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

KARSTEN FOGH, LARS IVERSEN, TROELS HERLIN and KNUD KRAGBALLE

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

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 B4 (LTB4), 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 B4 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 E2, 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 B4 and prostaglandin E2 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.

(Accepted February 22, 1993.)

K. Fogh, Department of Dermatology, Marselisborg Hospital, DK-8000 Aarhus C, Denmark.

| Ex Vivo Formation of LTB4 by Lesional Psoriatic Skin |

![Diagram]

Fig. 1. LTB4 levels expressed as ng/g 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. LTB4 was determined by RIA in fractions co-eluting with authentic LTB4 on RP-HPLC.
EX VIVO FORMATION OF 12-HETE BY LESIONAL PSORIATIC SKIN

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 octadeclsil (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 N₂ and resuspended in 100 μl 70% methanol.

EX VIVO FORMATION OF 15-HETE BY LESIONAL PSORIATIC SKIN

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.

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 LTBR, of 270% for both 12-HETE and 15-HETE and of 800% for PGE₂. However, a great variation in the stimulated PGE₂ 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 LTBR formation, bringing the mean level of LTBR 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 PGE₂ 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 keratolysis. 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 LTBR, PGE₂, 12-HETE, and 15-HETE during ex vivo incubation after stimu-
EX VIVO FORMATION OF PGE2 BY LESIONAL PSORIATIC SKIN

Fig. 4. PGE2 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 was determined by RIA in fractions co-eluting with authentic PGE2 on RP-HPLC.

ulation. The formation of LTβ is significantly inhibited by the 5-LO inhibitor RS43179, which, however, also led to a decrease in the formation of PGE2, whereas 12-HETE and 15-HETE were unaffected by RS43179.

The cellular source of LTβ 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 LTβ, from AA (17, 18). LTβ may be formed as a result of cell-cell interaction (i.e. LTβ 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). LTβ formed ex vivo may thus be derived directly from infiltrating neutrophils or from keratinocytes which have hydrolyzed LTα, released by neutrophils (20).

This study shows that lesional skin of psoriasis has the enzymatic capacity to form LTβ, 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 PGE2 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 present 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