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Note

Mass spectrometric analysis of endogenous diacylglycerols in normal and psoriatic skin

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The release of diacylglycerol (DAG) from a phosphoinositol precursor is thought to be an early event in the activation of inflammatory cells [1] and the DAGs and inositol phosphates so produced are believed to function as bioregulators, the former by activating protein kinase-C (PK-C) [2] and the latter by acting as second messengers in the release of intracellular Ca^{2+} [3]. Synthetic DAG molecules, especially oleoyl acetyl glycerol [4] and dioctanoyl glycerol [5] have been widely reported to mimic the mitogenic effects of the phorbol esters. Psoriasis is an inflammatory and proliferative skin disorder in which it has been reported that DAGs have a role to play in the activation and subsequent down-regulation of PK-C [6]. Few reports exist on the molecular nature of endogenous DAG species in man and we describe here our methods for their extraction and characterisation in samples from normal skin and from the skin lesions of volunteers with psoriasis.

EXPERIMENTAL

Surface stratum corneum samples were obtained from normal skin by abrading the heels of volunteers using a callus file and from psoriatic skin lesions by gentle abrasion with a scalpel blade. A 10-mg portion of each sample was extracted by homogenisation in glass homogeniser with 15 ml of a heptane–2-propanol (3:2, v/v) mixture. After a 3000 *g* spin the supernatant was evaporated to dryness and the residue dissolved in chloroform–2-propanol (2:1, v/v) prior to lipid class separation. This was performed according to the procedure of Kaluzny et al. [7]. In brief, two aminopropyl bonded solid-phase cartridge columns (Bond-Elut NH_2,

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Jones Chromatography, Glamorgan, U.K.) were washed with 10 ml each of chloroform, methanol and heptane, in that order, and the lipid extract was applied to the first column in a 2:1 (v/v) mixture of chloroform–2-propanol. The column was eluted with a total of 4 ml of the same solvent and this eluate collected and dried down under nitrogen. After redissolving the residue in heptane it was applied to a second washed aminopropyl bonded column. This latter was eluted successively with 4 ml heptane, 8 ml diethyl ether–dichloromethane–heptane (1:10:89, v/v/v), 12 ml ethyl acetate–heptane (1:9, v/v) and 8 ml ethyl acetate–heptane (1:3, v/v). This procedure retained the phospholipids on the first cartridge column and led to four separate fractions containing cholesterol esters, triglycerides, cholesterol and diglycerides, respectively. Monoglycerides remained on the second column and could be recovered with a chloroform–methanol (2:1, v/v) elution.

The DAG fraction thus obtained was purified on silica gel thin-layer chromatography (TLC) using a solvent system of chloroform–acetone (96:4, v/v) and Merck 5721 silica gel 60 plates (BDH, Poole, U.K.), in order to separate the 1:3 from the 1:2 DAGs. The 1:2 DAGs were eluted from the silica gel with chloroform and derivatised to the *tert.*-butyldimethylsilyl (TBDMS) ethers using N-(*tert.*-butyldimethylsilyl)-N-methyl-trifluoroacetamide [8] (MTBSTFA) reagent. The resulting silyl ethers were finally re-chromatographed on silica gel TLC, in order to remove excess reagent, using the same plates and a solvent system consisting of heptane–diethyl ether (9:1, v/v) and, again, recovered by elution with chloroform. In all cases the TLC plates were developed with a 1% solution of 1,6-diphenylhexatriene in heptane which gives rise to fluorescent products with lipids under long-wavelength UV light [9].

All solvents were of HPLC grade and were obtained from Fisons PLC (Loughborough, U.K.) and all lipid standards from Sigma (Poole, U.K.). All other reagents were obtained from Aldrich (Gillingham, U.K.). The mass spectrometric (MS) analyses were performed on a VG Analytical (Manchester, U.K.) Model 305 mass spectrometer operated in electron-impact (EI) mode at 40 eV ionisation potential. The gas chromatograph was a Varian Assoc. (Walton-on-Thames, U.K.) Model 3400 equipped with a cooled on-column injector. The gas chromatographic columns were 12 m × 0.33 mm fused-quartz capillary columns (Thames Chromatography, Maidenhead, U.K.) coated with SE-30 stationary phase in our own laboratory. The column was directly interfaced to the mass spectrometer source and operated at temperatures between 210 and 330 °C.

RESULTS AND DISCUSSION

The lipid class separation method using prepacked aminopropyl solid-phase columns is capable of efficient resolution of the DAGs from the other lipids extracted from skin. The spontaneous isomerisation of the endogenous 1:2 DAGs to the thermodynamically more stable 1:3 molecules, which takes place to a small extent (approximately 5%), on extraction and purification, made a TLC separation step necessary after the class separation. Relatively large volumes of solvents were employed in this study as stratum corneum is a lipid-rich tissue; other

matrices may be efficiently extracted with smaller amounts. In the initial TLC system employed here the R_F values of the 1:2 and 1:3 isomers were 0.46 and 0.56, respectively. Provided the derivatisation was carried out immediately following this separation, further isomerisation was prevented on account of the stability of the resulting silyl ethers.

