INCREASED LEVELS OF 6-oxo-PGF_{1α} IN HUMAN SKIN FOLLOWING ULTRAVIOLET B IRRADIATION

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- 1 Clinically normal human abdominal skin in 11 subjects, was irradiated with three times the minimal-erythema dose of ultraviolet B (u.v. B) irradiation.
- 2 Erythema was assessed visually, and exudate was recovered by a suction bulla technique from normal skin, and from skin at 6, 24 and 48 h after irradiation.
- 3 Erythema was moderate at 6 h, but increased to a maximum at 24 h, which was maintained at 48 h.
- 4 6-oxo-PGF_{1α} was markedly raised at 6 h, moderately raised at 24 h, but had returned to control levels at 48 h, when the erythema was still maximal.
- 5 Prostaglandin I₂, the precursor of 6-oxo-PGF_{1α} and 6-oxo-PGF_{1α} may, therefore, play a part in the early inflammatory process after u.v. B irradiation, but is unlikely to be responsible for the erythema still present at 48h.

Introduction

We have previously demonstrated increased levels of arachidonic acid, and prostaglandins D₂, E₂, F_{2α} in exudates from human skin up to 24 h after irradiation with u.v. B, but, the concentrations of these substances fell to control levels at 48 h, even though erythema was still maximal (Black *et al.*, 1980). Prostaglandin I₂ (PGI₂) is a potent vasodilator in man (Dusting *et al.*, 1977), and raised concentrations of its chemical hydrolysis product, 6-oxo-PGF_{1α} have been found in inflammatory processes in animals (Humes *et al.*, 1977) and in rheumatoid joint fluid in man (Brodie *et al.*, 1980). Since 6-oxo-PGF_{1α} also appears to possess significant vasodilator activity in human skin (Higgs *et al.*, 1979) we have measured 6-oxo-PGF_{1α} levels in skin exudate up to 48 h after u.v. B irradiation, as it is possible PGI₂ and 6-oxo-PGF_{1α} may contribute to the production of u.v. B erythema, and might explain its persistence at 48 h after irradiation.

The results of the 6-oxo-PGF_{1α} levels have been communicated to the British Pharmacology Society meeting on 10–12 September, 1980.

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Methods

Subjects

Eight male and three female fair-skinned subjects aged 43–71 years, were irradiated on untanned, clinically normal lower abdominal skin. The subjects had healed minor skin disease at other sites (hand and foot eczema), had no history of photosensitivity and were not taking oral antihistamines, corticosteroids, or non-steroidal anti-inflammatory drugs. The Institute's Ethical Committee approved the experiments, and each subject gave prior consent.

Irradiation

Two Phillips TL 20W/12 suntubes, with a major emission at wavelengths between 290–320 nanometres, and with an irradiance of 180 μW cm⁻² in the u.v. B range at a distance of 30 cm were used to irradiate each subject's abdomen. Three times the minimal erythema dose was given to an area approximately 20 cm². Erythema was assessed visually as absent, minimal, moderate or deep red.

Inflammatory exudate

Exudate was obtained from erythematous skin at 6, 24 or 48 h after u.v. B irradiation, using continuous suction at 200 mmHg below atmospheric pressure (Black *et al.*, 1977). Control samples were obtained from non-irradiated abdominal skin of each subject.

Extraction

Exudate (200 μ l) from individual samples was equilibrated with 25 ng each of 3,3',4,4',-tetra deuterio 6-oxo-PGF_{1 α} , PGE₂ and PGF_{2 α} (d₄ 6-oxo-PGF_{1 α} , d₄ PGE₂, d₄ PGF_{2 α}), and 100 ng 5,6,8,9,11,12,14,15 octadeuterio arachidonic acid (d₈ arachidonic acid) in 1.0 ml 15 μ M indomethacin in 95% aqueous ethanol. The samples were stored at -20°C prior to extraction.

The exudates, after dilution at 10 ml with distilled water and acidification to pH 3 with 0.1 M hydrochloric acid, were applied to 10 ml volume Amberlite XAD-2 columns, and successfully eluted with 15 ml of distilled water, and 5 ml n-heptane. These fractions were discarded. Prostanoids and arachidonic acid were recovered from the XAD-2 by elution with 10 ml of methanol, which was evaporated to dryness under nitrogen.

Thin-layer chromatography

The methanol residues were subjected to preparative thin-layer chromatography on silica gel G(200 $\times$ 100 $\times$ 0.25 mm on glass—Merck) using ethyl acetate/acetone/glacial acetic acid (90:10:1 by volume) as the eluting solvent. Zones corresponding with authentic 6-oxo-PGF_{1 α} plus PGF_{2 α} , PGE₂ and arachidonic acid were scraped from the plates, and eluted twice with methanol which was evaporated to

dryness at 40°C under vacuum. Further separation of 6-oxo-PGF_{1 α} and PGF_{2 α} was obtained on thin-layer chromatography using the eluting solvent, chloroform/methanol/acetic acid/water (90:10:1:0.65 by volume).

Preparation of derivatives for gas-liquid chromatography

The vacuum dried residues containing arachidonic acid, PGF_{2 α} and PGE₂ were converted to their corresponding methyl ester, methyl ester trimethylsilyl ether and methyl ester, *o*-methyloxime, trimethylsilyl ether derivatives respectively, as described previously (Black *et al.*, 1980). The 6-oxo-PGF_{1 α} was converted to its corresponding methyl ester, *o*-methyloxime, trimethylsilyl ether as detailed previously (Hensby *et al.*, 1979).

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

Quantitative multiple ion detection GC-MS was used for the estimation of arachidonic acid and prostanoids. Either a Finnigan 3200 or a Finnigan 4000 instrument equipped with a 1.5 m $\times$ 2 mm glass column packed with 3% OV-1 was used. The details of the equipment used are described elsewhere (Black *et al.*, 1980; Hensby *et al.*, 1979; Brodie *et al.*, 1980).

