TIME COURSE CHANGES IN LEVELS OF ARACHIDONIC ACID AND PROSTAGLANDINS D₂ E₂ F_{2α} IN HUMAN SKIN FOLLOWING ULTRAVIOLET B IRRADIATION

A.K. BLACK, N. FINCHAM, M.W. GREAVES & C.N. HENSBY*

Institute of Dermatology, Homerton Grove, London, E9 6BX

- 1 Clinically normal human abdominal skin was irradiated with three minimal-erythema doses of ultraviolet B irradiation, (u.v.B).
- 2 Erythema was assessed visually, and exudate recovered by a suction bulla technique from normal skin, and at 10 min, 2, 6, 18, 24 and 48 h after irradiation.
- 3 Erythema was barely visible at 2 h, but increased to a maximum at 24 h, which was maintained at 48 h.
- 4 Increased arachidonic acid and prostaglandin E₂, F_{2α} and D₂ concentrations in the exudate, measured by gas chromatography-mass spectrometry, accompanied the developing erythema, with the maximal rise of arachidonic acid, prostaglandin E₂ and D₂ occurring at the height of the erythema at 24 h.
- 5 At 48 h, still at the height of the erythema response, arachidonic acid and PGE₂ levels had returned to near normal.
- 6 Concentrations of arachidonic acid and of its products from the cyclo-oxygenase pathway, parallel the development of u.v.B. erythema in the first 24 h.

Introduction

We have previously demonstrated increased amounts of substances resembling arachidonic acid (AA), prostaglandin E₂ (PGE₂) and prostaglandin F_{2α} (PGF_{2α}) in exudate from human skin 6 and 24 h after irradiation with u.v.B (290–320 nm), but the concentrations of these agents fell to control levels at 48 h, even though erythema was still maximal (Black, Greaves, Hensby & Plummer, 1978). Studies at 24 h confirmed these rises by quantitative combined gas-chromatography-mass-spectrometry (GC-MS) (Black, Greaves, Hensby & Warin, 1978). These results, therefore, supported earlier observations that ultraviolet (u.v.) irradiation evokes increased prostaglandin-like activity in human skin (Sondergaard & Greaves, 1970; Snyder & Eaglstein, 1974). We have, therefore, studied the time-course of AA, PGE₂ and PGF_{2α} concentrations in exudates from normal skin up to 48 h after three minimal-erythema doses of u.v.B. irradiation.

Due to the apparent complexity of the relationship of u.v.B. erythema to arachidonic acid and its pre-

viously measured metabolites, it is possible that other vasoactive metabolites of arachidonic acid may be also involved. Since skin homogenates form prostaglandin D (Nugteren & Hazelhof, 1973), and intradermally injected prostaglandin D₂ (PGD₂) produced a long-lasting dose-related erythema in human skin and potentiates the increased vascular permeability due to histamine (Flower, Harvey & Kingston, 1976), we have also measured levels of PGD₂ in exudates from u.v.B. irradiated skin.

Methods

Subjects

Seventeen female and thirty male fair-skinned subjects aged 18–78 years (mean 46.8 ± 2.3 years) were irradiated on untanned clinically normal lower abdominal skin. The subjects, some with localised skin disease, had no history of photosensitivity, and were not taking any oral anti-histamines, corticosteroids or non-steroidal anti-inflammatory drugs. The Institute Ethical Committee approved the experiments and each subject gave prior informed consent.

Present address: *Centre International de Recherches Dermatologique, Sophia Antipolis, 06560 Valbonne, France.

Irradiation

Four F.S.20 Westinghouse sun tubes, with a major emission at 290–320 nm, and an intensity of $430 \mu\text{W cm}^{-2}$ below 320 nm at a distance of 30 cm, were used to irradiate each subject's abdomen at this distance with u.v.B. irradiation. Three minimal-erythema doses were given to an area approximately 60 cm^2 . The resulting erythema was assessed visually as absent, minimal, moderate or deep red (0, 1, 2, or 3 respectively).

Inflammatory exudate

Exudate from erythematous skin was obtained by continuous application of suction at 200 mm Hg below atmospheric pressure (Black, Greaves, Hensby, Plummer & Eady, 1979), at 2, 6, 18, 24 and 48 h after irradiation. In addition 10 min samples were obtained by raising the suction blisters on normal skin first, then irradiating, and removing the bulla fluid 10 min from the completion of irradiation since suction blisters take an average of 2 h to develop. Control samples were obtained from the contralateral non-irradiated abdominal skin of each subject.

