Relationship between granulocyte elastase and C3a under protamine dosing in on-pump cardiac surgery

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Abstract

Objective: The complement cascade and granulocytes are activated in on-pump cardiac surgery. If activation of complement directly regulates granulocytes, granulocyte elastase (GEL) should increase significantly after protamine administration. We examined the effect of protamine on granulocytes by protamine administration and observation of the effect on GEL and C3a.

Methods: Thirty patients who underwent coronary artery bypass grafting were randomly assigned to two groups. In 15 patients, protamine was administered 5 min after the termination of cardiopulmonary bypass, and was administered 35 min after cardiopulmonary bypass in the other 15 patients. All patients were perfused with heparin-coated circuits and received 300 IU/kg heparin and 3 mg/kg protamine. GEL and C3a concentrations were measured at 7 time points.

Results: GEL concentrations increased significantly just before aortic declamping and did not increase significantly after protamine administration. C3a concentrations, however, did not increase during cardiopulmonary bypass and did increase significantly after protamine administration.

Conclusions: This study indicates that GEL does not increase after protamine administration and that complement concentration does not directly affect GEL release.

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1. Introduction

During cardiopulmonary bypass (CPB), blood is exposed to artificial surfaces, and is subjected to mechanical trauma, hemodilution, shear stress forces, and action of potent drugs [1–6]. Those could lead to activation of complements, cytokines, coagulofibrinolysis, and granulocytes [2–7]. Activated granulocytes release granular protein into the circulation [6]. Granulocyte elastase (GEL), a serine protease stored in the azurophilic granules of neutrophils, is released and causes tissue injury. Therefore, the evaluation of GEL is important to understand the mechanism of postperfusion syndrome.

Complement is activated during CPB and protamine administration leads to further complement activation after CPB [8,9]. However, the effect of protamine administration on granulocyte remains unknown [6]. In addition, few investigations have been conducted whether granulocytes could be activated by complement or not [6,10]. If granulocytes are activated through complement activation, GEL should increase significantly after protamine administration.

Therefore, we studied the effects of protamine on C3a and plasma GEL concentrations in two groups assigned by different times of protamine administration.

2. Patients and methods

Thirty patients, who underwent elective coronary artery bypass grafting (CABG), were randomized into two groups (Table 1). Protamine was administered 5 min after termination of CPB in 15 patients (group A) and 35 min after CPB in the other 15 patients (group B). Anticoagulation or antiplatelet therapy was discontinued 7 days before the operation. All patients were given 300 IU/kg of heparin, and CPB was started when activated clotting time (ACT) was above 400 s. Additional heparin was given if ACT was below 400 s. All patients received 3 mg/kg of protamine for 5 min and no additional protamine was given. Steroid was not given before, during and after operation. This study was approved by the Medical Ethical Committee of our hospital, and informed consent was obtained from all patients.

3. Operation

Patients were premedicated with ranitidine, morphine sulfate, and scopolamine hydrobromide, then subjected to
anesthesia with fentanyl, propofol, and vecuronium. Left internal mammary artery, saphenous vein graft (SVG) and radial artery graft (RAG) were harvested after median sternotomy. After CPB was established and ascending aorta was clamped, all distal and proximal anastomoses of grafts were performed under cardiac arrest.

### 4. Extracorporeal circuits

All components of the CPB circuit were coated with covalently bonded heparin (Carmeda Bioactive Surface, Medtronic, Anaheim, CA, USA). The CPB circuit consisted of a hollow-fiber membrane oxygenator (Maxima CBMAX-PF, Medtronic), a soft shell venous reservoir (Maxima CB1386, Medtronic), a cardiotomy reservoir (CB1351, Medtronic), a 40-μM arterial filter (CBM-40, Medtronic), and a centrifugal pump (Bio pump CBBP-80, Medtronic). The circuits were primed with a mixture of 1300 ml of lactated Ringer’s solution, 250 ml of human serum albumin (250 mg/ml), 200 ml of mannitol (200 mg/ml), and 100 ml of sodium bicarbonate (84 mg/ml). Standard ascending aortic cannulation and right atrial cannulation were performed. During CPB, a nonpulsatile flow of 2.4 L/min per m² body surface area was maintained under moderate systemic hypothermia. The mean arterial pressure was maintained in the range from 50 to 80 mmHg during CPB. The left ventricle was vented by cannulation via the right superior pulmonary vein. Cold blood cardioplegia was administered in the antegrade and retrograde fashion during aortic clamping.

