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Note

15-Hydroxyeicosatetraenoic acid as a major eicosanoid in nasal secretions: assay by high-performance liquid chromatographic–radioimmunoassay and gas chromatographic–mass spectrometric procedures

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The nasal provocation test has been used as an in vivo model for studying the release of chemical mediators into nasal secretions and to assess their role in allergic rhinitis [1]. Histamine, kinins, tame esterase [2], lyso platelet activating factor (PAF)-acether [3] and arachidonic acid (AA) metabolites are released after challenges with allergen [4] or provocative stimulti [5] It has been reported that prostaglandin D_2 (PGD_2) and peptide-leukotrienes (p-LTs) are the major cyclooxygenase and lipoxygenase metabolites, respectively, of AA released into nasal lavage fluid. However, no data have been reported concerning other arachidonate metabolites such as monohydroxylated eicosatetraenoic acids (HETEs). Recent in vitro studies have shown that 15-HETE is the major arachidonic acid metabolite synthesized in human nasal and tracheal epithelial cell cultures [6,7]. 15-HETE is a potent inflammatory mediator [8] and agonist of tracheal mucus secretion [9]. These findings suggest

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that 15-HETE could be released in vivo by the nasal mucosa as a new potential mediator in allergic rhinitis. However, to be able to study the role of 15-HETE in nasal allergy, we must first establish the levels of this metabolite in nasal lavage fluid from healthy volunteers. Identification of 15-HETE was carried out by use of high-performance liquid chromatographic–radioimmunoassay (HPLC–RIA) and gas chromatographic–mass spectrometric (GC–MS) techniques. Basal levels of PGD_2, LTB_4 and p-LTs were also obtained.

EXPERIMENTAL

Subjects

Eleven healthy male and female volunteers (aged 20–45 years) were studied. None of them had a history of asthma or allergy.

Chemicals

Tritiated standards were purchased from Amersham (Amersham, U.K). C_{18} cartridges were obtained from J.T Baker (Deventer, The Netherlands) HPLC solvents were supplied by Scharlau (Barcelona, Spain). Trifluoroacetic acid, triethylamine, hexane, light petroleum and methyl formate were obtained from Fluka (Buchs, Switzerland). N,O-Bis(trimethylsilyl)trifluoroacetamide and N-methyl-N-1-nitrosoguanidine were supplied by Sigma (St. Louis, MO, U.S.A.) and were used in the derivatization procedure in GC–MS

Nasal lavage

A volume of 4 ml of saline solution (0 9% sodium chloride) was instilled into each nostril while the subject extended his neck approximately 30° from the horizontal and abstained from breathing or swallowing. After 10 s, volunteers expelled the mixture of saline and nasal secretions into polypropylene tubes as reported by Naclerio et al. [1]. Collected nasal washes were stored at −80°C until extraction

Extraction

Lipoxygenase metabolites were extracted using a modification of the method reported by Salari and Steffenrud [10]. Samples were directly processed through C_{18} cartridges, which were washed with 10 ml of distilled water LTs and HETEs were finally eluted with 5 ml of methanol–water (90 10, v/v). Dried residues were resuspended in acetonitrile and stored at −80°C until assay. PGD_2 was extracted as previously described [11].

Radioimmunoassay

Determinations in duplicate of 15-HETE, PGD_2, LTB_4 and p-LT were carried out with commercial assay system kits (Amersham) according to the supplier's instructions. The cross-reactivities of the p-LT antiserum with LTC_4,

LTD_4 and LTE_4 were 100, 46 and 64%, respectively The accuracy of 15-HETE determinations was assessed by the parallelism test. Nasal lavages were assayed at serial dilutions between 1 2 and 1 128 for four different samples. Values were corrected for reversed-phase C_{18} extraction recoveries.

