Institutional report - Cardiopulmonary bypass

Effect of a neutrophil elastase inhibitor on acute lung injury after cardiopulmonary bypass

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Abstract

Cardiopulmonary bypass (CPB) has been implicated as a cause of acute lung injury (ALI) in cardiac surgical patients. We used a bronchoscopic microsampling (BMS) probe to examine alveolar biochemical constituents and evaluated the effect of sivelestat sodium hydrate, a novel synthesized polymorphonuclear (PMN) neutrophil elastase inhibitor, on ALI induced by CPB. Twelve patients undergoing aortic valve replacement were treated with either sivelestat 0.2 mg/kg/h (sivelestat group, n=6) or 0.9% saline (control group, n=6) from the start of surgery. Samples were collected by the BMS probe at three time points: after tracheal intubation, 1 h after CPB introduction, and 3 h after CPB termination. Pulmonary function was assessed perioperatively. There were no differences in baseline characteristics. The concentration of PMN elastase was significantly suppressed in the sivelestat group, compared with the control group (P=0.001). The sivelestat group also had lower levels of interleukin-6 and interleukin-8. Alveolar–arterial oxygen difference markedly increased, and a worsening of the PaO2/FIO2 ratio indicated severe impairment after CPB. However, sivelestat attenuated the pattern of physiological deterioration of gas exchange. Sivelestat may attenuate neutrophil elastase or proinflammatory cytokines, and improve pulmonary dysfunction in patients undergoing CPB.

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Keywords: Cardiopulmonary bypass; Inflammatory mediators; Neutrophil elastase; Sivelestat; Bronchoscopic microsampling

1. Introduction

Cardiopulmonary bypass (CPB) induces a systemic inflammatory response called post-pump syndrome. It causes activation of the complement, neutrophil, coagulation, fibrinolytic and kallikrein cascades, along with the synthesis of proinflammatory cytokines [1, 2]. Although several organs can be involved, the lung is often the first organ damaged, making this a major complication of CPB [3]. It has been reported that activated neutrophils, which accumulate in the lung and release chemical mediators, such as polymorphonuclear (PMN) neutrophil elastase, play an important role in post-pump syndrome. PMN elastase is an extremely harmful enzyme and appears to mediate neutrophil extravasation, tissue infiltration, and endothelial cell injury.

Sivelestat sodium hydrate [sivelestat; ONO-5046-Na (C20H21NNaO5S-Na-4H2O, molecular weight, 528.51); Elaspol®; Ono Pharmaceutical Co, Osaka, Japan] – sodium N-(2-[4-(2,2-dimethylpropiolonyloxy) phenylsulfonylamino] benzoyl) aminoacetate tetrahydrate – is a novel, specific inhibitor of PMN elastase [4]. The beneficial effects of sivelestat on postperfusion lung, ischemia-reperfusion and endothelial cell injuries have been demonstrated in several experiments [5, 6].

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Assessment of the pulmonary biochemical environment by bronchoalveolar lavage (BAL) can provide valuable pathophysiologic information [7]. Ishizaka and co-workers developed a bronchoscopic microsampling (BMS) probe to measure the concentration of alveolar biochemical substances in the target bronchus and peripheral regions. They demonstrated the probe’s ability to monitor pulmonary biochemical events during acute respiratory distress syndrome (ARDS) [8]. Therefore, we used a BMS probe in our clinical study to evaluate the efficacy of sivelestat against lung injury caused by CPB.

2. Patients and methods

Patients were enrolled into the study at the Nippon Medical School Hospital (Tokyo, Japan), following protocol approval by our Institutional Review Board and informed consent. Patients were included if they were at least 20 years of age and scheduled to undergo elective aortic valve replacement surgery. Exclusion criteria included the presence of cardiogenic pulmonary edema, emergency or urgent cases, pre-existing asthma or chronic obstructive pulmonary disease, and major chest wall abnormalities.

Patients were randomized into two groups using a computer-generated randomization table. The control group received 0.9% saline (n=6), and the sivelestat group received a continuous infusion of sivelestat after tracheal intubation. Sivelestat (500 mg) was dissolved in 50 ml of...
5% glucose solution and administered intravenously at a rate of 0.2 mg/kg/h for 24 h. The drug administration was blinded to the surgical and anesthesiologic teams. Neither steroids nor other anti-inflammatory drugs were used in this study.

