Increased interleukin-8 in epithelial lining fluid of collapsed lungs during 1-lung ventilation for thoracotomy

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ABSTRACT

The present study was designed to evaluate inflammatory changes in collapsed lungs during 1-lung ventilation, using the assistance of a bronchoscopic microsampling probe. Serial albumin and interleukin (IL)-8 concentrations in epithelial lining fluid (ELF) were measured in 7 patients undergoing resection of lung tumors. The samples were taken after induction of anesthesia (baseline), thirty minutes after 1-lung ventilation was started (point 2), just before resuming 2-lung ventilation (point 3), and thirty minutes after 2-lung ventilation was restarted (point 4). The albumin and IL-8 concentrations in ELF were significantly increased at point 2 and point 3, respectively, and remained to be high, compared to the baseline. The increase in IL-8 at point 3 was correlated with the interval of 1-lung ventilation, however, none developed specific acute lung injury. These findings suggest that inflammatory changes can occur on the epithelium of collapsed lung even in patients underwent successful and standard thoracic surgery.

Key words: bronchoscopic microsampling, ARDS, permeability pulmonary edema, thoracic surgery, re-expansion pulmonary edema

INTRODUCTION

It has been reported that the prevalence of acute lung injury (ALI) and/or acute respiratory distress syndrome (ARDS) after lung resection ranges from 2.45-3.7% [1, 2]. Among them are patients who developed an ALI/ARDS in a collapsed lung after 1-lung ventilation during thoracic surgery [3,4]. Re-expansion pulmonary edema (REPE) has been included in the category of ALI/ARDS. REPE is a rare complication of thoracic surgery during 1-lung ventilation, but is an important clinical situation [3-5]. Several possible mechanisms have been proposed for the pathophysiology leading to the onset of REPE, including ALI/ARDS following pulmonary resection. Mechanical stimuli [6,7], a decrease in surfactant [8], or the release of biochemical mediators [7, 9] could also increase pulmonary microvascular permeability in a model of REPE, resulting in pulmonary edema [6-9]. In these experimental [6-9] and human studies [10,11], increased neutrophil accumulation and neutrophil elastase levels in the lungs or edema fluid were demonstrated. Furthermore, interleukin (IL)-8 production is enhanced in the re-expanded lung and associated with the development of REPE in these experimental [7] and clinical studies [10,11].

We hypothesized that inflammatory changes may occur in the collapsed lung of patients undergoing lung resection with 1-lung ventilation, even in those who do not

develop ALI/ARDS. Thus, we designed a study to evaluate airway inflammation of the collapsed lung in patients who underwent 1-lung ventilation for lung tumor resection. The present study was focused on serial measurements of inflammatory mediators using a novel technique, bronchoscopic microsampling (BMS), to obtain epithelial lining fluid (ELF)[12], during a thoracosurgery.

MATERIALS AND METHODS

The study protocol was approved by the Human Ethics Committee of Shinshu University Hospital, and written informed consent was obtained from each patient. Seven patients who were diagnosed with malignant tumor in a lung and planned to receive thoracic surgery were enrolled in the present study. Radical operations including lobectomy or partial resection were scheduled under combined epidural and general anesthesia. Meperidine hydrochloride, 1mg/kg (i.m.), and 0.5 mg (i.m.) atropine sulfate were administered 1 hour before anesthesia, which was induced and maintained by intermittent infusion of 2μg/kg fentanyl, 10 mg midazolam, and 4 mg vecronium. Percutaneous arterial oxygen saturation and brachial arterial pressure were continuously monitored. The lungs were ventilated with a 10mg/kg tidal volume, and the respiratory frequency was adjusted to maintain a partial tension of carbon dioxide (Pa_{CO2}) within

the normal range before one lung ventilation. For all subjects, 1-lung ventilation was performed to facilitate the surgical procedure for resecting the lung tumor.

The experimental schedule is summarized in Figure 1. Bronchoscopic sampling of the ELF was performed at 4 points: after induction of anesthesia just before 1-lung ventilation (baseline; point 1), 30 minutes after 1-lung ventilation was started (point 2), just before resuming 2-lung ventilation (point 3), and 30 minutes after resuming 2-lung ventilation (point 4) (Figure 1).

