Br. J. Pharmac. (1984), **83**, 313–317

Leukotriene B_4-like material in scale of psoriatic skin lesions

S.D. Brain[1], R.D.R. Camp, F.M. Cunningham, P.M. Dowd, M.W. Greaves & A. Kobza Black

Wellcome Laboratories for Skin Pharmacology, Institute of Dermatology, St. John's Hospital for Diseases of the Skin, Homerton Grove, London E9 6BX

1 Acidic lipid extracts of scale from the lesions of the skin disease, psoriasis, were purified by straight phase high performance liquid chromatography (h.p.l.c.). Assay of fractions by an agarose microdroplet chemokinesis method showed the presence of biologically active material that co-eluted with standard leukotriene B_4 (LTB_4).

2 LTB_4-like chemokinetic activity was also detected in fractions collected on reversed phase h.p.l.c. of psoriatic scale extracts that were initially purified by straight phase h.p.l.c.

3 No LTB_4-like activity was detected after similar purification of scale obtained by abrasion of large areas of normal skin.

4 The LTB_4-like material found in extracts of psoriatic scale may play a role in the pathogenesis of the neutrophil infiltrate which characterizes psoriasis.

Introduction

The common chronic skin disease, psoriasis, is characterized by epidermal proliferation and inflammatory changes, including intraepidermal neutrophil infiltration which has been reported to be one of the earliest events in the evolution of lesions (Chowaniec *et al.*, 1981). The generation of neutrophil chemoattractants within the epidermis may be important in the induction of this neutrophil infiltrate. Several lipoxygenase metabolites of arachidonic acid exhibit chemotactic activity towards neutrophils *in vitro*, leukotriene B_4 (5S, 12R-dihydroxy-6, 14-*cis*-8, 10-*trans*-eicosatetraenoic acid, LTB_4) being the most potent (Turner *et al.*, 1975; Goetzl & Sun, 1979; Palmer *et al.*, 1980). The presence of biologically active amounts of LTB_4- and monohydroxyeicosatetraenoic acid (monoHETE)-like material in fluid from chambers attached to abraded psoriatic skin lesions has been reported (Brain *et al.*, 1982; 1983a). In addition several monoHETE compounds have been conclusively identified by gas chromatography-mass spectrometry (g.c.-m.s.) in extracts of chamber fluid and superficial scale from lesional psoriatic skin (Camp *et al.*, 1983). The present paper concerns the analysis of scale samples from psoriatic lesions and from the skin of normal volunteers for LTB_4-like material.

Part of this work has been communicated to the British Pharmacological Society (Brain *et al.*, 1983b).

Methods

Volunteers

Volunteers were patients with untreated plaque psoriasis or healthy Institute staff receiving no medication. Volunteers had given informed consent, and the project was approved by the Institute Ethical Committee. The surface of psoriatic lesions or the entire back of each normal volunteer was gently abraded with a scalpel blade, and the samples so obtained were stored at −20 °C.

Sample purification

In preliminary experiments, 4 lesional psoriatic scale (150–160 mg) and 5 normal skin scale (10–70 mg) samples were each added to 6 ml ethyl acetate and 6 ml 0.1 M sodium acetate buffer, pH 3.5 and vortex-

[1] Present address: Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons, Lincoln's Inn Fields, London WC2A 3PN.

mixed. After centrifugation and removal of the upper organic phase, two further partitions with ethyl acetate were carried out. The organic phases were pooled, evaporated and vacuum desiccated. To remove non-polar material, the residue was partitioned between 4 ml *n*-heptane and 3 ml methanol. The upper phase was discarded, the lower layer evaporated, and the residue subjected to straight phase high performance liquid chromatography (h.p.l.c.). In this system a Nucleosil 50–5 μm silica column (25 cm × 4.9 mm i.d.) was eluted with hexane/propan-2-ol/ methanol/acetic acid (88:7:5:0.1, by vol.) at 1 ml min^{-1}. Effluent fractions (1 ml) were collected, evaporated and the residues redissolved in 0.3 ml Eagle's Minimal Essential Medium (MEM) buffered to pH 7.4 with 30 mM N-2-hydroxyethylpiperazine - N'-2-ethanesulphonic acid (HEPES) buffer. The chemokinetic activity of each fraction, and a ten-fold dilution, was determined by using an agarose microdroplet chemokinesis method.

