Effects of various exogenous stimuli on the metabolism of eicosanoids in the lung and airway: the role of 15-hydroxyeicosatetraenoic acid

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15-hydroxyeicosatetraenoic acidの役割

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Effects of various exogenous stimuli on the metabolism of eicosanoids in the lung and airway: the role of 15-hydroxyeicosatetraenoic acid

Takahide Nagase

INTRODUCTION

Eicosanoids are physiologically active metabolites of arachidonic acid and include thromboxanes, prostaglandins, leukotrienes, and hydroxyeicosatetraenoic acids. Although many investigators have examined the metabolism of thromboxane B_2, prostaglandins, leukotrienes, 5-hydroxyeicosatetraenoic acid (5-HETE), and 12-hydroxyeicosatetraenoic acid (12-HETE) in the respiratory system, little is known about the effect and metabolism of two major eicosanoids, i.e. 15-hydroxyeicosatetraenoic acid (15-HETE) and 11-dehydrothromboxane B_2 (11-dehydro-TXB_2), in the lung.

15-HETE is produced from arachidonic acid by the action of 15-lipoxygenase and the capacity to produce 15-HETE has been attributed to human and animal airway epithelial cells (1-4). Some studies indicate that pulmonary epithelial cells selectively oxidize arachidonic acid to 15-lipoxygenase metabolites (5). 15-HETE has recently been reported to be a potent inflammatory agonist for secretion of mucus and to cause chemotaxis of inflammatory cells (6).

11-dehydro-TXB_2 has been recently identified as a suitable parameter for monitoring thromboxane production *in vivo* (7). Though thromboxane B_2 (TXB_2) has been used extensively to monitor thromboxane A_2 (TXA_2) synthesis, the utility of TXB_2 measurement has been questioned on the basis of the artifactual production of TXB_2 during sample collection (8,9). Since 11-dehydro-TXB_2 appears to be unaffected by sample collection procedures, the measurement of 11-dehydro-TXB_2 can provide more valid assessment of TXA_2 production.

The aim of this work was to investigate the effects of various exogenous stimuli on the metabolism of 15-HETE and 11-dehydro-TXB_2 in the lung and airway. I chose to study the effects of 1) smoking exposure, 2) different modes of mechanical ventilation, i.e., high frequency oscillatory ventilation and conventional mechanical ventilation, and 3) endothelin-1 administration.

Some investigators have studied the effect of cigarette smoking on metabolism of TXB_2, leukotriene B_4 (LTB_4) and 5-HETE in the lung (10,11). However, there are very few reports describing the effects of smoking exposure on metabolism of the other eicosanoids. Thus, I investigated the effects of cigarette smoking on metabolism of two eicosanoids, 15-HETE and 11-dehydro-TXB_2, in the lung.

Recently, there has been much interest in high frequency oscillatory ventilation (HFOV), using high frequencies and small tidal volumes (12-17). Though this new mode of the ventilation was extensively studied with regard to more efficient gas exchange, it has also been reported that HFOV may alter mucociliary transport (18). The influence of HFOV on eicosanoid metabolism in the lung has been examined by several investigators, but the results were not unanimously agreeable. While the effects of HFOV on plasma concentrations of TXB_2 and 6-keto-prostaglandin $F_1\alpha$ have been studied (19-21), little is known about its effect on other species of eicosanoid, such as 15-HETE or 11-dehydro-TXB_2. I hypothesized that HFOV applied to the lower airways might change eicosanoid metabolism of the trachea and provide hitherto unrecognized advantages over conventional

mechanical ventilation (CMV). I therefore investigated the effects of HFOV on tracheal release of 15-HETE, 11-dehydro-TXB_2 and protein in anesthetized dogs using a double-balloon endotracheal catheter.

Endothelin-1 (ET-1), a novel potent vasoconstrictor peptide (22), has been shown to cause a bronchoconstriction (23). Other investigators have reported that eicosanoids and oxygen radicals are involved in airway inflammation and hyperreactivity. 15-HETE is a potent inflammatory agonist for secretion of mucus and chemotaxis of inflammatory cells (6). TXA_2 plays an important role in the metabolism of eicosanoids in the lung (24). Meltzer *et al.* (25) reported that superoxide generation of neutrophils in asthmatic patients was significantly higher than that in normal subjects. The present study was undertaken to determine if ET-1 is involved in the metabolism of eicosanoids as well as oxygen radical formation in the airway as a contributing factor for the development of bronchial hyperreactivity and inflammation.

Finally, I summarized these experiments and attempted to establish the role of these eicosanoids as a parameter reflecting the magnitude of responses to the exogenous stimuli to the lung and airway.

MATERIALS AND METHODS

Measurement of 15-HETE and 11-dehydro-TXB_2

15-HETE was measured by radioimmunoassay ([^{3}H]15-HETE, TRK-920, Amersham, UK). The sample was extracted and purified using a C_{18} column (Bond Elut C_{18}, Analytichem International, Harbor City, USA) (26). The eluate with ethyl acetate was dried and reconstituted in a buffer prior to an assay (Fig. 1). The efficacy of this procedure was checked using high performance liquid chromatography (LC6A, Shimadzu, Japan) and the recovery rate of 15-HETE was 88.2%.

11-dehydro-TXB_2 was also measured by radioimmunoassay ([^{125}I]11-dehydro-TXB_2, NEK-042, Du Pont, Boston, USA). The heparinized sample was extracted and purified using a C_{18} column (Bond Elut C_{18}) (26). After the eluate with ethyl acetate was dried and reconstituted in a buffer, it was allowed to sit for 2 hours at 37°C in order to be converted to the open form (Fig. 2).

LTB_4 was measured by radioimmunoassay ([^{3}H]LTB_4, Amersham, UK) after being purified with a C_{18} column (26).

The concentration of protein was measured by the method of Lowry *et al.* (27) using bovine serum albumin as a standard.