The patients' lungs were continuously ventilated throughout the procedure via a Bodai Suction Safe^{TM} Swivel Y connector. The inspired O_2 concentration was set at 100%, and other ventilator settings remained unchanged during the procedure. The design of the BMS probe and the ELF sampling method has been described elsewhere [12]. Briefly, a commercially available BMS probe (OD, 2.4mm) loaded with absorptive material (BC-401C; Olympus; Tokyo, Japan) was directed to the inferior bronchus of the resected lung through the channel of the bronchoscope. The catheter was advanced to the subsegmental bronchus and placed in contact with the epithelial surface for 10 seconds. Three samples without blood contamination were obtained for each time point. Serial ELF samples were obtained from the same distal subsegmental bronchus in each patient. The ELF samples were stored at -80°C until assayed. Arterial blood

samples were collected at the same time point as ELF samples, and were centrifuged at 3,000 rpm for 10 min at 4°C, and the supernatant was stored at -80°C until the samples were assayed.

The absorbed material collected with the BMS probe and the blood samples were subjected to enzyme linked immunosorbent assays (ELISAs) for IL-8 and albumin. The human albumin concentrations in the extracts were also measured by a colorimetric method (Beckman, Fullerton, CA). The original concentrations of these mediators in the ELF were calculated with a correction of the wet-to-dry ratio of the absorptive material and used for analysis [12]. Measurements were performed by the laboratory staff at Bio Medical Laboratories (BML) Inc (Tokyo, Japan) who were unaware of the clinical situation and profile of the patients.

DATA ANALYSIS

The data values in the text and figures were expressed as the means ± SEM. The changes of parameters in the ELF and peripheral blood during and after 1-lung ventilation were compared using the Wilcoxon signed rank test. Each variable was tested for correlations with the duration of 1-lung ventilation using simple linear regression analysis (Pearson's correlation). All statistical analyses were performed with

the use of a Windows-compatible software program (Stat Flex ver. 5.0, Artech Ltd., Osaka, Japan). P values < 0.05 were considered to be significant for all statistical analyses.

RESULTS

The seven patients' characteristics, pulmonary function test results, and P/F ratio at baseline are shown in Table 1. The patients included 4 females and 3 males, and their mean age was 65.5 yr, ranging from 52-79. The FEV1.0/FVC% of all 7 patients was within the normal range. However, in patient 1, the %VC was slightly low because of a history of lung resection for a metastatic lung tumor due to thyroid cancer 6 years prior to the present surgery. Three patients (patient. 1, 3, and 4) were ex-smokers. The diagnosis and surgical methods for each patient are shown in Table 2. Two patients had metastatic lung tumors, and the others had primary lung cancer. Three patients underwent video assisted thoracoscopic surgery (VATS), and the other 4 patients underwent open lung resection. The duration of the operation, anesthesia, and 1-lung ventilation and infusion, as well as blood loss and transfusion volumes are summarized in Table 3. The duration of 1- lung ventilation was 213±45 min. Allogenic blood transfusions were administered to two patients (patient 1 and 5), representing total volumes of 520 ml, respectively. The total infusion volumes were 1980 ±267 ml, and the urine volumes were 593±100 ml. Patient 1 developed profuse bleeding, so we did not perform examinations for points 3 and 4 for this patient. All 7 patients were successfully treated with surgery, and none developed ALI/ARDS after the operation.

The plasma concentrations of albumin and IL-8 are shown in Figure 2. The serum concentrations of albumin were significantly decreased at points 2 and 3. The serum concentrations of IL-8 increased at both points 3 and 4, and there was a significant difference between point 4 and the level at baseline.

The concentrations of albumin and IL-8 in ELF are shown in Figure 3. Compared with the baseline (point 1), the albumin concentrations in the ELF were significantly increased at point 2 and remained stably high at points 3 and 4. The IL-8 concentrations in the ELF were significantly increased at both points 3 and 4. After 2-lung ventilation was re-started (point 4), the albumin and IL-8 concentrations in the ELF tended to decrease, however, they still remained significantly higher than the baseline level at point 4.

