultures determined with an affinity purified LTA_4 hydrolase antibody. Keratinocytes were incubated with cyclosporin A (CsA) for 24 or 72 h. Proteins (15 µg) separated on SDS-PAGE (9% polyacrylamide gel) were transferred to a nitrocellulose membrane by electroblotting, and the LTA_4 hydrolase antibody was used for immunodetection. *Lane 1*: control, *lane 2*: 0.1 µg/ml CsA for 24 h, *lane 3*: 1 µg/ml CsA for 24 h, *lane 4*: 0.1 µg/ml CsA for 72 h, *lane 5*: 1.0 µg/ml CsA for 72 h.

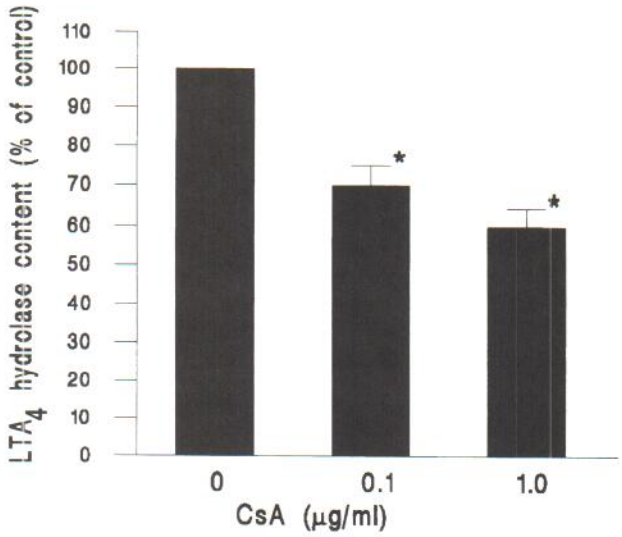

Fig. 4. LTA_4 hydrolase content in subconfluent cultured keratinocytes incubated for 72 h with cyclosporin A (CsA) (0.1, 1.0 µg/ml) or vehicle (ethanol) of CsA. The total protein concentration was measured and the LTA_4 hydrolase content was expressed in µg LTA_4 hydrolase/mg total protein. The LTA_4 hydrolase was detected by Western analysis and quantified by densitometry. One hundred per cent LTA_4 hydrolase corresponds to 2.3 ± 0.4 µg/mg protein. Results are expressed as the percentage of controls (mean ± SEM, $n=4$). * = $p < 0.05$.

of LTA_4 hydrolase per mg protein (30.1% at 0.1 µg/ml and 40.0% at 1.0 µg/ml) (Figs. 3 and 4). As assessed by trypan blue exclusion, the decreased amount of LTA_4 hydrolase was not due to a cytotoxic effect of CsA. The percentage of viable cells was 98 % ± 1, 97% ± 1 and 98% ± 1 after 72 h incubation with vehicle, 0.1 µg/ml CsA and 1.0 µg/ml CsA, respectively (mean ± SEM, $n=4$). Also, the decrease in keratinocyte proliferation cannot explain the reduced LTA_4 hydrolase content, because this was expressed per mg protein.

Normal levels of LTA_4 hydrolase were found in cultures treated with other anti-inflammatory and anti-proliferative compounds. Thus, incubations of keratinocyte cultures with 1,25 $(OH)_2$ D_3 (10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} M), all-trans retinoic acid (10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} M), eicosatrienoic acid (10^{-6}, 10^{-7}, 10^{-8} M), dexamethasone (10^{-5}, 10^{-6}, 10^{-7} M), IFN-γ (10 and 100 units/ml) or methotrexate (0.1, 1.0, 10 µg/ml) for 24 or 72 h did not result in any significant changes in the amount of LTA_4 hydrolase per mg protein (data not shown). Furthermore, the activity of the LTA_4 hydrolase (ng LTB_4/culture dish) was not changed after 24 h incubation with these compounds (data not shown).

