Activation of a Neutrophil-Derived Inflammatory Response in the Airways During Cardiopulmonary Bypass

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Cardiopulmonary bypass (CPB) is believed to cause postoperative lung dysfunction. To more closely examine the inflammatory processes occurring in the airways during CPB, we serially measured inflammatory mediators, with the assistance of a new bronchoscopic microsample probe, in 11 patients undergoing repair of aortic arch aneurysms. Epithelial lining fluid (ELF) and arterial blood were sampled simultaneously after induction of anesthesia, at the time of pulmonary reperfusion, and at the end of surgery. A decrease in the Pao_2/Fio_2 ratio was observed at the end of surgery ($P = 0.029$). Although the ELF concentrations of interleukin (IL)-8, IL-6, and neutrophil elastase had increased significantly at the end of surgery (median = 23,200, 1818, and 12,900 μg/mL, respectively), they did not correlate with the degree of hypoxemia. Neutrophil elastase increased significantly at the time of pulmonary reperfusion, before IL-8 and IL-6, and independently of blood transfusions. At the end of surgery, IL-6 in ELF correlated with total blood transfusion volume ($\rho = 0.731$, $P = 0.011$). These results indicate that a neutrophil-derived inflammatory response is activated in the airway in the early phase of CPB.
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Postoperative respiratory failure, apparent as hypoxemia, a common complication of cardiac surgery performed under cardiopulmonary bypass (CPB) (1), correlates with major morbidity and mortality (2,3). A higher incidence of prolonged mechanical ventilation has been reported in patients undergoing repair of aortic arch aneurysms (4). Circulating humoral and cellular factors account primarily for the inflammatory processes, such as activation of neutrophils and complement system, associated with exposure to foreign material during and after CPB, and the subsequent pulmonary injury (5). However, other events, including ischemia and reperfusion, abundant allogenic transfusions, or bacterial and endotoxin translocation from the hypoperfused gut, contribute to triggering this process (6,7). In view of the pivotal role played by systemic proinflammatory cytokines, such as interleukin (IL)-6 and IL-8, (7,8) monitoring of their concentrations may help us to understand the clinical status of patients and design treatments.

Several studies have confirmed that resident lung cells are the source of inflammatory mediators involved in the pulmonary dysfunction observed after CPB (9,10). While monitoring of cytokines in bronchoalveolar lavage (BAL) fluid was useful for following the development of pulmonary dysfunction after CPB (11), this method is limited by its invasive nature. A less invasive method of pulmonary epithelial lining fluid (ELF) collection would obviously be preferred in high-risk surgical patients. We have developed a bronchoscopic microsample probe and described its use for the serial assessment of intrapulmonary events in critically ill patients (12) and for the evaluation of the therapeutic effects of pharmaceuticals (13).

The aim of the current study was to test the feasibility of the bronchoscopic microsample method in patients who underwent prolonged CPB for the repair of aortic arch aneurysms. Serial measurements of several mediators with this new method would help to monitor the CPB-activated inflammatory response in the airways and could facilitate future analysis of airway cytokines and post-CPB pulmonary dysfunction.

METHODS

The study protocol was approved by the IRB of Keio University Hospital, and informed consent was obtained from each patient. We prospectively studied 10 men and 1 woman who underwent elective aortic arch repair during the study period. Meperidine hydrochloride, 1 mg/kg, i.m., and atropine sulfate, 0.5 mg, i.m., were administered 1 h before anesthesia, which was induced and maintained by continuous infusions of fentanyl, midazolam, and vecuronium.

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Percutaneous arterial oxygen saturation, brachial arterial pressure, and continuous thermodilution cardiac output (Vigilance, Edwards Lifesciences, Irvine, CA) were continuously monitored. The lungs were ventilated with a 10-mL/kg tidal volume, and the respiratory frequency was adjusted to maintain a $Paco_2$ within the normal range. No positive end-expiratory pressure was applied during CPB to facilitate the surgical procedure. The CPB circuit included a centrifugal pump (HC-011S, Terumo, Tokyo, Japan) and a heparin-coated membrane oxygenator (Affinity NT oxygenator, Medtronic, Inc., MN) and heat exchanger. Ultrafiltration (Capiox Hemoconcentrator CX-HC11S, Terumo, Tokyo, Japan) was systematically performed. Anticoagulation was performed with porcine heparin in an initial dose of 300 U/kg, and neutralized with protamine. Additional heparin administration and the protamine dose needed for its neutralization were calculated by monitoring of the activated coagulation time. Blood was transfused to maintain a hemoglobin concentration >8 g/dL. Adrenergic agonists, including dopamine, dobutamine, and norepinephrine were used to support the systemic blood pressure and promote urinary output. The patients were sedated postoperatively with a continuous propofol infusion, and mechanically ventilated for at least 12 h in the intensive care unit. The hemodynamic fluid and ventilatory management were left to the discretion of attending physicians. No steroids, antiprotease, nonsteroidal anti-inflammatory drugs, aprotinin, aminocaproic acid, or tranexamic acid were administered during the study period. Chest radiographs were obtained immediately after the operation and daily thereafter until the patients left the intensive care unit, to monitor the development of acute lung injury, diagnosed according to the criteria of the American–European Consensus Conference (14).