The relationships between the measured parameters (albumin and IL-8) and clinical factors (duration of anesthesia, length of the operation, 1-lung ventilation time and water balance) were analyzed. We found that the level of IL-8 in the ELF at point 3 was significantly correlated with the duration of 1-lung ventilation (Figure 4). Otherwise, we did not find any relationship between the IL-8 or albumin concentrations with other clinical parameters. In addition, there was no correlation between the IL-8 concentrations in the plasma and ELF.

DISCUSSION

We analyzed serial albumin and IL-8 levels in ELF of non-ventilated and collapsed lungs by using BMS in this study. We found that albumin concentrations in ELF were significantly increased in the collapsed lung 30 min after the initiation of 1-lung ventilation, followed by a subsequent increase in IL-8 in the ELF. In addition, the increases in IL-8 in ELF after 1-lung ventilation were significantly correlated with the duration of 1-lung ventilation.

Although several studies have emphasized the sub-clinical presence of inflammation or pathophysiological changes on the lung epithelium in several disease states [13,14], the time course of chemical mediators in the airway of non-ventilated and collapsed lungs during 1-lung ventilation has not been studied. In general, bronchoalveolar lavage (BAL) can provide valuable information about the biochemical status in the diseased lung [15-17], especially in cases of ALI/ARDS. However, it is difficult to perform BAL during 1-lung ventilation in patients treated with thoracic surgery. Recently, BMS was developed and has been applied to obtain ELF for several pulmonary diseases [12, 18-23]. Ishizaka *et al*. [12, 18] analyzed various cytokines, including IL-8, in patients with ARDS using this method, and reported that measurements of biomarkers in ELF were useful for monitoring the inflammatory

responses in patients with ALI/ARDS. Thus, this method is a novel and alternative tool for evaluating airway inflammation that is less invasive than BAL. In addition, the procedures were able to provide repeated samplings. In the present study, the ELF sampling procedure were completed within 10 min in each case, and no adverse hemodynamic or respiratory events, including desaturation, were observed during and after sample collection. Thus, we emphasize that BMS is a useful tool for serial evaluations of the inflammatory response in the airway, even in patients who undergo thoracic surgery.

It is noteworthy that the increase in albumin concentration in ELF of the collapsed lung was observed earlier than the increase in IL-8 and the increase in albumin (point 2) occurred just 30 min after the initiation of the surgical procedure. Since the time was too short, we thought that surgical stimulation might be independent of the increased albumin in the ELF. It is likely that the collapse of the lung itself led to the increases in albumin on the bronchial epithelium. The serum albumin concentration significantly decreased at the same point. Thus, the increased albumin in the ELF could be related to increased permeability or decreased absorption. It remains unknown that collapsed lung and just 30 min surgical stimuli can induce an increased permeability across epithelium. In addition, analysis of other pro-inflammatory cytokines including tumor necrosis

factor $-\alpha$, IL-1β and IL-6 et al, were lacking in the present study. Other biochemical markers may help to identify the pathophysiological significance at the each time of clinical points. In addition, it has been speculated that there is decreased pulmonary lymph flow or bronchial circulation in the collapsed lung [8]. Thus, we have to consider a possibility that absorbance of albumin on the bronchial wall was reduced in the collapsed lung, which partially contributed to the increased albumin in ELF. Indeed, the level of albumin in ELF decreased after a cessation of 1-lung ventilation.