DISCUSSION

This study shows that non-toxic concentrations of CsA inhibit the capacity of cultured keratinocytes to form LTB_4 by inhibiting the amount of the LTA_4 hydrolase enzyme, whereas CsA has no direct effect on LTA_4 hydrolase activity. The decrease in LTA_4 hydrolase was expressed per mg protein and, therefore, not secondary to the decreased keratinocyte proliferation induced by CsA. Accordingly, the anti-proliferative compounds 1,25-$(OH)_2$ D_3, all-trans retinoic acid, dexamethasone and methotrexate did not change the LTA_4 hydrolase levels in

keratinocyte cultures. The effect of CsA on LTA_4 hydrolase levels was only detectable after prolonged incubation, but it remains to be determined whether it is caused by decreased synthesis and/or accelerated degradation of the enzyme. At a CsA concentration of 1.0 μg/ml the LTA_4 hydrolase content was inhibited by 40%. It is, therefore, of interest that treatment with CsA 14 mg/kg body weight/day results in a CsA concentration of 2.8 μg/ml in involved psoriatic epidermis (25). Although lower CsA doses (2.5–5.0 mg/kg/day) are usually used for the treatment of psoriasis, these results indicate that the tissue concentrations of CsA obtained during CsA treatment can inhibit the levels of keratinocyte LTA_4 hydrolase. Accordingly, treatment with CsA 14 mg/kg body weight/day for 7 days decreases the LTB_4 content by 64% in lesional psoriasis (26). Taken together, these findings are compatible with the idea that CsA may owe part of its anti-psoriatic effect to a decrease of LTA_4 hydrolase levels and thereby the LTB_4 synthesis in the epidermis. Even though a slight inhibition of keratinocyte LTA_4 hydrolase levels was detected at a CsA concentration of only 0.1 μg/ml, lymphocytes are apparently more sensitive to CsA (27, 28). Therefore our results do not argue against the notion that CsA acts primarily as an immunosuppressant in psoriasis.

RP 64699 has previously been demonstrated to be a LTA_4 hydrolase inhibitor in porcine leukocytes, with an IC_{50} of 1.5 μM (18). This is in accordance with our finding of an IC_{50} of 0.7 μM in cultured keratinocytes. It was also demonstrated that incubation with 1.0 and 10 μM RP 64699 for 24 h resulted in a marked decrease in cell viability and proliferation, as evaluated by trypan blue exclusion and the MTT assay (data not shown). Therefore, RP 64699 appears not to be a candidate for the treatment of inflammatory skin diseases.

All other compounds tested in this study did not modulate the expression or activity of keratinocyte LTA_4 hydrolase. The lack of effect of all-trans retinoic acid on the LTA_4 hydrolase activity after 24 h incubation is in accordance with a previous study by Hamasaki et al. (29) who showed that RA induced LTC_4 synthase activity, but not LTA_4 hydrolase activity in rat basophilic leukemia-1 cells. Methotrexate has previously been suggested to suppress neutrophil LTA_4 hydrolase activity (30, 31). The inhibitory effect of methotrexate in neutrophils was demonstrated in freshly isolated neutrophils obtained from patients with rheumatoid arthritis treated with a single dose of methotrexate. $1,25(OH)_2$-vitamin D_3 can in combination with transforming growth factor β1 upregulate the 5-LO activity and expression in the human monocytic cell line Mono Mac 6, while $1,25(OH)_2$-vitamin D_3 alone only had a minor effect (32). In the present study, $1,25(OH)_2$-vitamin D_3 had no effect on either the amount or the activity of LTA_4 hydrolase. Dexamethasone has been shown to inhibit LTA_4 hydrolase activity in differentiated HL-60 cells, but not in nondifferentiated HL-60 cells (33). This difference between differentiated and nondifferentiated cells may explain the lack of effect of dexamethasone in subconfluent keratinocyte cultures, which mainly consist of undifferentiated cells. IFN-γ failed to modulate the LTA_4 hydrolase activity and content in cultured keratinocytes, which is in contrast to endothelial cells. After pretreatment for 72 h IFN-γ (100 U/ml) increased their LTA_4 hydrolase activity by approximately 100% (34). Dietary supplementation with eicosapentaenoic acid and eicosatrienoic acid has also been shown to inhibit LTB_4 formation in human and rat neutrophils. An inhibition of the LTA_4 hydrolase has been suggested to be their mechanism of action (35, 36). This inhibition is probably mediated via the two 5-LO products of eicosapentaenoic acid and eicosatrienoic acid, LTA_5 and LTA_3, by covalent coupling to the LTA_4 hydrolase (37-39). The absence of detectable 5-LO activity in undifferentiated cultured keratinocytes (11, 12, 40) may explain why eicosatrienoic acid had no effect on keratinocyte LTA_4 hydrolase.