On MS analysis, the TBDMS ethers showed EI mass spectra, in good agreement with the literature [10], with a base peak at the *m/z* value corresponding to the $[M-57]^+$ ion and prominent ions at lower masses corresponding to losses of the acyl side-chains. Fig. 1 shows the results of two selected ion recording runs, one on normal heel stratum corneum extracts and the other on psoriatic stratum corneum extracts. Eight principal DAGs were identified, by comparison with authentic standards, and the ion monitored at *m/z* 675.54 probably corresponds to a species containing an 18:2 side-chain. Only traces (<1%, data not shown) of species were found at times and masses expected for the appearance of arachidonate containing DAGs. Table I presents the mean relative peak heights of these

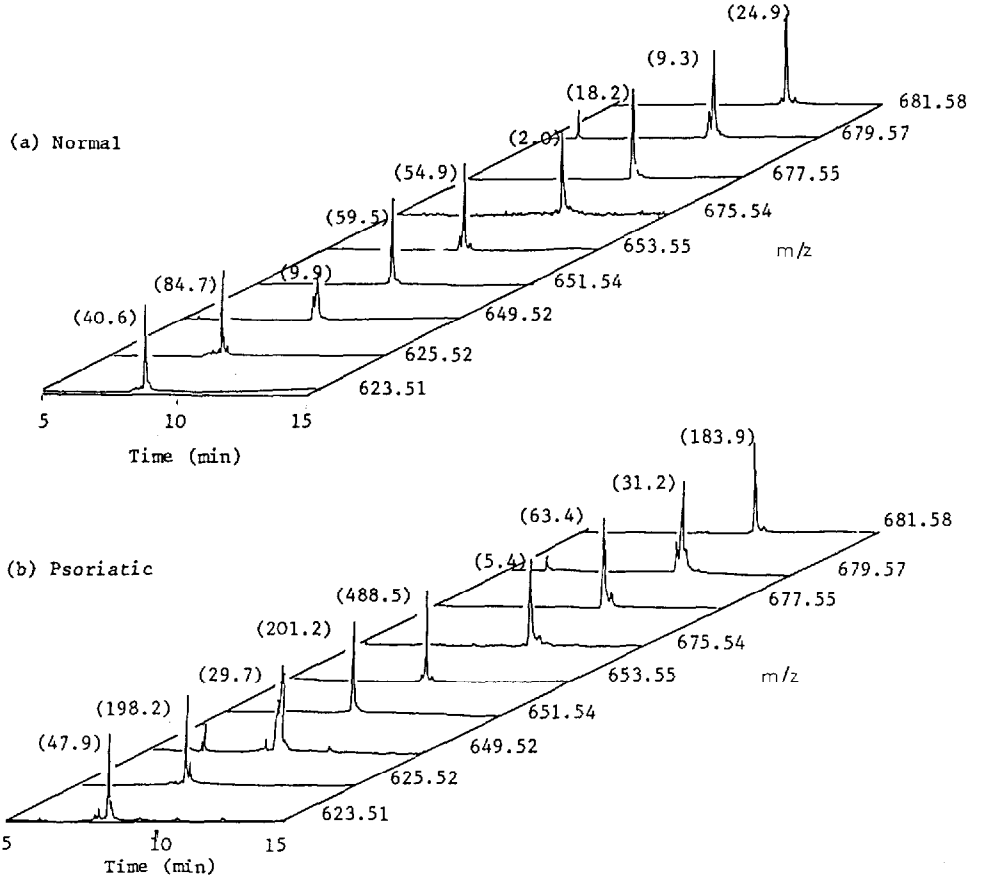

Fig. 1. Selected ion traces for extracted and derivatised DAGs from (a) normal and (b) psoriatic stratum corneum. The intensities of each ion (arbitrary units) are indicated in parentheses on the diagram.

TABLE I

COMPARISON OF THE INTENSITIES OF THE IONS MEASURED FOR THE EIGHT IDENTIFIED DAGs IN NORMAL AND PSORIATIC STRATUM CORNEUM EXTRACTS

m/z	Species	Relative intensity (mean ± S.D.) (%)	
		Psoriatic	Normal
623.51	16:0 16:1	7.3 ± 6.8	13.6 ± 0.6
625.52	16:0 16:0	21.5 ± 5.7	26.9 ± 2.6
649.52	16:0 18:2	2.4 ± 1.2	3.0 ± 0.2
651.54	16:0 18:1	16.1 ± 10.0	17.1 ± 2.7
653.55	16:0 18:0	28.2 ± 9.0	17.1 ± 1.3
677.55	18:1 18:1	10.3 ± 5.3	10.3 ± 4.9
679.57	18:0 18:1	2.9 ± 0.8	4.5 ± 1.9
681.58	18:0 18:0	11.4 ± 6.1	7.4 ± 1.0

eight DAG ions and it is clear that no major difference could be distinguished between results from normal and psoriatic stratum corneum extracts.

The present study did not aim to achieve a quantitative assay for these DAG species, for which rigorous internal standardisation would be required and this would be all the more important if analysis of the polyunsaturated fatty acid containing species was required. The present study only detected traces of arachidonic acid substituted DAGs and future work will concentrate on improving detection of these molecules. However, the procedures described here provide a method for the analysis, at a molecular level, of the DAGs present in endogenous biological sources, a process which has only too infrequently been applied in this field. A notable exception is the work published by Kennerly [11] who analysed the DAGs produced by purified rat mast cells and who also found only a small proportion of arachidonate containing DAGs in lipids released from stimulated cells. The literature contains several, conflicting, accounts of the structural features required by DAGs for activation of PK-C. Oleoyl acetyl and dioctanoyl glycerol [12] have been favourite choices to mimic the effects of phorbol esters in this regard but structural requirements for activation are in dispute [13]. The work described here demonstrates the need for further studies to determine the ability of the described endogenous DAGs to activate PK-C. In addition, the source of the identified compounds and their accessibility to intracellular domains under normal and pathological conditions remain to be established.

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