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Modulation of Eicosanoid Formation by Lesional Skin of Psoriasis: An *Ex vivo* Skin Model

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The purpose of the present study was to develop an *ex vivo* skin model to determine the capacity of lesional skin of psoriasis to form leukotriene B_4 and other eicosanoids. Keratomed skin samples were incubated in the presence of the calcium ionophore A23187 and arachidonic acid for 45 min at 37°C. After extraction of lipids, eicosanoids were determined by quantitative reversed-phase high-performance liquid chromatography in combination with specific radioimmunoassays. We found that stimulation of skin samples with A23187 and arachidonic acid increased the amount of leukotriene B_4 4.0-fold. The 12-lipoxygenase product, 12-hydroxy-eicosatetraenoic acid, and the 15-lipoxygenase product, 15-hydroxy-eicosatetraenoic acid, were both increased 2.7-fold. The cyclooxygenase product, prostaglandin E_2, was increased 8.0-fold. Similar incubations using psoriatic scales did not result in formation of eicosanoids. Incubations with the 5-lipoxygenase inhibitor RS43179 inhibited the formation of leukotriene B_4 and prostaglandin E_2 without significantly affecting the formation of 12-hydroxy-eicosatetraenoic acid and 15-hydroxy-eicosatetraenoic acid. These results reveal that lesional psoriatic skin *ex vivo* has the enzymatic capacity to increase the levels of eicosanoids. This provides an *ex vivo* skin model to determine whether putative lipoxygenase inhibitors are able to modulate the formation of eicosanoids in psoriatic skin. *Key word: 5-lipoxygenase inhibition.*

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Eicosanoids formed as enzymatic products of arachidonic acid (AA), leukotriene B_4 (LTB_4), 12-hydroxy-eicosatetraenoic acid (12-HETE) and prostaglandin E_2 (PGE_2) are potent mediators of cutaneous inflammation (1–6). These eicosanoids have been found in biologically active amounts in scales and samples of lesional psoriatic skin (7–10). The lipoxygenase (LO) products LTB_4 and 12-HETE can induce epidermal neutrophil accumulation and epidermal hyperproliferation after topical application (1, 4, 6). The cyclooxygenase (CO) product PGE_2 is able to cause vascular dilatation and has the capacity to amplify the inflammatory effect of other mediators such as LTB_4 (3). On the other hand 15-hydroxy-eicosatetraenoic acid (15-HETE), a 15-LO product of AA also present in psoriatic skin (9, 10), has the capacity to inhibit the formation of LTB_4 and 12-HETE (11, 12) and the chemotactic effect of LTB_4 (13). Intralesional injections of 15-HETE have been shown to improve psoriasis (14) and experimental arthritis in dogs associated with an inhibition of LTB_4 formation (15). These results show that 15-HETE may be an important regulator of inflammation involving LTB_4. The cellular source of eicosanoids in psoriatic skin has not yet been established. It has been shown that enzyme preparations of psoriatic skin and suspensions of normal epidermal keratinocytes have the capacity to form LTB_4, PGE_2, 12-HETE and 15-HETE (16–18). Therefore, it would be of interest to know whether lesional psoriatic skin has the capacity to form these eicosanoids. In the present study, we used keratomed slices of lesional psoriatic skin to determine its capacity to form eicosanoids *ex vivo*.

MATERIALS AND METHODS

Keratome biopsies

Scales and keratome biopsy specimens were obtained from patients with stable psoriasis vulgaris as previously described in detail (10). Briefly, scales were gently removed and skin biopsy specimens were obtained by tangential shaving of the lesional skin using a motordriven keratome. The keratome was set to obtain biopsies measuring 0.2 mm in thickness. All samples were immediately placed in icecold RPMI 1640 and kept on ice until analysis.

