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Topical steroid treatment reduces arachidonic acid and leukotriene B_4 in lesional skin of psoriasis

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1 Topical clobetasol propionate or vehicle ointment was applied daily for 3 days to psoriatic plaques on eight patients.
2 Skin chamber exudates from untreated, steroid and vehicle treated lesions were assayed for arachidonic acid (AA), leukotriene B_4 (LTB_4), prostaglandin E_2 (PGE_2) and 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) before, and at 24 h and 72 h after treatment.
3 Significant reductions in AA and LTB_4 were observed at 72 h in steroid treated lesions.
4 The reduction in 12-HETE levels observed after steroid treatment was not statistically significant. PGE_2 levels in lesional psoriatic skin were unaltered.
5 The reduction of AA, and LTB_4 was associated with clinical improvement of psoriasis.

Keywords psoriasis steroids arachidonic acid leukotriene B_4 12-hydroxyeicosatetraenoic acid

Introduction

The molecular mechanisms underlying the pathogenesis of psoriasis, an inflammatory and epidermal proliferative skin disorder, are not well understood. Neutrophil migration to the epidermis is an early cellular event in the evolution of the psoriasis lesion (Civatte, 1924; Jablonska *et al.*, 1982) and this process is thought to be mediated, at least in part, by neutrophil chemotactic and chemokinetic lipoxygenase products of arachidonic acid (AA). Such lipoxygenase products include leukotriene B_4 (LTB_4) and 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) which are present in increased amounts in lesional psoriatic skin compared with non-lesional skin (Hammarstrom *et al.*, 1975; Barr *et al.*, 1984a; Brain *et al.*, 1984). Small amounts of AA and 12-HETE are found in non-lesional psoriatic skin (Barr *et al.*, 1984a). Topical application of 12-HETE and LTB_4 produces inflammatory changes in human skin, including the formation of epidermal neutrophil microabscesses (Camp *et al.*, 1984; Dowd *et al.*, 1985).

Anti-inflammatory steroids are thought to act, at least in part, through the synthesis of an inhibitor of phospholipase A_2, lipocortin, leading to a reduction in the release of AA (Blackwell *et al.*, 1980; Hirata *et al.*, 1980; Parente *et al.*, 1984). A decrease in AA and 12-HETE levels in steroid treated lesional psoriatic skin has been reported (Hammarstrom *et al.*, 1977). The present study was designed to re-examine the activity of a potent topical corticosteroid on arachidonic acid, 12-HETE and PGE_2 levels in the lesional skin of psoriatic patients, and to extend previous data to include measurements of the potent neutrophil chemoattractant lipoxygenase product LTB_4. The relationship of molecular changes to clinical improvement in the treated skin was also determined.

Methods

Patients

Eight patients (six males) with stable bilateral lesions of psoriasis, which were symmetrical in

site, equal in severity and involved the upper and lower limbs, consented to the study. The patients had no oral or active topical medication for at least 2 weeks prior to the study. The protocol was approved by the Institute Ethics Committee. The age of the patients ranged from 25 to 57 years (mean 38 years) and the duration of their psoriasis from 2 to 37 years (mean 9 years). The extent of the disease varied from 20 to 50% of total body surface area and the severity of the lesions was graded for scaling, thickness and erythema on a 0 to 3 scale (none = 0; slight = 1; moderate = 2; severe = 3). Clinical assessments were made before and at 24 h and 72 h after the start of therapy. Topical clobetasol propionate (0.05%; Dermovate, Glaxo) was applied to plaques on one limb once daily without occlusion for 3 days. The total amount of ointment used was less than 30 g. The base ointment (Glaxo) containing propylene glycol, white soft paraffin and serbitan sesquioleate was applied to plaques on the opposite limb for the same duration. Untreated sites were located on the same side of the body as base treated lesions, but not directly opposite treated plaques. All applications were made by one person (E.W.). No treatment was given to the remainder of the psoriatic lesions during the 3 days of the study.

Collection of chamber fluid samples

Exudates from abraded sites on lesional skin were obtained using skin chambers as previously described (Wong *et al.*, 1984). Briefly, skin was gently abraded with a scalpel blade until a glistening surface and minimal punctate bleeding was obtained. Cylindrical chambers were glued over the abrasions and filled with 1 ml phosphate buffered saline (PBS). After 5 min the fluid was removed and replaced with a further 1 ml of PBS, which was left in contact with the skin for 30 min. Two skin chamber fluid samples were obtained from lesional skin before the start of treatment and from untreated lesional skin and plaques treated with either clobetasol propionate or base ointment, at 24 h and 72 h. The samples were centrifuged immediately after collection to sediment erythrocytes, leucocytes and platelets and 0.8 ml of each sample was then stored at −70° C prior to analysis.

