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Short Communication

In vivo transformation of arachidonic acid into 12-hydroxy-5,8,10,14-eicosatetraenoic acid by human nasal mucosa

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ABSTRACT

A method for the determination of 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) by gas chromatography–mass spectrometry (GC–MS) in samples obtained from healthy subjects by nasal lavage is presented. HETEs were extracted from samples obtained by nasal lavage using C_{18} solid-phase cartridges. The purification of 12-HETE and 5-HETE was carried out in two consecutive steps of purification by reversed-phase high-performance liquid chromatography. Methylated and trimethylsilylated fractions were separated by GC–MS with electron-impact ionization. The production of 12-HETE by human nasal mucosa was confirmed by GC–MS.

INTRODUCTION

Nasal mucosa from human subjects has been used as an *in vivo* model to study the synthesis and release of arachidonic acid (AA) in inflammatory reactions [1]. 15-Hydroxy-5,8,11,13-eicosatetraenoic acid (15-HETE) has been reported as the major metabolite of AA detected in nasal lavage samples from healthy subjects [2]. This agrees with the *in vitro* production of 15-HETE and other 15-lipoxygenase metabolites derived from the oxidation of 15-hydroperoxyeicosatetraenoic acid (15-HPETE) such as 8,15-diHETEs, 8,15-leukotrienes (8,15-LTs) and 14,15-diHETEs described in cultured nasal [3], bronchial [3] and tracheal epithelial cells [4]. These cells also produce small amounts of 12-HETE and 8-HETE. However, there are no data about the *in vivo* synthesis and release of these products by the respiratory airways of humans.

The aim of this study was to identify 12-HETE and 5-HETE in human nasal secretions by gas chromatography–mass spectrometry (GC–MS) to complement the earlier observation of 15-HETE in nasal lavage samples.

EXPERIMENTAL

Samples

Nasal lavage samples were obtained from healthy volunteers who had refrained from taking anti-inflammatory drugs for at least two weeks before the study, according to the method of Naclerio *et al.* [5]. Briefly, with the head tilted

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backwards, 4 ml of normal saline (0.9% NaCl) were instilled into each nostril while the subjects neither breathed nor swallowed. After 10 s, the subjects expelled the mixture of saline and nasal secretion into polypropylene tubes. Samples were stored at -80°C until extraction.

Extraction procedure

Samples were centrifuged at 20 000 *g* (4°C) for 20 min and 2.5-ml aliquots of the supernatants were processed, without modification of pH, through C_{18} cartridges (Baker, Phillipsburg, NJ, USA), which were washed with 10 ml of water. HETE compounds were finally eluted with 5 ml of methanol [6], which was evaporated to dryness under vacuum. Tritiated standards [14 pg/ml 15-HETE, 22 pg/ml 12-HETE, 14 pg/ml 5-HETE and 25 pg/ml leukotriene B_4 (LTB_4)] were added to the supernatants of centrifuged nasal washes when the extraction recoveries were evaluated.

Purification by HPLC

For the subsequent GC–MS identification, a pool of extracted nasal lavage samples was subjected to two consecutive HPLC purification steps. Two Spectroflow 400 pumps (Applied Biosystems, Ramsey, NJ, USA) were used. The biological extract was chromatographed on a Spherisorb ODS-2 column (250 mm × 4.6 mm I.D., 10 μm; Phase Separations, Deeside, UK) isocratically eluted with methanol–water–trifluoroacetic acid–triethylamine (75:25:0.1:0.05, v/v) at a flow-rate of 1.5 ml/min [7]. The retention times of the HETE compounds were established with tritiated standards, recording the tritium response with a radioactivity detector (Raytest Ramona, Issomess, Straubenhardt, Germany). After collection, evaporation and lyophilization, the 12-HETE and 5-HETE fractions were rechromatographed on the same column using water–acetonitrile–tetrahydrofuran–acetic acid (44:36:20:0.05, v/v) as a mobile phase at a flow-rate of 1.5 ml/min [8].

Gas chromatography–mass spectrometry

The twice-purified 12-HETE and 5-HETE fractions were methylated with diazomethane in diethyl ether for 15 min at room temperature in the dark and then silylated with N,O-bis(trimethylsilyl)trifluoroacetamide for 1 h at 60°C, as described previously [9]. MS studies were carried out using a Hewlett-Packard (Palo Alto, CA, USA) Model 5995 gas chromatograph–quadrupole mass spectrometer equipped with an OV-1 glass capillary column (25 m × 25 mm, film thickness 0.33 μm). The GC conditions were: column oven temperature, 190°C; injector, 260°C; column temperature programmed from 190 to 280°C at 3°C/min; and flow-rate of carrier gas (helium), 22.5 ml/min. The mass spectrometer was operated under electron–impact conditions at 70 eV ionization energy. The dwell time for selected-ion monitoring (SIM) was 210 ms with a cycle time of 1.3 s.

RESULTS

Recoveries of tritiated 15-HETE, 12-HETE, 5-HETE and LTB_4 standards added to the nasal lavage samples were 97.1 ± 2.1, 95.9 ± 1.6, 95.9 ± 0.7 and 101.2 ± 1.7%, respectively (Table I).