Extraction

Exudate ($100 \mu\text{l}$) from individual samples was equilibrated with 25 ng each 3, 3', 4, 4'-tetra deuterio PGE_2 ($\text{d}_4 \text{PGE}_2$) and 3, 3', 4, 4'-tetra deuterio $\text{PGF}_{2\alpha}$ ($\text{d}_4 \text{PGF}_{2\alpha}$) and 50 ng 5, 6, 8, 9, 11, 12, 14, 15 octa deuterio arachidonic acid (d_8 arachidonic acid) in 1.0 ml of $150 \mu\text{M}$ indomethacin in 95% aqueous ethanol. Where possible samples were immediately extracted; if not, they were stored at -20°C prior to extraction.

The exudates, acidified with hydrochloric acid (0.1 M) to pH 4.0, were extracted three times with an equal volume or redistilled ethyl acetate. The pooled organic phase was dried under vacuum at 40°C , using a rotary evaporator.

Thin-layer chromatography

The resulting organic residues were dissolved in 100–200 μl methanol, and subjected to preparative thin-layer chromatography (t.l.c.) on silica gel G glass-backed plates ($100 \times 200 \times 0.25 \text{ mm}$, Anderman) using the F VI solvent system of Anderson (1969) (ethyl acetate, acetone, acetic acid 90: 10: 1 by volume). The fractions corresponding to authentic arachidonic acid, prostaglandins E_2 , $\text{F}_{2\alpha}$ and D_2 were eluted twice with methanol. The PGD_2 assay is based on the conversion of PGD_2 by sodium borohydride reduction into $\text{PGF}_{2\alpha}$ which is then quantitatively assayed by GC-MS. The silica zone containing PGD_2 was eluted twice with 5 ml methanol, with centrifugation between extraction steps to remove trace amounts

of silica gel. The pooled methanol was evaporated to dryness under vacuum at 40°C and the residues redissolved in 1 ml of methanol containing 1–2 mg sodium borohydride. The samples were cooled to 0 to 5°C (on ice/ NaCl), left for 15 min, and then allowed to come to room temperature and then 3, 3', 4, 4'-tetra deuterio $\text{PGF}_{2\alpha}$ 25 ng was added to the solution in 2 ml of distilled water, and the resulting solution acidified to pH 4.0 with 0.1 M hydrochloric acid. The samples were then re-extracted twice with 2–3 volumes of ethyl acetate. The pooled ethyl acetate was taken to dryness under vacuum at 40°C . Reduction of the 11-keto group in PGD_2 with methanolic sodium borohydride results in two C-11 hydroxy epimers of $\text{PGF}_{2\alpha}$ (11α and 11β), but the ratio of 11α (natural $\text{PGF}_{2\alpha}$) to the 11β epimer is at least 95:5 (or greater) (C.N. Hensby unpublished observations). However, the PGD_2 reduction products do not separate on the t.l.c. system employed, unlike the PGE_2 sodium borohydride reduction products (9α hydroxyl: $\text{PGF}_{2\alpha}$; 9β hydroxyl: $\text{PGF}_{2\beta}$). Furthermore, both the 11α and 11β epimers of $\text{PGF}_{2\alpha}$ have almost identical gas chromatographic and mass spectrometric characteristics when analysed as their methyl esters, trimethylsilyl ether derivatives, and can be considered as identical species for the assay (Hensby, 1974).

The residues from the ethyl acetate were re-chromatographed on t.l.c. plates impregnated with 3% silver nitrate using ethyl acetate, acetone, glacial acetic acid (80: 20: 1 by volume). This separates $\text{PGF}_{2\alpha}$ from $\text{PGF}_{1\alpha}$ and 13, 14 dihydro $\text{PGF}_{2\alpha}$ etc.—which may be produced during the sodium borohydride reduction step (e.g. from 15 oxo- PGE_2 and 15 oxo-13, 14 dihydro PGE_2). The $\text{PGF}_{2\alpha}$ zone was again removed and eluted twice with methanol which was evaporated to dryness as described above. The residue was then dissolved in water acidified to pH 4 and re-extracted with ethyl acetate, to remove any colloidal silver that may be extracted from t.l.c. plates. The ethyl acetate was then evaporated as described below.

