The effect of etretinate on the cyclo-oxygenase and lipoxygenase products of arachidonic acid metabolism in psoriatic skin

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- 1 Eight psoriatic patients were treated with etretinate (50 mg daily) for 6 weeks.
- 2 Skin chamber exudates from involved and uninvolved skin were assayed for arachidonic acid, 12-HETE, PGE₂ and for neutrophil chemokinetic activity co-chromatographing with leukotriene B₄, before and at weekly intervals during therapy.
- 3 Pre-treatment concentrations of arachidonic acid, 12-HETE and leukotriene B₄-like chemokinetic activity but not of PGE₂ were elevated in involved skin when compared to uninvolved skin.
- 4 The concentrations of arachidonic acid and 12-HETE declined during therapy but changes in PGE₂ were minimal. LTB₄-like activity was detectable in involved skin both before and after etretinate treatment.
- 5 Clinically, scaling and infiltration improved but erythema was still evident.

Keywords psoriasis etretinate arachidonic acid 12-hydroxyeicosatetraenoic acid leukotriene B₄

Introduction

Psoriasis is a complex multifactorial inflammatory skin disease characterised by epidermal hyperplasia, neutrophil migration through the epidermis and dilatation of the dermal papillary vessels. Elevated levels of arachidonic acid, 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) and prostaglandin E₂ (PGE₂) are found in lesional psoriatic skin (Hammarstrom *et al.*, 1975). The presence of elevated levels of chemokinetic activity closely resembling leukotriene B₄ (LTB₄), a dihydroxy lipoxygenase product of arachidonic acid, has also been reported (Brain *et al.*, 1982, 1984). LTB₄, and to a lesser extent 12-HETE, are neutrophil chemoattractants (Goetzel *et al.*, 1977; Borgeat & Samuelsson, 1979). Their inflammatory properties *in vivo* have also been demonstrated using intradermal injection of LTB₄ and 12-HETE (Camp *et al.*, 1983a; Dowd *et al.*, 1983). These substances could therefore be important in mediating the migration of neutrophils in the

psoriatic epidermis. Etretinate, a synthetic aromatic derivative of vitamin A, taken orally has been reported in controlled studies to be effective in the treatment of chronic plaque psoriasis (Lassus, 1980). The effect of etretinate on arachidonic acid and its metabolites in psoriatic skin has not been reported. The aim of this study was to determine the effects of etretinate on arachidonic acid, 12-HETE, PGE₂ and LTB₄ in psoriatic skin and to correlate this with clinical response.

Methods

Patients

Eight patients (five males and three females) with chronic plaque psoriasis gave informed consent to the study. The protocol was approved

by the Institute Ethical Committee. Each patient was given 50 mg etretinate daily. Active topical and systemic drugs had been stopped for at least 2 weeks before the experiment began and none of the patients had received PUVA or cytotoxic therapy previously. Their ages ranged from 25 to 55 years with a mean of 39 years. The duration of their psoriasis ranged from 5 to 36 years with a mean of 25 years. The extent of their psoriasis was assessed by plotting the affected areas on a prestamped figure chart. The lesions were graded for scaling, infiltration and erythema on a 1–4 scale (none = 1; slight = 2; moderate = 3; severe = 4). Clinical assessments were carried out before and at weekly intervals during etretinate therapy.

Collection of samples

Exudate from involved and uninvolved skin was collected using the skin chamber technique (Brain *et al.*, 1984). Different sites were chosen for collection of chamber fluid each week. The anatomical region selected for sampling varied among the patients depending on the distribution of the psoriasis. The skin was gently abraded until a glistening layer was seen. A cylindrical acrylic chamber (210 mm²) was glued with cyanoacrylate adhesive to the skin and the abraded surface washed with 1 ml phosphate buffered saline pH 7.4 for 2 min. The chamber was refilled with 1 ml phosphate buffered saline which was removed 30 min later and centrifuged to sediment any contaminating red cells. The supernatant (0.7 ml) was equilibrated with deuterated internal standards: 200 ng [5,6,8,9,11,12,14,15-²H] arachidonic acid and 25 ng each of [3,3',4,4'-²H] PGE₂, [5,6,8,9,11,12,14,15-²H] 12-HETE, for mass spectrometry. Exudates were stored at -70°C until analysed.