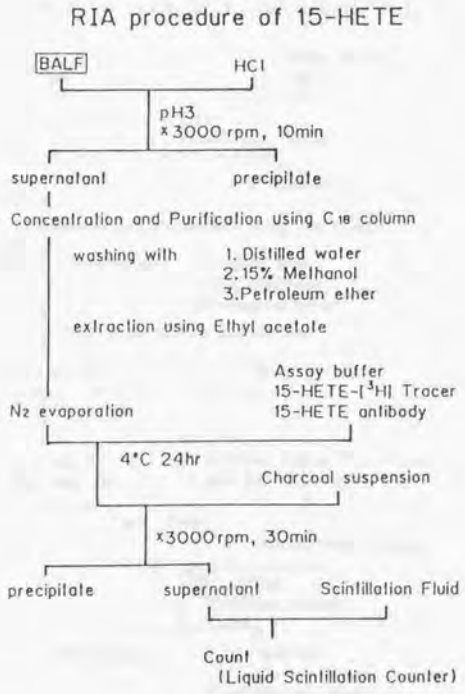

Fig. 1 Radioimmunoassay procedure of 15-HETE.

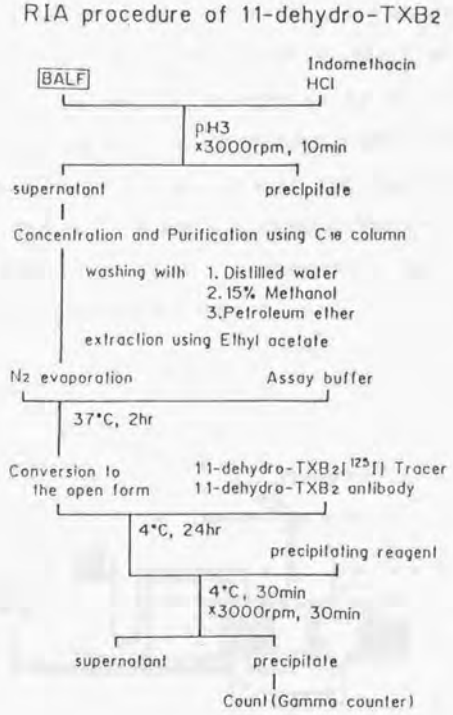

Fig. 2 Radioimmunoassay procedure of 11-dehydro-TXB_2.

Experiment 1

Twelve female Wistar rats aged between 5 and 6 weeks were chosen and the smoking group (SG, n=6) was compared to the non-smoking group (NSG, n=6). Cigarette smoke exposure was performed using Hamburg-II inhalation apparatus (Heinr.Borgwaldt, Hamburg, FRG) and the dose was 10 cigarettes in 30 min (28) (Fig. 3). Bronchoalveolar lavage (BAL) was conducted by the method of Brain (29) 30 min after smoking exposure and the recovered BAL fluid was centrifuged at 3000 rpm for 10 min. The levels of 15-HETE and 11-dehydro-TXB_2 of the supernatant were measured by radioimmunoassay in each sample.

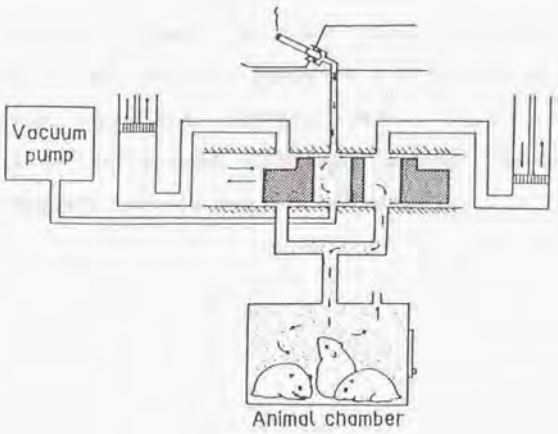

Fig. 3 Schematic diagram of Hamburg-II inhalation apparatus. By means of a dual pump system the smoke is transferred from the smoke chambers into the cylindrical inhalation chamber, where the smoke is simultaneously diluted with air. The puff volume is adjusted by means of a flowmeter and a constant concentration of the smoke-air mixture supplied is guaranteed.

Experiment 2

A double-balloon endotracheal catheter designed to isolate a tracheal segment was constructed. This catheter system differed slightly from Kirsch and Nadel's (30). In the current experiment, the lung was ventilated through a separate tube to facilitate the application of HFOV.

I anesthetized six mongrel dogs weighing 12 to 14kg with pentobarbital sodium (30mg/kg, i.v.), cut along the anterior midline of the lower two thirds of the extra thoracic trachea and pulled the cut edges widely apart. Utmost caution was paid to minimize tissue damage due to the operation. I then inserted a conventional tracheal tube connected to a ventilator (Model 607, Harvard Apparatus, South Natick, USA) and the double-balloon catheter to make an isolated segment of the trachea for instillation of saline. The tip of this catheter was located under the larynx and its position was verified by using a bronchoscope (Type B-3, Olympus, Japan). Fig. 4 illustrates intratracheal instillation using this system. The volume of the isolated segment between the two balloons was 5 ml (31).

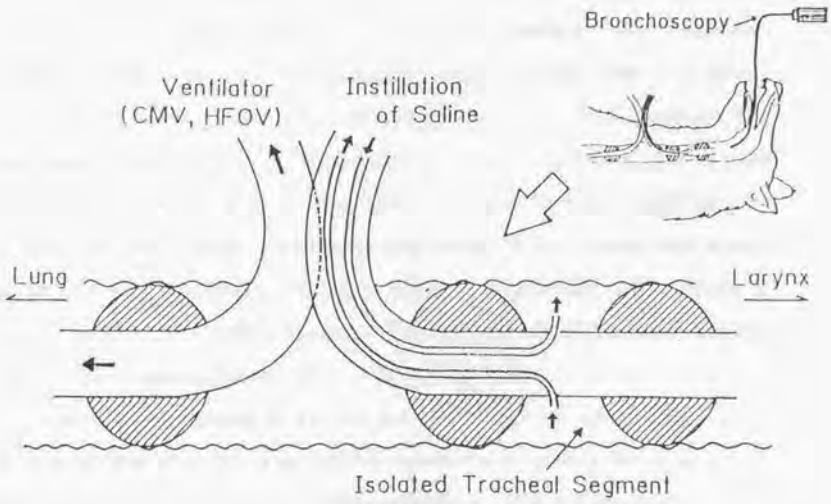

Fig. 4 Schematic diagram of intratracheal instillation using double-balloon catheter system. The lung was ventilated through the separate tube. Saline was instilled into an isolated tracheal segment. The position of this catheter was verified by using a bronchoscopy.