### 5. Data collection and measurements

Blood sampling was obtained at the following 7 time points in both groups: before operation (Pre); 60 min after the initiation of CPB (CPB60); immediately before aortic declamping (Ao pre); 10 min after aortic declamping (Ao10); 15, 45, and 75 min after the termination of CPB (Off15, Off45, Off75).

The hematocrit was measured with an automatic cell counter (MAXX-M Retic, Beckman Coulter, Fullerton, CA, USA). Plasma was separated from blood cells by centrifugation at 3000×g for 10 min, and stored at −80 °C until analysis. C3a was measured by radioimmunoassay (Biotrak, Amersham Pharmacia Biotech, Piscataway, NJ, USA). GEL was measured by enzyme immunoassay (granuloelastase EIA, Sanwa Kagaku Kenkousyo, Nagoya, Japan). The obtained values were collected for hemodilution and normalized to the hematocrit before the operation.

### 6. Statistical analysis

Data were analyzed using standard computer software (Statview 5.0; SAS Institute, Cary, NC, and Super ANOVA 1.11; Abacus Concepts, Berkeley, CA, USA). All results were reported as the mean ± SE of mean. Student’s t-test was used for comparison of preoperative and intraoperative values between the two groups. A two-factor repeated measures analysis of variance was performed to evaluate differences between the groups. Contrast analysis was done in comparison of the same group. For correlation analyses, Pearson’s correlation coefficient was calculated with corresponding P-values. A P-value of less than 0.05 was considered statistically significant.

### 7. Results

#### 7.1. C3a concentration

The C3a concentration increased significantly 60 min after the initiation of CPB. Although the C3a concentration was maintained higher than baseline, it did not increase after aortic declamping. At 15 min after the termination of CPB, the C3a increased significantly in group A (481 ± 41–833 ± 86 ng/ml, P < 0.01), but did not increase in group B (Fig. 1). The C3a increased at the 45-min time point in group B (394 ± 24–764 ± 67 ng/ml, P < 0.01). In both groups, a significant increase in C3a concentrations was observed after protamine administration. There was significant

![Fig. 1. Changes in C3a concentration. The values are shown as the mean ± SE of mean. Closed circles indicate group B and open circles present group A. Samples were obtained at the following time points: before operation (Pre); 60 min after the initiation of cardiopulmonary bypass (CPB60); immediately before aortic declamping (Ao pre); 10 min after aortic declamping (Ao10); and 15, 45, and 75 min after termination of cardiopulmonary bypass (Off15, Off45, Off75). Arrow shows time of protamine administration.](https://academic.oup.com/ejcts/article-abstract/28/3/431/465112)
difference between groups A and B 15 min after CPB (group A vs. group B: 833 ± 86 vs. 394 ± 24 ng/ml, P < 0.01).

7.2. GEL concentration

GEL increased gradually throughout the time course during and after CPB. GEL concentrations were significantly higher just before aortic declamping, as compared to preoperative concentrations (group A, 58 ± 3–207 ± 29 μg/ml, P < 0.01; group B, 52 ± 4–225 ± 53 μg/ml, P < 0.01), and this high concentration was maintained after CPB (Fig. 2). No significant differences between groups A and B at any time point existed. The GEL concentrations in groups A and B at the 45-min time point were 375 ± 27 and 404 ± 50 μg/ml, respectively (P = 0.62). The GEL concentration was not affected by the time of protamine administration. The highest values were obtained at 75 min after the termination of CPB in both groups (group A, 400 ± 29 μg/ml; group B, 426 ± 45 μg/ml).

