The effect of inhaled 15-(s)-hydroxyeicosatetraenoic acid (15-HETE) on airway calibre and non-specific responsiveness in normal and asthmatic human subjects

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ABSTRACT: 15-hydroxyeicosatetraenoic acid (15-HETE) is the predominant oxidative metabolite of arachidonic acid in human lung. We have studied its effects on airway calibre and non-specific bronchial responsiveness (NSBR) in eight normal and eight asthmatic subjects. 15-HETE, at doses up to 70 nmol, had no effect on airway calibre in either group of subjects. However, 3 h after its administration, 15-HETE reduced NSBR in the normal subjects (geometric mean methacholine $PD_{40} \dot{V}p_{30}$ increased by 2.29-fold from baseline compared with a corresponding 1.14-fold increase after diluent, $p<0.05$). Similarly, 4 h after inhaled 15-HETE, the spontaneous increase in NSBR in the asthmatics was completely inhibited (geometric mean histamine $PD_{40} \dot{V}p_{30}$ decreased significantly to 0.41-fold of baseline after diluent ($p<0.01$) compared with a 1.1-fold increase after 15-HETE, $p<0.01$). These data suggest 15-HETE may play an autacoid role in airway function.
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15-(s)-hydroxyeicosatetraenoic acid (15-HETE) is the predominant oxidative metabolite of arachidonic acid in normal and asthmatic airways [1]. Either *in vitro* or *in vivo*, the release of 15-HETE from airways of patients with atopic asthma can be greatly enhanced upon allergen challenge [2, 3]. The demonstration that prostaglandin generating factor of anaphylaxis derived from the secretory granule of human mast cells can also stimulate 15-HETE release by human airways *in vitro* [4] provides further evidence for this mediator being released in allergic reactions involving human lung. The most likely cellular origin for 15-HETE are airways epithelial cells and eosinophils, both of which have been shown to possess 15-lipoxygenase activity [5, 6].

In vitro, 15-HETE is a weak contractile agonist of human isolated bronchial muscle and guinea-pig lung parenchyma [7]. In different cell systems, 15-HETE either activates [8, 9] or inhibits [10–14] the 5-lipoxygenase pathway and, therefore, may be able to modulate the generation of leukotrienes. In dogs, inhalation of 15-HETE causes an influx of neutrophils and mast cells into the airways, alveolar oedema, increased respiratory fluid loss and mucus secretion. The demonstrations of physiologically effective levels of 15-HETE in canine mucus and its increase when the airways were exposed to exogenous arachidonic acid or hypoxia, indicate that this lipid product is a pro-inflammatory mediator in this animal [15].

Evidence for the release and pharmacological activity of 15-HETE suggests that it may contribute to the inflammatory events of asthma. Since there is no information on the effect of this mediator on human airway function *in vivo*, we have studied the effects of inhaled 15-HETE, on baseline airway calibre and the response of the airways to inhaled methacholine in normal subjects and to histamine in asthmatics.

Methods

Subjects

Eight asthmatic and eight normal subjects, who were all nonsmokers, participated in the study (table 1). The normal subjects took no medication and had no symptoms of asthma. All asthmatic subjects were symptomatic during the month preceding the study and had a 20% variability of forced expiratory volume in one second (FEV_1) and/or peak expiratory flow. They took regular inhaled salbutamol and inhaled corticosteroid, with the former being withheld for 8 h and the latter for 48 h before each study day. Three of the normal and seven of the asthmatic subjects were atopic when defined by positive skin prick tests (>2 mm wheal response) to two or more allergens: grass pollen, *Dermatophagoides pteronyssinus* and cat dander (Bencard, Brentford, Middlesex, UK). The study was approved by the Southampton University and Hospitals Ethical Sub-Committee, and written informed consent was obtained from each subject.

Table 1. – Subjects and characteristics

	Normal n=8	Asthmatic n=8
Age yr	31.4±2.5	37.5±5.6
Sex M/F	8/0	5/3
Atopy +/-	3/5	7/1
FEV_1 % pred	105.9±5.2	78.6±3.2
PD_{20} FEV_1* µmol	26.31 (2.60–143.95)	0.2 (0.078–0.47)

Values for age and FEV_1 are mean±SEM. *PD_{20} FEV_1: geometric mean provocation dose of methacholine causing a 20% fall in baseline FEV_1 in normal subjects (n=7 as one subject failed to show any appreciable fall in FEV_1 even after the highest dose of methacholine) and that of histamine in asthmatic subjects, with range in parentheses. FEV_1: forced expiratory volume in one second.

