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STRUCTURAL IDENTIFICATION OF PLATELET ACTIVATING FACTOR IN PSORIATIC SCALE

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Platelet activating factor was isolated from the lesional scale of psoriatic patients using the method described by Bligh and Dyer (8). The extract was subjected to thin layer chromatography, and the region of the plate co-migrating with platelet activating factor removed. A portion of each sample was assayed for aggregating activity using washed guinea-pig platelets and the remainder treated with phospholipase C, derivatised, and subjected to reversed phase high performance liquid chromatography. Fractions were analysed for platelet activating factor using capillary gas chromatography-mass spectrometry. Nanogram quantities of platelet activating factor were recovered from 100 mg scale and both the C16 and C18 alkyl substituents were present in the ratio 3 : 1, C16 : C18.

Platelet activating factor (PAF-acether) is a lipid mediator of allergic and inflammatory reactions that is produced from a wide variety of cell types (1,2). The structure of PAF-acether derived from animal cells has been shown to be 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine, in which the chain length of the alkyl substituent can vary (3,4). The PAF-acether released from isolated human polymorphonuclear leukocytes (PMNs) following stimulation with the ionophore A23187 has been variously reported to be a mixture of the hexadecyl and octadecyl homologues in proportions between 2.5 to 5 :1 (5), and as the pure hexadecyl form (6). We have recently described the identification of PAF-acether in the lesional skin of patients with psoriasis, an inflammatory and proliferative skin disorder characterised by an early intraepidermal accumulation of PMNs (7). The structure of this material has now been confirmed using capillary gas chromatography-mass spectrometry (GC-MS), and the nature of the mixture of the alkyl ether side chains determined.

ABBREVIATIONS:
PAF-acether, platelet activating factor; GC-MS, gas chromatography-mass spectrometry; TLC, thin layer chromatography; HPLC, high performance liquid chromatography; TBDMS, tert-butyldimethylsilyl ether; SIR, selected ion recording; PMN, polymorphonuclear leukocyte.

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MATERIALS AND METHODS

Isolation of PAF-acether. 130 - 180 mg samples of psoriatic scale were obtained from patients, who had given informed consent, by gentle abrasion with a scalpel blade. No patient had received medication for at least two weeks prior to scraping, and, in each experiment scale from separate individuals was used. Lipids were extracted from psoriatic scale according to the method of Bligh and Dyer (8). The extract was then applied to a silica gel thin layer chromatography (TLC) plate (Silica gel G 500 uM; Anachem), and developed in chloroform:methanol:acetic acid:water (50:25:10:4 v/v) at room temperature. The region of the plate containing PAF-acether was scraped off and extracted with chloroform:methanol:water (1:2:0.8 v/v). After centrifugation (200 g;10 mins), sufficient water and chloroform were added to the supernatant to achieve a final solvent composition of 2:2:1.8 (v/v) and the mixture shaken. The lower layer was recovered, and 90 % removed for the platelet aggregation assay. The remainder was hydrolysed with phospholipase C (50 units per incubation, consisting of 1 ml aqueous borate buffer, pH 8.0, containing 22 umole Ca^{++} and 4 ml ether) for 30 mins at 37°C (9) and subjected to reversed phase high performance liquid chromatography (HPLC) on a Spherisorb ODS column eluted with 96:4, methanol:water (v/v). The fraction containing the 1-O-alkyl-2-acetyl-sn-glycerols was then treated with a novel reagent, the tert-butyldimethylsilyl enol ether of pentane-2:4-dione in order to produce the corresponding tert-butyldimethylsilyl (TBDMS) ethers. The reagent was prepared according to the procedure of Veysoglu and Mitscher (10). The dried product from the HPLC system was treated with 100 ul of a 0.25 M solution of the enol silylether in N,N'dimethylformamide (DMF) together with a catalytic amount of p-toluene-sulphonic acid (10 ul of a 5 mg ml^{-1} solution in DMF) for 10 hrs at room temperature. The reaction mixture was partitioned between water and heptane and the derivatives recovered from the heptane layer in a form appropriate for GC-MS analysis. This procedure has the advantage of producing only volatile and water soluble by-products which do not interfere in the subsequent analysis.