IL-8 was also increased in both ELF and plasma in the present study. Because surgical stimuli might stimulate the production of IL-8 in the targeted lung, it is easily speculated that the increased IL-8 in ELF and plasma could be due to direct procedure of thoracic surgery. Furthermore, another ventilated lung is exposed to a hyperoxic condition and received dynamic hyperinflation, which also might influence the increase in serum IL-8. However, a significant increase in IL-8 was observed earlier in the ELF (point 3) than in the plasma (point 4) and was not correlated with the level in plasma at either point. These findings suggest that the IL-8 in ELF was produced on the bronchial epithelium in the collapsed lung, rather than supplies from the systemic flow to the bronchial wall. Thus, there were various factors in the 1-lung ventilation that contributed to the IL-8 production in ELF, such as surgical stimuli, the hyperoxic (or

hypoxic) conditions of the lungs, ventilation-perfusion mismatch, fluid replacement, anesthetic agents, and other multiple stresses, etc. In the present study, the increases of IL-8 in ELF after 1-lung ventilation were significantly correlated with the duration of the 1-lung ventilation. This finding suggests that surgical stimuli or anesthetic procedures may be mainly responsible for stimulating IL-8 production by the bronchial epithelium.

In conclusion, the elevation of IL-8 subsequent to increased albumin in ELF was observed in the collapsed lungs during 1-lung ventilation. The clinical importance of these findings is unclear for standard thoracic surgery; however, these responses could be a trigger for post-operative pulmonary complications in patients receiving 1-lung ventilation.

Conflict of Interest; There are no conflicts of interest for this study.

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pulmonary Mycobacterium avium complex disease. *Respiration* 76:338-343.

TABLES

TABLE 1. Clinical characteristics of the seven patients

Pt No.	Age (yrs)	Sex	Height (cm)	Weight (kg)	Smoking (pack-years)	%VC (%)	FEV1 %pred	FEV1 (ml)	PaO2/FiO2 (baseline)
1	79	M	175	65	37	64.6	75.7	1560	246
2	59	F	149	53	None	102.6	75	1800	480
3	52	M	174	65	14	133	72.6	3660	372
4	77	M	156	52	17.5	101.3	84.7	2550	421
5	73	F	154	57	None	86.9	79.2	1520	323
6	72	F	140	45	None	119.5	84.5	2070	464
7	57	F	154	54	None	112.2	78.2	2150	227

TABLE 2. Diagnosis and surgical procedures performed for each patient

Pt (No.)	Diagnosis	Procedure
1	NSCLC (adenocarcinoma, T2N0M0)	Left lower lobe lobectomy
2	Metastatic Lung Tumor (ovarian cancer)	Left upper lobe partial resection (VATS)
3	Metastatic Lung Tumor (MFH)	Left upper lobe partial resection (VATS)
4	NSCLC (giant cell carcinoma,T3N0M0)	Left upper lobe lobectomy
5	NSCLC (adenocarcinoma, T1N0M0)	Right upper lobe lobectomy
6	NSCLC (adenocarcinoma, T1N0M0)	Right middle lobe lobectomy (VATS)
7	NSCLC (adenocarcinoma, T2N2M0)	Right lower lobe lobectomy

NSCLC: non-small cell lung cancer MFH: malignant fibrous histiocytoma VATS: Video assisted thoracoscopic surgery

TABLE 3. Surgical Characteristics and the Need for Blood Transfusion

Pt (No.)	Duration of anesthesia (min)	Length of operation (min)	1-lung ventilation time (min)	Blood loss (ml)	Blood transfusion (ml)	Replacement fluid (ml)	Water balance (ml)
1	492	427	395	550	520	2550	+ 1970
2	270	187	150	160	0	1200	+ 690
3	178	99	60	120	0	1150	+ 750
4	437	370	235	380	0	2350	+ 1500
5	361	280	No	1720	520	3050	+ 1120
6	388	305	230	114	0	1813	+ 979
7	356	293	205	40	0	1750	+ 660

water balance = (replacement fluid + blood transfusion) – (loss of blood + urine output)

FIGURE LEGENDS

Figure 1: Time course and sampling points in this experimental protocol

ELF: epithelial lining fluid

Figure 2: Time courses of albumin and interleukin-8 (IL-8) concentrations in plasma.

Values are shown as the means ±SE. ＊p<0.05, vs point 1

Figure 3: Time courses of albumin and interleukin-8 (IL-8) concentrations in epithelial lining fluid (ELF).