Representative chromatograms of tritiated standards run under the HPLC conditions used to purify 12-HETE and 5-HETE from nasal lavage samples are shown in Fig. 1. These conditions allow a complete separation of HETE compounds and as the mobile phases are sufficiently volatile, the collected fractions can be readily evaporated dryness.

The relative SIM response of the six ions selected for the identification of 12-HETE in a nasal lavage pool is shown in Fig. 2. The twice-

TABLE I

PERCENTAGE RECOVERIES OF 15-HETE, 12-HETE, 5-HETE AND LTB_4 FROM NASAL LAVAGE SAMPLES USING C_{18} BAKER CARTRIDGES

Eicosanoid	Recovery (mean ± S.D., *n* = 6) (%)
15-HETE	97.1 ± 2.1
12-HETE	95.9 ± 1.6
5-HETE	95.9 ± 0.7
LTB_4	101.2 ± 1.7

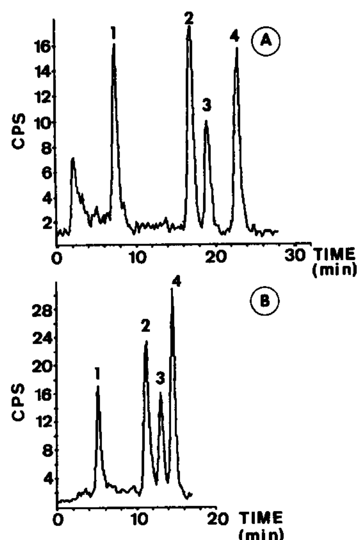

Fig. 1. Reversed-phase HPLC separation of tritiated 15-HETE, 12-HETE and 5-HETE standards, using a 10-μm Spherisorb ODS-2 column. (A) Mobile phase, methanol–water–trifluoracetic acid–triethylamine (75:25:0.1:0.05, v/v). (B) Mobile phase, water–acetonitrile–tetrahydrofuran–acetic acid (4:36:20:0.05 v/v). The flow-rate was 1.5 ml/min in both instances. Peaks: 1 = LTB_4; 2 = 15–HETE; 3 = 12-HETE; 4 = 5-HETE.

purified 12-HETE fraction showed a positive response at the ions characteristic of the trimethylsilylmethyl ester of 12-HETE [10] at m/z 406 (M^+, molecular peak), 391 (M^+ − 15, loss of CH_3 group), 375 (M^+ − 31, loss of CH_3O group), 295 (M^+ − 111, base peak arising from

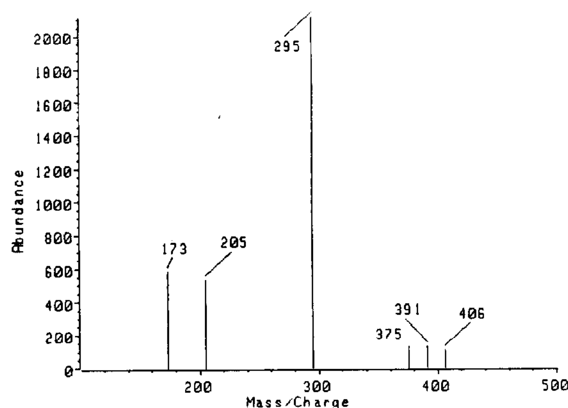

Fig. 2. Multiple-ion SIM recording of the methyl ester, trimethylsilyl ether derivative of a twice-purified HPLC fraction corresponding to the retention time of 12-HETE in a sample from a pool of nasal lavage samples.

loss of CH_2–CH=CH–CH–$(CH_2)_4$–CH_3), 205 (295 − trimethylsilyl (TMSiOH) group) and 173 [295 − (TMSiOH + CH_3OH)]. The specific SIM response for these m/z values at the expected GC retention time of 12-HETE as well as the peak area ratios for the authentic standard of 12-HETE and the peak from nasal lavage supernatants were almost identical, confirming the identification of 12-HETE in nasal lavage samples. In contrast, no ion response was found at the retention time of 5-HETE.

DISCUSSION

The data reported here identify 12-HETE in nasal lavage samples obtained from healthy subjects, indicating that human nasal mucosa synthesizes 12-HETE *in vivo*. This agrees with previously reported studies on the *in vitro* biosynthesis of 12-HETE by airway epithelial cells [3,4]. Neither the enzyme responsible for 12-HETE synthesis nor its cellular origin are known, but it seems reasonable to attribute the presence of 12-HETE to 12-lipoxygenase activity, although it is well established that in some cellular types such as reticulocytes, 15-lipoxygenase is capable of generating both 12-HETE and 15-HETE [11]. As the epithelial cells isolated from nasal lavage samples produce 12-HETE, presumably the 12-HETE released into nasal secretions derives from the nasal epithelial cells.

Although the biological importance of 12-lipoxygenase activity in human nasal mucosa is uncertain, it has been reported that 12-HETE is a potent secretagogue released by mucosa [12,13] similar to 15-HETE [14]. It has also been proposed that 12-lipoxygenase activity could be responsible for the formation of an epoxide intermediate leading to the generation of 8,15-leukotrienes [15].

It now seems appropriate to study the *in vivo* modulation of HETEs in human subjects to evaluate the effects of pharmacological agents on mucous secretion and their possible clinical implications. Further studies will be carried out to assess the pathophysiological role of 12-lipoxygenase metabolites in nasal mucosa.

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