Initially, in order to confirm the time course of sampling, 5 ml of sample warmed to 37°C was instilled into an isolated tracheal segment of this catheter system for 5, 7, 10, and 20 min in random sequence during CMV and HFOV. I then recovered the instilled saline and measured its volume and concentration of protein and 15-HETE. Subsequently, I sampled the isolated tracheal segment for 10 min consecutively during CMV and HFOV. After intratracheal instillation of saline (IIS), I recovered the sample and stored it at -70°C. HFOV (stroke volume= 6 ml/kg, f= 10 Hz, bias flow= 5 l/min) and CMV (stroke volume= 12ml/kg, f= 0.25 Hz) were performed in random sequence. I also obtained blood samples from the femoral artery during HFOV and CMV, using a syringe containing indomethacin. The plasma samples were stored at -70°C till measurement of 11-dehydro-TXB_2.

Then, I performed a bilateral vagotomy at the upper level of the trachea and IIS was again conducted during HFOV and CMV for 10 min respectively in random sequence. The 10-minute periods of each form of ventilation were used both before and after vagotomy.

Two eicosanoids, i.e. 15-HETE and 11-dehydro-TXB_2, were measured by radioimmunoassay in each IIS sample. We also measured the concentration of 11-dehydro-TXB_2 in the plasma samples.

Experiment 3

In vivo: A bolus of phosphate buffer saline (PBS) containing synthetic ET-1 (Peptide Institute Inc., Japan) was given intravenously through a tail vein in female Wistar rats weighing 140-160 g. The dose of ET-1 was 0.25 nmol/kg of body weight (Endothelin-1 group A, ETG-A, n=9) or 1 nmol/kg of body weight (Endothelin-1 group B, ETG-B, n=9). A comparative volume of PBS (control group, CG, n=9) and 1 mmol/kg of histamine (histamine group, HG, n=9) were, respectively, given in the same fashion. Bronchoalveolar lavage (BAL) was conducted by the method of Brain (28) 20 min after i.v. administration, and the BAL fluid was centrifuged. The levels of 15-HETE, 11-dehydro-TXB_2 and LTB_4 of the supernatant were measured by radioimmunoassay. Photon counts of the generation of oxygen radicals by BAL cells (10^6 cells) were measured using lucigenin-dependent chemiluminescence with the photon-counter (Biolumat LB9505, Berthold, FRG) following maximum stimulation by phorbol myristate acetate (1 μg) (32).

In vitro: Female Wistar rats (140-160g) were sacrificed by exsanguination under the anesthesia with sodium pentobarbital. The lungs were removed and were homogenized in 10 volumes of ice-cold PBS with an overhead stirrer. The incubation mixture contained lung homogenate (1 g of wet weight) and varying concentrations of ET-1 (0, 1 and 10 nM, n=4, respectively) in 10 ml of PBS. The reaction was carried out at 37°C for 20 min, and was terminated by acidification to pH 3 with 2N HCl. The amount of 15-HETE was measured by radioimmunoassay after being purified with a C_{18} column (26).

Data Analysis

Statistical analyses were performed using Student's t test in *Experiment 1*, Wilcoxon test in *Experiment 2* and one-way analysis of variance test in *Experiment 3*, respectively. To carry out statistics, I used SAS program (SAS Institute, Cary, USA). P values less than 0.05 were taken as significant and data are expressed as mean ± standard error of mean (SEM).

RESULTS

Experiment 1

Recovered 15-HETE significantly increased in SG (SG: 490±109pg, NSG: 191±63pg, P<0.05), while there was no significant difference in recovered protein (SG: 2.64±0.50mg, NSG: 2.23±0.10mg) or 11-dehydro-TXB_2 (SG: 128±13pg, NSG: 156±7pg) (Fig. 5,6). There was a significant negative correlation between recovered 15-HETE and 11-dehydro-TXB_2 (r= −0.720, P<0.01) (Fig. 7).

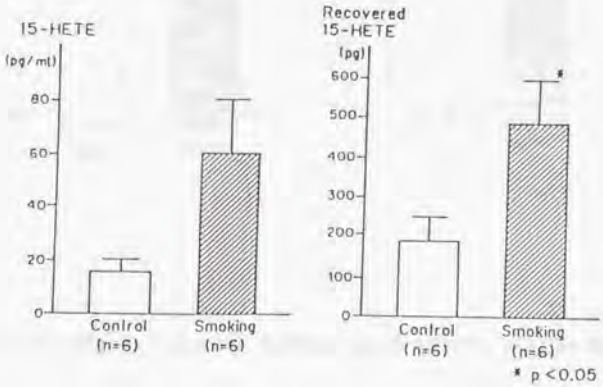

Fig. 5 Influence of cigarette smoking on 15-HETE in BAL fluid.

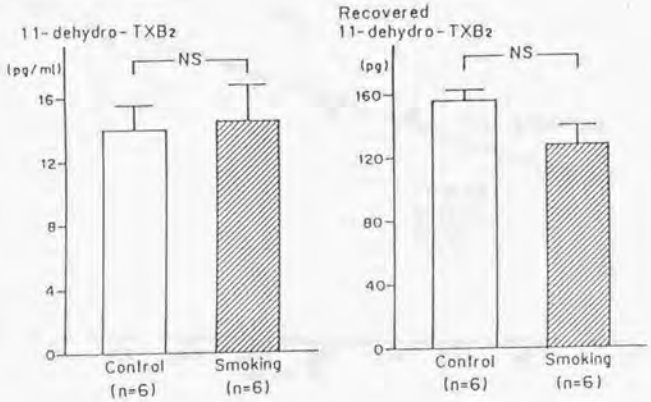

Fig. 6 Influence of cigarette smoking on 11-dehydro-TXB_2 in BAL fluid.