Values are shown as the means±SE. ＊p<0.05, vs point 1

Figure 4: IL-8 concentrations in the ELF at point 3 and the duration of 1-lung ventilation.

n=6; r=0.90, P <0.05

Figure 1

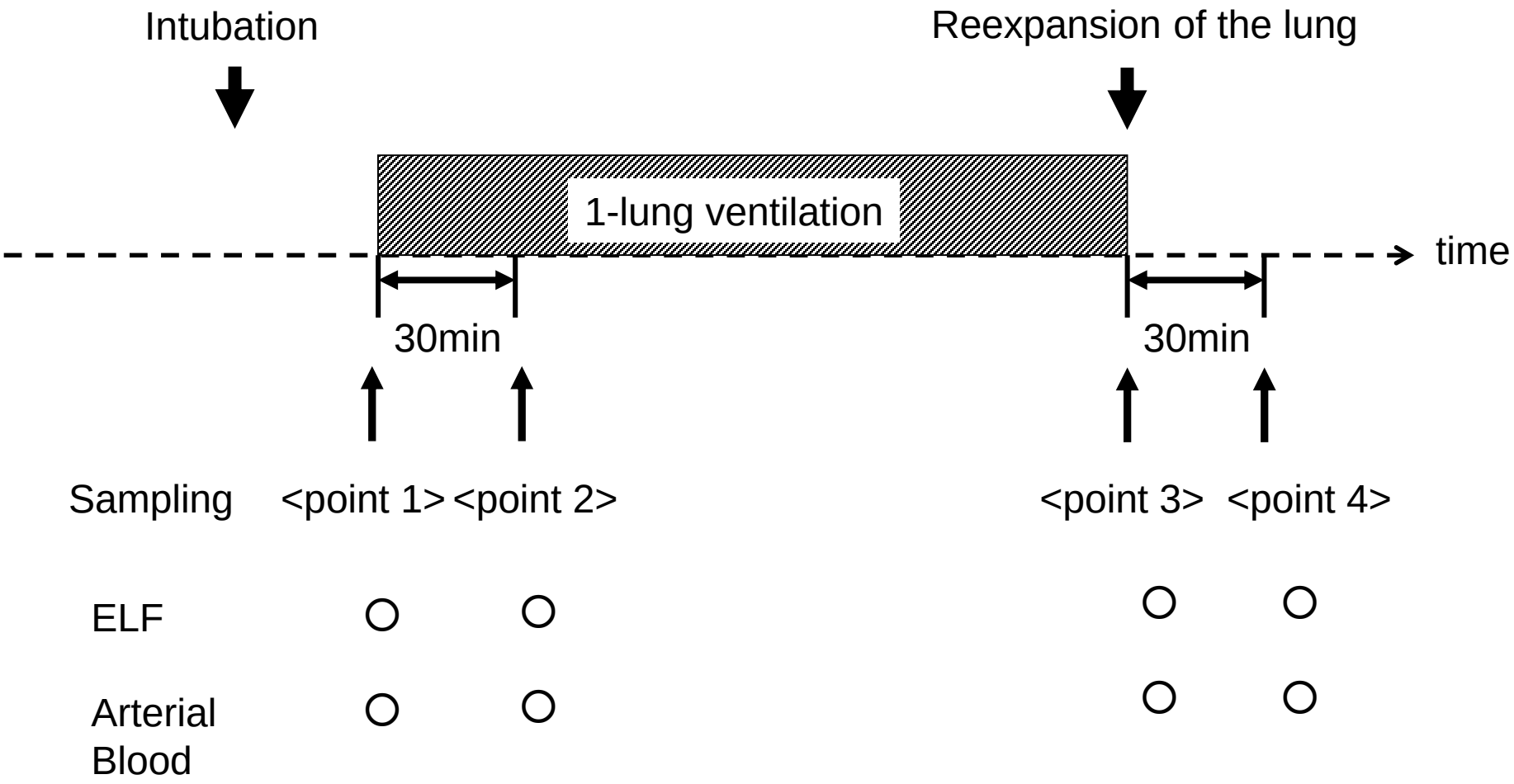

Intubation
Reexpansion of the lung
1-lung ventilation
time
30min
30min
Sampling
<point 1>
<point 2>
<point 3>
<point 4>
ELF
Arterial Blood