Analysis of chamber fluids

The samples were acidified to pH 3.5 with acetic acid (20 µl) and the arachidonic acid metabolites extracted into ethyl acetate. The solvent was removed under a stream of nitrogen and the lipid desiccated under vacuum. The extracted lipid was resuspended in 0.4 ml 0.1 M phosphate buffer, pH 8.4, and partitioned twice against 0.8 ml chlorobutane which extracted arachidonic acid and 12-HETE. The aqueous phase was acidified to pH 3.5 and extracted with ethyl acetate to recover LTB₄ and PGE₂. The arachidonic acid and 12-HETE extracts were further purified by redissolving in metha-

nol (0.5 ml) and heptane (0.7 ml). The upper (heptane) layer was discarded and the methanol phase containing arachidonic acid and 12-HETE was taken to dryness under nitrogen. The residue was treated with 50 µl ethereal diazomethane containing 10% methanol for 10 min and the resulting methyl esters separated by high pressure liquid chromatography (h.p.l.c.) on a LiChrosorb-Si60 column, 250 × 4 mm, eluted with hexane:propan-2-ol:methanol (100:1:0.5) at 1.5 ml/min. Fractions corresponding to the methyl esters of arachidonic acid and 12-HETE were collected. Methyl arachidonate was further purified by reverse phase h.p.l.c. (Hawk *et al.*, 1983). The final ethyl acetate extracts containing LTB₄ and the prostaglandins were chromatographed on a LiChrosorb-Si60 column, 250 × 4 mm, eluted with chlorobutane : acetonitrile : methanol : acetic acid (60:40:3:0.3) and fractions corresponding to LTB₄ and PGE₂ collected.

Arachidonic acid, 12-HETE and PGE₂ were assayed by gas chromatography-mass spectrometry (GC-MS). The conditions used for arachidonic acid and PGE₂ were as previously described (Hawk *et al.*, 1983). Samples from h.p.l.c. containing 12-HETE methyl ester were dissolved in 25 µl N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) and heated to 50°C for 30 min to give the trimethylsilylether-methyl ester. The 12-HETE derivative was chromatographed on a 12.5 m × 0.3 mm OV-17 capillary column at 210°C with helium as carrier gas. The mass spectrometry and operating conditions were as previously described (Hawk *et al.*, 1983). The ions monitored for 12-HETE and the internal standards were m/z 295 and 301 respectively.

H.p.l.c. fractions corresponding to authentic LTB₄ were taken to dryness and redissolved in 0.3 ml Eagle's minimal essential medium buffered to pH 7.4 with 20 mM HEPES. Each was analysed for LTB₄ equivalents by the agarose microdroplet chemokinesis assay (Smith & Walker, 1980) using human leucocyte suspensions containing 70–80% neutrophils, prepared as previously described (Camp *et al.*, 1983b). The chemokinetic activity in test samples was assayed directly against a standard LTB₄ dose response curve. Results of the LTB₄ assay were not corrected for losses during extraction and purification. Recovery of LTB₄ was generally 30–50%.

Statistical analysis

Data were analysed by paired *t*-tests, Wilcoxon's sign rank test and two-way analysis of variance.

Table 1 Concentrations (ng/ml, mean $\pm$ s.d.) of mediators in the chamber fluid from untreated psoriatic skin. Paired *t*-test, *n* = 8.

	<i>Involved</i>	<i>Uninvolved</i>	<i>Significance</i>
Arachidonic acid	210 $\pm$ 112	83 $\pm$ 50	<i>P</i> = 0.018
12-HETE	38.7 $\pm$ 16	13.6 $\pm$ 6.3	<i>P</i> = 0.002
PGE ₂	7.2 $\pm$ 4.6	12.5 $\pm$ 5.9	<i>P</i> = 0.062

Multiple comparisons between treatment means and zero time control means were tested by Dunnett's procedure. Best fit linear regression lines were calculated.

Results

Pretreatment

The concentrations of arachidonic acid, 12-HETE and PGE₂ in exudates from psoriatic plaque and uninvolved skin before starting etretinate therapy are shown in Table 1. Plaque

contained 2.5 to 3 times more arachidonic acid and 12-HETE than uninvolved skin but PGE₂ levels in lesional and non lesional areas were similar.

LTB₄-like chemokinetic activity was found in exudate from plaque of seven of the eight patients, mean concentration 43 $\pm$ 40 pg/ml exudate (mean $\pm$ s.d., *n* = 8). Low levels of LTB₄-like activity were found in exudates from uninvolved skin of two patients; the remaining samples from uninvolved skin contained no detectable (< 9 pg LTB₄ equivalents per sample) chemokinetic material. The difference between plaque and uninvolved skin was significant (*P* < 0.001, Wilcoxon's sign rank test).

Figure 1 Mean concentrations in involved (●) and uninvolved (○) exudates, *n* = 8 except day 42 when *n* = 7. Calculated linear regression line (—) and the corresponding 95% confidence limits (---) involved, (.....) uninvolved are shown to one side of the regression line only. The regression coefficients and significance of the slopes are shown to the right of the graphs. Treatment means from 7 to 42 days were compared to control mean (0 day) using Dunnett's procedure for multiple comparisons. * is *P* < 0.05.