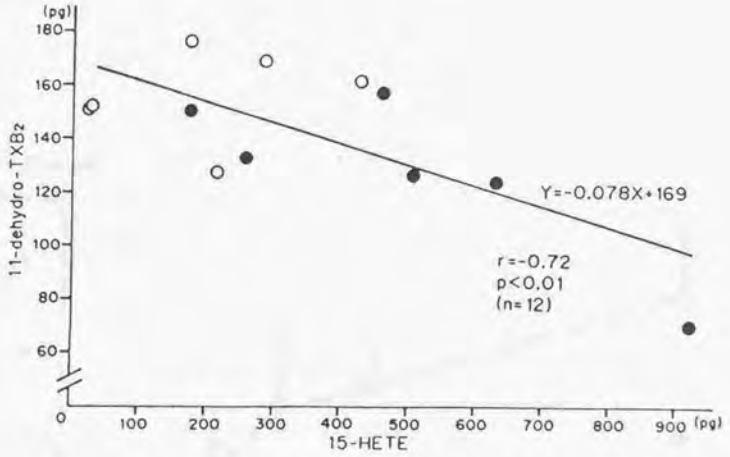

Fig. 7 Correlation between recovered 15-HETE and 11-dehydro-TXB_2. Open circles show the control group (n=6) and closed ones the smoking group (n=6).

Experiment 2

The time course of changes in protein concentration of the IIS sample is shown in Fig. 8 (HFOV/CMV (mg/ml), 5min; 0.199±0.013/0.339±0.063, 7min; 0.260±0.041/0.481±0.144, 10min; 0.311±0.010/0.528±0.105, 20min; 0.587±0.038/0.721±0.145, respectively).

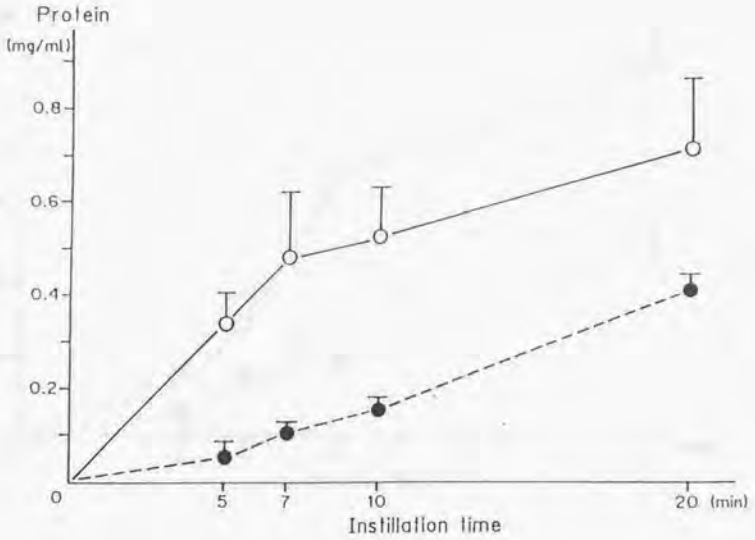

Fig. 8 Changes with instillation time in protein concentration of IIS samples during HFOV (closed circles, n=4) and CMV (open circles, n=5).

Fig. 9 illustrates the time course of changes in 15-HETE concentration of the same IIS sample (HFOV/CMV (pg/ml), 5min; 23±3/64±20, 7min; 56±4/139±37, 10min; 82±7/325±111, 20min; 191±41/475±46, respectively). The recovery rates of IIS for 10 min during HFOV and CMV were 87.6% and 86.9%, respectively.

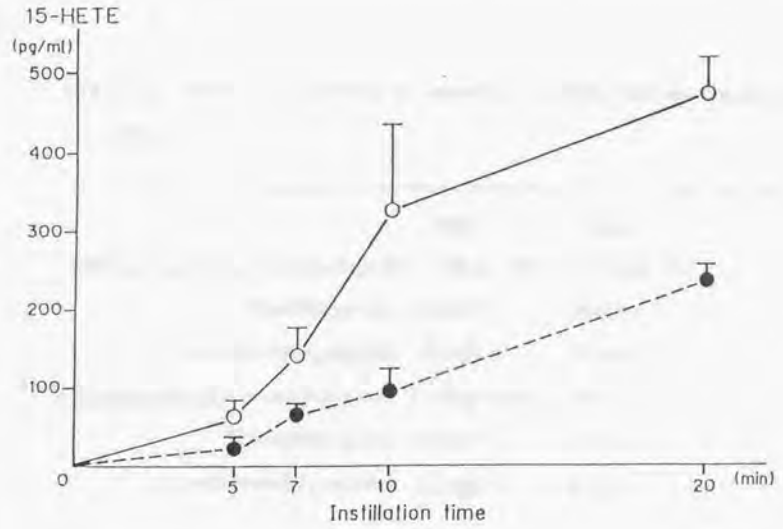

Fig. 9 Changes with instillation time in 15-HETE concentration of IIS samples during HFOV (closed circles, n=4) and CMV (open circles, n=5).

I achieved comparable gas exchange during HFOV and CMV (HFOV/CMV, PaO_2 (mmHg); 115.5±16.3/114.7±11.1, $PaCO_2$ (mmHg); 35.7±1.7/36.2±1.1, pH; 7.418±0.095/7.408±0.112, respectively).

Table 1 summarizes the data obtained in this study.

Table 1. The concentrations of protein, 15-HETE, and 11-dehydro-TXB_2 in IIS sample.

		HFOV	CMV
IIS(vagi intact)	protein(mg/ml)	0.322±0.084*	0.514±0.107
	15-HETE(pg/ml)	87±67*	286±184
	11-dehydro-TXB_2(pg/ml)	11.9±1.4	25.8±8.0
IIS(vagotomized)	protein(mg/ml)	0.371±0.159	0.368±0.108
	15-HETE(pg/ml)	42±14*	120±103
	11-dehydro-TXB_2(pg/ml)	12.6±2.0	8.9±1.3

The data are expressed as mean ± SEM (n=6). The asterisks indicate statistical significance from CMV with $P<0.05$. IIS: Intratracheal instillation of saline.