Figure 2

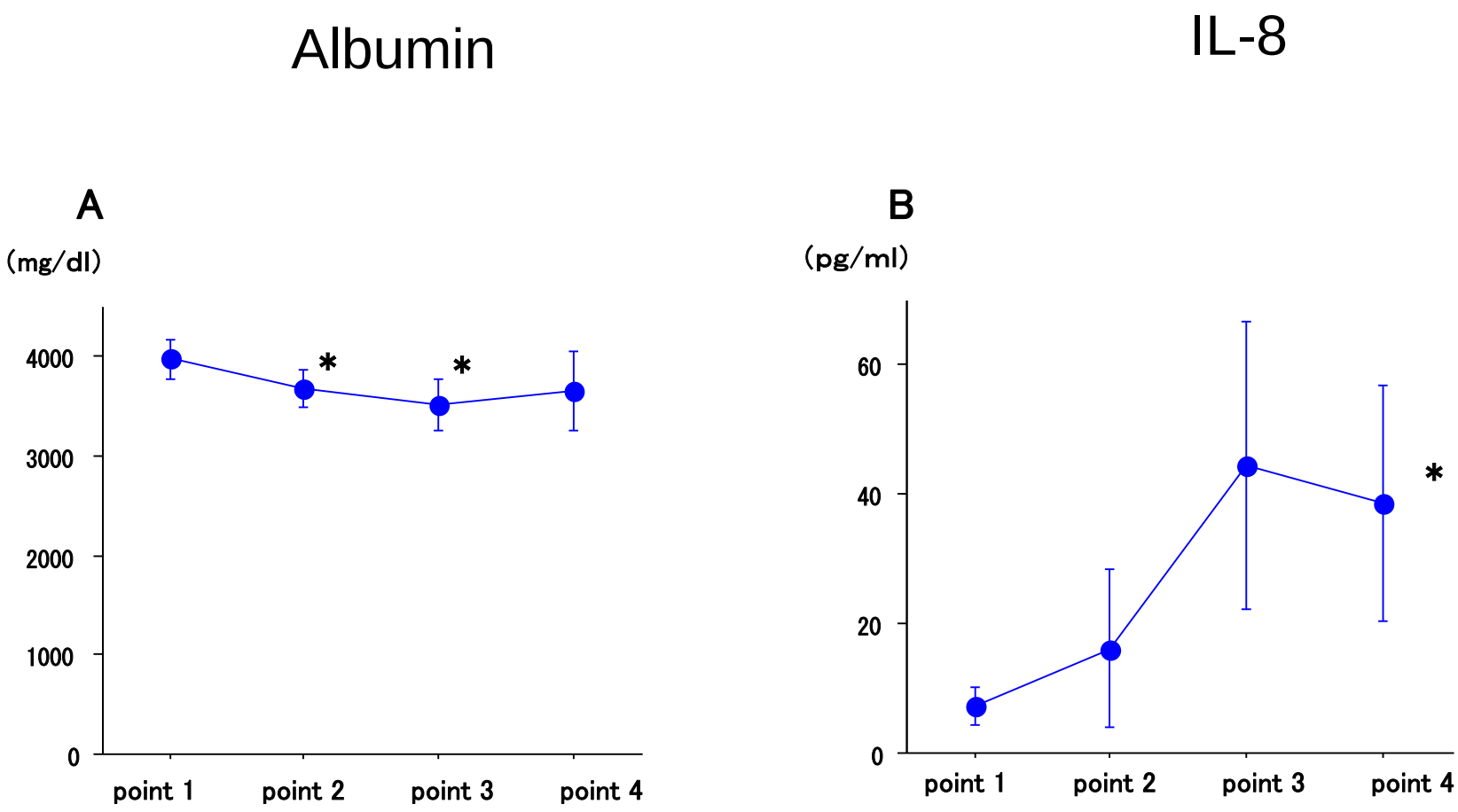