Measurements

Airway calibre was recorded both as the forced expiratory volume in one second (FEV_1) and as the maximum expiratory flow at 70% of baseline vital capacity below total lung capacity (TLC) during a partial forced expiratory manoeuvre ($\dot{V}p_{30}$). Both measurements were derived from flow-volume curves produced on a rolling seal, flow rate-dependent spirometer (Morgan Spiroflow, P.K. Morgan Ltd, Kent, UK) connected to an 85B desk top computer *via* an 82940A GP-10 interface (Hewlett Packard, Wokingham, Berkshire, UK). After a period of normal tidal breathing, flow-volume curves were obtained by asking the subject to expire maximally into a mouthpiece connected to the spirometer from end tidal inspiratory capacity to residual volume (RV). In this way a partial expiratory flow-volume curve was obtained from which $\dot{V}p_{30}$ was derived. On reaching RV, the subject inspired to TLC and then expired maximally back to RV, allowing a measurement of FEV_1 to be recorded. Blood pressure measurements were made using a random zero sphygmomanometer and the heart rate recorded by radial artery palpation.

Drug administration

Methacholine chloride (Sigma, Poole, Dorset, UK; MW 195.7) was prepared in 0.9% sodium chloride (saline) to produce a range of doubling concentrations of 1–256 mg·ml^{-1}. Histamine acid phosphate (MW 307.14) was similarly prepared with concentrations ranging from 0.03–8 mg·ml^{-1}. 15-hydroxyeicosatetraenoic acid (Orbit Laboratories Ltd, Bangor, Gwynedd, UK) was dissolved as a stock solution in absolute alcohol (Eli Lilly, Surrey, UK) at a concentration of 2.42 mg·ml^{-1} and stored at -20°C under nitrogen until used. On the 15-HETE study day, an aliquot of the stock solution was diluted in isotonic (0.15 M) sodium phosphate buffer, pH 7.4, to produce a solution containing 1 mM 15-HETE. This

solution, together with its serial dilutions prepared in isotonic sodium phosphate buffer containing 500, 250, 125 and 62.5 µM 15-HETE, were used for the first study on normal subjects, and 100, 10 and 1 µM for the study on asthmatics. All solutions were stored at -4°C until 10 min prior to inhalation, when the desired concentration to be administered was left at room temperature. Similarly, on the placebo day, an aliquot of absolute alcohol was mixed with isotonic sodium phosphate buffer and subsequent serial dilutions of this solution were prepared in the same diluent to give the same number of solutions, as on the corresponding 15-HETE study day. The control vehicle solution for the 15-HETE and placebo challenges consisted of 0.15 M sodium phospate buffer and 10% ethanol, and that for the methacholine and histamine challenges was 0.9% saline.

The aqueous solutions were administered as aerosols generated from a starting volume of 2.0 ml in a disposable Inspiron Mini-Neb nebulizer (CR Bard Int., Sunderland, UK), connected to a Rosenthal-French dosimeter (Bethesda Hospital, Rochester, New York, USA) driven by compressed air at a pressure of 138 kPa. Subjects wearing a nose-clip, were instructed to take ten consecutive breaths *via* a mouthpiece, slowly from functional residual capacity (FRC) to TLC without breath-holding in between breaths [16]. The dosimeter setting was adjusted so that this procedure generated 70 µl of aerosol of mass median particle diameter 4.7 µm to the mouth. This delivered doubling doses of 15-HETE from 4.5–70 nmol to the normal subjects, tenfold increasing doses from 0.07–70 nmol to the asthmatic subjects, doubling doses of methacholine from 0.36–91.65 µmol and histamine from 0.0068–1.82 µmol.

Study design

The study was carried out in two phases:
Phase I. This initial phase of the study was conducted on normal subjects outside the pollen season on two occasions separated by at least 21 (range 21–60) days. Bronchoprovocation tests were performed at the same time of day on each of the two occasions. Because of the potential risks of administering a compound which had not previously been given by this route to humans, this phase of the study was conducted in an open and non-randomized fashion. Preceding the dose-response study with each of the three agonists (15-HETE, placebo phosphate buffer and methacholine), measurements of FEV_1 and $\dot{V}p_{30}$ were made before and after inhalation of the vehicle solution. If the FEV_1 did not fall by >10% of the baseline value after the administration of vehicle, the dose-response study with one of the three agonists was performed. 15-HETE and the matched placebo were administered in a series of doubling doses at 11 min intervals, followed by measurements of $\dot{V}p_{30}$ and FEV_1 at 2, 5 and 10 min after inhalation of each dose. Similarly, a series of doubling doses of methacholine were administered at intervals of 4 min and measurements of $\dot{V}p_{30}$ and FEV_1 made at 1 and 3 min after each inhalation of this agonist. The inhalations and measurements were

continued until the FEV_1 had fallen by >20% of the post-vehicle value, or until the maximal dose of agonists (70 nmol for 15-HETE, 91.65 µmol for methacholine) had been administered.