Incubation procedure and lipid extraction

Before incubation, the keratome specimens were chopped into 2–3 mm^2 pieces, weighed and mixed thoroughly. Fifty mg samples in duplicate were incubated in 3 ml RPMI 1640 using a shaking water bath. Control samples were 1) extracted without incubation and without addition of stimuli or inhibitor, 2) incubated without stimuli for 45 min at 0°C and 3) incubated without stimuli for 45 min at 37°C. Stimulated samples were pre-incubated in the presence of AA (25 μM, final concentration) and/or RS43179 (Lonapalene, Syntex; 25 μM, final concentration) for 5 min at 37°C. Then A23187 (10 μM, final concentration) was added and incubation carried out for 45 min at

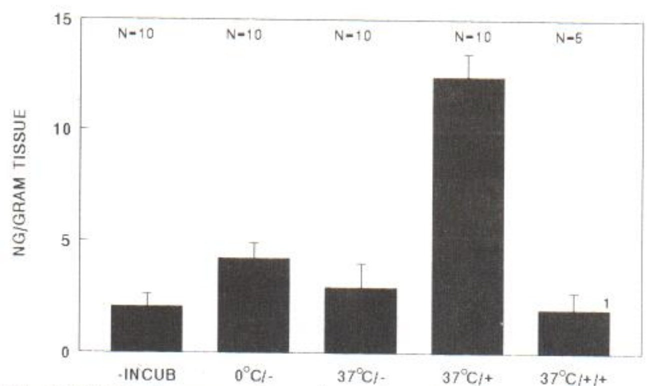

Fig. 1. LTB_4 levels expressed as ng/gram tissue (mean ± standard error of the mean (SEM) of N experiments assayed in duplicate). −INCUB denotes samples extracted without incubation and without addition of stimuli. 0°C/− denotes incubation without stimuli for 45 min at 0°C. 37°C/− denotes incubation without stimuli for 45 min at 37°C. 37°C/+ denotes incubation for 45 min at 37°C in the presence of A23187 (10 μM) and AA (25 μM). 37°C/+/+ denotes incubation for 45 min at 37°C in the presence of A23187 (10 μM), AA (25 μM) and RS43179 (25 μM). 1: 37°C/+ vs 37°C/+/+: $p < 0.05$. LTB_4 was determined by RIA in fractions co-eluting with authentic LTB_4 on RP-HPLC.

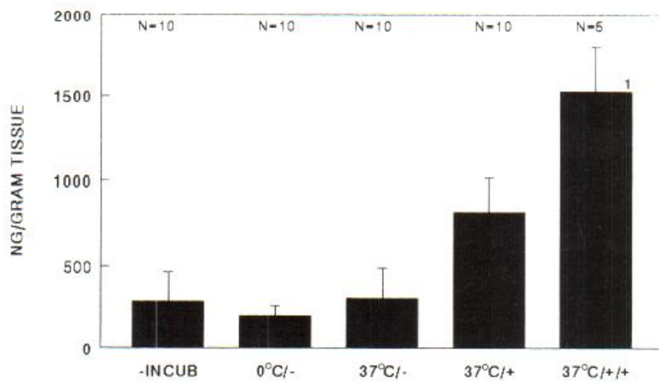

Fig. 2. 12-HETE levels expressed as ng/gram tissue (mean ± standard error of the mean (SEM) of N experiments assayed in duplicate). −INCUB denotes samples extracted without incubation and without addition of stimuli. 0°C/− denotes incubation without stimuli for 45 min at 0°C. 37°C/− denotes incubation without stimuli for 45 min at 37°C. 37°C/+ denotes incubation for 45 min at 37°C in the presence of A23187 (10 μM) and AA (25 μM). 37°C/+/+ denotes incubation for 45 min at 37°C in the presence of A23187 (10 μM), AA (25 μM) and RS43179 (25 μM). 1: 37°C/+ vs 37°C/+/+: $0.05 < p < 0.1$. 12-HETE was quantified by integrated optical density during RP-HPLC.

37°C. All reactions were terminated by the addition of 2 volumes of icecold methanol. Homogenization was performed and lipids were extracted on octadecylsilyl (ODS) silica columns and eluted exactly as described previously (10). The methanol fraction containing arachidonic acid metabolites was taken to dryness under a stream of N_2 and resuspended in 100 μl 70% methanol.