Mass Spectrometry. Mass spectrometric analysis was carried out using a VG Analytical Ltd., 305 mass spectrometer with a 2025 data system. A Pye-Unicam 204 gas chromatograph housing a 25 m x 0.33 mm fused silica capillary column, coated by us with SE-30 stationary phase, and directly coupled to the mass spectrometer source, was used throughout. EI ionisation at 40 ev was used, and selected ion recording (SIR) was carried out using accelerating voltage switching under computer control. Samples were injected onto the column using a falling needle injector and appropriate column temperatures between 240° and 260° C were used.

Platelet Aggregation. 20 mls of blood was collected from anaesthetised guinea-pigs by cardiac puncture into EDTA (0.5 ml anticoagulant for 20 mls blood) and platelet rich plasma (PRP) obtained by centrifugation at 375 g for 20 mins. The platelet pellet formed by centrifugation of PRP at 1400 g for 15 mins was resuspended in an equal volume of Tyrode-gelatin buffer, pH 6.5, containing no calcium (11). After centrifugation, washed platelets were treated with aspirin (0.1 mM) for 15 mins, recentrifuged, and suspended at a concentration of 3×10^9 ml^{-1} in Tyrode gelatin- buffer containing no calcium. 50 ul of platelet suspension was then added to 350 ul Tyrode-gelatin buffer, pH 7.4, and stirred at 900 rpm and 37°C in the cuvette of a Payton aggregometer. Creatine phosphate and creatine phosphokinase in saline were added to the cuvette at final concentrations of 0.7 mM and 39.3 U ml^{-1} respectively, 30 secs prior to addition of either sample or standard PAF-acether (Cambridge Research Biochemicals Ltd.; a 3 : 1 mole:mole mixture of the C16:0 and C18:0 ethers) in saline containing 0.25 % essentially fatty acid free bovine serum albumin. Aggregation was measured as an increase in light transmission in duplicates, and the responses obtained after addition of extracted scale samples compared to a dose response curve to standard PAF-acether.

Unless otherwise stated, all chemicals were obtained from Sigma or Aldrich Chemical Companies.

RESULTS

Measurements of the recovery of radioactive PAF-acether showed that more than 78% was present in the extracts after TLC, at which stage the bioassay was performed, and over 47% was recovered after HPLC and derivatisation immediately prior to GC-MS analysis.

The PAF-acether was extracted from the TLC plates and 90 % of each sample was examined for biological activity using washed guinea-pig platelets. 10 % was subjected to reversed phase HPLC, derivatised and analysed on GC-MS for PAF-acether. Figure 1a is a representative trace showing the aggregating activity obtained in one psoriatic scale extract. 0.3 ml BSA/saline was added