HPLC purification

After evaporation to dryness, the extraction residues were resuspended in the chromatographic buffer and injected directly into a Kontron (Zurich, Switzerland) HPLC system coupled to a 2211 Superrack fraction collector (LKB, Bromma, Sweden). An Ultrasphere IP (5 μm) column (250 mm$\times$4.6 mm I.D.) was used for the complete separation of 15-HETE from the other lipoxygenase metabolites present in nasal washes. The column was eluted isocratically with methanol–water–trifluoroacetic acid–triethylamine (80 20 0 1 0 05, v/v) as described by Eskra et al [12]. The flow rate was 1.0 ml/min. In order to assess the specific immunoreactivity present in biological samples, 2-min eluates were collected during the HPLC analysis. After lyophilization, each fraction was subjected to RIA, as indicated below Alternatively, and only for GC–MS identification purposes, eluent fractions from a nasal lavage pool were also collected at the retention time of 15-HETE, previously established for the tritiated analogue by the use of a RayTest Ramona (Isomess, Straubenhardt, F.R.G.) radioactivity detector, directly coupled to the HPLC system. The residues obtained after lyophilization of eluates were resuspended in 250 μl of water at pH 3.4 (adjusted with acetic acid)–acetonitrile (45 55, v/v) and subjected to an additional HPLC purification. A Spherisorb ODS 2 column (300 mm$\times$3.9 mm I D) was eluted isocratically with the above chromatographic buffer at 1.0 ml/min. Finally, residues obtained after lyophilization were derivatized for GC–MS analysis.

Gas chromatography–mass spectrometry

The dry residues obtained after two consecutive HPLC purification steps were methylated and silylated as reported previously [13] Mass spectrometric identification was carried out by using a Hewlett-Packard 5995 gas chromatograph–mass spectrometer equipped with an OV-1 glass capillary column (25 m$\times$0.25 mm I.D.; film thickness 0.33 μm) programmed from 190 to 280°C at 3°C/min. The injector temperature was 280°C. Helium was used as the carrier gas at the optimal flow-rate. Electron-impact mass spectra were recorded at 70 eV. The dwell time for selected ion monitoring (SIM) was set at 210 ms with a cycle time of 1 3 s.

RESULTS

Concentrations of 15-HETE and the other arachidonic acid metabolites measured by direct RIA in nasal washes from healthy volunteers are given in

TABLE I

CONCENTRATIONS OF EICOSANOIDS IN NASAL SECRETIONS OBTAINED BY DIRECT RIA

Volunteer No	Concentration (pg/ml)			
	15-HETE	p-LTs	LTB_4	PGD_2
1	591	332	85	57
2	589	340	104	48
3	141	110	45	64
4	281	357	89	77
5	994	555	86	53
6	2096	252	104	54
7	865	280	55	74
8	1221	215	64	223
9	1976	590	42	155
10	2702	287	57	104
11	2924	445	71	83
Mean ± S D	1398 ± 891	342 ± 142	73 ± 22	90 ± 54

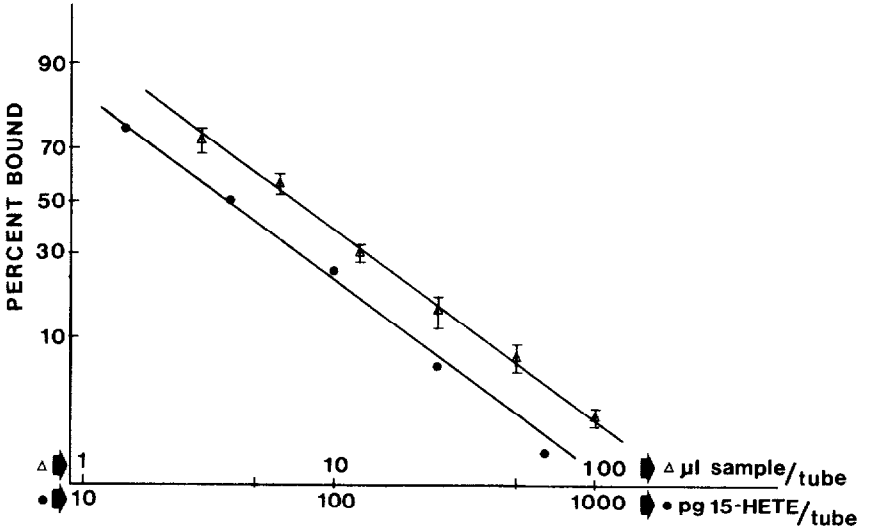

Fig 1 Linear response curves obtained from (•) 15-HETE standard and (△) nasal lavage fluid ($n = 4$, mean ± S D) assayed with serial dilutions from 1 2 to 1 128

Table I. These results indicate that 15-HETE is the major metabolite released by the nasal mucosa in vivo.