Analysis of chamber fluids

a. *LTB_4* One chamber fluid of each duplicate from lesional skin was analysed for LTB_4. Each sample (0.8 ml) was acidified and extracted three times with 0.8 ml ethyl acetate. The ethyl acetate extracts were pooled, evaporated under nitrogen and the residues purified by high performance liquid chromatography (h.p.l.c.) using a Nucleosil 50-5 silica column (125 × 4 mm), eluted with hexane:propan-2-ol:methanol:acetic acid (88:7:5:0.1 v/v) at 1 ml min^{-1}. The h.p.l.c. fractions co-chromatographing with authentic LTB_4 were evaporated to dryness and assayed for LTB_4 in an agarose microdroplet neutrophil chemokinesis assay (Smith & Walker, 1980). Recovery of LTB_4 after extraction and purification was 55–60%.

b. *AA, 12-HETE and PGE_2* The remaining duplicate sample was equilibrated with 80 ng of 14-HETE, 200 ng [5,6,8,9,11,12,14,15-^{2}H] AA and 25 ng [3,3′,4,4′-^{2}H] PGE_2 for mass spectrometry. The samples were extracted three times with ethyl acetate and the residues partitioned between phosphate buffer (pH 8.4) and chlorobutane (×2) to obtain the AA and mono-HETEs in the organic phases (Barr *et al.*, 1984a). The remaining aqueous phases were acidified to pH 3.5 and extracted with ethyl acetate to recover PGE_2.

The AA and mono-HETE fraction was methylated with methanolic-ethereal (1:9) diazomethane (100 µl × 2) and the tert-butyldimethylsilyl (tBDMS) ether derivatives were prepared (Woollard & Mallet, 1984). These were subjected to reversed phase high-performance liquid chromatography on a Spherisorb analytical S5 ODS column eluted with methanol:water (925:75 v/v) at a flow rate of 1 ml min^{-1}. Fractions containing the arachidonic acid methyl ester and the monohydroxy fatty acid methyl ester tBDMS ether derivatives were collected at the relevant times.

The arachidonic acid methyl ester fractions were analysed by gas chromatography-mass spectrometry (GC-MS) on a VG Analytical model 305 single focussing magnetic sector mass spectrometer. Quantification was carried out by monitoring the ions at m/z 318 and 326 ($M^{+\cdot}$) for methyl arachidonate and the deuterated internal standard respectively (Barr *et al.*, 1984a).

12-HETE was quantified according to the method of Woollard & Mallet (1984). GC-MS of the hydrogenated compounds was carried out by incorporating a reduction chamber in the gas chromatography column, and the α cleavage ions m/z 301 (12-HETE) and m/z 329 (14-HETE internal standard) were monitored.

Ethyl acetate extracts containing PGE_2 were purified by h.p.l.c. and the amount of PGE_2 present determined by gas chromatography-mass spectrometry as previously described (Hawk *et al.*, 1983; Barr *et al.*, 1984b).

Statistics

The paired *t*-test was used to compare data from lesional skin before and after treatment with topical steroids or base ointment. The Wilcoxon signed ranks test was used to compare LTB_4 data before and after treatment. The level of significance for each of the statistical methods was taken to be $P < 0.05$ on a two-tailed hypothesis.

Results

Eicosanoid concentrations

The concentrations of AA, 12-HETE, LTB_4 and PGE_2 in untreated, steroid and base treated psoriatic lesions are shown in Figure 1 a,b,c,d. There was no significant difference between the concentration of AA in the untreated sites at the different time points nor between base and untreated lesions at 24 h and 72 h. A significant reduction in the concentration of AA was observed in the steroid treated sites at 72 h (128 ± 40 ng ml^{-1}; $P = 0.04$) but not at 24 h (129 ± 41 ng ml^{-1}) when compared to corresponding values in the base treated lesions (300 ± 65; 312 ± 86 ng ml^{-1}; 72 h and 24 h respectively).

