Vol. 136, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1986 Pages 169-176

# STEREOCHEMICAL DIFFERENCE BETWEEN 12-HYDROXY- 5,8,10,14-EICOSATETRAENOIC ACID IN PLATELETS AND PSORIATIC LESIONS

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Received February 19, 1986

Stereochemical analysis of 12-hydroxy-5,8,10,14-eicosatetraenoic acid derived from the lesional scale of patients with psoriasis is reported. Resolution of the C-12 hydroxyl enantiomers was carried out by high pressure liquid chromatography of their diastereomeric methyl ester dehydroabietyl urethane derivatives. The 'psoriasis derived" compound was shown to be stereochemically distinct from the platelet 12(S)-enantiomer as its derivative co-chromatographed with the 12(R)-diastereomer. © 1986 Academic Press, Inc.

12-HETE is a major proinflammatory metabolite of arachidonic acid in human skin and has been assumed to be formed by the action of an epidermal 12 - lipoxygenase enzyme similar to that found in platelets (1). It is generally accepted therefore, that the stereochemical constraints of lipoxygenase reactions are adhered to and that the product formed in human skin is 12(S)-HETE, identical to that produced by the platelet enzyme (1,2).

The lesional skin of patients with psoriasis exhibits an increased ability to synthesize 12-HETE from exogenous arachidonic acid in vitro (3) and also contains markedly higher concentrations of these compounds in vivo (4). Recent evidence has shown that the amount of 12-HETE present in scale derived from lesional skin is sufficient to cause erythema on topical application to human skin (5), or to cause polymorphonuclear leukocyte chemokinesis in vitro (5) and may be partly responsible for the inflammatory aspects of psoriasis.

If inhibition of 12-HETE synthesis is to be evaluated as a mode of action of anti-inflammatory drugs in the treatment of psoriasis the

Abbreviations: 12-HETE, 12-hydroxy- 5, 8, 10, 14-eicosatetraenoic acid; DHA, dehydroabietyl-

0006-291X/86 $1.50
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nature of the arachidonic acid conversion must be confirmed. Therefore it has been necessary to establish first whether the 'psoriasis derived' 12-HETE originates solely via a specific biosynthetic mechanism, or was also the result of a less specific autoxidative process occurring on the large surface area of the lesional psoriatic skin. Distinction between these routes of formation can be made by determining the contribution of the constituent (S) and (R) hydroxyl enantiomers in the 12-HETE recovered. This communication describes the use of diastereomeric methyl ester DHA urethane derivatives for the stereochemical analysis of 12-HETE by HPLC and reports the findings of this analysis applied to 12-HETE derived from lesional psoriatic skin.

## MATERIALS AND METHODS

(+)-DHA amine (~90%) and 4-dimethylaminopyridine (purum) were obtained from Fluka (Glossop, UK); trichlorosilane (95%) was from Aldridge (Gillingham, UK); ethylchloroformate (96.5%) and triethylamine (99%) used in the preparation of the DHA isocyanate, were from BDH (Poole, UK) and arachidonic acid was from Sigma London (Poole, UK). All solvents were of HPLC grade and were purchased from Fisons (Loughborough, UK). Sep Pak™ silica cartridges were obtained from Millipore (Harrow, UK).

### Synthesis of Dehydroabietyl Isocyanate

(+)-DHA amine was purified by two recrystallisations of its acetate salt from toluene as described (6). After regeneration of the amine by reaction with sodium hydroxide (6M) the product was extracted two times with one volume of ether, was then washed three times with water. Evaporation of the ether under reduced pressure left a pale yellow viscous amine which crystallised on cooling. DHA isocyanate was then prepared after conversion of the amine to the ethyl carbamate using the method described by Pirkle and Hoekstra (7). Vacuum distillation of the product was carried out and the viscous fraction distilling between 218 - 220°C (6 mbar) was collected. Care was taken to avoid contamination of the DHA isocyanate by viscous side-products.

Confirmation of the structure of DHA isocyanate was obtained by electron impact mass spectrometry. Mass spectra were acquired using a VG 305 analytical model single focusing magnetic sector mass spectrometer interfaced to a VG 2025 data system. Sample introduction was via the probe inlet.

### Synthesis of 12-HETE Enantiomers

#### Racemic 12-HETE

Racemic 12-HETE was prepared by reaction of arachidonic acid with singlet oxygen and purified as described previously (8), using a modification of the method of Porter *et al* (9).