On the first occasion subjects underwent a dose-response study with inhaled methacholine (0.36–91.65 µmol) to assess their baseline non-specific airways responsiveness. Once the airway calibre had returned to baseline (approximately two hours later), a dose-response study was performed with 15-HETE and any change in airway calibre followed as FEV_1 and Vp_{30} for 6 h. Airways responsiveness to methacholine was determined at 3, 6 and 24 h after inhaling the final concentration of 15-HETE. On the second occasion, the same protocol was repeated with 15-HETE replaced by repeated inhalations of placebo (sodium phospate buffer). Blood pressure and pulse rate were recorded at 3 min following the inhalation of each dose of 15-HETE and placebo.

Phase II. This latter phase of the study was conducted on asthmatic subjects, again outside the pollen season on two occasions separated by at least ten days (range 10–21 days). Since none of the normal subjects developed any adverse effects to the inhaled eicosanoid, 15-HETE and the matched placebo were administered double-blind, cross-over and in random order in this second phase of the study. The protocol was slightly modified from that used in phase I, in that 15-HETE was administered at tenfold increasing doses from 0.07–70 nmol. Because of a worldwide reduced availability of Food and Drug Administration (FDA) approved methacholine for bronchial challenge, bronchial responsiveness was followed in this phase of the study using histamine provocation undertaken before and at 2, 4 and 24 h after inhalation of 15-HETE or placebo. A number of studies have shown a close correlation between histamine and methacholine reactivities as indices of non-specific bronchial responsiveness [17–19]. The technique used for histamine challenge was identical to that used in phase I with methacholine.

Data analysis

The data from the two phases of the study were analysed separately. All figures refer to the mean±SEM, unless otherwise stated, and the p<0.05 level of significance was accepted. Changes in airway calibre in response to the different agonists were expressed as percentage change from the post-vehicle baseline values. For both FEV_1 and Vp_{30}, the lowest of the values recorded for each dose of agonist was used for analysis. Dose-response curves to methacholine or histamine were constructed by plotting the percentage change in FEV_1 and VP_{30} against the cumulative dose of methacholine or histamine on a logarithmic scale. The provocation dose (PD) value was derived by linear interpolation and geometric mean calculated for the group. Thus the $PD_{40} Vp_{30}$ is the dose of methacholine or histamine causing a 40% fall in Vp_{30} from post-vehicle baseline and the $PD_{20} FEV_1$, a 20%

fall in FEV_1. One of the normal subjects failed to demonstrate any appreciable fall in FEV_1 with any of the methacholine challenges and, therefore, only seven subjects were used to analyse the PD_{20} data in this group. With eight of the 56 methacholine challenges, the FEV_1 fell by <20% even after the inhalation of the highest dose of methacholine (91.65 µmol). In these situations, the dose-response curves were extrapolated to the next doubling doses to obtain an estimate of the PD value. If a PD value could still not be obtained, this final dose was used as an approximate lower estimate of the PD value [20, 21].

Student's t-tests were used to compare paired data of baseline Vp_{30} and FEV_1 prior to the inhalation of each agonist on the two study days. The effect of 15-HETE on airway calibre was compared with placebo by t-test statistics on the difference in the percentage change of FEV_1 and Vp_{30} between the corresponding doses of 15-HETE and placebo. Two-way analysis of variance (ANOVA) was used to analyse: 1) the PD values obtained on the placebo day for assessing any significant diurnal variations in methacholine or histamine responsiveness; and 2) the changes in Vp_{30} and FEV_1 for assessing the effect of inhaled 15-HETE and placebo on baseline airway calibre, any significance was further analysed by least significant differences. The effect of 15-HETE on methacholine responsiveness in the normal subjects and histamine responsiveness in the asthmatics was analysed by: 1) paired Student's t-tests on the difference between the log PD values at baseline and at each of the subsequent time points post 15-HETE or placebo on the two study days; and 2) two-way ANOVA on the change in log PD values from baseline at each time point on the two study days and any significance was further analysed by least significant difference. The relationship between the change in $PD_{40} Vp_{30}$ and that in baseline Vp_{30} were examined by least squares linear regression.