Four samples (each of 75 mg) of lesional psoriatic scale were then further purified by consecutive straight and reversed phase h.p.l.c., as follows. Acidic lipids in each scale sample were extracted into ethyl acetate as described above, and, following evaporation, each organic residue was partitioned between 2 ml 0.1 M sodium phosphate buffer, pH 8.5, and 2 ml 1-chlorobutane.Monohydroxy fatty acids and less polar material are preferentially soluble in the chlorobutane layer whilst LTB_4 and more polar material remain in the aqueous phase. After a further partition with 2 ml 1-chlorobutane, the aqueous layer was acidified by addition of 2 ml 0.1 M sodium acetate buffer, pH 3.5, and extracted 3 times with 4 ml ethyl acetate. The pooled ethyl acetate phases were evaporated, vacuum desiccated and subjected to straight phase h.p.l.c. as described above. For each sample, a fraction co-eluting with standard LTB_4 was collected, evaporated and re-purified by reversed phase h.p.l.c. on a Spherisorb S50DS column (25 cm × 4.9 mm i.d.) eluted with methanol/water/acetic acid (80:20:0.01, by vol.) at 1 ml min^{-1}. Fractions (1 ml) were collected, evaporated and redissolved in 0.3 ml MEM for assay of chemokinetic activity. The profile of biological activity was compared with that in four 75 mg samples of normal scale purified in an identical manner. In order to obtain the required amount of normal scale, material from a number of volunteers was pooled. To ensure that there was no contamination of biological samples with standard LTB_4, fractions (1 ml) with elution times similar to LTB_4 were collected, evaporated and assayed for chemokinetic

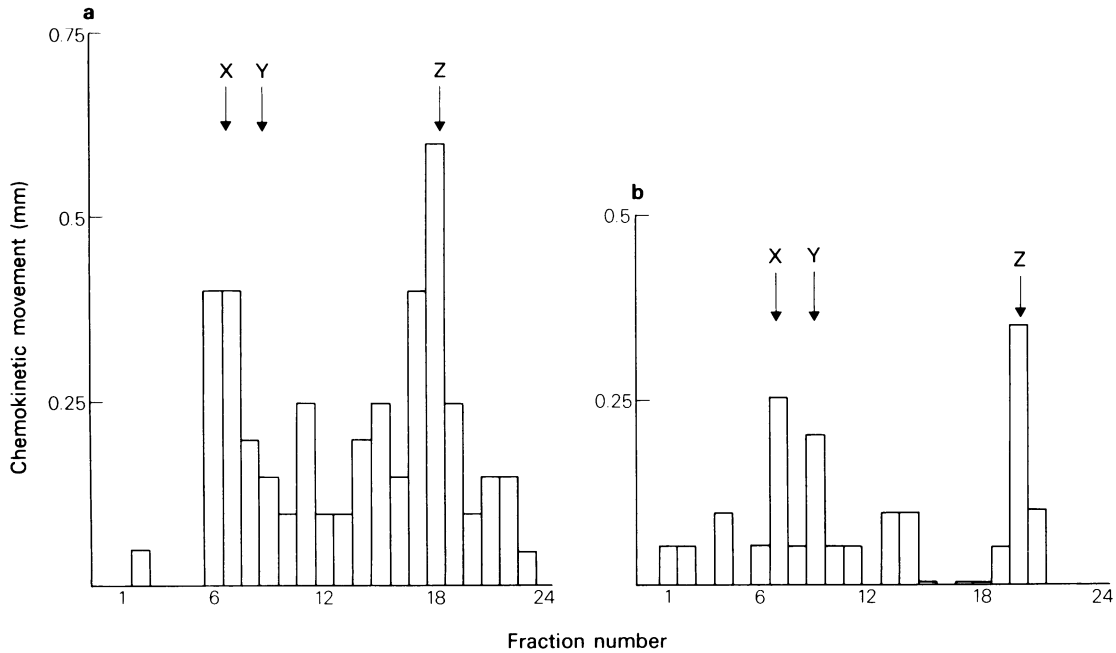

Figure 1 Chemokinetic activity in extracts of psoriatic scale (150 mg) after purification by straight phase h.p.l.c. The results of two experiments (a and b) are shown. Each h.p.l.c. fraction was evaporated and redissolved in 0.3 ml MEM. The profiles shown were obtained when ten fold dilutions were assayed for chemokinetic activity. Activity has been expressed as the distance moved by PMNs less random movement in the presence of MEM alone. The mean of duplicate observations is given for each h.p.l.c. fraction. Elution times of standard 12-HETE (X), 5-HETE (Y) and LTB_4 (Z) are given.

activity, after blank injection of h.p.l.c. solvent. This procedure was performed after each injection of standard LTB_4, before purification of scale extracts.