12(S)-HETE

12(S)-HETE was prepared by incubation of arachidonic acid with washed human platelets according to the method of Hammarstrom et al. (10). After acidification of the incubate (pH 3.5) and extraction with ethyl acetate the organic phase was evaporated under nitrogen at 40°C and the residue treated in the same manner as the 'psoriasis derived' 12-HETE as described next.

'Psoriasis Derived' 12-HETE

Psoriatic scale samples (196 - 610 mg) were obtained by gentle abrasion of skin lesions. Each sample was mixed with sodium acetate buffer (6ml, pH 3.6, 0.1M) and extracted two times with an equal volume of ethyl acetate. The pooled extract was evaporated to dryness under nitrogen at 40°C. The residues from these and platelet incubates were then subjected to partition between sodium phosphate buffer (3 ml, pH 8.4, 0.1M) and 1-chlorobutane (3 ml) to remove polar lipids. The organic phase was removed, evaporated to dryness under nitrogen and the non-polar lipids removed by partitioning between heptane (4 ml) and methanol (3 ml). The methanol layer containing the monohydroxy fatty acids was subjected to straight phase HPLC on a semi-preparative Spherisorb S5 W column using hexane : propan-2-ol : methanol : acetic acid (1070 : 5 : 15 : 1 by vol) as the eluting solvent. 12-HETE was collected and evaporated to dryness under nitrogen.

Derivatisation

Sample residues were methylated with methanol : ethereal diazomethane (1 : 9 v/v) and the methyl esters of the platelet and 'psoriasis derived' 12-HETE were then purified by reversed phase HPLC on an analytical Spherisorb S5 ODS column using methanol : water (9 : 1 v/v) as the eluting solvent. Racemic 12-HETE methyl ester needed no purification prior to further analysis.

Formation of DHA urethane derivatives

The DHA urethane derivative of the 12-HETE methyl ester samples was prepared by an adaption of the method of Corey and Hashimoto (11). The methyl ester was reacted in glass vials (1.75 ml vol) with DHA isocyanate (0.38 M) and 4-dimethylaminopyridine (0.26 M) in dichloromethane (200 ul) at 60°C for 65 hr. The solvent was evaporated under a stream of nitrogen, the products dissolved in ether:hexane (5:1 v/v) and the solution then applied to a silica Sep Pak™ column. The Sep Pak™ column was then eluted consecutively with hexane (5 ml) and diethyl ether (5 ml). The methyl ester DHA urethane derivative which was recovered from the diethyl ether fraction after evaporation was then subjected to analysis by HPLC.

Steric Analysis

Analysis of the diastereomeric methyl ester DHA urethane derivatives was carried out by HPLC on a Spherisorb S5W column (25cm x 4.9mm)eluted with hexane: isopropanol: methanol (400:1:1 by vol) at 1 ml.min$^{-1}$. UV absorbance was monitored at 236 nm.

## RESULTS

The double bond in the allylic 12 - hydroperoxy group formed by the reaction of singlet oxygen with arachidonic acid under the conditions used is known to have the trans configuration identical to that produced by the 12 - lipoxygenase enzyme (9). However in this reaction abstraction of the hydrogen at C-10 and the substitution of the hydroperoxy group at C-12 are not stereoselective and both 12(S)- and 12(R)-hydroperoxy- 10trans, 5,8,14cis- eicosatetraenoic acid are formed (12).

After reduction of the hydroperoxy group, resolution of the resultant racemic 12-HETE was possible by HPLC of the diastereomeric methyl ester DHA urethane derivatives (Fig 1). Purification of the diastereomers was carried out by collection of the eluting compounds and each was obtained with less than 3% contamination by its epimer, as determined by HPLC of the purified compounds.

Due to the close elution of the two diastereomers, derived from either 12(R)- or 12(S)-HETE, identification of each was carried out by sequential HPLC analyses of the platelet 12(S)-HETE derivative co-