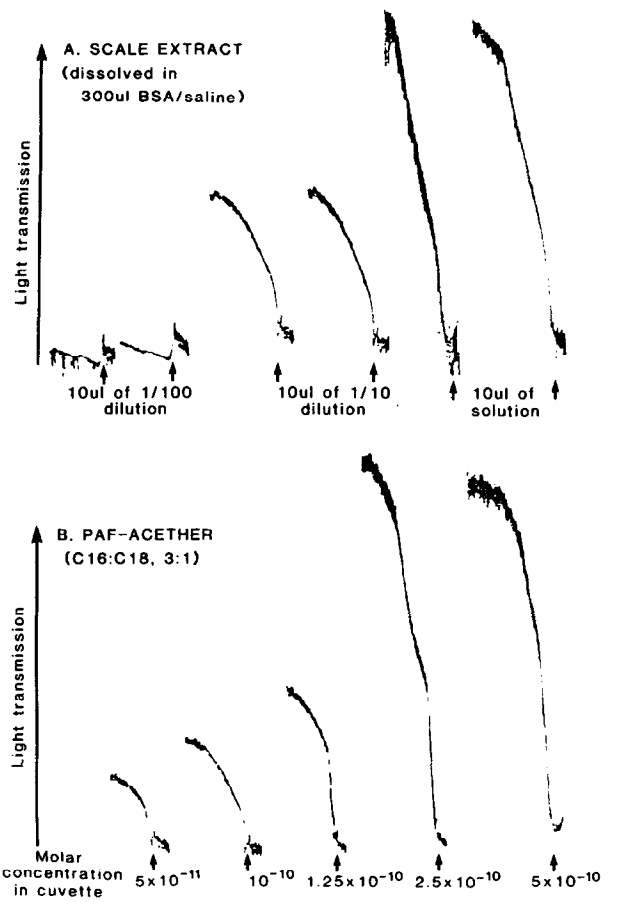

Figure 1. Aggregation of washed guinea-pig platelets, pretreated with aspirin (0.1mM) and creatine/creatine phosphokinase (0.7 mM; 39.3 U ml^{-1}) in response to (A) psoriatic scale extract and (B) standard PAF-acether (C16 : C18, 3:1).

to the dried extract after TLC and 1 : 10 and 1 : 100 dilutions made. 10 ul of each sample was added to the stirred platelet suspension in duplicate, and the increase in light transmission compared to that obtained with a dose response curve to standard PAF-acether (a 3 : 1 mixture as above; Figure 1b). A shape change but no aggregation was routinely observed at concentrations of 10^{-11} M PAF-acether, measurable responses being obtained at concentrations above 2.5 x 10^{-11} M, and reaching a maximum between 10^{-10} and 2.5 x 10^{-10} M. Addition of samples prepared from psoriatic scale produced an aggregation response that was indistinguishable from that induced by standard PAF-acether.

Figure 2 illustrates the full mass spectra obtained for the tBDMS derivatives of C16:0 and C18:0 species of PAF-acether as well as for the PAF-acether isolated from psoriatic scale. SIR of the $[M-57]^+$ ions was carried out for both authentic material and the scale derived product, and measurement of the respective peak heights suggested that the scale derived PAF-acether contained both C16:0 and C18:0 alkyl side chains in the ratio of 3 : 1. Approximate quantities recovered from 100 mg scale were between 0.5 and 3 ng (n = 3). There was no evidence in the scale extracts for the presence of PAF-acether species with other alkyl chain lengths or of unsaturated groups such as C16: 1 (5).

DISCUSSION

Although there have been numerous studies concerning the release of PAF-acether from a variety of animal and human cell types (12), reports of the *in vivo* detection of PAF-acether in man are limited (13,14,15). We have recently reported the presence of PAF-acether in scale derived from patients with the inflammatory and proliferative skin disorder psoriasis (7). The structural identity of this material has now been confirmed using a combination of capillary GC-MS and platelet aggregation. Nanogram quantities of PAF-acether were recovered from 100 mg samples of lesional psoriatic scale, and both the C16:0 and C18:0 alkyl side chains were found to be present in the approximate ratio of 3 :1. No evidence of alkyl-ether side chains of other lengths or degrees of unsaturation was seen. The full mass spectra of the derivatives we