Further characterization of LTB_4-like material in psoriatic scale was achieved by obtaining an ethyl acetate extract of bulked scale (500 mg) as described above. The organic residue was partitioned between *n*-heptane and methanol, and the evaporated methanol phase purified by the straight phase h.p.l.c. system described above. A fraction corresponding to the elution time of standard LTB_4 was collected, evaporated, vacuum desiccated and stored in methanol. A portion of the sample was evaporated and redissolved in MEM and the chemokinetic activity determined. The remaining methanol solution was then evaporated and sufficient MEM added to give biological activity corresponding to that of 2.9×10^{-9} M LTB_4. Appropriate dilutions were made and the dose-response curve thus obtained was compared with that of standard LTB_4 (8.9×10^{-11}–2.9×10^{-9} M; 3–96 pg per well) in the same assay.

Chemokinesis assay

Chemokinetic activity for human peripheral blood polymorphonuclear leucocytes (PMNs) was assayed by the agarose microdroplet chemokinesis method (Smith & Walker, 1980). Chemokinetic activity has been expressed as the distance moved by PMNs (mm) or as LTB_4 equivalents when h.p.l.c. fractions co-eluting with standard LTB_4 were assayed against

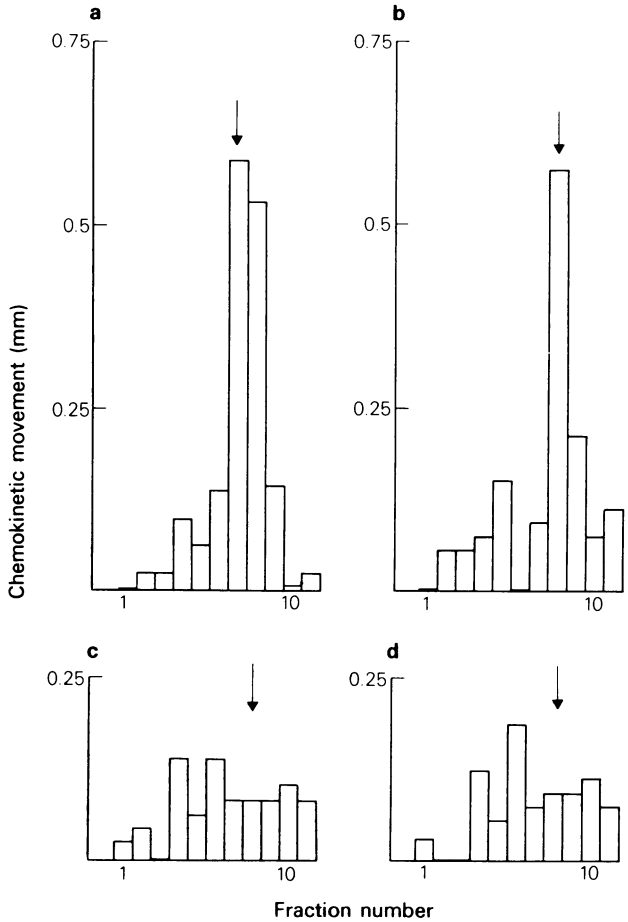

Figure 2 Chemokinetic activity in reversed phase h.p.l.c. fractions after preliminary purification of two psoriatic scale extracts (a and b) and two normal scale extracts (c and d) by straight phase h.p.l.c. Each fraction was evaporated, redissolved in 0.3 ml MEM and assayed without further dilution. Activity has been expressed as the distance moved by PMNs less random movement in the presence of MEM alone. The mean of duplicate observations is given for each h.p.l.c. fraction. Arrows show the elution times of standard leukotriene B_4.

standard LTB_4 (3.0×10^{-11}–3.0×10^{-9} M; 1–100 pg per well).

Materials

Organic solvents, 12-HETE, 5-HETE and materials used in the agarose microdroplet chemokinesis assay were obtained as described (Camp *et al.*, 1983) except for Indubiose A37 (Uniscience, London, U.K.). LTB_4 was a gift from Dr J. Rokach, Merck Frosst, Point-Claire Dorval, Canada. 1-Chlorobutane (h.p.l.c. grade) was obtained from Fisons, Loughborough and all other reagents from B.D.H., Poole.