The anti-psoriatic effect of CsA is believed to be a result of immuno-suppression. In the present study CsA, in contrast to other anti-inflammatory and anti-proliferative compounds, inhibited keratinocyte LTA_4 hydrolase at doses obtained in the epidermis during treatment of psoriasis. Since LTA_4 hydrolase activity results in the formation of pro-inflammatory LTB_4, part of the anti-psoriatic effect of CsA may be due to an anti-inflammatory effect.

REFERENCES

1. Fogh K, Herlin T, Kragballe K. Eicosanoids in acute and chronic psoriatic lesions. Leukotriene B_4, but not 12-hydroxy-eicosatetraenoic acid is present in biologically active concentrations in acute and guttate lesions. J Invest Dermatol 1989; 92: 837–841.
2. Brain S, Camp R, Dowd P, Black AK, Greaves M. The release of leukotriene B_4-like material in biologically active amounts from the lesional skin of patients with psoriasis. J Invest Dermatol 1984; 83: 70–73.
3. Grabbe J, Czarnetzki BM, Rosenbach T, Mardin M. Identification of chemotactic lipoxygenase products of arachidonate metabolism in psoriatic skin. J Invest Dermatol 1984; 82: 477–479.
4. Camp RDR, Coutts AA, Greaves M, Kay AB, Walport MJ. Responses of human skin to intradermal injection of leukotrienes C_4, D_4 and B_4. Br J Pharmacol 1983; 80: 497–502.
5. Soter NA, Lewis RA, Corey EJ, Austen KF. Local effects of synthetic leukotrienes (LTC_4, LTD_4, LTE_4 and LTB_4) in human skin. J Invest Dermatol 1983; 80: 115–119.
6. Chan CC, Duhamel L, Ford-Hutchinson A. Leukotriene B_4 and 12-hydroxyeicosatetraenoic acid stimulate proliferation in vivo in the guinea pig. J Invest Dermatol 1985; 85: 333–334.
7. Borgeat P, Samuelsson B. Arachidonic acid metabolism in polymorphonuclear leukocytes: effects of ionophore A23187. Proc Natl Acad Sci 1979; 76: 2148–2152.
8. Sun FF, McGuire JC. Metabolism of arachidonic acid by human neutrophils. Characterization of the enzymatic reactions that lead to the synthesis of leukotriene B_4. Biochim Biophys Acta 1984; 794: 56–64.
9. Jakschik BA, Kuo CG. Characterization of leukotriene A_4 and B_4 biosynthesis. Prostaglandins 1983; 25: 767–782.
10. Iversen L, Ziboh VA, Shimizu T, Ohishi N, Rådmark O, Wetterholm A, et al. Identification and subcellular localization of leukotriene A_4 hydrolase activity in human epidermis. J Dermatol Sci 7; 191–201: 1994.
11. Sola J, Godessart N, Vila L, Puig L, Moragas JM de. Epidermal cell-polymorphonuclear leukocyte cooperation in the formation of leukotriene B_4 by transcellular biosynthesis. J Invest Dermatol 1992; 98: 333–339.
12. Iversen L, Fogh K, Ziboh VA, Kristensen P, Schmedes A, Kragballe K. Leukotriene B_4 formation during human neutrophil keratinocyte interactions: evidence for transformation of leukotriene A_4 by putative keratinocyte leukotriene A_4 hydrolase. J Invest Dermatol 1993; 100: 293–298.
13. Iversen L, Kristensen P, Nissen JB, Merrick WC, Kragballe K. Purification and characterization of leukotriene A_4 hydrolase from human epidermis. FEBS Lett 1995; 358: 316–322.
14. McGee J, Fitzpatrick F. Enzymatic hydration of leukotriene A_4.