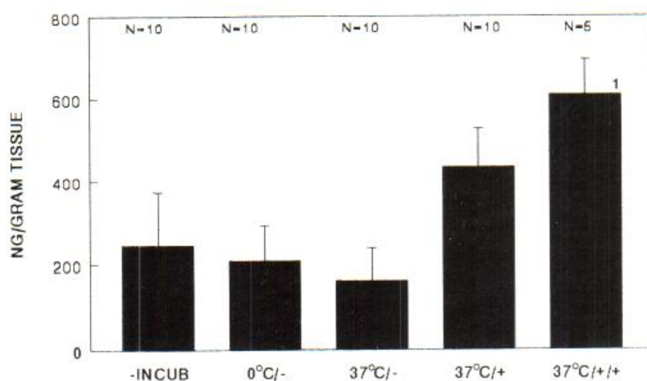

Fig. 3. 15-HETE levels expressed as ng/gram tissue (mean ± standard error of the mean (SEM) of N experiments assayed in duplicate). −INCUB denotes samples extracted without incubation and without addition of stimuli. 0°C/− denotes incubation without stimuli for 45 min at 0°C. 37°C/− denotes incubation without stimuli for 45 min at 37°C. 37°C/+ denotes incubation for 45 min at 37°C in the presence of A23187 (10 μM) and AA (25 μM). 37°C/+/+ denotes incubation for 45 min at 37°C in the presence of A23187 (10 μM), AA (25 μM) and RS43179 (25 μM). 1: 37°C/+ vs 37°C/+/+: $0.05 < p < 0.1$. 15-HETE was quantified by integrated optical density during RP-HPLC.

Reversed-phase high-performance liquid chromatography (RP-HPLC) and radioimmunoassays for LTB_4 and PGE_2

RP-HPLC was carried out as previously outlined (10). Extracted lipids were separated by RP-HPLC on a Hypersil C_{18} column eluted isocratically with methanol/water/acetic acid, 70:30:0.01 (by vol). The eluent was monitored by a UV detector producing the UV absorption as a function of wavelength for each chromatographic peak. 12-HETE and 15-HETE were quantified by integrated optical density at 235 nm as described (10). The eluent was collected by a fraction collector programmed to collect 1 ml fractions. LTB_4 and PGE_2 were quantified by RIA as previously described in detail (10). Briefly, eluate fractions co-chromatographing with authentic LTB_4 or PGE_2 were evaporated under a stream of N_2 and resuspended in assay buffer. The RIA analyses were performed in accordance with the manufacturer's instructions.

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). Statistical significance was assessed by Wilcoxon's rank sum test. A p value below 0.05 was considered significant.

RESULTS

Figs. 1–4 show eicosanoid levels obtained from *ex vivo* incubations of lesional psoriatic skin. Eicosanoid levels obtained from incubations without incubation/addition of stimuli, samples incubated without addition of stimuli for 45 min at 0°C, and samples incubated without addition of stimuli for 45 min at 37°C were not significantly different (Figs. 1–4). Incubations for 45 min at 37°C in the presence of A23187 or AA alone did not result in any significant change in eicosanoid levels (data not shown). From the figures it can be seen that the addition of both A23187 and AA resulted in a marked increase in all four eicosanoids measured. Relative to incubations for 45 min at 0°C without stimuli, we observed an increase on an average of 400% for LTB_4, of 270% for both 12-HETE and 15-HETE and of 800% for PGE_2. However, a great variation in the stimulated PGE_2 values was observed. Incubation times shorter than 45 min (5, 15 and 30 min) led to no significant stimulation of eicosanoid formation (data not shown). Consequently, incubations were carried out at 45 min. Incubations with A23187, AA and the 5-LO inhibitor RS43179 resulted in a significant inhibition of LTB_4 formation, bringing the mean level of LTB_4 down to control values (Fig. 1). Similar incubations resulted in no statistically significant ($0.5 < p < 0.1$) increase in the levels of 12-HETE (Fig. 2) or 15-HETE (Fig. 3). The levels of PGE_2 were significantly lower in samples incubated with RS43179 (Fig. 4). To determine whether eicosanoids can be synthesized by psoriatic scales, we obtained scales by gentle abrasions of the lesional skin before keratoming. Fifty mg scale samples were then incubated under the same experimental conditions as the keratomed samples. Under these experimental conditions stimulation with A23187 and AA did not cause any change in the levels of eicosanoids after stimulation (data not shown).