Results

Effect on airway calibre in normal and asthmatic subjects

Baseline values of FEV_1 and Vp_{30} did not differ significantly between the 15-HETE and placebo study days. In both the normal and asthmatic subjects, inhalation of 15-HETE caused no respiratory sensations or cough at any of the doses administered. There were also no significant changes in blood pressure or heart rate after 15-HETE inhalation. Inhaled 15-HETE, in cumulative doses of 135.8 nmol in normal subjects and 77.8 nmol in asthmatic subjects, caused no significant change in Vp_{30} or FEV_1 when compared with placebo (figs 1 and 2). However, in the asthmatic subjects inhaled 15-HETE resulted in a significant fall in baseline Vp_{30} at doses of 0.07 and 70 nmol (p<0.05), and both inhaled 15-HETE at doses of 0.7–70 nmol and the corresponding placebo solutions also caused a significant fall in baseline FEV_1 (p<0.05) (fig. 2).
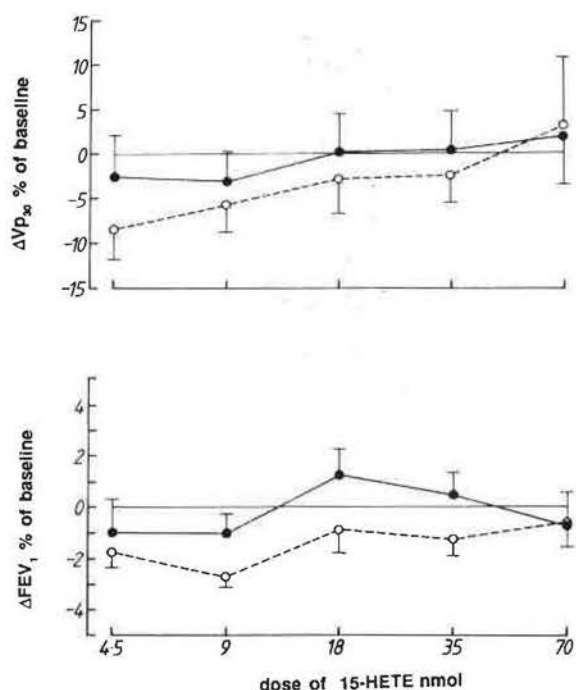
Fig. 1. – The effect of increasing doses of inhaled 15-HETE (solid circles) and a matched placebo (open circles) on airway calibre in eight non-asthmatic subjects. Changes in airway calibre were expressed as a percentage of the lowest post-diluent FEV_1 or $\dot{V}p_{30}$. Respective baseline values (mean±SEM) of FEV_1 on 15-HETE and placebo days were 3.75±0.21 and 3.75±0.22 l, respectively, and of $\dot{V}p_{30}$ were 106.12±7.13 and 108.21±13.24 $l \cdot min^{-1}$, respectively. 15–HETE: 15-(s)-hydroxyeicosatetraenoic acid; FEV_1: forced expiratory volume in one second; $\dot{V}p_{30}$: expiratory flow rate at 70% of baseline vital capacity below total lung capacity during a partial forced expiratory manoeuvre.
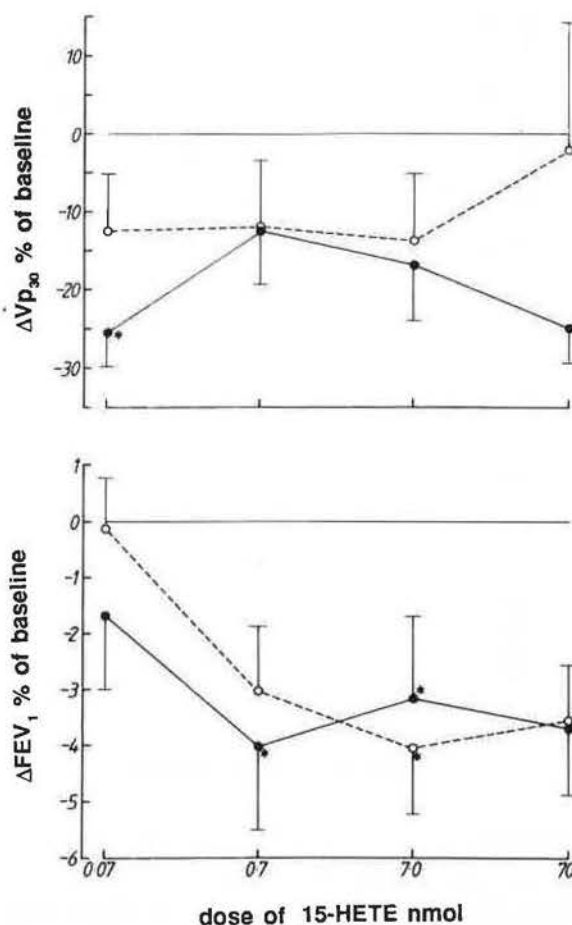

Fig. 2. – The effect of increasing doses of inhaled 15-HETE (solid circles) and a matched placebo (open circles) on airway calibre in eight asthmatic subjects. Changes in airway calibre were expressed as a percentage of the lowest post-diluent FEV_1 or $\dot{V}p_{30}$. *: $p<0.05$ when compared with baseline. Respective baseline values (mean±SEM) of FEV_1 on 15-HETE and placebo days were 2.45±0.21 and 2.44±0.24 l, respectively and of $\dot{V}p_{30}$ were 55.99±6.76 and 59.33±12.3 $l \cdot min^{-1}$, respectively. For abbreviations see legend to figure 1.