Purification and characterization of a novel epoxide hydrolase from human erythrocytes. J Biol Chem 1985; 260: 12832–12837.
15. Ohishi N, Izumi T, Minami M, Kitamura S, Seyama Y, Ohkawa S, et al. Leukotriene A_4 hydrolase in the human lung. Inactivation of the enzyme with leukotriene A_4 isomers. J Biol Chem 1987; 262: 10200–10205.
16. Haeggström J, Bergman T, Jörnvall H, Rådmark O. Guinea-pig liver leukotriene A_4 hydrolase. Purification, characterization and structural properties. Eur J Biochem 1988; 174: 717–724.
17. Iversen L, Deleuran B, Hoberg AM, Kragballe K. LTA_4 hydrolase in human skin: decreased activity, but normal concentration in lesional psoriatic skin. Evidence for different LTA_4 hydrolase activity in human lymphocytes and human skin. Arch Dermatol Res 1996; 288: 217–224.
18. Labaudinière R, Hilboll G, Leon-lomeli A, Lautenschläger HH, Parnham M, Kuhl P, Dereu N. ω-[(ω-Arylalkyl)aryl]alkanoic acids: a new class of specific LTA_4 hydrolase inhibitors. J Med Chem 1992; 35: 3156–3169.
19. Orning L, Krivi G, Fitzpatric FA. Leukotriene A_4 hydrolase. Inhibition by bestatin and intrinsic aminopeptidase activity establish its functional resemblance to metallohydrolase enzymes. J Biol Chem 1991; 266: 1375–1378.
20. Orning L, Krivi G, Bild G, Gierse J, Aykent S, Fitzpatrick FA. Inhibition of leukotriene A_4 hydrolase/aminopeptidase by captopril. J Biol Chem 1991; 266: 16507–16511.
21. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dey binding. Anal Biochem 72; 248–254: 1976.
22. Maycock AL, Anderson MS, DeSousa DM, Kuehl FA Jr. Leukotriene A_4: preparation and enzymatic conversion in a cell-free system to leukotriene B_4. J Biol Chem 1982; 257: 13911–13914.
23. Fogh K, Herlin T, Kragballe K. In vitro inhibition of leukotriene B_4 formation by exogeneous 5-lipoxygenase inhibitors is associated with enhanced generation of 15-hydroxyeicosatetraenoic acid (15-HETE) by human neutrophils. Arch Dermatol Res 1988; 280: 430–436.
24. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. J Immunol Methods 1983; 65: 55–63.
25. Fisher GJ, Duell EA, Nickoloff BJ, Annesley TM, Kowalke JK, Ellis CN, et al. Levels of cyclosporine in epidermis of treated psoriasis patients differentially inhibit growth of keratinocytes cultured in serum free versus serum containing media. J Invest Dermatol 1988; 91: 142–146.
26. Ellis CN, Gorsulowsky DC, Hamilton TA, Billings JK, Brown MD, Headington JT, et al. Cyclosporine improves psoriasis in a double-blind study. JAMA 1986; 256: 3110–3116.
27. Cooper KD, Baadsgaard O, Ellis CN, Duell E, Voorhees JJ. Mechanisms of cyclosporin A inhibition of antigen presenting activity in uninvolved and lesional psoriatic epidermis. J Invest Dermatol 1990; 94: 649–656.
