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THE RELATIONS OF LUNG INJURY AFTER CARDIOPULMONARY BYPASS AND INFLAMMATORY REACTION, ESPECIALLY NEUTROPHIL ELASTASE

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Abstract We have proved that Sivelestat preserved lung function after cardiopulmonary bypass (CPB) in the rabbit model. We now report the therapeutic efficacy of Sivelestat in the clinical case. From July 2005, 16 patients who underwent aortic arch replacements were enrolled in this study. Diagnosis included dissected aortic aneurysm in 6 patients, true aortic arch aneurysm in 8, and traumatic aortic arch aneurysm in 1 patient. We randomly divided these patients into two groups. In the Pre-Group (Pre:n=8), infusion of Sivelestat (0.2mg/kg/hr) was started before the operation; in the Post-Group (Post:n=8), it was started after the operation. Serum elastase activity, interleukin-8 (IL-8) levels were measured before the operation, before cessation of CPB and at the end of operation. Blood gas analyses were measured before the operation, at one and three hours after the CPB and the next morning. Because the preoperative P/F ratio (arterial PO_2/FiO_2) varies with each case, the value of the P/F ratio at one hour after the CPB was calculated for 100%. Elastase activity of both groups were increased at the end of CPB (Pre:8.9±10.6, Post:4.6± 3.5), then returned to baseline level at end of operation (NS). IL-8 of both groups were increased at end of CPB, then in the Pre-Group decreased to 59.3± 25.0 pg/ml, but in the Post-Group increased to 97.9±45.7 pg/ml (p=0.09). The P/F ratio in the Pre-Group was well maintained from post CPB to next morning, but in the Post-Group was decreased three hours after the CPB ($p<0.05$). In conclusion, these findings showed that Sivelestat reduced the inflammatory reaction associated with cardiopulmonary bypass, and prevented the pulmonary dysfunction caused by inflammatory reaction.

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Key words: SIRS; cardiopulmonary bypass; cardiac surgery; neutrophil elastase

Introduction

In cardiac surgery, surgical stress and cardiopulmonary bypass (CPB) cause systemic inflammatory response syndrome (SIRS) that affects several organs[1]. SIRS causes various kinds of complications and has an influence on operative results. One of the most important mechanisms in the initial phase of SIRS is priming, activation and sequestration of polymorphonuclear neutrophils (PMNs). Then PMNs will adhere to activated endothelium and release various cytotoxic contents. Neutrophil elastase is one of these cytotoxic contents, and an extremely cytotoxic protease, which degrades not only connective tissue components but also fibrinogen, coagulation factors and complements[2].

We established a new endotoxin (LPS)-induced acute lung injury (ALI) model after CPB in the rabbit[3]. LPS functions as a substitute of surgical stress, and combination of low dose LPS and CPB caused ALI in rabbits. On the other hand, only low dose LPS or CPB did not cause ALI. Sivelestat (sodiumN- [2-[4- (2,2-dimethyl- propionyloxy) phenylsulfonylamino] benzoyl] aminoacetate tetrahydrate) is a specific inhibitor of neutrophil elastase with a small molecular weight. In this model, the continuous administration of Sivelestat inhibited PMN aggregation in proximal vessels and PMN

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accumulation, and it attenuated the respiratory and hemodynamic devastation.

The purpose of this study was to determine whether or not pretreatment with a neutrophil elastase inhibitor (Sivelestat) can prevent the acute lung injury by bypass in clinical cases.

Methods

From July 2005 to July 2006, 16 patients who underwent aortic arch replacements or hemiarch replacements were enrolled in this study. Diagnoses were dissected aortic aneurysm in 6 patients, true aortic arch aneurysm in 8 and traumatic aortic arch aneurysm in 1 patient. We randomly divided these patients into two groups (Pre-Group and Post-Group). Diagnoses in the Pre-group included 3 cases of dissected aortic aneurysm type A and 5 true aortic arch aneurysms; diagnoses in the Post-group included 4 dissected aortic aneurysm type A, three true aortic arch aneurysms and 1 traumatic aortic rupture. Three hemiaortic arch replacements and 5 total aortic arch replacements were performed in the Pre-group, 8 total aortic arch replacements were performed in the Post-group. Operation time, CPB time and aortic clamp time in the Post-group were relatively longer than the Pre-group, but there were no significant differences between the groups. The age at operation in the pre-group was older than the post-group ($P<0.05$). The clinical and operative profiles of the patients are summarized in Table 1.