2.1. Operative technique and management

All patients received midazolam (0.1 mg/kg), vecuronium bromide (0.1 mg/kg), and fentanyl (2 μg/kg) intravenously. Anesthesia was maintained with inhalation of 1.0–2.0% sevoflurane, and intermittent intravenous administration of fentanyl (2 μg/kg). Radial and central venous catheters were introduced. After tracheal intubation, the lungs were ventilated with intermittent positive pressure ventilation. Tidal volume, respiratory frequency, and the fraction of inspired oxygen (FiO₂) were adjusted to maintain an arterial PaCO₂ of 35–40 mmHg and an arterial oxygen saturation of more than 95%. Sternotomy was performed in all patients. The extracorporeal circuit consisted of a roller pump, a capillary membrane oxygenator, a hard-shell cardiotomy reservoir, and an arterial filter (MERA Excellung HP, Senko Medical Instrument Co, Ltd, Tokyo, Japan). The circuit was primed with electrolyte solution (750 ml), mannitol (200 ml), albumin (50 ml), sodium bicarbonate (40 mEq), and cefazolin sodium (6 g). Neither aprotinin nor antifibrinolytic agents were used. Heparin was initiated at a dose of 2 mg/kg and adjusted accordingly to achieve a target activated clotting time of 400 s or more. CPB was established and conducted at normothermia (35–36 °C). Mechanical ventilation was stopped after cardiopulmonary arrest and continuous positive airway pressure (3–4 cmH₂O) was performed during CPB.

After weaning from CPB, patients underwent 1–2 min of manual ventilation to restore mechanical ventilation. FiO₂ was 0.5, inspiratory/expiratory ratio was 1:2, and the respiratory rate was 12–15 breaths/min. After chest closure, patients were transferred to the intensive care unit and ventilated. Tidal volume was applied at 8 ml/kg of body weight and continuous positive airway pressure ranged from 4 to 5 cmH₂O.

Alveolar biochemical constituents were taken after tracheal intubation (Pre CPB), at 1 h after CPB initiation (during CPB), and at 3 h after CPB termination (post CPB). The ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen (PaO₂/FiO₂ ratio) was assessed as oxygenation perioperatively.

2.2. Bronchoscopic microsampling procedure

The BMS probe (Olympus Co, Tokyo, Japan) and sampling procedure have been described in detail by Ishizaka et al. [8]. In brief, a fiberoptic bronchoscope (Olympus) was inserted into the trachea and, after flushing with air to minimize sample contamination, advanced to a right main bronchus. The BMS probe was maintained in position for 10 s to absorb the epithelial lining fluid. The probe was cut at a 3-cm distal position from its tip and placed in a previously weighed container and frozen at −80 °C until use. A diluted solution containing the frozen probe by adding 2 ml of saline was centrifuged for 10 min at 1500 rpm, and the supernatant collected. All determinations of cytokines in the alveolar biochemical constituents were carried out by a technician blinded to treatment allocation. Interleukin (IL)-6 (Human IL-6 chimiluminescent enzyme immunoassay (CLEIA); Fujirebio, Tokyo, Japan) was measured by CLEIA. In addition, PMN elastase (PMN Elastase kit; Merck immunoassay, Darmstadt, Germany) and IL-8 (BIOSOURE IL-8 EASIA kit; BioSource Europe S.A., Nivelles, Belgium) were measured using enzyme-linked immunoabsorbent assays. Concentrations of the alveolar biochemical constituents obtained by the BMS probe were expressed by unit volume of epithelial lining fluid after correction for the dilution factor.

All data were expressed as the mean ± standard deviation (S.D.). The changes in the levels of PMN elastase, cytokines and PaO₂/FiO₂ ratio were performed by a log-transformation and comparison of two groups was performed by a repeated measures ANOVA within factor time of measurement. A Student’s unpaired t-test was used to compare other continuous variables between the two groups.

3. Results

Baseline anthropometric characteristics and clinical parameters of the two groups were similar and are shown in Table 1. There were no deaths or major complications, and no patient had to undergo re-operation or be re-admitted after intensive care unit discharge. There were no significant differences in the time-to-extubation and clinical outcomes between the two groups.

In the control group, the levels of PMN elastase in the alveolar biochemical constituents showed a dramatic increase after CPB initiation, and were maintained at high levels 3 h after CPB termination (Fig. 1). In contrast, the sivelestat group had lower concentrations of PMN elastase during and after CPB. The differences in PMN elastase levels between the sivelestat and control groups were found to be significant (P = 0.001).

The levels of IL-6 in the alveolar biochemical constituents were also increased during CPB in the control group, followed by a decrease 3 h after CPB termination (Fig. 2). However, the sivelestat group suppressed the elevation of IL-6 levels during CPB, followed by a slight increase after CPB. These changes were significant (P = 0.042) between the sivelestat and control groups.

Baseline IL-8 levels in the alveolar biochemical constituents were found to be increased in both treatment groups.

<table>
<thead>
<tr>
<th>Table 1 Clinical and operative data</th>
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<tr>
<td>Control</td>
</tr>
<tr>
<td>Age (years)</td>
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<tr>
<td>Gender (male/female)</td>
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<tr>
<td>Left ventricular ejection fraction (%)</td>
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<tr>
<td>Pulmonary function</td>
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<td>%VC</td>
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<td>FEV₁%</td>
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<td>Operation time (min)</td>
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<tr>
<td>CPB time (min)</td>
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<td>Intraoperative fluid balance (ml)</td>
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Data are presented as means ± standard deviation. VC, vital capacity; FEV₁, forced expiratory volume; CPB, cardiopulmonary bypass.
(Fig. 3). After CPB initiation, these levels increased in the control group, and remained elevated from baseline at 3 h after CPB. The sivelestat group maintained similar IL-8 levels throughout the study. The changes in IL-8 levels were statistically significant between the two groups ($P=0.022$).