Preparation of derivatives for gas-liquid chromatography

- Methyl esters: Authentic prostaglandins, arachidonic acid and biological samples were vortexed for 60 s with ethereal methanolic (9:1 v/v) diazomethane. The samples were rapidly taken to dryness under a stream of dry air and the methylation step repeated.
- Oximation: The methyl esters of authentic PGE_2 and PGE_2 zones from the t.l.c. plates were further converted to the corresponding 9-O-methyl oxime by reacting overnight at room temperature with 50–100 μl methyloxime hydrochloride (5 mg ml^{-1} in pyridine). The pyridine was removed under a stream of air.

Table 1 Derivatives and the ions monitored by GC-MS for quantitation of arachidonic acid, PGF_{2α} (PGD₂) and PGE₂.

Compound	Derivative	Ion fragment	M/Z protium	M/Z deuterium
Arachidonic acid	Methylester	M ⁺	318	326
PGF ₂ (+PGD ₂ reduction products)	Methylester trimethylsilyl ether	[M - (90 + 71)] ⁺	423	427
PGE ₂	Methylester 9-O methyloxime trimethylsilyl ether		295	299

- (c) Trimethylsilylation: The desiccated residues of authentic PGF_{2α} methyl ester, the PGF_{2α} zone and the above oximation products were reacted with 50 μl *N,N*-bis (trimethylsilyl)-tri-fluoroacetamide (BSTFA: Sigma) using the method of Thompson, Los & Horton (1970).

Combined gas-liquid chromatography-mass spectrometry (GC-MS)

This was performed on a Finnigan 9610 gas chromatograph equipped with a glass column (1.5 m × 2 mm) packed with 3% OV-1 on Supelcoport interfaced with a Finnigan 4000 mass spectrometer. The system was operated using a Finnigan series 6000 data system. The gas chromatograph was operated at 200–250°C with a carrier gas (helium) flow rate of 20 ml min⁻¹. The mass spectrometer was operated at 25eV electron energy, a pre-amplifier setting of 10⁻⁸ amps/V, ioniser temperatures of 300°C and electron multiplier at 1800 volts. Table 1 shows that M/Z values monitored. Calibration lines for protium/deuterium ratios versus protium concentrations were constructed and used to quantitate the endogenous arachidonic acid and prostaglandin concentrations in the biological samples.

Solvents and chemicals

All solvents were analytical grade or redistilled before use. Pyridine and methyloxime hydrochloride were obtained from Aldrich.

Statistical analyses were by Student's *t*-test.

Results

Erythema

Erythema was barely visible at 2 h, of moderate degree at 6 h, and maximally deep red at 24 h and 48 h. It then gradually faded with associated increase in pigmentation.

Arachidonic acid and prostaglandins

The concentrations of arachidonic acid and prostaglandins D₂, E₂ and F_{2α} measured by gas-liquid chromatography-mass spectrometry following irradiation of human skin by u.v.B irradiation are shown on Table 2. Prostaglandin E₂ levels show a progressive significant elevation at 2, 6, 18 and 24 h, but a fall to near normal was present at 48 h. Prostaglandin F_{2α} was raised at 24 h. Prostaglandins D₂ concentrations were elevated at 6, 18 and 24 h (*P* < 0.05 at 6 and 24 h).

Arachidonic acid levels were also elevated at 2, 6, 18 and 24 h, (*P* < 0.05 at 2, 18 and 24 h), but fell to near control levels at 48 h.

Discussion

The present findings indicate that irradiation of human skin with u.v.B activates arachidonic metabolism, and increases the concentrations of prostaglandins measured in exudates from erythematous skin up to 24 h after irradiation. At the time of still-maximal erythema at 48 h, PGE₂ and arachidonic acid levels had declined to near control levels. Black *et al.* (1978a) previously showed that the levels of prostaglandin F_{2α}, were raised at 24 h but returned to near control levels at 48 h. Irradiation of human skin with u.v.C. produces elevation of PGE₂, PGF_{2α} and arachidonic acid levels in exudate at 6, 18 and 24 h, with a maximum at 18 h. (Camp, Greaves, Hensby, Plummer & Warin, 1978). The continued raised levels of PGE₂ at 48 h after u.v.C. irradiation might explain the still present u.v.C. erythema at that time. Increased prostaglandin D₂ levels have not previously been shown in human inflamed skin but their presence was to be expected following release of arachidonic acid.