Results

After purification of 4 lesional psoriatic scale samples (150–160 mg) by straight phase h.p.l.c., chemokinetic activity was measurable in fractions co-eluting with standard LTB_4 and the two monohydroxy fatty acids, 12-HETE and 5-HETE, which are poorly separated by this system. Figure 1 shows two representative h.p.l.c. profiles. Assay of the active material in h.p.l.c. fractions co-eluting with LTB_4 against standard LTB_4, revealed that the 4 psoriatic scale samples contained a mean of 435 pg LTB_4 equivalents per 100 mg scale (range 180–800 pg per 100 mg). These values have not been corrected for losses incurred during purification. The presence of chemokinetically active amounts of monoHETE-like material in straight phase h.p.l.c. fractions of psoriatic scale is also demonstrated in Figure 1, and agrees with published data (Hammarstrom *et al.*, 1975;Camp *et al.*, 1983). The 5 normal scale samples (10–70 mg) were purified by straight phase h.p.l.c. and the evaporated fractions redissolved in 0.3 ml MEM. Assay of each fraction without further dilution led to variable profiles of chemokinetic activity, and where active material was seen to elute in the region of LTB_4 (in 3 out of 5 samples) broad peaks were obtained which lacked the discrete shape of those seen on purification of psoriatic samples.

In order to clarify whether the chemokinetic activity in normal scale could be attributed to LTB_4-like material, 4 samples (75 mg each) were extracted, partitioned and purified by the 2 h.p.l.c. systems described. Effluent fractions from the second h.p.l.c. system contained no significant chemokinetic activity. In contrast, 4 psoriatic scale samples (75 mg each), when purified by the same two h.p.l.c. systems, all contained LTB_4- like material. Representative profiles of biological activity are shown in Figure 2. When the active fractions were assayed against standard LTB_4 the mean value of LTB_4 equivalents per 100 mg scale was calculated to be 353 pg (range 188–712 pg).

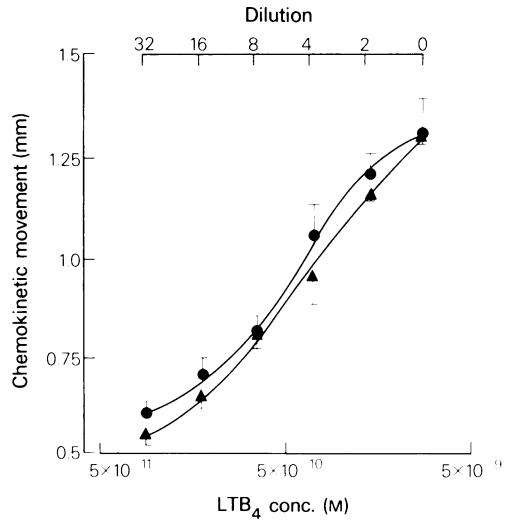

Figure 3 Dose-response curves to standard leukotriene B_4 (LTB_4, 8.9×10^{-11} to 2.9×10^{-9} M, ●) and the LTB_4-like material obtained on purification of psoriatic scale extracts by straight phase h.p.l.c. (▲). The concentration of standard LTB_4 is given on the lower horizontal axis and the dilution of LTB_4-like material from psoriatic scale on the upper horizontal axis. Results are expressed as mean ($n = 4$) with s.e.mean shown by vertical lines.

After purification of a bulked psoriatic scale sample (500 mg) by straight phase h.p.l.c. and assay of dilutions of the fraction co-eluting with LTB_4 for chemokinetic activity, as described, a log dose-response curve closely resembling that of standard LTB_4 was obtained (Figure 3). Similar results were obtained in a second such experiment.

Discussion

Straight phase h.p.l.c.-purified extracts of lesional psoriatic scale were shown to contain biologically active amounts of both LTB_4- and monoHETE- like material (Figure 1). Chemokinetic activity co-eluting with LTB_4 was also present after purification of psoriatic scale extracts by 2 consecutive h.p.l.c. systems (Figure 2a and b). Further evidence for the presence of a compound with similar biological properties to LTB_4 in psoriatic scale was provided by the close similarity between the dose-response curve generated by such material in straight phase h.p.l.c.-purified scale extracts, and that generated by standard LTB_4 (Figure 3).

The identity of the material causing the variable chemokinetic activity in straight phase h.p.l.c. fractions of normal scale extracts has not been deter-

mined. When normal scale was subjected to partition and purification by the two h.p.l.c. systems described, no chemokinetic activity co-eluting with standard LTB_4 was found (Figure 2c and d). This is in contrast to the results obtained with psoriatic scale where LTB_4-like material was observed consistently after both straight phase h.p.l.c. and straight phase followed by reversed phase h.p.l.c. We have therefore concluded that normal scale does not contain significant amounts of LTB_4.