Fig 1 shows the plots corresponding to a 15-HETE standard and nasal wash samples assayed with serial dilutions The reliability of the data obtained by

direct RIA was confirmed by the results of the parallelism test, as shown Further, the method was validated by immunochromatographic and GC–MS procedures.

The immunochromatogram of 15-HETE obtained from a nasal lavage pool is shown in Fig 2. The major peak of immunoreactivity coelutes with tritiated 15-HETE standard. No response was detected at this elution time on the ultraviolet profile monitored at 235 nm (data not shown)

Single-ion monitoring (SIM) of a nasal lavage purified fraction collected at the retention time of 15-HETE is shown in Fig 3. A positive response at the characteristic ions of the trimethylsilylmethyl ester of 15-HETE was observed for ions at m/z 406, 335 and 225 Peak-area ratios corresponding to the selected ions for the authentic standard of 15-HETE and the biological sample were almost identical

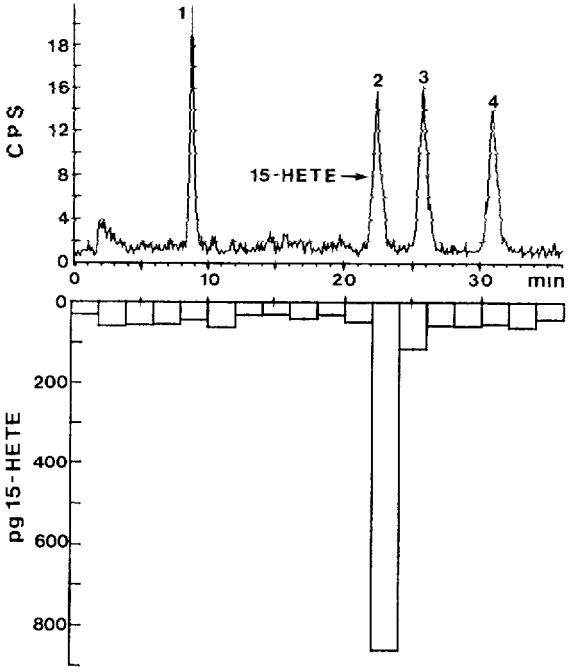

Fig 2 Top HPLC separation of tritiated (1) LTB_4, (2) 15-HETE, (3) 12-HETE and (4) 5-HETE using an Ultrasphere IP column Mobile phase methanol–water–trifluoroacetic acid–triethylamine (80 20 0 1 0 05, v/v) at a flow-rate of 1 0 ml/min Bottom 15-HETE immunochromatogram corresponding to 2 ml of collected eluates of nasal lavage pool under the same conditions The slight delay in the appearance of immunoreactive species relative to the radioactive species profile above is due to the connecting lines

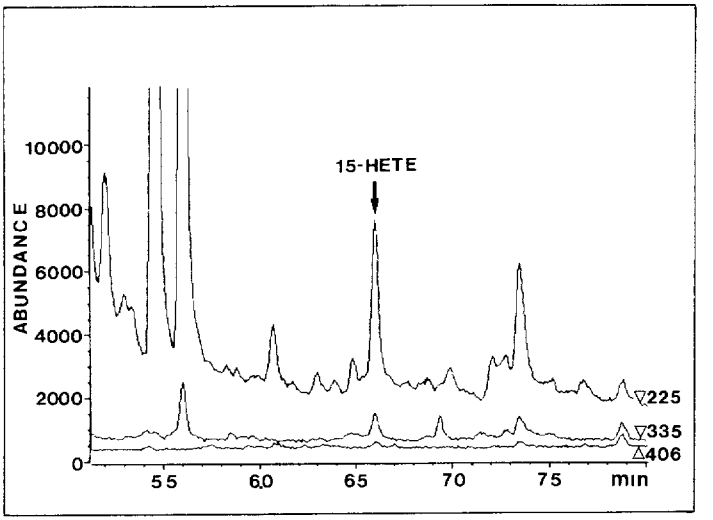

Fig 3 Single ion monitoring of an HPLC fraction from nasal lavage showing a positive response for 15-HETE at its characteristic m/z values (225, 335 and 406) and retention time

DISCUSSION

These preliminary results are in agreement with previously reported in vitro studies by Henke et al [6], which showed 15-lipoxygenase activity in nasal epithelial cells stimulated with AA in the presence of calcium A23187 ionophore or 15-hydroperoxyeicosatetraenoic acid (15-HPETE).