DISCUSSION

The present study shows that lesional psoriatic skin, but not psoriatic scales, has the capacity to synthesize LTB_4, PGE_2, 12-HETE, and 15-HETE during *ex vivo* incubation after stim-

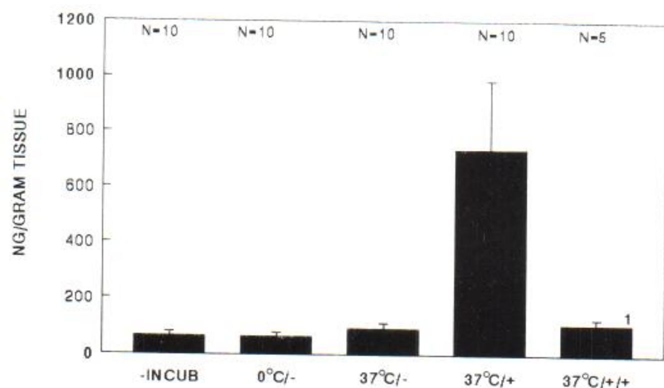

Fig. 4. PGE_2 levels expressed as ng/gram tissue (mean ± standard error of the mean (SEM) of N experiments assayed in duplicate). −INCUB denotes samples extracted without incubation and without addition of stimuli. 0°C/− denotes incubation without stimuli for 45 min at 0°C. 37°C/− denotes incubation without stimuli for 45 min at 37°C. 37°C/+ denotes incubation for 45 min at 37°C in the presence of A23187 (10 μM) and AA (25 μM). 37°C/+/+ denotes incubation for 45 min at 37°C in the presence of A23187 (10 μM), AA (25 μM) and RS43179 (25 μM). 1: 37°C/+ vs 37°C/+/+: $p<0.05$. PGE_2 was determined by RIA in fractions co-eluting with authentic PGE_2 on RP-HPLC.

ulation. The formation of LTB_4 is significantly inhibited by the 5-LO inhibitor RS43179, which, however, also led to a decrease in the formation of PGE_2, whereas 12-HETE and 15-HETE were unaffected by RS43179.

The cellular source of LTB_4 in the lesional skin is unknown. It has been shown that normal epidermis and normal dermis have the capacity to form 12-HETE and 15-HETE (12, 19), but in contrast to neutrophils, normal human epidermis/dermis has not convincingly been shown to synthesize LTB_4 from AA (17, 18). LTB_4 may be formed as a result of cell-cell interaction (i.e. LTB_4 may be formed by one cell type from precursors released by another cell type). It has been shown that psoriatic epidermis has an increased 5-LO activity compared to uninvolved skin (16). LTB_4 formed *ex vivo* may thus be derived directly from infiltrating neutrophils or from keratinocytes which have hydrolyzed LTA_4 released by neutrophils (20).

This study shows that lesional skin of psoriasis has the enzymatic capacity to form LTB_4 *ex vivo* and that this activity can be inhibited by a 5-LO inhibitor without any statistically significant increase in the formation of the LO products 12-HETE and 15-HETE. However, the formation of PGE_2 was also inhibited, suggesting that under these experimental conditions RS43179 is also a CO inhibitor. This observation underscores the importance of testing putative inhibitors of eicosanoid synthesis not only *in vitro* and in animal models, but also in diseased tissue.

The presented *ex vivo* model of eicosanoid formation in psoriatic tissue provides a model for studying the potency and selectivity of putative inhibitors of eicosanoid metabolism.