In the Pre-Group (Pre: n=8), infusion of Sivelestat (0.2 mg/kg/hr) was started at induction of anesthesia, and in the Post-Group (Post: n=8), infusion of Sivelestat (0.2 mg/kg/hr) was started after the operation. Infusion of drug was discontinued when the P/F ratio (arterial PO_2/FiO_2) recovered to over 200 or respiratory function became stable. Sivelestat was obtained from Ono Pharmaceutical Co., Ltd. (Osaka, Japan).

Serum elastase activity and interleukin-8 (IL-8) levels were measured before operation, at cessation of CPB and at the end of operation. Blood gas analyses were measured before the operation, at one and three hours after the CPB and the next morning. Because the preoperative P/F ratio (arterial PO_2/FiO_2) varies with each

Table 1. Patient's Characteristics and Perioperative data.

	Pre group	Post group	
Gender (Female: Male)	2 : 6	1 : 7	
Age	74 ± 4.5	62 ± 16.2	P<0.05
Diagnosis	DAA (typeA) : 3	DAA (typeA) : 4	
	TAA : 5	TAA : 3	
		TAA (traumatic rupture) : 1	
Operation	Hemiarch replacement : 3	Total arch replacement : 8	
	Total arch replacement : 5		
Operation time (minutes)	506 ± 127	711 ± 333	
CPB time (minutes)	254 ± 42	345 ± 96	
Aortic clamp (minutes)	163 ± 45	217 ± 80	
Rectum temp (℃)	25 ± 1.5	24 ± 2.0	
Duration of Sivelestat (hours)	116 ± 136	196 ± 129	
Tracheostomy	1	1	
Complication	0	Mediastinitis:1	
Mortality		1 (hospital death)	

DAA: dissected aortic aneurysm, TAA: True thoracic aortic aneurysm, CPB: cardiopulmonary bypass

case, the value of the P/F ratio at one hour after the CPB was calculated for 100%. ICU stays, hospital stay and duration of intubation were compared between the Pre-group and Post-group.

All values are reported as mean±STD. Two-way ANOVAs between the pre-group and post-group were used to analyze group and time effects and establish significant differences (Stat View 4.0 for Macintosh). The unpaired t statistic was used for comparing the ICU stay, hospital stay and duration of intubation between the groups. Differences were considered significant at a probability level of p less than 0.05.

Results

Hospital mortality occurred in one patient in the Post-group. One patient died as result of MRSA mediastinitis 4 months after surgery. Two patients (one patient in each group) had a tracheostomy at 33 days and 20 days respectively after operation because of respiratory dysfunction and cerebellar infarction.

P/F ratio of the Pre-group was well maintained until postoperative day 2 over the 100% of the base line. But in the post-group, P/F ratio decreased to 79% of the base line at three hours after the CPB and increased to 112% of the base line on postoperative day one (P<0.05) (Figure 1).

The plasma IL-8 level increased in both groups at cessation of CPB (69.1±38.4 vs 71.0±37.0 μ/ml) and progressively increased in the Post-group at end of operation (97.9±45.7 μ/ml). By contrast the IL-8 level in the Pre-group decreased at end of operation (59.3±25.0 μ/ml) (Figure 2). However there were no significant differences between groups.

Plasma elastase activity in the both groups gradually increased at cessation of CPB and decreased at the end of operation (Figure 3), and here as well there were no significant differences between groups.