Levels of 15-HETE determined in nasal lavage fluid from healthy subjects (see Table I) revealed the production of this metabolite in vivo by human nasal mucosa. The reliability of RIA determinations was established by the parallelism test (Fig. 1) and immunochromatographic studies (Fig. 2) Identification of 15-HETE was carried out by GC–MS using multiple ion detection. Although the biological levels were not sufficient to obtain a total electron-impact mass spectrum, the specific SIM responses obtained at the expected retention time of 15-HETE and the coincidence of the peak-area ratios in standard and biological samples confirm the presence on this 15-lipoxygenase metabolite in nasal secretions.

Hence we have described for first time the in vivo release of 15-HETE into nasal secretions, showing its predominance over other AA metabolites Although its physiological role has not been established in allergic rhinitis, several data suggest that 15-HETE could play a potential role in hypersensitivity reactions It has been reported that in vitro 15-lipoxygenase activity may be increased in the asthmatic lung [14] and increased 15-HETE levels have been detected in bronchoalveolar lavage from atopic patients after local antigen challenge [15]. Further studies will be carried out in order to assess the role of

this 15-lipoxygenase metabolite in the nasal allergy response using a nasal provocation test.

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REFERENCES

1 R M Naclerio, H L Meier, A Kagey-Sobotka, N F Adkinson, Jr , D A Meyers, P S Norman and L M Lichtenstein, Am Rev Respir Dis , 128 (1983) 597
2 R M Naclerio, D Proud, S P Peters, G Silber, A Kagey-Sobotka, N F Adkinson, Jr and L M Lichtenstein, Clin Allergy, 16 (1986) 101
3 N Meslier, G Braunstein, J Lacronique, J F Dessanges, F Rakotosihanaka, P Devillier, A Lockhart, J M Mencia-Huerta and H A Boushey, Am Rev Respir Dis , 137 (1988) 617
4 M S Brown, S P Peters, N F Adkinson, Jr , D Proud, A Kagey-Sobotka, P S Norman, L M Lichtenstein and R M Naclerio, Arch Otolaryngol Head Neck Surg , 113 (1987) 179
5 A G Togias, R M Naclerio, S P Peters, I Nimmagadda, D Proud, A Kagey-Sobotka, N F Adkinson, Jr , P S Norman and L M Lichtenstein, Am Rev Respir Dis , 133 (1986) 1133
6 D Henke, R M Danilowicz, J F Curtis, R C Boucher and T E Eling, Arch Biochem Biophys , 267 (1988) 426
7 J A Hunter, W E Finkbeiner, J A Nadel, E J Goetzl and M J Holtzman, Proc Natl Acad Sci U S A , 82 (1985) 4633
8 B Samuelsson, Science, 220 (1983) 568
9 Z Maron, J H Shelhamer and M Kaliner, J Clin Invest , 67 (1981) 1695
10 H Salari and S Steffenrud, J Chromatogr , 378 (1986) 35
11 I Ramis, J Serra, J Roselló, C Picado, E Gelpí and A Agustí Vidal, Prostaglandins Leukotrienes Essential Fatty Acids, 34 (1988) 109
12 J D Eskra, M J Pereira and M J Ernest, Anal Biochem , 154 (1986) 332
13 J Roselló, E Gelpí, M Rigaud, J Durand and J C Breton, Biomed Mass Spectrom , 8 (1981) 149
14 M Hamberg, P Hedqvist and K Radegran, Acta Physiol Scand , 110 (1980) 219
15 J J Murray, A B Tonnel, A R Brash, J L Roberts, P Gosset, C R Workman, A Capron and J A Oates, N Engl J Med , 315 (1986) 800