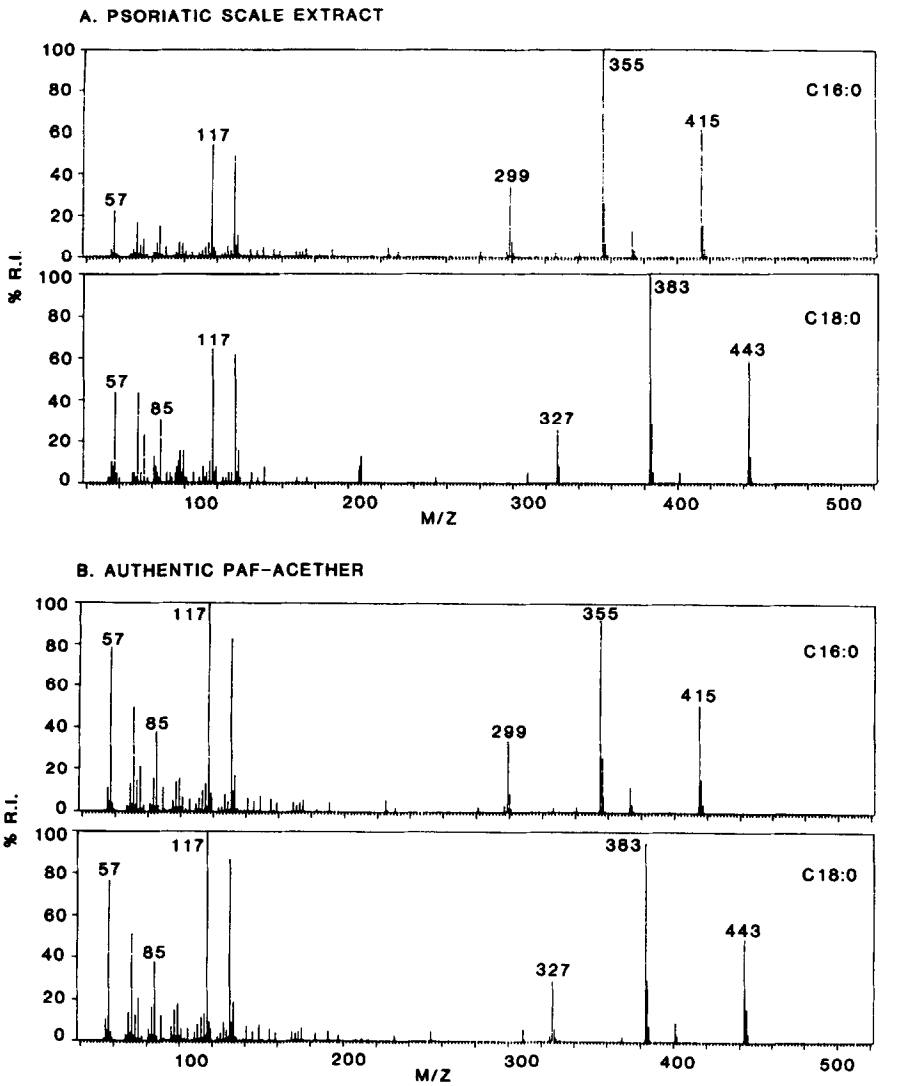

Figure 2. Electron impact mass spectra of the TBDMS derivatives produced from ,(A), extracted psoriatic scale and, (B), authentic PAF-acether. The peaks at m/z 415 and 433 represent the $[M-57]^+$ ions for the C16:0 and C18:0 ethers respectively.

have prepared provide confirmation of the structures of the two PAF-acethers present; the presence of isobaric 1-O-acyl-2-acetyl-glycerol TBDMS ethers can be discounted since these have significantly different EI mass spectra and different gas chromatographic retention times on our system.

The proportions of the C16:0 and C18:0 ether components we have found agree with those published by Satouchi et al (5) for the PAF-acether extracted from human neutrophils stimulated with the ionophore A23187. A recent paper by

Clay et. al.(6) has reported that the PAF-acether released from human neutrophils, stimulated with either A23187 or with opsonised zymosan, only contains the C 16:0 species, with no detectable traces of the C 18:0 or any unsaturated ethers. Pinckard et. al. (16) find evidence for at least five distinct species of PAF-acether in pooled extracts from neutrophils stimulated with formyl methionyl leucyl phenylalanine, although the two groups do agree that the predominant species released is the C 16:0 ether. Until the composition of PAF-acether released from stimulated leukocytes has been agreed, neutrophils, which are present in psoriatic lesions, cannot be discounted as a source of the PAF-acether we have extracted. An alternative source of this material could be found in the resident cell population, consisting primarily of keratinocytes and Langerhan cells, although the capacity of these cells to release PAF-acether has not yet been investigated, nor has the presence of the requisite precursor ether linked phosphocholines or the enzymes necessary to convert lyso-PAF to the active molecule been demonstrated. Although the role of PAF-acether in the development of a psoriatic lesion cannot be defined until the cell type responsible for the generation of the molecule is identified, the potent pro-inflammatory properties of PAF-acether as a vasodilator, permeability increasing agent and chemoattractant could contribute to the inflammatory changes observed in psoriasis, and the maintenance of an established lesion.

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