Bronchoscopic sampling of ELF was performed after induction of anesthesia (baseline), at re-establishment of pulmonary circulation (reperfusion), and at the end of surgery. Patients' lungs were continuously ventilated throughout the procedure via a Bodai Suction Safe™ Swivel Y connector (Sontek Medical, Hingham, MA). The inspired O_2 concentration was set at 100% and other ventilator settings remained unchanged during the procedure. The design of the bronchoscopic microsample probe and the ELF sampling method has been described elsewhere (12). Briefly, a commercially available bronchoscopic microsample probe (OD, 2.4 mm) loaded with absorptive material (BC-401C, Olympus, Tokyo, Japan) was directed to the right S4 or S5 bronchus through the channel of the bronchoscope. The catheter was advanced to the subsegmental bronchus and placed in contact with the epithelial surface for 5 s. Three samples without blood contamination were obtained at 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.

Table 1. Patient Characteristics and P/F Ratio

Patient no.	Age (yr)/sex	Height (cm)/weight (kg)	DOV (days)	Pao_2/Fio_2		
				BL	PR	ES
1	71/M	164/81	16	537	350	301
2	80/F	156/71	6	352	521	326
3	66/M	162/66	1	364	556	228
4	72/M	165/81	2	242	293	224
5	71/M	163/69	1	360	479	301
6	67/M	158/64	1	434	528	427
7	73/M	172/63	1	569	640	648
8	68/M	164/89	2	425	243	252
9	70/M	170/69	1	308	467	368
10	65/M	165/70	1	343	780	373
11	65/M	165/69	1	288	688	464

DOV = duration of ventilation, BL = baseline, PR = pulmonary reperfusion, ES = end of surgery.

Arterial blood samples for blood gas analyses were collected at the time of ELF sampling. The remainder of the blood samples was centrifuged at 3000 rpm for 10 min at 4°C, and the supernatant was stored at −80°C until assayed.

The absorbed material collected with the bronchoscopic microsample probe and the plasma samples underwent enzyme linked immunosorbent assay for IL-8, IL-6, and neutrophil elastase. The human albumin concentration in the extract was also measured by a colorimetric method (Beckman, Fullerton, CA). The original concentrations of these mediators in ELF were calculated with the correction of wet-to-dry ratio of the absorptive material and used for analysis. Measurement was performed by the laboratory staff unaware of the clinical situation and profile of the patients. All results of chemical mediator assay and albumin concentration are means of two measurements.

The results were expressed as means and SD or medians, where appropriate. Changes in Pao_2 were tested by one-way repeated measures analysis of variance and by paired *t*-test with Bonferroni correction. Time-dependent changes were analyzed with Friedman analysis and Wilcoxon's ranked sum test since the data were skewed. The correlation of log-transformed mediator concentrations with Pao_2/Fio_2 ratio (P/F ratio) or total volume of blood transfusion was analyzed with Spearman correlation coefficient. A P value <0.05 was considered significant.

RESULTS

One of the 12 patients enrolled was excluded from the analysis because of postoperative bleeding and need for surgical re-exploration. Data from one patient who needed prolonged postoperative ventilatory support for late-onset ventilator-associated pneumonia were included in this analysis. The postoperative course of the other 10 patients was uneventful. The demographic characteristics, duration of ventilation, and P/F ratio in the 11 patients included in the study are shown in Table 1. No patient developed postoperative bilateral

Table 2. Operative and Blood Transfusion Characteristics

Patient no.	Anesthesia time (min)	Operation time (min)	Total CPB time (min)	Aortic cross clamp time (min)	Blood transfusions (mL)	
					Total volume	Before reperfusion
1	550	445	290	198	0	0
2	540	320	170	110	0	0
3	575	434	268	170	520	0
4	555	474	280	152	2470	0
5	570	480	257	171	3510	0
6	428	350	183	112	4680	0
7	515	435	281	146	0	0
8	550	462	262	159	1820	260
9	685	553	300	168	6760	0
10	715	542	269	177	6760	0
11	712	602	365	250	7410	1040