The source of the increased prostaglandins measured has not yet been established. It may be epidermal, since human isolated epidermal cells obtained by a keratome synthesize prostaglandins *in vitro* (Gold, Jordan & Winkelmann, 1975). However,

a substantial percentage (3–44%) of light in the ultra-violet B range is transmitted into the dermis (Everett, Yeagers, Sayre & Olson, 1966), where it may stimulate prostaglandin production. Perhaps prostaglandins also originate from leucocytes (Higgs & Youlton, 1972) which are present in the u.v.B-irradiated skin (Pathak & Epstein, 1971).

The relationship of increased prostaglandin concentrations to u.v.B-induced erythema is also uncertain. Our observations suggest that the erythematous reaction up to 24 h is at least partly mediated by prostaglandins, since there was reasonable correlation between intensity of erythema, and changes in the concentrations of arachidonic acid, and prostaglandin E_2 , $F_{2\alpha}$ and D_2 . The ability of indomethacin (Black, Greaves, Hensby, Plummer & Warin (1978b) to totally suppress the evoked prostaglandin increase at 24 h, while only partially suppressing erythema,

supports only partial involvement of the prostaglandins in erythema production. At 48 h, continuance of the deep red erythema, with normal levels of PGE_2 and $PGF_{2\alpha}$, indicates that prostaglandins do not maintain the prolonged erythematous response, but it is possible the action of the PGs formed at 24 h was sustained until 48 h or that the levels were also raised in the intervening time between 24 and 48 h. Studies of other metabolites of arachidonic acid formed via the cyclo-oxygenase and lipoxygenase and other metabolic pathways may help to clarify the production of erythema induced by u.v.B irradiation.

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Table 2 The recoverable levels (mean $\pm$ s.e. mean) of PGE_2 , $PGF_{2\alpha}$, PGD_2 and arachidonic acid measured by GC-MS in exudates from control skin and at various time intervals after three minimal-erythema doses of u.v.B. irradiation

	Arachidonic acid (ng ml ⁻¹)	PGE_2 (ng ml ⁻¹)	$PGF_{2\alpha}$ (ng ml ⁻¹)	PGD_2 (ng ml ⁻¹)
Control	276.3 $\pm$ 19.5 <i>n</i> =47	17.06 $\pm$ 0.7 <i>n</i> =47	18.2 $\pm$ 1.1 <i>n</i> =46	21.8 $\pm$ 1.3 <i>n</i> =35
Time after irradiation (h)				
0.1	294.3 $\pm$ 45.7 <i>P</i> >0.7 <i>n</i> =7	20.2 $\pm$ 1.8 <i>P</i> >0.1 <i>n</i> =7	ND	17.9 $\pm$ 3.1 <i>P</i> >0.2 <i>n</i> =7
2	488.0 $\pm$ 83.2 <i>P</i> <.02 <i>n</i> =5	23.9 $\pm$ 1.9 <i>P</i> <0.005 <i>n</i> =5	ND	26.1 $\pm$ 5.7 <i>P</i> =3 <i>n</i> =5
6	360.2 $\pm$ 69.3 <i>P</i> >0.2 <i>n</i> =9	28.2 $\pm$ 3.3 <i>P</i> <0.02 <i>n</i> =9	ND	35.0 $\pm$ 4.7 <i>P</i> <0.05 <i>n</i> =7
18	540 $\pm$ 59.5 <i>P</i> <0.001 <i>n</i> =6	34.9 $\pm$ 3.4 <i>P</i> <0.005 <i>n</i> =6	ND	34.9 $\pm$ 6.7 <i>P</i> >0.1 <i>n</i> =6
24	785 $\pm$ 56.8 <i>P</i> <0.001 <i>n</i> =13	49.4 $\pm$ 5.2 <i>P</i> <0.001 <i>n</i> =13	32.4 $\pm$ 2.9 <i>P</i> <0.001 <i>n</i> =13	34.3 $\pm$ 4.8 <i>P</i> <0.05 <i>n</i> =10
48	232.9 $\pm$ 24.4 <i>P</i> >0.1 <i>n</i> =7	19.2 $\pm$ 0.8 <i>P</i> >0.05 <i>n</i> =7	ND	ND

P relative to control
ND Not done

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