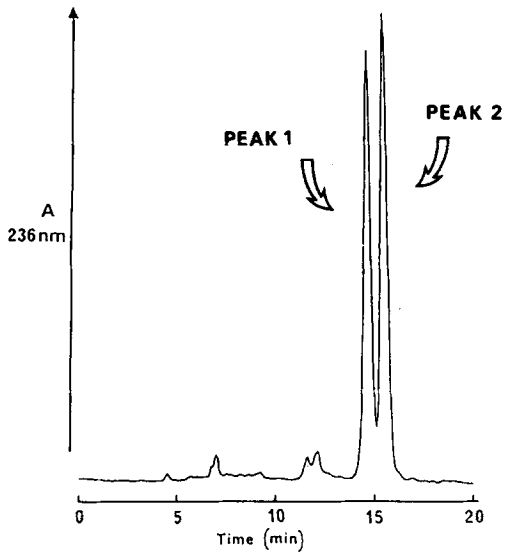


FIG. 1:
HPLC profile of the diastereomeric methyl ester DHA urethane derivatives of racemic 12-HETE. HPLC was carried out on a Spherisorb S5W column (25cm x 4.9mm) with the solvent system - hexane : propan-2-ol : methanol (400 : 1 : 1 by vol) at a flow rate of 1ml. $min^{-1}$. UV absorbance was monitored at 236 nm. The derivatives assigned PEAK 1 and PEAK 2 as shown were collected after HPLC.

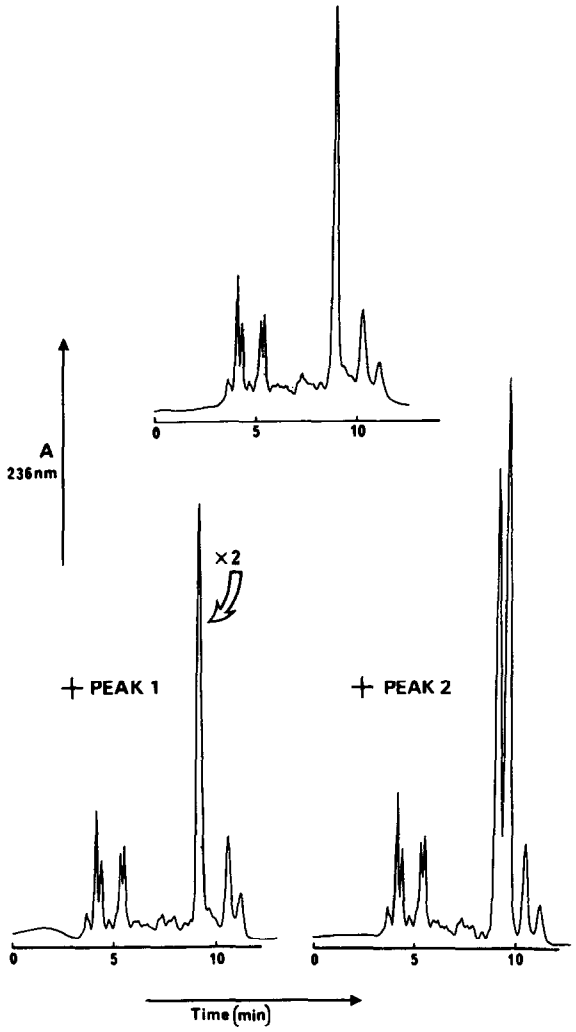


FIG. 2:
Sequential HPLC analyses of platelet 12(S)-HETE methyl ester DHA urethane derivative injected alone (top trace) and co-injected with aliquots of each of PEAK 1 and PEAK 2 (bottom traces). PEAK 1 is identified as the 12(S)-HETE due to its co-elution with platelet 12(S)-HETE derivative. PEAK 2 is derived from 12(R)-HETE.

injected with each purified diastereomer in turn (Fig 2). The platelet 12(S)-HETE derivative co-chromatographed with the earlier eluting, less polar component of the mixture indicating that this component was the 12(S) derivative (Peak 1) (Fig 2).

Analysis of the 'psoriasis derived' 12-HETE was carried out in the same manner using a pooled sample obtained from 6 patients and separate samples from 5 individual patients. In each case the major psoriatic scale derivative co-chromatographed with the later eluting, more polar component of the mixture, the 12(R)-HETE derivative (Peak 2) (Fig 3).