Change in methacholine responsiveness in normal subjects

There was no significant difference in baseline values of PD_{20} FEV_1 or PD_{40} $\dot{V}p_{30}$ between the 15-HETE and placebo vehicle study days. After inhaled placebo, PD_{20} FEV_1 and PD_{40} $\dot{V}p_{30}$ showed a tendency to increase across the 24 h of the study period (fig. 3). However, when compared with the baseline values by two-way ANOVA, none of these changes reached statistical significance. After inhalation of 15-HETE there was no significant change in PD_{20} FEV_1 when compared with corresponding values obtained after inhaled placebo at any of the three time points up to 24 h (fig. 3.) At 3 h after 15-HETE, geometric mean PD_{40} $\dot{V}p_{30}$ increased significantly when compared with the corresponding increase on the placebo day by paired t-test 2.29-fold increase from baseline vs 1.14-fold increase on the placebo day, $p<0.05$ (fig.3). However, no significant change in PD_{40} $\dot{V}p_{30}$ was observed when the data were analysed by two-way ANOVA. Subsequent increases in PD_{40} $\dot{V}p_{30}$ at other time points were higher than those on the placebo day but failed to reach statistical significance (fig. 3).

Change in histamine responsiveness in asthmatic subjects

There was no significant difference in baseline values of PD_{20} FEV_1 or PD_{40} $\dot{V}p_{30}$ between the 15-HETE and placebo study days. After inhaled placebo, PD_{20} FEV_1 decreased to 0.77-fold of baseline at 2 h (NS) and to 0.54-fold of baseline at 4 h ($p<0.05$ vs either baseline or 24 h) before returning to baseline at 24 h (fig. 4). PD_{40} $\dot{V}p_{30}$ followed a similar trend with significant falls at 2 and 4 h (0.42- and 0.41-fold of baseline corresponding to $p<0.05$ and $p<0.01$, respectively when compared with baseline) before returning to 0.66-fold of baseline at 24 h (NS vs baseline) (fig. 4). After 15-HETE inhalation, there was no significant change in PD_{20} FEV_1 when compared with corresponding values obtained after placebo at any of the three time points up to 24 h (fig. 3). However, at the 4 h time point, the change in PD_{40} $\dot{V}p_{30}$ from baseline was significantly different from that after placebo (1.1- and 0.41-fold of respective baseline values, $p<0.01$ by
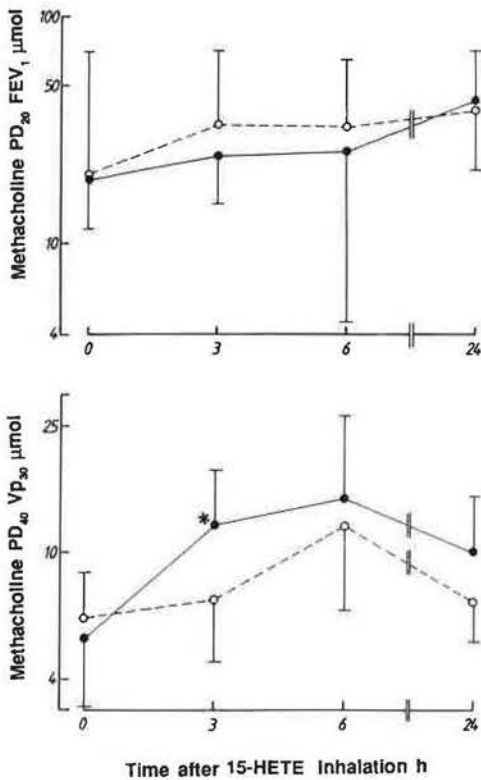
Fig. 3. – The time course of bronchial responsiveness to methacholine after inhalation of 15-HETE (solid circles) or placebo (open circles) in normal subjects. Values are geometric mean±SEM; n=7 for PD_{20} FEV_1 data; n=8 for PD_{40} $\dot{V}P_{30}$ data. *: p<0.05 (Student's paired t-tests); NS: (two-way analysis of variance) when changes from baseline values at the corresponding time points on the two study days were compared. PD_{20}, PD_{40}: provocation dose producing a 20%, 40% fall, respectively. For other abbreviations see legend to figure 1.
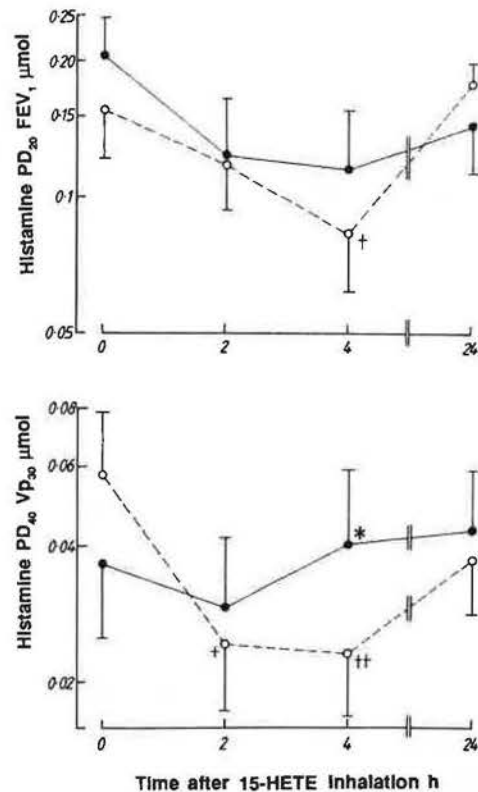