CPB = cardiopulmonary bypass.

infiltrates on roentgenographic examination. Significant changes in oxygenation were found by Friedman analysis ($P = 0.029$). Pao_2 decreased at the end of surgery compared with reperfusion ($P = 0.008$). However, nine patients were weaned from the ventilator on Day 1 or 2 after the operation, and were discharged from intensive care the next day. Table 2 summarizes the operative and blood transfusion characteristics. The mean duration of CPB was 266 ± 53 min. Allogenic blood transfusions were administered to eight patients, representing a mean total volume of 3085 ± 2925 mL. The majority of blood products were given at the time of weaning from CPB, though three patients were transfused before the onset of pulmonary reperfusion.

The ELF sampling procedure was completed within 10 min, and no adverse hemodynamic or respiratory event was observed. FIO_2 returned to previous levels immediately after the procedure in all patients. The concentrations of inflammatory mediators and albumin in ELF are shown in Figure 1. The changes in IL-8, IL-6, and neutrophil elastase in ELF were statistically significant ($P = 0.029$, 0.006, and 0.003, respectively). The median concentrations of IL-8 (23,200 pg/mL) and IL-6 in ELF (1,818 pg/mL) were significantly higher at the end of surgery than at baseline ($P = 0.008$ and 0.003, respectively). The median concentration of neutrophil elastase also increased significantly at the time of reperfusion (5000 μg/mL) and at the end of surgery (12,900 μg/mL) when compared with baseline ($P = 0.017$ and 0.004, respectively). The albumin concentration in ELF remained unchanged throughout the study period. The log-transformed measurements of IL-6, IL-8, and neutrophil elastase did not correlate with the P/F ratio (data not shown).

The plasma concentrations of IL-6 and IL-8 increased significantly after reperfusion and at the end of surgery when compared with baseline (Table 3). Although time-dependent increases in plasma concentrations in IL-6 and IL-8 were observed by Friedman analysis ($P = 0.001$ and 0.002, respectively), the difference between the concentrations at reperfusion versus at the end of surgery did not reach statistical significance.

No correlation was found between the concentrations of neutrophil elastase, IL-6, and IL-8 and P/F ratio. The log-transformed mean IL-6 concentration at the end of surgery correlated closely with total transfusion volume (Fig. 2, $\rho = 0.731$, $P = 0.011$).

DISCUSSION

This study is the first to show that neutrophil elastase increases at the time of pulmonary reperfusion after CPB, preceding the increase in IL-6 and IL-8. We also found significant increases in ELF IL-6, IL-8, and neutrophil elastase after CPB, an observation in agreement with previous studies. One report has highlighted the importance of the alveolar space as a source of inflammatory mediators, as several inflammatory mediators were increased in BAL fluid (9). Kotani et al. (10) have reported that the gene expression of IL-6, IL-8, and tumor necrosis factor-α were up to 8 times greater in alveolar macrophages than in plasma monocytes after CPB. In addition, the activation of mitogen-activated protein kinase in BAL after CPB, which leads to a variable inflammatory cytokine production in the air spaces, has been observed in an animal model (15). However, the time course of these responses during CPB has not been studied. Our observations suggest that neutrophil activation in the airway is the earliest stage of the inflammatory response induced by CPB. They further suggest that a neutrophil-derived inflammatory reaction plays a role in CPB-induced lung dysfunction, although the increase in concentrations of mediators did not correlate with the impairment of oxygenation in this study. The unchanged ELF albumin concentration (Fig. 1) and mild decrease in P/F ratio (Table 1) indicate that this severe reaction did not cause extensive endothelial or epithelial injury. Therefore, a concomitant anti-inflammatory reaction in the airway and in the systemic circulation might play a role in the mitigation of lung dysfunction (16). It