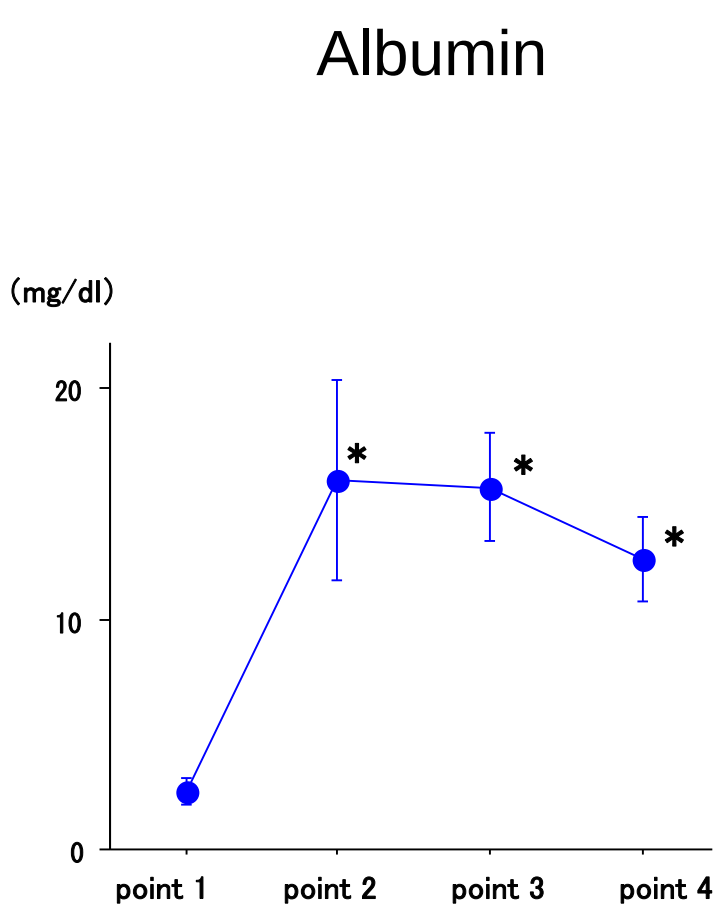