LTB_4 chemokinetic activity was significantly reduced by steroid treatment at 72 h (20 ± 4 pg ml^{-1}; $P = 0.02$) but not at 24 h (23 ± 4 pg ml^{-1}; $P = 0.08$) compared to both the base (35 ± 3 and 40 ± 6 pg ml^{-1}; 24 h and 72 h respectively) and untreated lesions (27 ± 4 and 30 + 4 pg ml^{-1}; 24 h and 72 h respectively) (Figure 1b). As with AA, there was no change in LTB_4 in the untreated controls at the different time points. However, base treatment appeared to elevate LTB_4 levels at 72 h compared to untreated control ($P = 0.04$).

The concentrations of 12-HETE and PGE_2 were not significantly affected by treatment with

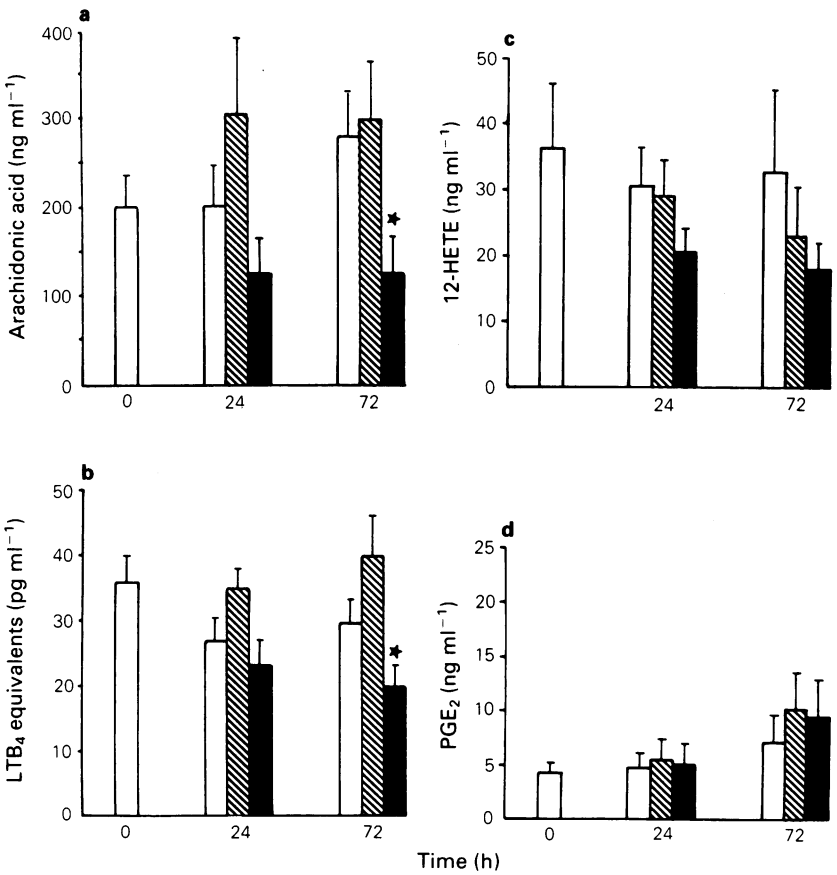

Figure 1 Concentrations of arachidonic acid (a), LTB_4 (b), 12-HETE (c), and PGE_2 (d) in untreated lesional psoriatic skin (□), base treated lesions (▨) and steroid treated lesions (■). Results are expressed as mean ± s.e. mean, $n = 8$. * = statistically significant difference *vs* base treated site ($P < 0.05$).

either steroid or base and, as with AA and LTB_4, there was no change in the levels of these metabolites in untreated sites during the period of the study (Figure 1 c and d).

Clinical assessment

At 24 h, steroid-treated lesions showed minimal detectable changes in scaling (three patients), erythema (five patients) and thickness (two patients), compared to base-treated lesions (scaling improvement seen in three) and untreated plaques. By 72 h, each of the eight patients showed a marked improvement in all the parameters tested in the steroid treated areas. In the base-treated lesions, a slight improvement was seen in scaling (6); erythema (3) and infiltration (4). The total clinical scores after 72 h of topical steroid treatment are shown in Table 1.