It shows the concentration of protein, 15-HETE and 11-dehydro-TXB_2 in tracheal fluid when the animals were maintained on HFOV or CMV. In dogs with the vagi intact, the concentration of 15-HETE in tracheal fluid was significantly decreased with HFOV to less than half of its concentration with CMV (Fig. 10). The protein level with HFOV was also significantly smaller than that with CMV (Fig. 11), but the concentration of 11-dehydro-TXB_2 was not significantly different.

After bilateral vagotomy, the concentration of 15-HETE tended to be further lowered both with HFOV and CMV. However, the concentration ratio of CMV/HFOV (vagi intact; 3.28, after vagotomy; 2.86) remained almost unchanged (Fig. 10). There were no significant differences in the protein and 11-dehydro-TXB_2 between HFOV and CMV.

The concentrations of plasma 11-dehydro-TXB_2 between HFOV and CMV were neither significantly different with vagi intact nor with vagi cut (HFOV/CMV (pg/ml), with vagi intact; 48.5±8.5/65.0±14.3, with vagi cut; 48.7±8.8/45.3±5.6, respectively).

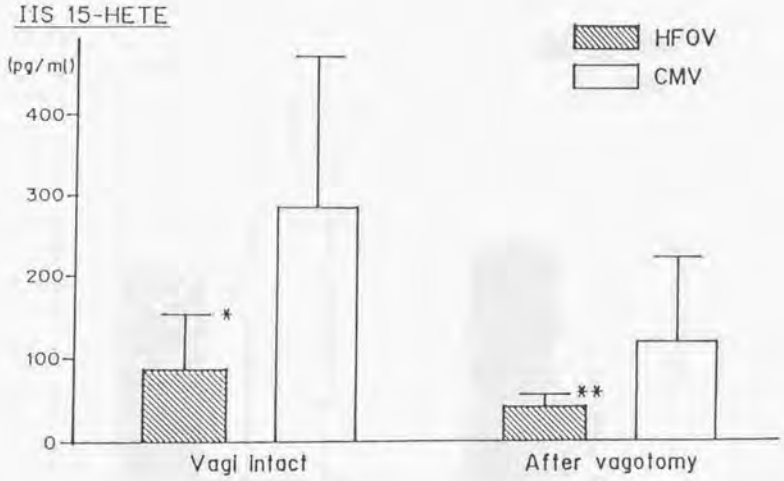

Fig. 10 The effect of HFOV on 15-HETE concentration of IIS samples with vagi intact and after vagotomy (n=6). The asterisk indicates statistical significance from CMV ($P<0.05$) with vagi intact. The double asterisk indicates statistical significance from CMV ($P<0.05$) after vagotomy.

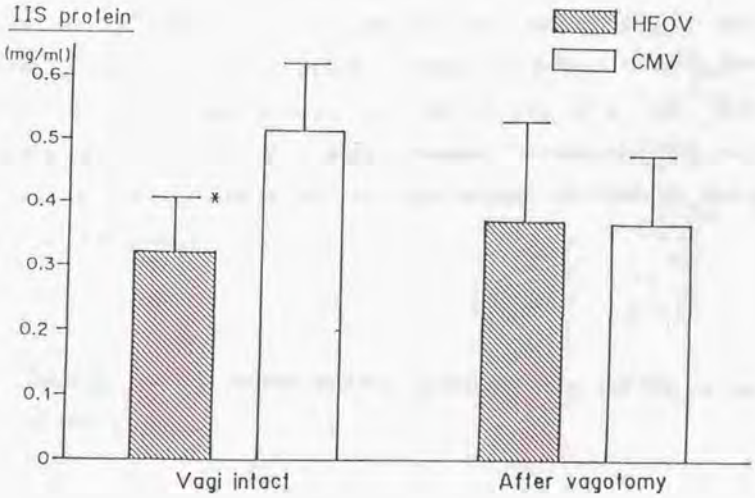

Fig. 11 The effect of HFOV on protein concentration of IIS samples with vagi intact and after vagotomy (n=6). The asterisk indicates statistical significance from CMV ($P<0.05$) with vagi intact.

Experiment 3

In vivo: The level of 15-HETE in the BAL fluid of ETG-B was significantly higher than that of the other groups (CG: 148±16, HG: 177±48, ETG-A: 217±58, ETG-B: 475±70** (pg/ml), respectively, **P<0.01 vs CG) (Fig. 12). The photon counts of oxygen radicals generation by BAL cells induced by 1 nmol/kg ET-1 were also significantly higher than those of the other groups (CG: 0.85±0.17, HG: 1.24±0.21, ETG-A: 1.24±0.14, ETG-B: 1.74±0.22** ($10^4/10^6$ cells), respectively, **P<0.01 vs CG) (Fig. 13). With regard to cell counts, cell types, protein, 11-dehydro-TXB_2, and LTB_4 in BAL fluid, there was no significant difference among these groups (Table 2).

Table 2. The cell counts, protein, 11-dehydro-TXB_2, and LTB_4 in BAL fluid of each group

		CG	HG	ETG-A	ETG-B
Cell	(10^5/mℓ)	5.45±1.96	9.12±1.63	2.36±0.43	2.97±0.69
Protein	(mg/mℓ)	0.216±0.019	0.343±0.099	0.238±0.024	0.197±0.019
11-$dTXB_2$	(pg/mℓ)	7.9±1.8	23.5±12.0	3.4±0.3	5.5±1.3
LTB_4	(pg/mℓ)	471±301	608±167	321±27	929±267

The data are expressed as mean ± SEM. 11-$dTXB_2$; 11-dehydro-TXB_2.
CG; Control group. HG; Histamine group. ETG-A; ET-1 (0.25 nmol/kg) group. ETG-B; (1 nmol/kg) group.