7.3. Correlation of C3a and GEL concentrations

The C3a and GEL concentrations were studied before (a) and after (b) protamine administration. As shown in Fig. 3, no consistent correlation between the C3a and GEL concentrations was identified (a: P < 0.05, R² = 0.07, b: P = 0.40, R² = 0.01).

8. Comment

The present study showed that protamine administration caused a sharp rise in C3a concentration as the previous studies. However, GEL expression differed from C3a expression after protamine administration in this study.

Fukutomi et al. have demonstrated that a linear relationship between CPB time and C3a or GEL concentration existed, and that the C3a and GEL concentrations correlated during CPB [10]. Thus, they have concluded that granulocytes may be activated through complement activation [10]. They did not, however, directly examine the correlation between C3a and GEL concentrations at different time points after protamine administration. The present results that GEL increased significantly just before aortic declamping, but C3a maintained the same level during CPB, suggest that GEL and C3a are differentially modulated. Furthermore, this study clarified that protamine administration causes a sharp rise in C3a concentrations without a change in GEL concentration. These results reveal that the GEL concentration may not be influenced by protamine administration. Thus, we indicate that complement activation does not directly influence GEL release in on-pump cardiac surgery.

Exposure of blood to an artificial surface can activate neutrophils and result in GEL release. The GEL concentration has been reported to be significantly lower in heparin-coated circuits, as compared to uncoated circuits [6,10,11]. The low level of GEL concentration at 60 min after CPB initiation is probably due to this inhibitory effect exerted by heparin-coated circuits. GEL increased gradually during CPB, and increased significantly just before aortic declamping. These results show that CPB itself can influence on increased expression of GEL, even when heparin-coated circuits are used. GEL concentration may increase significantly after aortic declamping, because GEL stored in the lung during cardiac arrest may enter into systemic circulation. In this study, we could not reveal the significant increase in GEL concentrations between before and after the aortic declamping, although the GEL concentration was higher after the aortic declamping.

As another major source of GEL, the importance of pulmonary circulation has been emphasized [12,13]. During CPB, blood flow into the pulmonary circulation is markedly reduced. Therefore, GEL concentration may increase after CPB, because GEL stored in the lung during CPB may enter into systemic circulation. The present results that GEL increased gradually after CPB may reveal contribution of pulmonary circulation to this GEL release. However, we cannot describe the source of GEL, because we did not sample from the pulmonary and coronary circulation.

9. Conclusion

The present study revealed that changes between C3a and GEL were differentially regulated in cardiac surgery using CPB, and it indicated that complement concentration does not directly affect granulocyte activity. Furthermore,
this study demonstrated that protamine administration does not influence total GEL protein expression.

10. Study limitations

The durations of surgery and CPB were long in this study. We considered that there were two reasons for these long durations. First, with advances in percutaneous coronary interventions, our patients referred for CABG often had diffuse coronary artery disease. It took relatively long time to obtain complete myocardial revascularization by bypassing multiple significant stenoses in the coronary vessels in such patients. Second, our Medical School Hospital is the institution for training young surgeons. Because the operator was not always only one, durations of surgery and CPB became long.

The long durations of surgery and CPB could affect the concentrations of C3a and GEL. As GEL concentration in the lung is considered to continue to increase during CPB, long-time CPB may influence the high GEL concentration near the end of CPB. Long-time CPB necessitates a higher dose of heparin that results in the increased amount of heparin–protamine complex. The increase of heparin–protamine complex may also affect concentrations of C3a and GEL. Furthermore, the activating mechanism of inflammatory mediators and relationship between these mediators have not been investigated completely yet. Thus, it is suggested that various factors affect these mediators including C3a and GEL, when the durations of surgery and CPB are long. The limitations of this study are such that the results were obtained in the relatively small number of patients and the effects of the operation and CPB times were not evaluated. Further study is necessary to evaluate the precise role of C3a and GEL concentrations in the large number of patients.

References