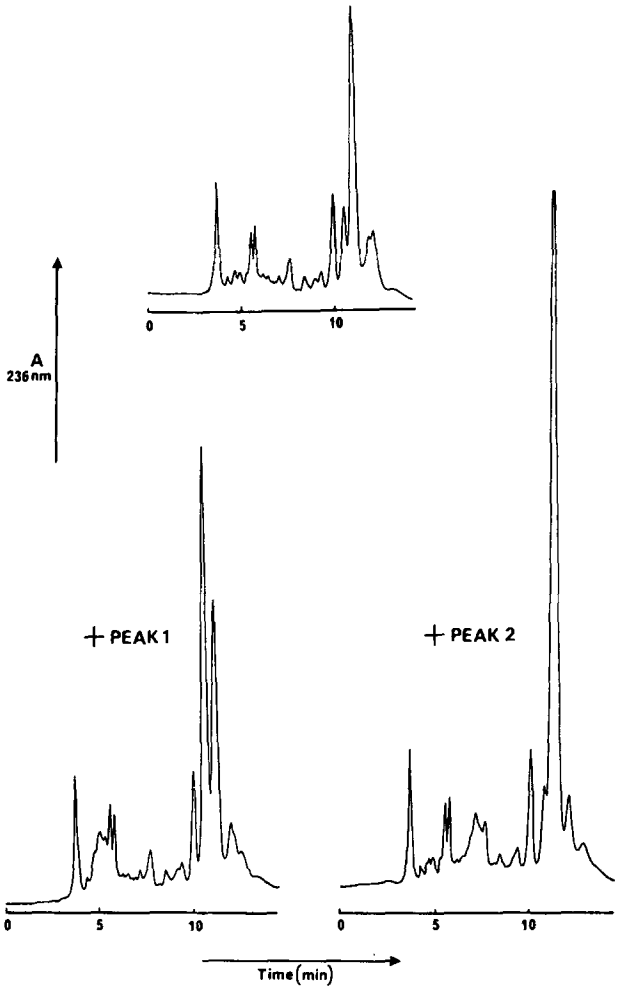


FIG. 3:
Sequential HPLC analyses of 'psoriasis derived' 12-HETE methyl ester DHA urethane derivative injected alone (top trace) and co-injected with aliquots of each of the derivatives of 12(S)- and 12(R)-HETE (bottom traces). "Psoriasis derived' 12-HETE was obtained from a pooled sample of lesional scale (196 - 610 mg) from six patients with psoriasis. The methyl ester DHA urethane derivative co-elutes with the 12(R)-HETE derivative.

## DISCUSSION

The results obtained indicate that 12-HETE present in the scale of patients with psoriasis is not 12(S)-HETE as is widely assumed and suggest that its enantiomer, 12(R)-HETE, is the predominant stereoisomer found. The possibility that the isomer present is a double bond stereoisomer is unlikely as trans, trans conjugated dienes would be expected to separate during the initial chromatographic steps (13).

Previous results have shown that oxidation of a number of fatty acids occurs in psoriatic skin and that in addition to 12-HETE small amounts

of the other conjugated diene monohydroxy eicosatetraenoic acids are present (8). The wide range of substrates and products present suggested that autoxidation may account, in part, for the increase in 12 - HETE concentrations. However, the predominance of a single diastereomeric derivative is evidence that autoxidation of free arachidonic acid contributes minimally to the 12 - HETE present in scale and that a specific biosynthetic process is involved.

The likelihood that 'psoriasis derived' 12 - HETE is synthesized enzymically in the skin is also supported by the findings that high speed supernatants of human skin homogenates convert arachidonic acid into 12 - HETE (3,14) and that both guinea pig (15) and mouse epidermis (16) contain a 12- lipoxygenase - like enzyme. However distinct differences were found between the properties of both enzymes and those of the 12 - lipoxygenase in platelets. In particular, 12-HETE synthesised by mouse skin homogenates originates largely from a particulate enzyme suggested to be a cytochrome $P_{450}$ dependent system similar to that in rat liver (16). Arachidonic acid is known to be metabolised by a rat liver cytochrome $P_{450}$ dependent enzyme which forms monohydroxy eicosatetraenoic acids with the correct cis, trans diene configuration, possesses stereoselectivity with regard to its oxygenated products, is inhibited by 5,8,11,14-eicosatetraynoic acid and possesses other mechanistic similarities to the lipoxygenase reaction (17, 18). In view of the diverse nature and origin, yet functional similarity of these enzymes, crucial differences in the mechanism of hydrogen abstraction and oxygenation may be responsible for formation of the stereochemically distinct 12-HETE found in psoriatic plaque.

It will be of interest to further characterise the nature and site of the enzyme and to determine whether differences in its stereoselectivity are important to the biochemistry of the normal or diseased epidermis. In particular, care must be taken in extrapolating results of drugs defined as 12-lipoxygenase inhibitors on the basis of platelet studies, to their use as inhibitors of the skin enzyme(s).

ACKNOWLEDGEMENTS

Thanks are due to Mr. G. Ramos for his valuable technical assistance, to my clinical colleagues for collection of psoriatic skin scale and to the Medical Research Council of Great Britain for supplying funds for the VG 305 model Mass Spectrometer.

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