The reduction in $\text{PaO}_2/\text{FiO}_2$ ratio after CPB was strongly related to the increase in IL-8, IL-6 or the elevation in PMN elastase. There was a statistically significant negative correlation between $\text{PaO}_2/\text{FiO}_2$ ratio and the levels of IL-8 ($R=0.78$, $P=0.004$), IL-6 ($R=0.71$, $P=0.013$) or concentration of PMN elastase ($R=0.71$, $P=0.02$) in these patients. $\text{PaO}_2/\text{FiO}_2$ ratio decreased in both groups after CPB (Fig. 4). However, sivelestat reduced this deterioration during the observation period ($P=0.054$).

4. Discussion

The BMS probe is less invasive than the standard BAL method in patients undergoing cardiac surgery, and the procedure presented in this study can be repeated without saline administration into the lungs. The BMS probe was developed by Ishizaka et al. [8] to collect and measure the alveolar biochemical constituents. They were able to detect increased concentrations of IL-6 and neutrophil elastase in diffuse pulmonary diseases, such as ARDS and acute lung injury (ALI). Changes in cytokine concentrations reflected the severity of disease. In the present study, we were able to safely measure cytokine and PMN elastase...
levels using the BMS probe, thus evaluating the efficacy of sivelestat.

Although the incidence of severe morbidity and mortality induced by CPB has markedly decreased with recent improvements in CPB procedures [9, 10], an inflammatory response during CPB, such as the activation of complement, neutrophils and cytokines induced by a blood–foreign body surface reaction, cannot be avoided [1, 2]. Neutrophil activation increases the affinity of adhesives to the endothelium through the expression of adhesion molecules. These activated neutrophils accumulate in the tissue, producing superoxide radicals and chemical mediators, such as PMN elastase. Sivelestat is a novel, specific neutrophil elastase inhibitor with a low-molecular weight. It has the advantage of achieving close contact with neutrophils and substrates without rapid inactivation by superoxide [4]. It has been reported that sivelestat administration reduced plasma neutrophil elastase levels by competitively inhibiting the activity of secreted neutrophil elastase without direct inhibition on its release [4]. Furthermore, Nakatani et al. [6] suggested that sivelestat acted directly on neutrophils, thus suppressing the production and secretion of activated elastase, as well as inactivating extracellular neutrophil-secreted elastase. In experimental studies, the plasma levels of neutrophil elastase and cytokines (IL-6 or IL-8) initiated by extracorporeal circulation were significantly reduced by a high dose of sivelestat (15 mg/kg/h or 100 µmol/l) [4, 11].

Previous clinical studies have shown the activation of complement and increases in cytokine and neutrophil elastase levels in the plasma [12] or BAL fluid [13] during CPB. In the present study, the substantially increased IL-6, IL-8, and PMN elastase levels after CPB induction in the control group were consistent with previous studies. In contrast, the concentration of PMN elastase was suppressed and elevations of IL-6 and IL-8 levels were attenuated by continuous sivelestat infusion before the start of CPB. Aggravated oxygenation after CPB was also improved in the sivelestat group. Our results indicate that the effect of sivelestat leads to the amelioration of pulmonary damages caused by CPB. Previously, sivelestat has been shown to markedly inhibit the increases in pulmonary extravascular water volume, interstitial and intra-alveolar edema, and plasma exudation in alveoli [5]. Kotani et al. [13] recently showed that the pulmonary concentrations of IL-8 after disconnection from CPB were significantly correlated to the changes in arterial oxygenation at the end of surgical intervention. We also demonstrated that the reduction in PaO2/FiO2 ratio was strongly related to the increase in IL-8, IL-6 or the elevation in PMN elastase.

The present study provides evidence for an anti-inflammatory effect of sivelestat in vivo but not for prevention of post-pump ALI because the postoperative course was similar between the two study groups. This may be due to a lack of power to detect such differences in outcomes or to the fact that the occurrence of ALI is multifactorial. It should be noted that the number of patients involved in this study was small and the preoperative patient risks were relatively low. This study would provide information concerning these changes and enable another larger trial including higher risk patients to be planned with appropriate sample size analysis being conducted based on the findings observed in this study.

In conclusion, the continuous infusion of sivelestat before the start of CPB suppressed the production of PMN elastase and attenuated IL-6 and IL-8 elevations in the pulmonary biochemical substances, resulting in improved oxygenation. Sivelestat may appear to have anti-inflammatory effects in patients undergoing CPB.

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