REFERENCES

1. Camp RDR, Jones RR, Brain S, Woollard P, Greaves M. Production of intraepidermal microabscesses by topical application of leukotriene B_4. J Invest Dermatol 1984; 82: 202–204.
2. Soter NA, Lewis RA, Corey EJ, Austen KF. Local effects of synthetic leukoktriene (LTC_4, LTD_4, LTE_4, and LTB_4) in human skin. J Invest Dermatol 1983; 80: 115–119.
3. Archer CB, Page CP, Juhlin L, Morley J, MacDonald DM. Delayed synergism between leukotriene B_4 and prostaglandin E_2 in human skin. Prostaglandins 1987; 33, 799–805.
4. Dowd PM, Black AK, Woollard PM, Camp RDR, Greaves MW. Cutaneous responses to 12-hydroxy-eicosatetraenoic acid (12-HETE). J Invest Dermatol 1985; 84: 537–541.
5. Kragballe K, Desjarlais L, Voorhees JJ. Leukotrienes B_4, C_4 and D_4 stimulate DNA synthesis in cultured human epidermal keratinocytes. Br J Dermatol 1985; 113: 43–52.
6. Bauer FW, van de Kerkhof PCM, Maassen-De Grood RM. Epidermal hyperproliferation following the induction of microabscesses by leukotriene B_4. Br J Dermatol 1986; 114: 409–412.
7. Hammarström S, Hamberg M, Samuelsson B, Duell EA, Stawski M, Voorhees JJ. Increased concentrations of non-esterified arachidonic acid, 12L-hydroxy-5,8,10,14-eicosatetraenoic acid, prostaglandin E_2 and prostaglandin $F_{2\alpha}$ in epidermis of psoriasis. Proc Natl Acad Sci USA 1975; 72: 5130–5134.
8. 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.
9. Duell EA, Ellis CN, Voorhees JJ. Determination of 5, 12, and 15-lipoxygenase products in keratomed biopsies of normal and psoriatic skin. J Invest Dermatol 1988; 91: 446–450.
10. Fogh K, Herlin T, Kragballe K. Eicosanoids in acute and chronic psoriatic lesions. Leukotriene B_4, but not 12-hydroxy-eicosatetraenoic acid (12-HETE) is present in biologically active amounts in acute guttate lesions. J Invest Dermatol 1989; 92: 837–841.
11. Vanderhoek JY, Bryant RW, Bailey JM. Inhibition of leukotriene biosynthesis by the leukocyte product 15-hydroxy-5,8,11,13-eicosatetraenoic acid. J Biol Chem 1980; 255: 10064–10066.
12. Kragballe K, Pinnamaneni G, Desjarlais L, Duell EA, Voorhees JJ. Dermis-derived 15-hydroxy-eicosatetraenoic acid inhibits epidermal 12-lipoxygenase activity. J Invest Dermatol 1986; 87: 494–498.
13. Ternowitz T, Fogh K, Kragballe K. 15-Hydroxy-eicosatetraenoic acid (15-HETE) specifically inhibits LTB_4-induced chemotaxis of human neutrophils. Skin Pharmacol 1988; 1: 93–99.
14. Fogh K, Søgaard H, Herlin T, Kragballe K. Improvement of psoriasis vulgaris after intralesional injections of 15-hydroxy-eicosatetraenoic acid (15-HETE). J Am Acad Dermatol 1988; 18: 279–285.
15. Fogh K, Hansen ES, Herlin T, Knudsen V, Henriksen TB, Bünger C, et al. 15-Hydroxy-eicosatetraenoic acid (15-HETE) inhibits carragheenan-induced experimental arthritis and reduces synovial fluid leukotriene B_4 (LTB_4). Prostaglandins 1989; 37: 213–228.
16. Ziboh VA, Caselbolt TL, Marcelo CL, Voorhees J. Biosynthesis of lipoxygenase products by enzyme preparations from normal and psoriatic skin. J Invest Dermatol 1984; 83: 426–430.
17. Ziboh VA, Tamara L, Casebolt BS, Marcelo CL, Voorhees JJ. Lipoxygenation of arachidonic acid by subcellular preparation from murine keratinocytes. J Invest Dermatol 1984; 83: 248–251.
18. Grabbe J, Rosenbach T, Czarnetzki BM. Production of LTB_4-like chemotactic arachidonate metabolites from human keratinocytes. J Invest Dermatol 1985; 85: 527–530.
19. Kragballe K, Desjarlais L, Duell EA, Voorhees JJ. In vitro synthesis of 12-hydroxy-eicosatetraenoic acid is increased in uninvolved psoriatic epidermis. J Invest Dermatol 1986; 87: 47–52.
20. Sola J, Godessart N, Vila L, Puig L, de Moragas M. Epidermal cell-polymorphonuclear leukocyte co-operation in the formation of leukotriene B_4 by transcellular biosynthesis. J Invest Dermatol 1992; 98: 333–339.