The ICU stay and duration of intubation were

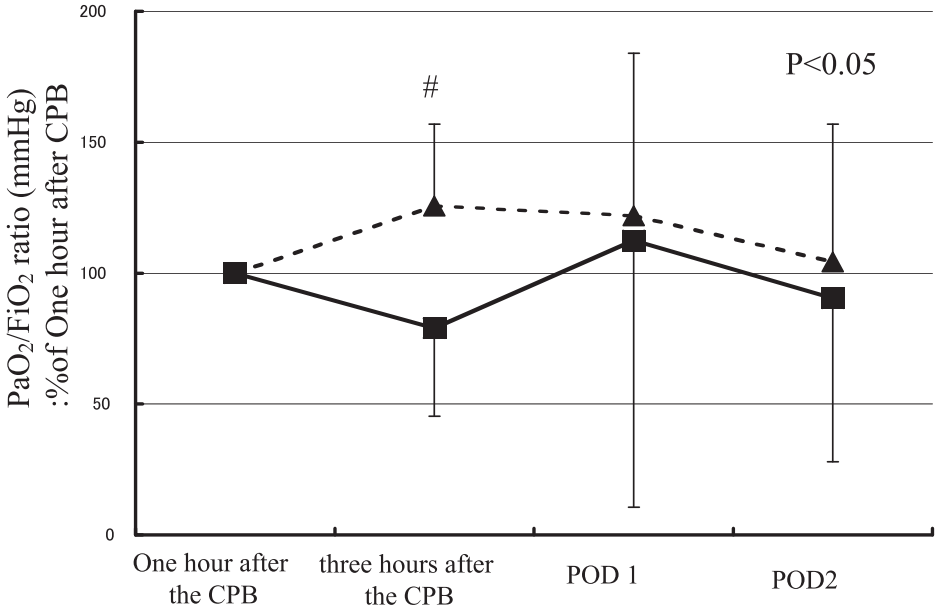

Figure 1 PaO_2 (arterial)/FiO_2 ratio: P/F (mmHg) (% of one hour after the CPB) Arterial PO_2 was measured before operation, and at one and three hours after the CPB and next morning, and then P/F ratio was calculated using arterial PO_2 and FiO_2. A value of the P/F ratio at one hour after the CPB was calculated for 100%. Filled triangles indicate Pre-Group, filled squares indicate Post-Group. Values are the mean±standard deviation (SD).

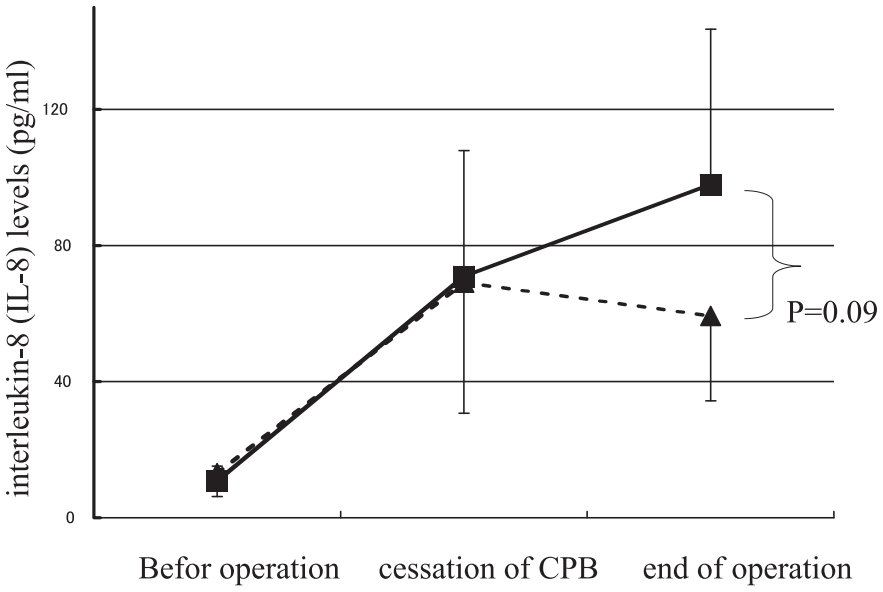

Figure 2 Change in plasma IL-8 level. Symbols as in Figure 1.