The presence of LTB_4-like material in extracts of lesional, but not normal scale is consistent with the possibility that this material plays a role in the pathogenesis of the psoriatic neutrophil infiltrate. These results support the previous finding of LTB_4-like material in extracts of chamber fluid from abraded psoriatic lesions (Brain *et al.*, 1982; 1983a). Topical application of 5–500 ng LTB_4 to the skin of human volunteers produces localized erythema and swelling, and histological examination shows intraepidermal neutrophil microabscesses with some similarities to those seen in acute pustular psoriasis (Camp *et al.*, 1984). Neutrophils are however capable of producing LTB_4 upon stimulation (Borgeat & Samuelsson, 1979), and it is not yet clear whether the release of LTB_4-like material in psoriatic lesions is a primary phenomenon or occurs after neutrophils have infiltrated the skin. In addition, the structure of the LTB_4-like material found in extracts of psoriatic scale has, as yet, not been confirmed as being identical to that of authentic LTB_4 by physicochemical methods such as g.c.-m.s. Analysis by ultraviolet absorbance spectrophotometry or g.c.-m.s. requires relatively large amounts of material; experiments using the latter technique are however in progress. Determination of the exact role of the LTB_4-like material in psoriasis must await its structural identification, and the availability of specific lipoxygenase inhibitors or receptor antagonists.

This work was supported in part by grants from The Wellcome (S.D.B. and F.M.C.) and The Dunhill Trust (A.K.B.). We are grateful to S. Mistry, V. McIntosh and J. Isaacs for skilled assistance.

References

BORGEAT, P. & SAMUELSSON, B. (1979). Arachidonic acid metabolism in polymorphonuclear leukocytes: effect of calcium ionophore A23187. *Proc. natn. Acad. Sci. U.S.A.*, **76**, 2148–2152.

BRAIN, S.D., CAMP, R.D.R., DOWD, P.M., KOBZA BLACK, A., WOOLLARD, P.M., MALLET, A.I. & GREAVES, M.W. (1982). Psoriasis and leukotriene B_4 (letter). *Lancet*, **ii**, 762–763.

BRAIN, S.D., CAMP, R.D.R., DOWD, P.M., KOBZA BLACK, A., WOOLLARD, P.M., MALLET, A.I. & GREAVES, M.W. (1983a). Release of leukotriene B_4 (LTB_4) and monohydroxyeicosatetraenoic acids (HETEs) from the involved skin of patients with psoriasis. In *Leukotrienes and other Lipoxygenase Products*. ed. Piper, P.J. pp. 248–254. Chichester: Wiley.

BRAIN, S.D., CAMP, R.D.R., MALLET, A.I., WOOLLARD, P.M. & GREAVES, M.W. (1983b). Hydroxy fatty acid lipoxygenase products are present in superficial psoriatic scale. *Br. J. Pharmac.*, Proc. Suppl. **79**, 359P.

CAMP, R.D.R., MALLET, A.I., WOOLLARD, P.M., BRAIN, S.D., KOBZA BLACK, A. & GREAVES, M.W. (1983). The identification of hydroxy fatty acids in psoriatic skin. *Prostaglandins*, **26**, 431–448.

CAMP, R.D.R., RUSSELL JONES, R., BRAIN, S., WOOLLARD, P. & GREAVES, M.W. (1984). Production of intra-epidermal micro-abscesses by topical application of leukotriene B_4. *J. Invest. Dermatol.*, (in press).

CHOWANIEC, O., JABLONSKA, S., BEUTNER, E.H., PRONIEWSKA, M., JARZABEK-CHORZELSKA, M. & RZESA, G. (1981). Earliest clinical and histological changes in psoriasis. *Dermatologica*, **163**, 42–51.

GOETZL, E.J. & SUN, F.F. (1979). Generation of unique monohydroxyeicosatetraenoic acids from arachidonic acid by neutrophils. *J. exp. Med.* **150**, 406–411.

HAMMARSTROM, S., HAMBERG, M., SAMUELSSON, B., DUELL, E.A., STAWISKI, M. & VOORHEES, J. (1975). 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. natn. Acad. Sci. U.S.A.*, **72**, 5130–5134.

PALMER, R.M.J., STEPNEY, R.J., HIGGS, G.A. & EAKINS, K.E. (1980). Chemokinetic activity of arachidonic acid lipoxygenase products on leukocytes of different species. *Prostaglandins*, **20**, 411–418.

SMITH, M.J.H. & WALKER, J.R. (1980). The effects of some antirheumatic drugs on an *in vitro* model of human polymorphonuclear leucocyte chemokinesis. *Br. J. Pharmac.* **69**, 473–478.

TURNER, S.R., TAINER, J.A. & LYNN, W.S. (1975). Biogenesis of chemotactic molecules by the arachidonate lipoxygenase system of platelets. *Nature*, **257**, 680–681.

(*Received March 30, 1984.*)