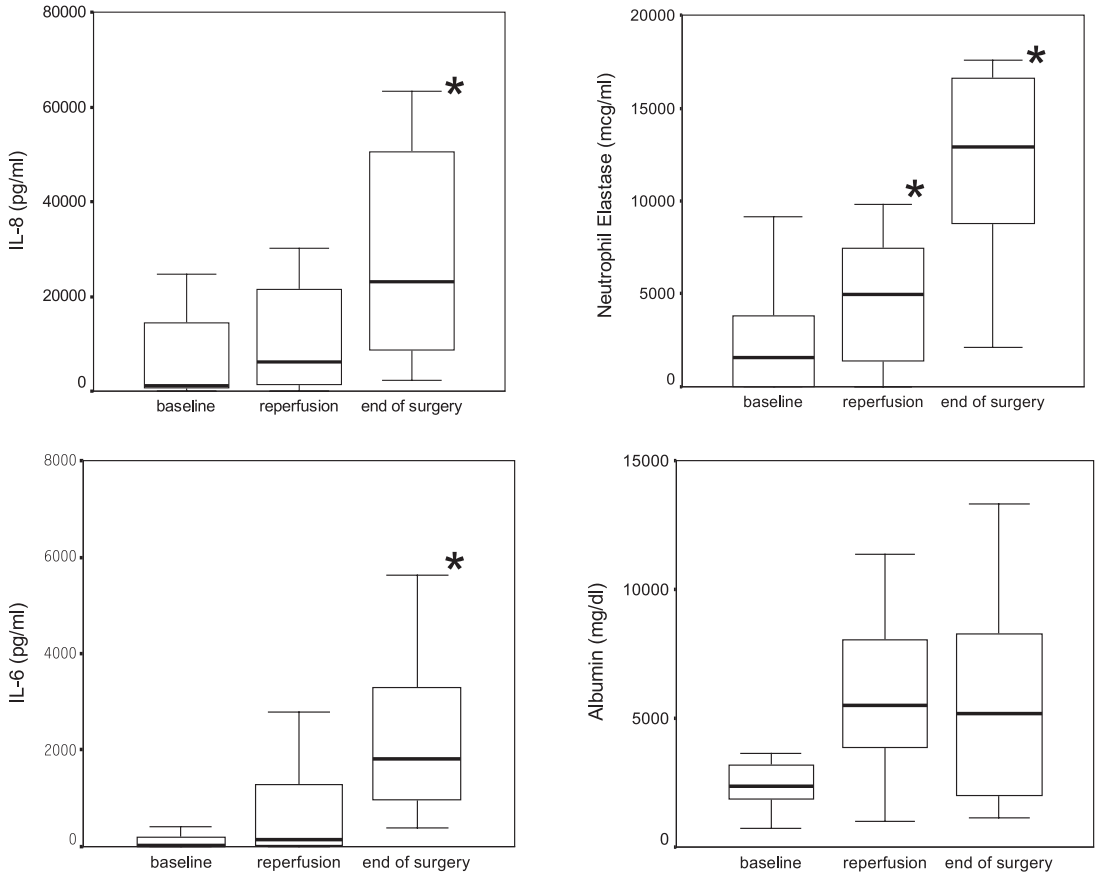

Figure 1. The concentrations of interleukin (IL)-8, IL-6, neutrophil elastase, and albumin in epithelial lining fluid (ELF) collected by the microsampling method are shown in the box graph. The data are presented as medians, 5, 25, 75, and 95%, and means (closed box). *$P < 0.05$ by Wilcoxon's ranked sum test versus baseline.

Table 3. Plasma Levels of Interleukin (IL)-6 and IL-8

	IL-6 (pg/mL)			IL-8 (pg/mL)		
Patient no.	Baseline	Reperfusion	End of surgery	Baseline	Reperfusion	End of surgery
1	1.2	446.0	92.9	0.0	36.7	18.4
2	2.4	159.0	223.0	0.0	21.1	44.4
3	1.5	1.2	73.9	0.0	0.0	326.0
4	2.3	5.1	77.1	0.0	0.0	14.2
5	0.9	177.0	71.9	0.0	26.7	27.1
6	0.8	192.0	71.0	0.0	32.4	0.0
7	2.2	66.9	224.0	0.0	29.8	53.1
8	3.1	20.9	151.0	0.0	36.1	17.8
9	2.0	6.9	202.0	0.0	29.4	44.6
10	0.6	39.3	92.5	0.0	21.4	36.3
11	1.6	143.0	52.4	0.0	23.4	0.0
Median	1.6	66.9*	92.5*	0.0	26.7*	27.1*

BL = baseline, PR = pulmonary reperfusion, ES = end of surgery.

* $P < 0.05$ when compared with baseline.

is noteworthy that, despite the absence of postoperative lung injury, IL-6 and neutrophil elastase were higher in ELF at the end of surgery than the peak concentrations we previously observed in seven patients with acute respiratory distress syndrome (median, 342.2 pg/mL and 9829.1 μg/L, respectively). (12) This may be an indication that lungs exposed to CPB become susceptible to even subtle stimuli, such as infection or mechanical over-distension, that can induce further cytokine upregulation.