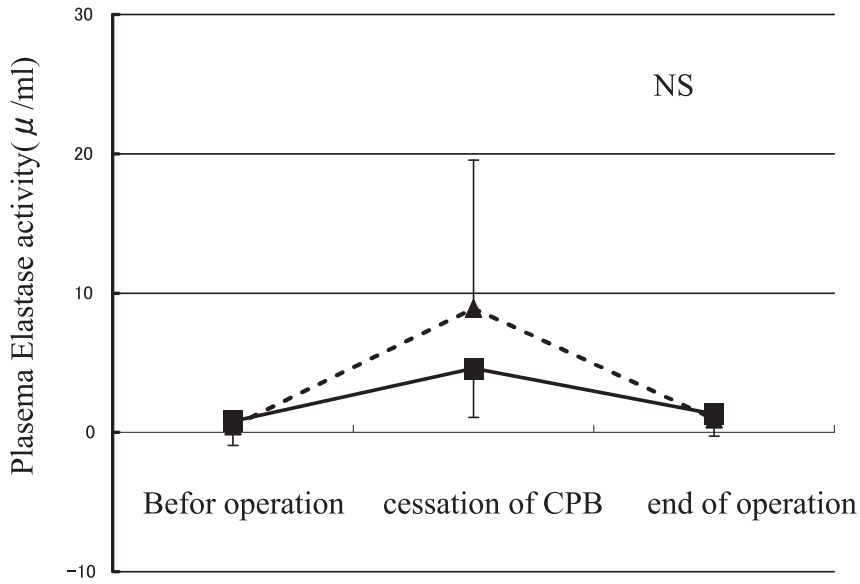

Figure 3 Change in plasma elastase activity. Symbols as in Figure 1.

201±291 hours, and 72±106 hours respectively in the Pre-group and 302±244 hours, and 152±134 hours in the Post-group. And hospital stay was 48±42days in the pre-group and 59±38 days in the post-group (Figure 4:a b c). ICU stay, hospital stay and duration of intubation were relatively shorter in the pre-group than in the post group, but there were no significant differences between the groups.

Discussion

Systemic inflammatory response syndrome (SIRS) is caused by operative stress, and that causes various kinds of complications[4]. Relatively

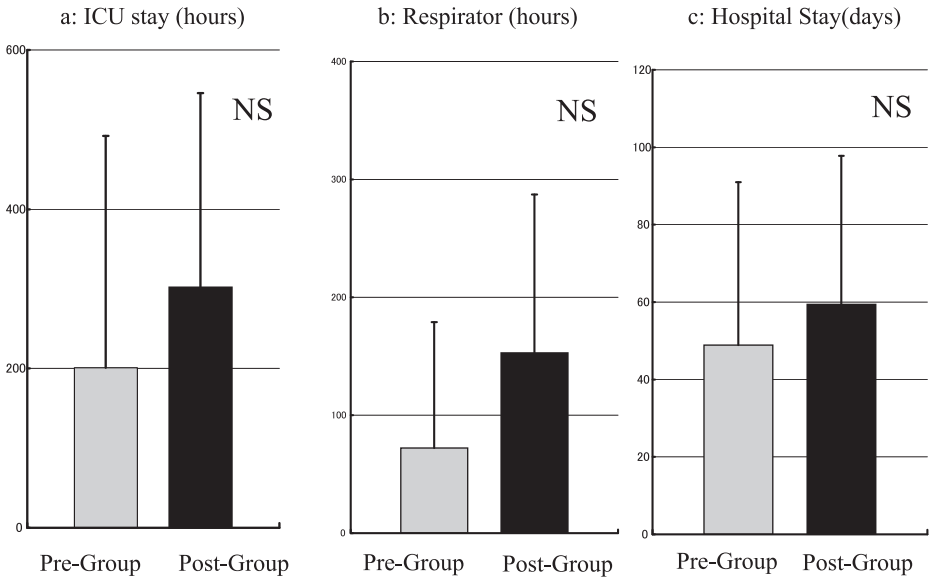

Figure 4 a: ICU stays (hours) b: duration of intubation (hours) c: hospital stay (days)

stronger inflammatory reaction occurs during cardiac surgery in comparison to general surgery because of the influence of CPB as well as greater surgical stress[5,6]. The greater operative stress creates a stronger inflammatory reaction, and then it causes more postoperative complications. In particular in cardiac surgery, there is a lot of stress for patients during aortic arch replacement for dissecting aortic aneurysm and true aortic arch aneurysm. We therefore usually use hypothermia during the extracorporeal circulation to protect the brain and other organs of the body because the CPB time and operation time are longer than for other cardiac operations. One of sever postoperative complications after aortic arch replacement is acute lung injury (ALI) that was defined by the American European Consensus Conference[7]. Although ALI after cardiac surgery was shown to develop in 0.4 to 3% of CPB patients, mortality remained high, between 15 to 70% [8-11] and longer mechanical ventilated support was needed.