Fig. 4. – The time course of bronchial responsiveness to histamine after inhalation of 15-HETE (solid circles) and placebo (open circles) in eight asthmatic subjects. Values are geometric mean±SEM. +p<0.05 when compared with corresponding values at baseline on placebo day (two-way analysis of variance); ++: p<0.01 when compared with baseline value on placebo day (two-way analysis of variance); *: p<0.01 (Student's paired t-tests) and <0.05 (two-way analysis of variance) when changes from baseline values at the corresponding time points on the two study days were compared. For abbreviations see legends to figures 1 and 3.

paired t-test and <0.05 by two-way ANOVA). No significant differences in the changes of PD_{40} $\dot{V}p_{30}$ after 15-HETE inhalation were observed at the two other time points (fig. 3). There was no correlation between the change in PD_{40} $\dot{V}p_{30}$ after 15-HETE and the change in baseline $\dot{V}p_{30}$ in either group of subjects.

Discussion

In this study we have demonstrated that inhaled 15-HETE has no direct effect on two indices of airway calibre, FEV_1 and $\dot{V}p_{30}$, in either normal or asthmatic subjects. The lack of a significant bronchoconstrictor response to this mediator is in keeping with the *in vitro* finding that 15-HETE is a weak contractile agonist of human airways, being ten times less potent than histamine [7]. None of the subjects noticed any increased sputum production in the 24 h following 15-HETE inhalation, or any other adverse effects. However, in both normal and asthmatic subjects inhalation of 15-HETE produced small but significant reductions in bronchial responsiveness to methacholine and histamine, respectively, when changes in airway calibre to the provoking agents were measured as $\dot{V}p_{30}$ but not as FEV_1.

In this study the cumulative dose of 15-HETE used

was 135.8 nmol in the normal subjects and 77.8 nmol in asthmatic subjects. With the nebulization procedure used, approximately 13.6 and 7.8 nmol of 15-HETE would be delivered to the lungs of the respective subjects, corresponding to 10% of the dose delivered to the mouth. These doses are approximately 10–20 times higher than that administered to mongrel dogs by JOHNSON *et al.* [15] who reported mucus hypersecretion and inflammatory changes in the airways of these animals. At the high doses used we were unable to show any evidence that this mediator has the capacity to reduce airway calibre in the normal subjects. The apparent small bronchoconstrictor effect of 15-HETE seen in the asthmatics was also observed with inhaled placebo (fig. 2) suggesting a non-specific response to the cold challenge of the nebulized solutions (which were kept in ice until 10 min prior to their administration) in hyperreactive subjects.