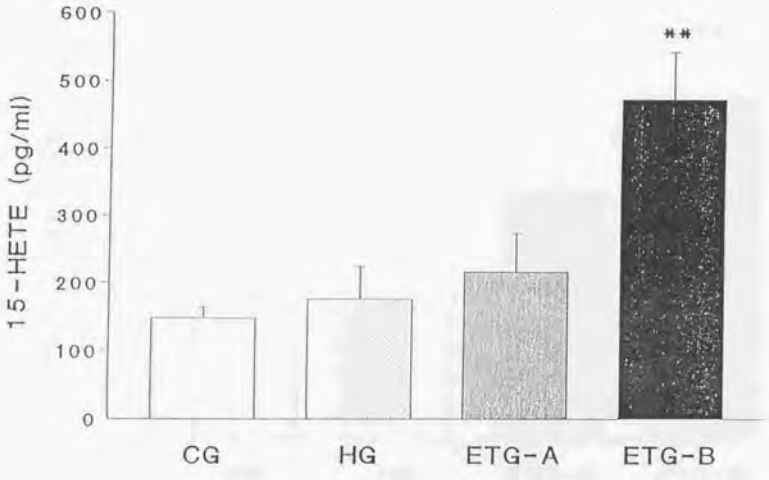

Fig. 12 The concentration of 15-HETE in BAL fluid. CG; Control group. HG; Histamine group. ETG-A; ET-1 (0.25 nmol/kg) group. ETG-B; (1 nmol/kg) group. **P<0.01 between CG and ETG-B.

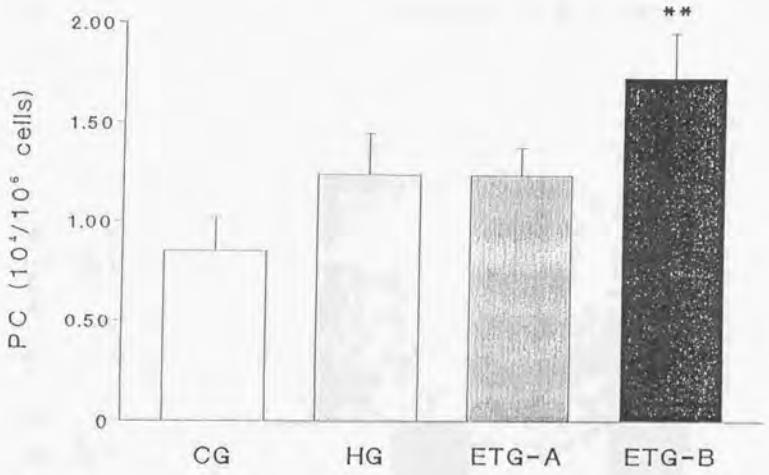

Fig. 13 The photon counts (PC) of oxygen radicals generation by BAL cells. CG; Control group. HG; Histamine group. ETG-A; ET-1 (0.25 nmol/kg) group. ETG-B; (1 nmol/kg) group. **P<0.01 between CG and ETG-B.

In vitro: The 15-lipoxygenase activity was stimulated dose-dependently by the addition of ET-1 (0 nM: 4.05±0.46, 1 nM: 5.53±0.59, 10 nM: 8.05±0.63 (ng/g of wet weight), respectively). A statistically significant difference was observed between control and 10 nM concentration of ET-1 ($P<0.01$, Fig. 14).

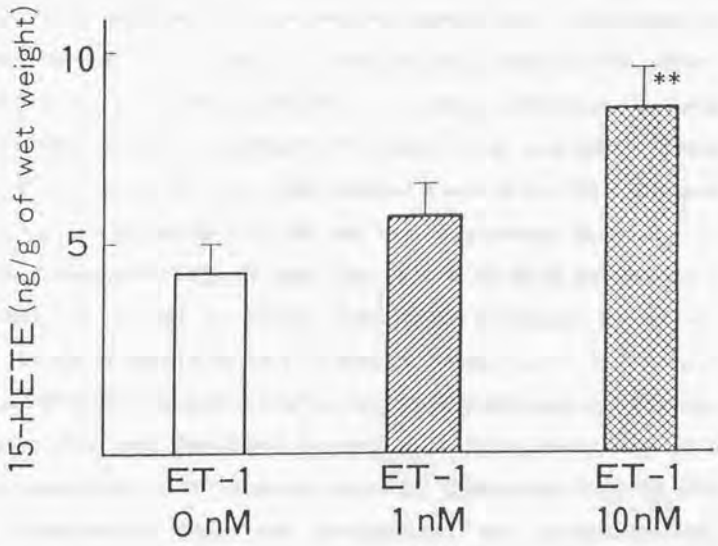

Fig. 14 The level of 15-HETE in the lung homogenate with 0, 1 and 10 nM ET-1. **P<0.01 between 0 and 10 nM ET-1.

DISCUSSION

In the current study, I observed that various exogenous stimuli affected 15-HETE metabolism in the lung and airway, while the variation of 11-dehydro-TXB_2 was not significant. These findings suggest that 15-HETE could be a potential parameter to assess lung and airway disorders.

In *Experiment 1*, I investigated the effects of cigarette smoking on metabolism of two eicosanoids, 15-HETE and 11-dehydro-TXB_2, in the lung. Recovered 15-HETE significantly increased in the smoking group, while there was no significant difference in recovered protein or 11-dehydro-TXB_2. With regard to the effect of smoking on eicosanoid metabolism, some investigators have reported that smoking exposure increased TXB_2 and LTB_4 in BAL fluid (10,11). However, very few reports focused on the influence of smoking on the metabolism of two major eicosanoids, i.e. 15-HETE and 11-dehydro-TXB_2, in the lung. Since 15-HETE has recently been reported to be a major eicosanoid produced by airway epithelial cells and to be an inflammatory mediator, the increase in BAL 15-HETE may indicate acute lung injury induced by smoking. 11-dehydro-TXB_2 was unchanged by smoking, while there are some studies reporting that smoking exposure increased TXB_2 in BAL fluid. Considering that the measurement of 11-dehydro-TXB_2 provides a better understanding of the biological role of TXA_2, smoking might not influence the production of TXA_2 in the distal lung. I observed a significant negative correlation between recovered 15-HETE and 11-dehydro-TXB_2. 15-HETE and 11-dehydro-TXB_2 are eicosanoids produced from arachidonic acid by different enzyme pathways, 15-lipoxygenase and cyclooxygenase,

respectively. It might be possible that an increased 15-HETE production depressed 11-dehydro-TXB_2 production, and vice versa. In summary, acute cigarette smoke exposure increased the concentration of 15-HETE in recovered BAL fluid. The presence of 15-HETE in BAL fluid may indicate lung injury induced by smoking.