One of the most important mechanisms in the initial phase of SIRS and ALI is priming, activation and sequestration of polymorphonuclear neutrophils (PMNs). SIRS with CPB is most likely caused by contact between blood and the artificial surfaces of the perfusion circuit. This interaction activates complement, although the exact role of complement in this process is not completely understood. This reaction, activated complement, makes PMNs prime, and when a second attack such as endotoxemia, hypoxia, deep hypothermia and ischemia occurs, PMNs will adhere to activated endothelium and release various cytotoxic contents[2,5]. Those are elastase, myeloperoxidase (MPO) and reactive oxygen species (ROS). When the patient is in a state of endotoxemia, hypoxia and ischemia, CPB may activate PMNs, inducing severe ALI after cardiovascular surgery. One of these cytotoxic contents is neutrophil elastase, which is an extremely cytotoxic protease, and has an important role in the ALI.[12].

We established a new endotoxin (LPS)-induced acute lung injury (ALI) model after CPB in the rabbit[3]. LPS functions as a substitute of surgical stress, and combination of low dose LPS and CPB caused ALI in rabbits. This LPS dosage, 0.5 μg/kg, is one percent of the usual quantity that

causes an endotoxin-induced lung injury. On the other hand, only low dose LPS or CPB did not cause ALI. Low dose LPS made PMNs prime, and interaction between blood and artificial surface worked as second attack following ALI. Sivelestat is a specific inhibitor of neutrophil elastase with a small molecular weight[13,14]. In this model, the continuous administration of Sivelestat inhibited PMN aggregation in proximal vessels and PMN accumulation, and it attenuated the respiratory and hemodynamic devastation. Sivelestat can reduce the extremely vicious cycle leading to the development of SIRS and ALI.

In clinical cases, a beneficial effect of Sivelestat has not been uniformly fully accepted because the clinical significance of this CPB and dissected aortic aneurysm related pulmonary injury varies widely, ranging from a clinically undetectable condition to very severe pulmonary failure. These varieties of lung dysfunction probably depend on the level of whole body inflammatory response, but these varieties of clinical course have not as yet been well studied. In addition, the clinical use of Sivelestat for ALI has been approved in Japan after a pulmonary disorder outbreak. Judging from the developmental mechanism of ALI, earlier administration of Sivelestat, such as before operation, should be more effective, as we have shown in our rabbit ALI model. However, there has been no clinical study to determine whether pretreatment with Sivelestat before CPB can prevent the progression to ALI after CPB.

In this study, we compared the effectiveness of Sivelestat between two groups. Infusion of Sivelestat was started at induction of anesthesia in the pre-group and after the operation in the post-group. A value of the P/F ratio at one hour after the CPB was calculated for 100%, because there is a variation in the preoperative P/F ratio for each case. Pretreatment with Sivelestat inhibited the fall of the P/F ratio from one hour after the operation to postoperative day two. But administration of Sivelestat after the operation did not inhibit a fall of P/F ratio at three hours after the operation, and the next morning the P/F ratio recovered to the pretreatment group. Although the pretreatment of Sivelestat did not suppress the accumulation of the the neutrophils and inflammatory response in the lung field, it probably reduced the spread to the inflammatory response by elastase like cutting of the vicious cycle. In the Post-Group, P/F ratio returned to the same level as the Pre-Group despite the postoperative dosage administration. During the operation, activated neutrophils released elastase that caused endothelial cell damage, and this reaction took almost 24 hours in the endotoxin-induced intestinal model described by Gott JP et al[15]. Even if considering that neutorphils and monocytes were recruited from blood to alveoli at a much earlier stage, administration of Sivelestat after the operation had efficacy for inhibiting progression to ALI.

IL-8 level in the Pre-group was relatively lower than the Post-group at three hours after the CPB (P=0.09). It was thought that operative and CPB stress caused inflammatory reaction, and then cytokine was released by macrophage[16]. Neutrophils were activated by cytokine, but Sivelestat inhibits apoptosis of neutrophils and also creates vicious cycle as elastase accelerates activation of the neutrophil[17].

The clinical use of Sivelestat had no effect for ALI, because administration of Sivelestat after acute lung injury demonstrated that there was no significant effect on 28-day all-cause mortality or duration of mechanical ventilation[18]. We did not find any differences for the length of ICU stay, hospital stay or duration of intubation between the Pre-Group and Post-Group. A limitation in this study was the lack of a control group for comparison in which Shivelestat was not infused.

Conclusion

These findings showed that Sivelestat reduced

the inflammatory reaction associated with cardiopulmonary bypass, and prevented the pulmonary dysfunction caused by inflammatory reaction.

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