28. Khandke L, Krane JF, Ashinoff R, Staiano-Coico L, Granelli-Piperno A, Luster AD, et al. Cyclosporine in psoriasis treatment. Inhibition of keratinocyte cell-cycle progression in G_1 independent of effects on transforming growth factor α/epidermal growth factor receptor pathways. Arch Dermatol 1991; 127: 1172–1179.
29. Hamasaki Y, Abe M, Matsumoto S, Ichimaru T, Hara N, Miyazaki S. Specific induction of LTC_4 synthase by retinoic acid in rat basophilic leukemia-1 cells. Int Arch Allergy Immunol 1994; 103: 260–265.
30. Leroux JL, Damon M, Chavis C, De Paulet AC, Blotman F. Effects of a single dose of methotrexate on 5- and 12-lipoxygenase products in patients with rheumatoid arthritis. J Rheumatol 1992; 19: 863–866.
31. Sperling RI, Coblyn JS, Larkin JK, Benincaso AI, Austen KF, Weinblatt ME. Inhibition of leukotriene B_4 synthesis in neutrophils from patients with rheumatoid arthritis by a single oral dose of methotrexate. Arthritis Rheum 1990; 33: 1149–1155.
32. Brungs M, Rådmark O, Samuelsson B, Steinhilber D. Sequential induction of 5-lipoxygenase gene expression and activity in Mono Mac 6 cells by transforming growth factor β and 1,25-dihydroxyvitamin D_3. Proc Natl Acad Sci USA 1995; 92: 107–111.
33. Rådmark O, Medina JF, Macchia L, Barrios C, Funk CD, Larsson O, et al. Leukotriene A_4 hydrolase in two human cell lines. Adv Prostaglandin, Thromboxane and Leukotriene Res 1990; 21: 41–44.
34. Renkonen R, Ustinov J. Interferon-gamma augments hydrolysis of LTA_4 to LTB_4 by endothelial cells. Prostaglandins 1990; 39: 205–211.
35. James MJ, Gibson RA, Neumann MA, Cleland LG. Effect of dietary supplementation with n-9 eicosatrienoic acid on leukotriene B_4 synthesis in rats: a novel approach to inhibition of eicosanoid synthesis. J Exp Med 1993; 178: 2261–2265.
36. Sperling RI, Benincaso AI, Knoell CT, Larkin JK, Austen KF, Robinson DR. Dietary ω-3 polyunsaturated fatty acids inhibit phosphoinositide formation and chemotaxis in neutrophils. J Clin Invest 1993; 91: 651–660.
37. Evans JF, Nathaniel DJ, Zamboni RJ, Ford-Hutchinson AW. Leukotriene A_3. A poor substrate but a potent inhibitor of rat and human neutrophil leukotriene A_4 hydrolase. J Biol Chem 1985; 260: 10966–10970.
38. Rådmark O, Haeggström J. Properties of leukotriene A_4-hydrolase. Adv Prostaglandin, Thromboxane and Leukotriene Res 1990; 20: 35–45.
39. Nathaniel DJ, Evans JF, Leblanc Y, Léveillé C, Fitzsimmons BJ, Ford-Hutchinson AW. Leukotriene A_5 is a substrate and an inhibitor of rat and human neutrophil LTA_4 hydrolase. Biochem Biophys Res Commun 1985; 131: 827–835.
40. Janssen-Timmen U, Vickers PJ, Wittig U, Lehmann WD, Stark HJ, Fusenig NE, et al. Expression of 5-lipoxygenase in differentiating human skin keratinocytes. Proc Natl Acad Sci USA 1995; 92: 6966–6970.