Various modes of mechanical ventilation, e.g. HFOV or CMV, provide a mechanical stimulus for a lung. I investigated the effect of HFOV and CMV on the metabolism of eicosanoids in the lung. The most striking finding in *Experiment 2* was that the concentration of 15-HETE in tracheal fluid with HFOV was less than half of that with CMV. It has been shown by Johnson *et al.* (6) that 15-HETE is a potent agonist for secretion of glycoprotein-containing mucus from the canine trachea *in vivo*. Furthermore, it causes the chemotaxis of inflammatory cells into the lumen of the canine airway. These observations suggest that 15-HETE may have an important role in the evolution of inflammation in the tracheo-bronchial airways of dogs (6,33-35). Thus, it seems reasonable to assume that the decrease in the concentrations of protein and 15-HETE in tracheal fluid may be more beneficial to keep the airway intact.

The mechanism of the reduction of 15-HETE when applying HFOV remains unclear. The principal site of 15-HETE production is thought to be the airway lumen including the mucosa and glandular tissue (6). Therefore, one might assume that high-frequency oscillation of these tissues may result in smaller amount of 15-HETE secretion. In this study, peak pressure swing during the experiment was less prominent in HFOV than in CMV (18). Then, one could hypothesize that CMV might be a more invasive mode of

mechanical ventilation than HFOV. It might be possible that the mode of ventilation applied to the lower lung influences the production of such blood chemical substances affecting airway secretion of 15-HETE. It has been reported by Sirois *et al.* (24) that TXA_2 mediates the myotropic action of LTB_4 in the guinea pig lung and one can speculate that similar mechanisms may exist between airway secretion of 15-HETE and some eicosanoids in blood. To verify this notion, I measured plasma 11-dehydro-TXB_2 level. However, there was no significant difference between HFOV and CMV. I observed that this reduction tended to be enhanced by vagotomy and that the concentration ratio of CMV/HFOV remained unchanged. This finding suggests that the vagal tone is related to the regulation of the total amount of secretion both for HFOV and CMV, but not related to the reduced production of 15-HETE during high frequency oscillation of the lung and thorax. Finally, oscillatory movement of the tracheal portion in this experimental setup was less extensive than that in the ventilated portion of the lung. However, it is possible that the transmitted oscillation of the trachea may have contributed to the observed reduction of 15-HETE.

Although there have been several studies investigating the effects of HFOV on mucociliary transport and viscoelasticity of tracheo-bronchial secretions (18,36), very few have addressed the influence of HFOV on the secretion of chemical substances including 15-HETE or 11-dehydro-TXB_2 in the airway. Wetzel *et al.* (19) have reported that HFOV increases prostacyclin production and that the attenuation of pulmonary hypoxic vasoconstriction with HFOV might cause the increase in prostacyclin. Durante and

Van der Zee (20,21) measured plasma TXB_2 and concluded that application of HFOV did not result in a significant change of thromboxane production.

11-dehydro-TXB_2 has been recently identified as a suitable parameter for monitoring thromboxane production *in vivo*. Westlund et al. (7) have reported that 11-dehydro-TXB_2 gives a more reliable picture of metabolic events than TXB_2, which to a large extent reflects technical difficulties during blood sample collection. I measured 11-dehydro-TXB_2 concentration of tracheal fluid and plasma during HFOV and CMV and observed no significant difference between these two modes of ventilation. Thus, HFOV did not seem to influence tracheal secretion of thromboxane. Available data about thromboxane production in the circulating system are limited to the results obtained by measuring plasma TXB_2 (20,21). I confirmed that HFOV did not change thromboxane production significantly by measuring plasma 11-dehydro-TXB_2, a more accurate and reliable parameter of *in vivo* thromboxane metabolism.

I performed this study using a double-balloon catheter technique. Kirsch *et al.* (30) first described this technique and found it to give reproducible results. They reported that it allowed the analysis of chemotaxis in the living airway. We used a modified version of this technique in our study to analyse airway secretion during HFOV and CMV. In the current experiment, the lung was separately ventilated below the lower level of trachea and the upper tracheal segment was kept intact as much as possible. I confirmed the location of the double-balloon using a bronchoscopy and could sample the isolated tracheal segment.

We observed decreases in the concentrations of both protein and 15-HETE after vagotomy. The behaviour of this isolated tracheal segment before and after vagotomy suggests its function in accordance with the lower respiratory tract. The implication of this finding might be extended to the notion that the secretion of total airway including trachea may be similarly influenced by HFOV both in quality and in quantity. In summary, I investigated the effects of HFOV and CMV on canine tracheal secretion using modified endotracheal double-balloon catheter and observed a decrease in the concentration of 15-HETE in tracheal fluid with HFOV. HFOV may provide hitherto unrecognized advantage over CMV by reducing airway secretion of 15-HETE, a potent inflammatory mediator.