During treatment

The concentration of arachidonic acid in exudates from psoriatic plaque, but not from uninvolved skin, declined at a significant rate during etretinate therapy (Figure 1a), suggesting a greater effect of etretinate on free arachidonic acid levels in the involved psoriatic skin. At 35 days the arachidonic acid concentration in exudate from psoriatic plaque was significantly less than the pretreatment level.

Changes in 12-HETE during etretinate treatment reflected the changes seen with arachidonic acid. The 12-HETE in exudate from psoriatic plaque, but not the 12-HETE in uninvolved skin, declined at a significant rate (Figure 1b). As with arachidonic acid, etretinate has a greater effect on plaque lipoxygenase activity than on values in uninvolved skin.

The results for PGE₂ differed from those of arachidonic acid and 12-HETE in that there was no difference between exudates from plaque and uninvolved skin (Figure 1c). In both instances the decline in PGE₂ was minimal.

Chemokinetic activity co-chromatographing with LTB₄ was detected in 44 of 47 exudates collected from plaque, but was found in seven of 47 exudates from uninvolved skin. At 42 days, LTB₄-like chemokinetic activity was found in lesional skin of the seven remaining patients but in uninvolved skin of one patient only. There appears to be no decline in LTB₄-like activity in the plaque exudates during etretinate therapy (Figure 2).

Figure 2 LTB₄ levels in involved skin before and during etretinate therapy. Key: see Figure 1.

Clinical

Over the 6 week period there was diminution in scaling in seven of eight patients and in infiltration in three patients. Erythema persisted in all patients and generally became more visible because of the reduction in overlying scales. The percentage body involvement remained the same in all patients except one who developed new lesions while on etretinate. Side-effects were tolerable except for one

patient in whom treatment was stopped on the sixth week due to intense pruritus.

Discussion

The increased concentrations of arachidonic acid, 12-HETE and LTB₄ in skin chamber fluid from lesional psoriatic skin before etretinate treatment suggests abnormal metabolism of arachidonic acid by the lipoxygenase pathways in psoriasis. However, the increases in arachidonic acid and 12-HETE that we found using the skin chamber techniques were considerably lower than the increases found in keratome slices of psoriatic plaque and uninvolved epidermis (Hammarstrom *et al.*, 1975). Also, we found no increase in PGE₂ in the plaque areas in contrast to the earlier study on keratome slices. These differences in the observed degree of elevation of arachidonic acid metabolites in involved skin are probably a reflection of the skin sampling procedures used. The skin chamber technique collects exudate primarily from the abraded dermis and epidermis which is devoid of scales (Brain *et al.*, 1984) whilst keratome slices include considerable amounts of surface scale. We have previously shown (Camp *et al.*, 1983b) that scale contains significant amounts of arachidonate lipoxygenase products in psoriasis. Bleeding, if any, from the abrasion was minimal and had ceased by the end of the wash period. Furthermore, few or no leucocytes were found in the chamber fluid and there was no correlation between the chemokinetic activity in the chamber fluid and the number of leucocytes when present (Brain *et al.*, 1984).

Comparison of results between different patients showed wide variations, in the concentrations of arachidonic acid and 12-HETE in psoriatic skin, as has been reported previously (Hammarstrom *et al.*, 1975). Further details on the skin chamber technique as applied to the quantitative analysis of arachidonic acid metabolites in healthy and psoriatic skin, including reproducibility of results from different sites and patients, will be published elsewhere (Barr *et al.*, 1984).

The very small amount of LTB₄-like chemokinetic material in human skin makes its detection and analysis difficult. We used the agarose microdroplet neutrophil chemokinetic assay because of its sensitivity and improved its specificity by h.p.l.c., but clearly it is less precise and reproducible than GC-MS with addition of internal standards as in the other assays. Nevertheless, our results show increased frequency of detectable LTB₄-like material in

involved compared with uninvolved skin and we were unable to detect any decline during etretinate therapy.

Decline in concentrations of arachidonic acid and 12-HETE in lesional skin occurred concurrently with reduction in scaliness but the relationship of these pharmacological changes to clinical response is speculative.

A role of 5-lipoxygenase and 12-lipoxygenase products in psoriasis has been proposed (Hammarstrom *et al.*, 1975; Brain *et al.*, 1982) and inhibition of both these enzyme pathways by etretinate has been demonstrated (Fiedler-Nagy *et al.*, 1983; Bray, 1984). The mode of action of etretinate is not known but studies have revealed a complex variety of effects (marks *et al.*, 1984) which include a direct

action on neutrophil migration (Dubertret *et al.*, 1982) and inhibition of polyamine biosynthesis (Kaplan *et al.*, 1983). However, the reduction of arachidonic acid levels observed in the present study raises the possibility that etretinate acts at a stage prior to transformation of arachidonic acid. Further work with specific inhibitors of arachidonic acid metabolism is required to elucidate the role of cyclo-oxygenase and lipoxygenase products in psoriasis.

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