Albumin
A
(mg/dl)
4000
3000
2000
1000
0
*
*
point 1
point 2
point 3
point 4
IL-8
B
(pg/ml)
60
40
20
0
*
point 1
point 2
point 3
point 4

Figure 3


Albumin
(mg/dl)
20
10
0
*
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*
point 1
point 2
point 3
point 4

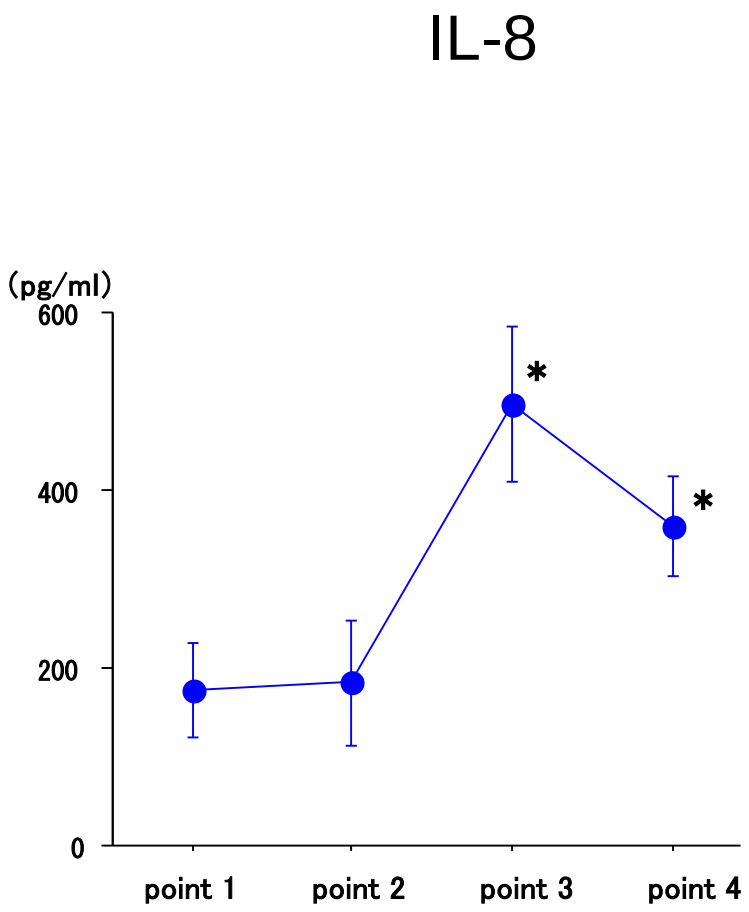
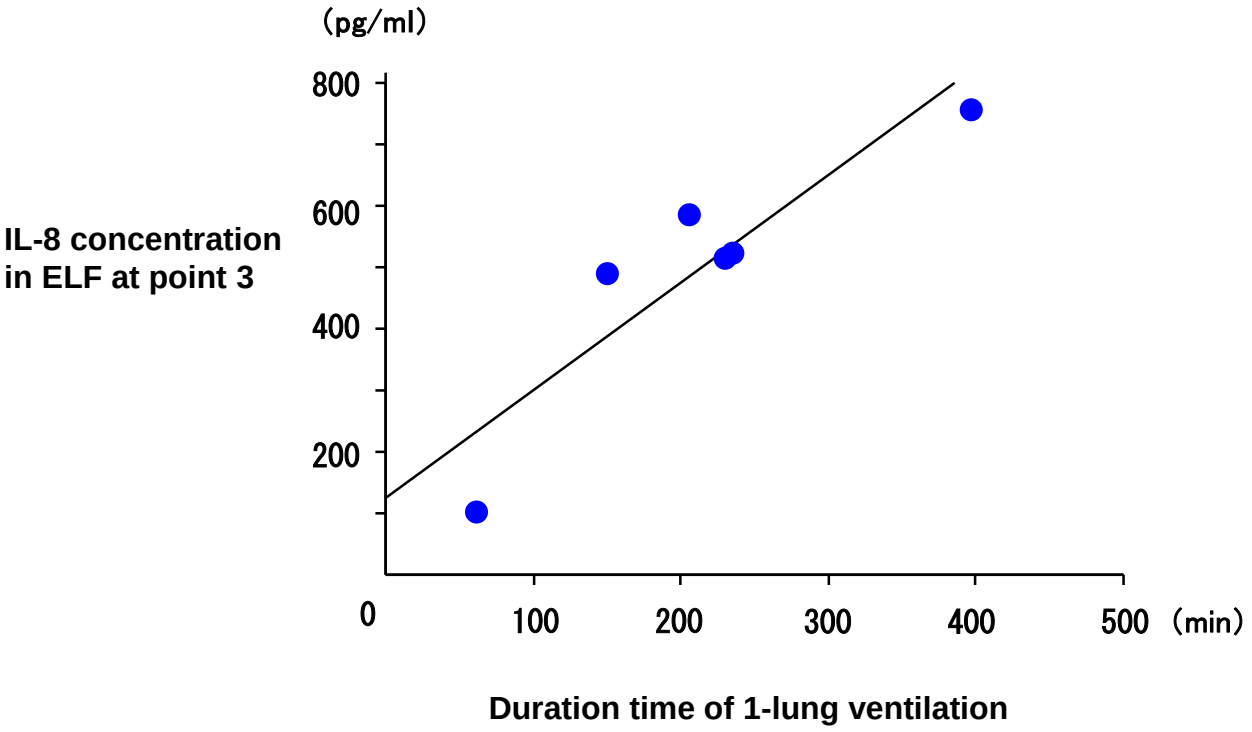

IL-8
(pg/ml)
600
400
200
0
*
*
point 1
point 2
point 3
point 4

Figure 4


(pg/ml)
800
600
400
200
0
100
200
300
400
500
(min)
IL-8 concentration in ELF at point 3
Duration time of 1-lung ventilation