The 21-amino acid polypeptide ET-1 is a potent constrictor of vascular smooth muscle (9) and has been shown to be a bronchoconstrictive agent (23). It has been reported by de Nucci *et al.* (38) that the administration of ET-1 (1 nM) releases prostacyclin and TXA_2 from rat isolated pulmonary circulation. However, there are very few reports of the effects of ET-1 on eicosanoids and oxygen radicals production in the airway space. I hypothesized that ET-1 could be involved in the metabolism of eicosanoids as well as oxygen radical formation in the airway as a contributing factor for the development of bronchial hyperreactivity and inflammation. In *Experiment 3*, a prominent finding is that the level of 15-HETE and oxygen radicals generation in BAL fluid of ET-1 treated animals (1 nmol/kg) was significantly higher than that of the other groups. The level of 15-HETE formation in the lung homogenate with 10 nM ET-1 was

significantly higher than that of the control. Thus, ET-1 has stimulating effects on the 15-lipoxygenase activity of the lung both *in vivo* and *in vitro*. BAL fluid obtained with Brain's method (29) contains metabolic products of the distal lung unit, especially bronchioli and alveoli. 15-HETE is a potent secretagogue of mucus from trachea *in vivo* (6). Furthermore, 15-lipoxygenase products are precursors of another proinflammatory compounds, lipoxins (37). It seems reasonable, therefore, to assume that the increase in the concentration of 15-HETE in BAL fluid induced by ET-1 administration (1 nmol/kg) may enhance the development of inflammation in the airways. I measured 11-dehydro-TXB_2 and LTB_4 in BAL fluid in each group and observed no significant difference among these groups. Thus, the effect of ET-1 seems specific for 15-lipoxygenase, and neither 5-lipoxygenase nor cyclooxygenase pathway was altered in this study.

Oxygen free radicals can damage lung tissues (39,40). Meltzer *et al.* (25) reported that superoxide generation in subjects with asthma was significantly higher as compared to that of normal subjects. One might then assume that the increase in superoxide generation by BAL cells observed in ET-1 administration (1 nmol/kg) may have some contributing roles in evoking airway hyperreactivity.

Recently, it has been suggested that ET-1 is synthesized in endocrine cells of human lung (41). In addition, it has been reported that ET-1 stimulates 15-HETE production from nasal mucosa in human (42). Our observation indicates that ET-1 may contribute to the inflammatory and hyperreactive process of lungs

by enhancing the release of 15-HETE and oxygen radicals in the distal lung unit.

Finally, the results of these three experiments suggest that 15-HETE reflects the lung and airway injury in response to exogenous stimuli and that 15-HETE may play an important role in the evolution of inflammation in the lung and tracheobronchial airways. Recently, it has also been reported that 15-HETE could be involved in the mechanism of bronchial asthma (43). It seems that 15-HETE could be a potential and important parameter to assess various disorders of the respiratory system, from the nasal airway to the distal lung unit.

Meanwhile, the various exogenous stimuli did not significantly affect 11-dehydro-TXB_2 in the lung and airway. While it has been reported that 11-dehydro-TXB_2 is a major, enzymic metabolite of TXB_2 in blood (7) and urine (44), little is known about the metabolism of TXB_2 in the airway and distal lung unit. Therefore, the role of 11-dehydro-TXB_2 as a parameter in the lung and airway warrants further investigation.

In summary, the observations of my work suggest that 15-HETE could be an important parameter to assess the inflammation and injury in the lung and airway.

CONCLUSION

In conclusion, I investigated the effects of various exogenous stimuli on the metabolism of 15-HETE and 11-dehydro-TXB_2 in the lung and airway. I conclude as described below.

1) The smoking exposure increased 15-HETE in BAL fluid in rats. The presence of 15-HETE in BAL fluid may indicate lung injury induced by smoking.

2) High frequency oscillatory ventilation decreased the concentration of 15-HETE in canine tracheal fluid compared to conventional mechanical ventilation. High frequency oscillatory ventilation may provide hitherto unrecognized advantage over conventional mechanical ventilation by reducing airway secretion of 15-HETE.

3) Intravenous bolus administration of endothelin-1 increased 15-HETE in BAL fluid in rats. Endothelin-1 exhibited a stimulatory effects on 15-lipoxygenase activity in lung homogenate. Endothelin-1 may contribute to the inflammatory and hyperreactive process of lungs by enhancing the release of 15-HETE in the distal lung unit.

These findings suggest that 15-HETE reflects the lung and airway injury in response to exogenous stimuli and that 15-HETE may play an important role in the evolution of inflammation in the lung and tracheobronchial airways. Thus, 15-HETE could be an important parameter to assess the inflammation and injury in the lung and airway in the presence of exogenous stimuli.

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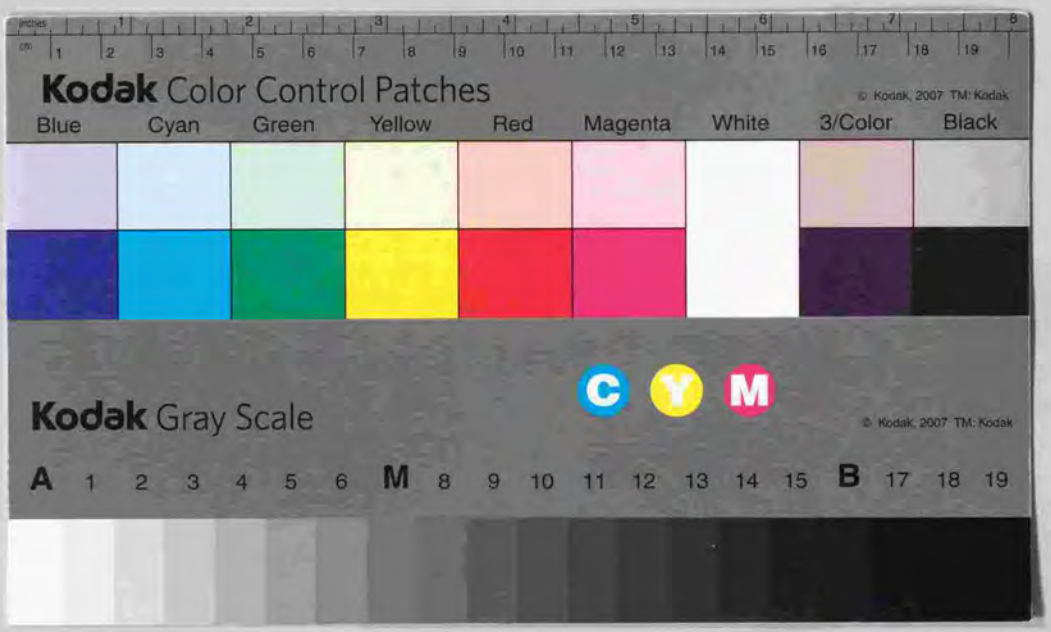
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