Solvents and chemicals

All solvents were of analytical grade or were redistilled before use. Statistical analysis was by Student's *t*-test.

Table 1 The recoverable levels in ng ml⁻¹ (mean $\pm$ s.e. mean) of 6-oxo-PGF_{1 α} , PGE₂, PGF_{2 α} and arachidonic acid measured by GC-MS in exudate from control skin and at various time intervals after three erythema doses of ultraviolet B irradiation.

	6-oxo-PGF _{1α}	PGE ₂	PGF _{2α}	Arachidonic acid
Control	8.7 $\pm$ 0.9 <i>n</i> = 11	17.1 $\pm$ 0.8 <i>n</i> = 11	17.0 $\pm$ 0.9 <i>n</i> = 11	213.1 $\pm$ 26.4 <i>n</i> = 11
Time after irradiation (h)				
6	43.0 $\pm$ 4.9 <i>n</i> = 4 <i>P</i> < 0.001	26.9 $\pm$ 1.1 <i>n</i> = 4 <i>P</i> < 0.001	24.9 $\pm$ 2.3 <i>n</i> = 4 <i>P</i> < 0.001	373.3 $\pm$ 28.1 <i>n</i> = 4 <i>P</i> < 0.005
24	14.5 $\pm$ 1.1 <i>n</i> = 4 <i>P</i> < 0.005	36.6 $\pm$ 2.8 <i>n</i> = 4 <i>P</i> < 0.001	28.4 $\pm$ 1.0 <i>n</i> = 4 <i>P</i> < 0.001	574.3 $\pm$ 86.0 <i>n</i> = 4 <i>P</i> < 0.001
48	80 $\pm$ 1.0 <i>n</i> = 3 <i>P</i> > 0.7	17.0 $\pm$ 0.5 <i>n</i> = 3 <i>P</i> > 0.9	20.5 $\pm$ 0.9 <i>n</i> = 3 <i>P</i> > 0.05	274.0 $\pm$ 25.7 <i>n</i> = 3 <i>P</i> > 0.2

n number of subjects; *P* relative to control.

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 of pigmentation.

Arachidonic acid and its metabolites

The concentration of arachidonic acid, 6-oxo-PGF_{1α}, PGE₂ and PGF_{2α}, measured in the exudates, are shown in Table 1. The levels of 6-oxo-PGF_{1α} were markedly raised at 6 h, moderately raised at 24 h, but had returned to control levels at 48 h. In this study, as in previous ones, the concentrations of arachidonic acid, PGE₂ and PGF_{2α} were also elevated at 6 h, but reached a maximum at 24 h, and returned to near control levels at 48 h (Black *et al.*, 1980).

Discussion

The present findings confirm that irradiation of human skin with u.v. B wavelengths activates arachidonic acid metabolism, and increases the concentrations of prostanooids measured in exudates from erythematous skin up to 24 h after irradiation. We have now shown markedly increased levels of 6-oxo-PGF_{1α} in an inflammatory skin response in man especially in the early stages of inflammation. Therefore, 6-oxo-PGF_{1α}, and its vaso active precursor PGI₂ may be an important mediator of this response, particularly at 6 h, and less so at 24 h. The results also suggest that 6-oxo-PGF_{1α} achieves its maximum concentration at an earlier time than the other prostaglandins measured. Therefore, the therapeutic effect of non-steroid anti-inflammatory drugs, may partly be due to their inhibition of synthesis of PGI₂ (Dusting *et al.*, 1978) as well as PGE₂.

The intense erythema at 48 h is unlikely to be due to PGI₂, because intradermal injections into humans in nanogram amounts, produce erythema lasting only 1 h (Higgs *et al.*, 1979), and 6-oxo-PGF_{1α} levels are only slightly elevated at 24 h and similar to control levels at

48 h. However, PGE₂ could be responsible for erythema between 24 and 48 h since we did not carry out determination of PGE₂ levels between 24 h, when the concentrations of PGE₂ was maximum, and 48 h. Although the arachidonic acid concentrations had returned to control values at 48 h, the rate of arachidonic acid turnover may have been high at this time. Thus we cannot exclude the possibility that other products of arachidonic acid metabolism, which we have not measured, might be involved in the later stages of the reaction.

The basal levels of 6-oxo-PGF_{1α} in normal skin may be due to a steady turnover, analogous to that of PGE_{2α} and PGF_{2α} in healthy skin. Administration of indomethacin, an inhibitor of prostaglandin synthetase, causes significant elevation of arachidonic acid levels, suggesting a significant metabolism of arachidonic acid via the cyclo-oxygenase pathway in normal skin (Black *et al.*, 1978).

The source of 6-oxo-PGF_{1α} in the inflammatory exudate is uncertain. It is probably derived from PGI₂ synthesised locally by endothelial cells (Weksler *et al.*, 1977) or perhaps by leucocytes (Blackwell *et al.*, 1978) which are present in small numbers in u.v. B irradiated animal skin (Eaglstien *et al.*, 1979).

It is not known how good the correlation is between concentrations of PGI₂ and its hydrolysis product 6-oxo-PGF_{1α} in the skin. However, after intravenous infusions of PGI₂, at increasing concentrations, there is not a proportionate increase in plasma 6-oxo-PGF_{1α} recovered (Hensby *et al.*, 1979).

Thus metabolism of PGI₂ by other pathways is possible (Johnson *et al.*, 1976) and this raises the possibility that actual levels of PGI₂ in our exudate samples were higher, and therefore, more significant, than the measured concentrations of 6-oxo-PGF_{1α} indicate.

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