The increase in bronchial responsiveness to histamine observed in our asthmatic subjects following the inhalation of placebo (fig. 4) probably reflects the circadian variation in bronchial responsiveness in this group of subjects. This is in agreement with the findings in a group of 15 asthmatic children whose geometric mean provocation concentration of histamine causing a 20% fall in

FEV$_1$ from baseline fell by >1 doubling dilution from 10:00–16:00 h [22]. These were approximately the times of our histamine challenges at baseline and 4 h. In this study, the magnitude of the fall in histamine PD$_{20}$ FEV$_1$ is also similar to that observed in our subjects (0.54-fold of baseline PD$_{20}$ FEV$_1$ at 4 h; 0.41-fold of baseline PD$_{40}$ V̇p$_{30}$ at 4 h) (fig. 4). An additional explanation for the apparent change in responsiveness is a rebound increase following the abrupt withdrawal of inhaled ß$_2$-agonist prior to each study day [23]. ß$_2$-agonist was administered at the completion of the histamine concentration-response study at the 4 h time point to reverse the induced bronchoconstriction. Subjects were allowed to take ß$_2$-agonist from then onwards until 8 h prior to the histamine challenge at 24 h. Thus it is not surprising that histamine responsiveness, whether measured as PD$_{20}$ FEV$_1$ or PD$_{40}$ V̇p$_{30}$, had returned to baseline values by 24 h (fig. 4) as rebound increase in responsiveness is maximal at 23 h following the cessation of long term ß$_2$-agonist therapy [23].

Considering the degree of mucosal inflammation and mucus hypersecretion reported in dogs after inhalation of 15-HETE, we were surprised to find in both normal and asthmatic subjects that this mediator decreased rather than increased non-specific bronchial responsiveness as measured by the provocative dose of methacholine or histamine causing a 40% fall in V̇p$_{30}$. Since PD$_{40}$ V̇p$_{30}$ is a more sensitive index of bronchial responsiveness than PD$_{20}$ FEV$_1$ [24], it is not surprising that the apparent protective effect of 15-HETE was observed when airway calibre was followed as V̇p$_{30}$ but not as FEV$_1$. Although the magnitude of the changes as small (2.29-vs 1.14-fold at 3 h in normal subjects and 1.11-vs 0.41-fold at 4 h in asthmatic subjects; changes in PD$_{40}$ V̇p$_{30}$ on 15-HETE day vs placebo day), (figs 3 and 4), they are similar to those reported in asthmatic subjects after treatment with inhaled corticosteroids [25, 26]. The change in responsiveness produced by 15-HETE is of the same magnitude as that observed in subjects with atopic asthma spontaneously during the pollen season [27] and following the inhalation of prostaglandin D$_2$ [28], and in normal subjects after inhalation of platelet-activating factor [29], albeit in an opposite direction. We cannot totally disregard the possibility that the observed change in PD$_{40}$ V̇p$_{30}$ was no greater than the natural variation of the measurement but this seems unlikely considering the consistent trend of 15-HETE in decreasing responsiveness to the two agonists when compared with placebo. In both the normals and asthmatics the reduction in responsiveness measured 3–4 h after 15-HETE was independent of whether histamine or methacholine was used as the provoking agonist. Finally, in both groups of subjects the geometric mean PD$_{40}$ V̇p$_{30}$ at all time points following inhalation of 15-HETE were higher than those after placebo but the difference only reached significance at 3–4 h.

The observation made in the present study of the effects of 15-HETE on airway calibre and responsiveness in humans contrasts strongly with the potent pro-inflammatory actions of this mediator in the canine airways [15]. Although in the canine model bronchial responsiveness was not assessed after the administration of 15-HETE, the predominance of neutrophils in the inflammatory infiltrate in this model is similar to that observed in the airways after exposure to ozone [30], allergen [31] and the dihydroxyeicosanoid, leukotriene B$_4$ [32], stimuli which all increase bronchial responsiveness. However, in human asthma it is the eosinophil rather than the neutrophil that has been incriminated most closely with acquired hyperresponsiveness [33–35], thereby highlighting a significant departure from the canine model. Although 15-HETE has been shown to be chemotactic for human neutrophils in $vitro$ [36], there is no evidence that it has a similar effect on eosinophils.

Repeated inhalation of high concentrations of methacholine at intervals of 24 h or less in non-asthmatic subjects has recently been reported to lead to attenuation of bronchonconstriction produced by this cholinergic agonist [37, 38]. This tachyphylactic response to repeated challenges might have contributed to the reduction in methacholine responsiveness after 15-HETE seen in the normal subjects. However, the magnitude of this reduction is significantly more than that after placebo (fig. 3), thus suggesting that 15-HETE plays an active role in modifying the bronchoconstriction response to methacholine in the human airway.