Table 1 Clinical scores of untreated *vs* steroid and base-treated lesions after 72 h of treatment

Patient	*Pretreatment*	*Base*	*Steroid*
DC	6	6	3
PR	7	6	2
EE	8	7	5
SM	7	4	2
SO	9	7	6
DR	5	4	4
NC	8	4	2
RF	7	5	2
Total	57	43	26

Discussion

Although there is considerable evidence from *in vitro* studies for the inhibition of phospholipase A_2 by corticosteroids, few studies have been carried out *in vivo* in human skin. Suppression of AA levels by systemic steroid therapy has been demonstrated in tetrahydrofurfuryl nicotinate (Trafuril)-inflamed skin (Black *et al.*, 1982) and following topical corticosteroid treatment in lesional psoriatic skin (Hammarstrom *et al.*, 1977). More recently, Norris *et al.* (1984) reported that phospholipase A_2 activity was diminished by topical steroid treatment. Our results provide further *in vivo* evidence that steroids can lower AA concentrations in psoriatic skin.

Steroid therapy does not, however, totally suppress AA. We observed a 46% reduction of AA by 72 h while Hammarstrom *et al.* (1977) reported a 57% fall at 24 h in psoriatic lesions. Previously, Black *et al.* (1982) observed that systemic steroid therapy did not affect basal levels of AA and PGE_2 in the skin. The interpretation of *in vivo* studies is complicated by the possible involvement of a number of mechanisms in the release of AA from phospholipids and its subsequent metabolism. Lipases other than phospholipase A_2 may release AA from phospholipids (Majerus *et al.*, 1980; Nishizuka, 1984), release from triglycerides or diacylglycerols can take place and AA can be reacylated into membrane phospholipids. The action of steroids on these pathways is uncertain. In addition, the steroid-induced phospholipase A_2 inhibitory protein, lipocortin, is only active in the dephosphorylated form (Hirata, 1981) and since the factors controlling phosphorylation-dephosphorylation of lipocortin *in vivo* are not known the pharmacological actions of steroids are difficult to predict.

The suppression of LTB_4 in lesional skin exudates by topical steroids is consistent with a previous study (Klickstein *et al.*, 1980) showing a reduction of LTB_4 in the joint fluid of arthritic patients after intra-articular injection of steroid. LTB_4 in psoriatic lesions was not, however, reduced to basal levels by steroid treatment, since measurable amounts were present in chamber fluid at 72 h and the levels of LTB_4 in the clinically normal skin of patients with psoriasis are below the detection limit of the assay.

In contrast to the findings of Hammarstrom *et al.* (1977), we were unable to demonstrate a significant reduction in 12-HETE after steroid treatment. This may be due to differences in experimental design since in the present study the corticosteroid was not applied under occlusive dressing. Occlusion alone, without steroid application, was reported by Hammarstrom and coworkers (1977) to produce lower AA and 12-HETE concentrations than in unoccluded, untreated psoriasis lesions. Occlusion may aid penetration of the steroid resulting in higher local concentrations of the drug, and a greater effect on AA levels. The concentration of PGE_2, unlike the AA lipoxygenase products does not appear to be elevated in lesional psoriatic skin, and no decrease in PGE_2 was seen with steroid therapy. Whether the lack of an evoked increase in PGE_2 explains the absence of demonstrable steroid suppression of the prostaglandin is unclear.

Care was taken in our study to eliminate regional differences between base and steroid treated sites by selecting symmetrical lesions on opposite limbs. That the concentrations of eicosanoids in untreated and base treated skin did not change significantly during the 72 h study

suggests that systemic effects resulting from absorption of the drug into the systemic circulation were unlikely to have occurred. Although base treatment alone has been shown to affect AA metabolism in human skin (Hammarstrom *et al.*, 1977; Black *et al.*, 1978) we did not detect significant differences in the concentrations of metabolites between base and untreated lesions at the various time points except at 72 h when LTB_4 appeared significantly higher in the base treated site than in the corresponding untreated control site ($P = 0.04$).

Very little clinical improvement in the lesions was observed at 24 h but at 72 h, marked improvement in steroid treated lesions was observed compared to the base and untreated lesions. Although the improvement seen at 72 h is concurrent with a 40% reduction in AA and a 44% reduction in LTB_4 it is not possible to say from this study whether the reduction in these metabolites preceded the clearing of psoriasis or whether they occurred as a consequence of improvement in the lesions. Use of specific lipoxygenase inhibitors or antagonists of the actions of lipoxygenase products will be crucial to the further elucidation of this problem.

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