Hauser et al. (17) reported that IL-6 was significantly increased in BAL at the time of removal of the aortic cross-clamp and remained increased for 24 h after pediatric open heart surgery. Although the sampling method and study population were different, our results showed a concordant time-dependent evolution. In the study of Hauser et al., a correlation was also observed between concentrations of serum IL-6 and postoperative mortality. While plasma IL-6 in the current study also increased in a time-dependent manner, the increase was considerably

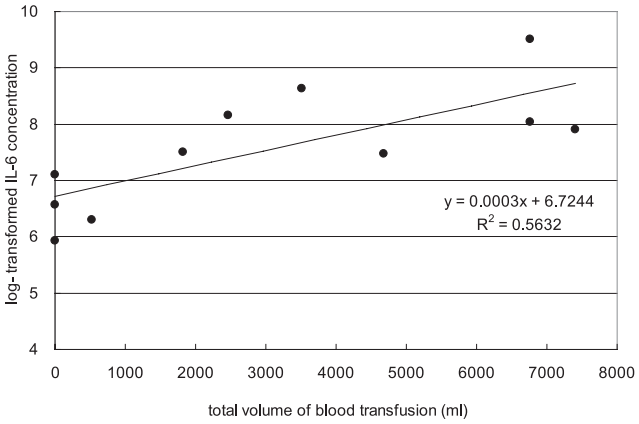

Figure 2. Correlation between log-transformed interleukin (IL)-6 concentration in epithelial lining fluid (ELF) and total blood transfusion volume.

less than that measured among the survivors in that study, probably due to differences in the patients' clinical conditions.

We also found that the increase in neutrophil elastase was independent of blood transfusion, since few blood products had been administered before pulmonary reperfusion (Table 2), whereas the increase in IL-6 in ELF at the end of surgery correlated with the volume of blood transfusion. Direct contact of the neutrophils with foreign materials or ischemia during CPB are mechanisms that are susceptible, alone or in combination, to activating neutrophils, and which warrant further investigation. Other mechanisms may also be responsible for activation of neutrophils during cardiac surgery.

While several studies emphasized the importance of lung epithelium as a source of inflammatory mediators (9,11), few clinical studies have detailed the evolution of these inflammatory changes, probably because of the lack of a safe procedure applicable to critically ill patients during CPB. While BAL has become a bedside procedure used to diagnose respiratory disorders, it causes temporary, though sometimes severe, O_2 desaturation (18). It is noteworthy that our bronchoscopic microsample technique often enabled us to collect ELF without the saline instillation that carries such risks as hypoxia or dissemination of infection. It also allowed us to understand the contribution of blood transfusions to the evolution of the inflammatory response in the airway, and its time course during and after CPB. This study will prompt further applications of bronchoscopic microsampling in the study of the pathophysiology of postoperative pulmonary dysfunction.

LIMITATIONS OF OUR STUDY

It might not be legitimate to compare measurements made in ELF obtained by the bronchoscopic microsample technique to that obtained by BAL, since the former is not diluted by saline. However, we (12) have previously demonstrated that the concentrations of biochemical substances obtained by bronchoscopic microsample showed a similar trend as those obtained by BAL in healthy subjects, and that it is a reliable method to grade the severity of lung injury in patients with acute respiratory distress syndrome (19), and in the diagnosis of lung cancer (20). Our study had a weak statistical power because of the small number of patients, perhaps explaining the discordant absence of correlation between ELF mediators and decreased arterial oxygenation after cardiac surgery (11). To exclude biases introduced by the surgical procedure and techniques, we limited our patient population to those operations that were elective and performed by a single surgeon. This design did not allow the inclusion of a control group, and our study was observational. However, the baseline values measured in each patient were used as intra-individual controls, allowing an assessment of the time-dependent changes as a means of mitigating this limitation. Mechanical ventilation could affect inflammatory mediator production, especially in critically ill patients. A previous report showed that ventilation with 15 mL/kg of tidal volume on zero positive end-expiratory pressure did not result in higher cytokine levels compared with 6 mL/kg in essentially normal patients' lungs (21). Thus, the ventilation applied in this study had little effect on cytokine production in the airway. Finally, fewer emigrated neutrophils might change the results, although cells were not counted since the bronchoscopic microsample method does not provide enough cells in the airspace.

In conclusion, our study indicates that neutrophil elastase, IL-8 and IL-6, in the airway increases during CPB. Neutrophil elastase increased before IL-8 and IL-6, and independently of blood transfusions, suggesting that CPB triggered a neutrophil-related inflammatory process in the airway. The bronchoscopic microsample method was safely used in multiple samplings and chemical analyses of pulmonary epithelial fluid, including intra-operatively in cardiac patients.

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