Although none of our subjects complained of any sputum production after inhalation of 15-HETE, we cannot exclude the possibility that hypersecretion of mucus did occur to increase the thickness of the periciliary fluid layer but not to a degree sufficient to give rise to symptoms or change in airway calibre. A small increase in the amount of fluid lining the airways might be sufficient to increase the diffusion barrier to inhaled histamine and methacholine. Chronic exposure of the canine airways to cigarette smoke [39] and sulphur dioxide [40] results in pulmonary inflammation accompanied by an increase in the number of neutrophils in the bronchoalveoar lavage fluid and mucus gland hypertrophy. These changes are accompanied by a reduction in airway responsiveness to inhaled but not to intravenous methacholine. Disparity between airway responsiveness measured by these two routes has been interpreted as mucus hypersecretion providing a barrier to the inhaled agonist. The unpleasant systemic side-effects of agents such as histamine and methacholine when administered intravenously to humans in high doses precluded us from undertaking a similar experiment.

Another possible explanation for our findings is that this 15-lipoxygenase product might have modulated the mechanisms underlying the pathogenesis of bronchial hyperresponsiveness. 15-HETE is an inhibitor of the 5-lipoxygenase pathway in human neutrophils [11] and T-lymphocytes [12] and, therefore, may reduce the formation of pro-inflammatory leukotrienes, which are considered to be putative mediators involved in the pathogenesis of asthma [41]. An anti-inflammatory action of 15-HETE has been shown by the improvements in the skin lesions of patients with psoriasis following the intralesional injection of this eicosanoid [42].

In conclusion, the present study has shown that 15-HETE by inhalation is able to produce a small

reduction in bronchial responsiveness to inhaled methacholine in normal subjects and to inhaled histamine in asthmatic subjects without having any direct effect on airway calibre. Whether or not this seemingly protective effect is due to the induction of a physical barrier or to the modification of the mechanisms underlying the pathogenesis of bronchial hyperresponsiveness deserves further evaluation.

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Effets de l'inhalation d'acide 15-(s)-hydroxyeicosatetraenoique (15-HETE) sur le calibre des voies aériennes et sur la réactivité non spécifique chez les sujets humains normaux et asthmatiques. CK.W. Lai, G.D. Phillips, J.R. Jenkins, S.T. Holgate.
RÉSUMÉ: Nous avons étudié les effets *in vivo* de l'inhalation de 15-HETE sur les voies aériennes de sujets humains normaux et asthmatiques. Au cours d'une étude ouverte et non randomisée, 8 sujets non asthmatiques ont subi à 2 jours différents une étude dose-réponse à l'inhalation de 15-HETE (4.5–70 nmol) le jour 1 et au diluant (tampon phosphate sodique) au jour 2. Les calibres des voies aériennes ont été suivis par le volume expiratoire maximum seconde (VEMS) et les débits expiratoires à 70% de la capacité vitale en dessous de la capacité pulmonaire totale au cours d'une manoeuvre d'expiration forcée partielle ($\dot{V}p_{30}$). La réactivité bronchique non spécifique a été exprimée comme la dose de provocation de methacholine entraînant une chute de 20% du VEMS de base (PD_{20}VEMS), ainsi que celle provoquant une chute de 40% du $\dot{V}P_{30}$ de base ($PD_{40} \dot{V}p_{30}$) respectivement, 3, 6 et 24 h après l'inhalation, soit du 15-HETE, soit du diluant. Un protocole légèrement modifié a été adopté lors d'une étude ultérieure en double aveugle, randomisée et avec permutation croisée, chez 8 sujets asthmatiques soumis à des doses de 15-HETE, s'étendant de 0.07–70 nmol, et chez qui la réactivité bronchique a été mesurée à l'histamine après 2, 4 et 24 h. Quoique le 15-HETE n'eut aucun effet sur le calibre des voies aériennes dans aucun des deux groupes de sujets, elle a augmenté la $PD_{40} \dot{V}p_{30}$ à la methacholine à 3 h chez les sujets normaux (augmentation géométrique de 2.29 fois les valeurs de base par comparaison avec une augmentation de 1.14 après diluant) (p<0.05). Chez les asthmatiques, la moyenne géométrique du $PD_{40} \dot{V}p_{30}$ à l'histamine a diminué de facon significative jusqu' à 0.41 fois la valeur de base 4 h après diluant (p<0.01), alors qu'elle augmentait de 1.1 fois après 15-HETE (p<0.01). L'on n'a pas observé de différence significative dans les modifications du $PD_{40} \dot{V}p_{30}$ à d'autres moments, ni de modification du PD_{20} VEMS à aucun des moments. Nous concluons que l'inhalation de 15-HETE n'a pas d'effet direct sur le calibre des voies aériennes chez les sujets normaux ou asthmatiques. D'autre part, elle produit une légère réduction de la réactivité bronchique non-spécifique chez les sujets non-asthmatiques, et elle inhibe l'augmentation spontanée de la réactivité bronchique chez les asthmatiques. Ces données suggèrent que le 15-HETE aurait un rôle autacoïde dans la fonction des voies aériennes.
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