J Anesth (1995) 9:324–328

Journal of
Anesthesia

Relationship between plasma neutrophil elastase and respiratory index of patients who had undergone cardiac surgery with cardiopulmonary bypass

TOSHIKO YUSA[1], ATSUSHI NOHARA[2], and MASAHIDE SUNAGAWA[2]

Department of Anesthesiology[1] and Hyperbaric Medicine[2], University of the Ryukyu, Faculty of Medicine, 207 Uehara, Nishihara, Okinawa, 903-01 Japan

Abstract: To evaluate the effects of cardiopulmonary bypass (CPB) on the release of polymorphonuclear leukocyte elastase (PMN-E) and postoperative pulmonary function, the perioperative plasma levels of PMN-E in α_1-antitrypsin complex (EAC) and hydrogen peroxide concentration in the expired breath were measured in eight patients who underwent cardiac surgery with CPB, and the relationship between EAC levels and the respiratory index (RI) was studied. Although PMN, EAC, and the ratio of EAC to neutrophil (E/N) were elevated significantly after surgery, alveolar–arterial oxygen difference ($A\text{–}aDO_2$) and respiratory index ($A\text{–}aDO_2/PaO_2$) did not change when compared with those of the preoperative period. Hydrogen peroxide concentration in the expired breath also did not change (below 2.5 $\mu mol \cdot l^{-1}$) during the perioperative period. These results suggest that the elevation of EAC immediately after cardiac surgery using CPB, which lasted less than 2 h, was not a cause of postoperative pulmonary disorder. However, there was a significant positive correlation between E/N ratio and respiratory index ($r = 0.67$, $P < 0.01$). Thus excessive release of PMN-E during CPB may be implicated in the etiology of postoperative respiratory dysfunction.

Key words: Neutrophil elastase, Elastase in α_1-antitrypsin complex, Cardiopulmonary bypass, Respiratory index, Expired breath hydrogen peroxide concentration

Introduction

Neutrophil elastase, which is proteinase, is the quantitatively predominant constituent of azurophilic granules of polymorphonuclear leukocyte (PMN) and is known to possess elastinolytic and collagenolytic properties capable of causing major tissue destruction at sites of inflammation [1]. Polymorphonuclear leukocyte elastase (PMN-E) is released from lysosomes, and an elevated level of PMN-E in plasma has been thought to be involved in pulmonary disorders, especially in adult respiratory distress syndrome (ARDS) [2]. Reduced oxygen species liberated by activated PMN are also involved in the injury process [3]. The hydrogen peroxide content of the expired breath from patients with ARDS was higher than that of other intensive care unit (ICU) patients [4].

During extracorporeal circulation such as hemodialysis and cardiac surgery with cardiopulmonary bypass (CPB), a marked increase of PMN-E in plasma was reported as a result of degranulation from mechanically traumatized blood [5]. In CPB, a post-bypass syndrome including pulmonary dysfunction ("post-perfusion lung") has been reported, and was considered to be mediated by increased plasma PMN-E activity [6]. Significant positive correlations between the plasma level of PMN-E and alveolar–arterial oxygen difference ($A\text{–}aDO_2$) [7,8], respiratory index ($A\text{–}aDO_2/PaO_2$) [9], or oxygenation index (PaO_2/FiO_2) [10] during the perioperative period have also been reported.

To evaluate the effects of CPB on the release of PMN-E and postoperative pulmonary function, we measured the plasma level of PMN-E in α_1-antitrypsin complex (EAC) and the hydrogen peroxide concentration in the expired breath, and evaluated the relationship between these values and the respiratory index (RI) in patients who underwent cardiac surgery with CPB.

Materials and methods

Materials

Following approval by the Committee on Human Research of University of the Ryukyu's, eight patients

Received for publication on September 7, 1994; accepted on May 19, 1995
Part of this work was presented at the 41st annual meeting of the Japan Society of Anesthesiology, Tokyo, April 14, 1994

who underwent elective cardiac surgery with CPB were included in this study. Six of these eight patients underwent aorto-coronary bypass and other two had aortic valve replacement. Their ages ranged from 32 to 70 years. Informed consent was obtained from each patient.

All patients were premedicated with morphine and scopolamine. Anesthesia was induced and maintained with high-dose fentanyl and diazepam supplemented with enflurane as required. Pancuronium was used as a muscle relaxant. Patients were perfused with membrane oxygenators and received corticosteroid intravenously at the start of CPB. They had controlled or assisted ventilation through an endotracheal tube until an early postoperative period in the ICU.

Methods

Heparinized and EDTA-anticoagulated arterial blood samples and expired breath samples were obtained before surgery (after induction of anesthesia; pre-op), at the end of the operation (post-op) and the following morning in the ICU when patients were breathing 100% O_2.

Blood gas analysis, measurements of hemoglobin (Hb), and hematocrit (Hct) were done from a heparinized arterial blood sample (using the CIBA Corning 278 Blood Gas System and a Corning 2500 Co-oximeter, Ciba Corning Diagnostics Corp., Tokyo, Japan). White blood cell counts were also done from an arterial blood sample. EAC was measured by an enzyme-linked immunoassay using the technique of Neumann [11].

To collect the expired gas, the breath condensate was obtained by passing the expired breath through 90 cm Tygon tubing submerged in an ice-water bath. The tubing was connected to the expiratory limb of a one-way exhalation valve. Expired gas was collected until a condensate of approximately 1 ml had formed (within 10 min). The condensate was then transferred to a polystyrene tube and immediately placed on ice.

Hydrogen peroxide in the breath condensate was assayed by the horseradish peroxide method (Sigma, Type II, SIGMA Chemical Company, MO, USA) using a spectrophotometer (Shimadzu UV 3000, Shimadzu Corporation, Kyoto, Japan) [12].

From the blood gas analysis data, the RI was calculated as the ratio of A–aDO_2 to PaO_2. The A–aDO_2 was calculated as follows: A–aDO_2 = $\{(760 - 47)FiO_2)\} - PaCO_2\ R^{-1} - PaO_2$, where the respiratory exchange coefficient (R) is 0.8 and FiO_2 is 1.0.

The ratio of EAC ($fg \cdot ml^{-1}$) to neutrophil ($cell \cdot ml^{-1}$) (E/N ratio) was also calculated to eliminate the effect of hemodilution during CPB.

Statistical analysis

Values were expressed as mean ± SE. Statistical analyses were performed using analysis of variance (ANOVA) with Dunnett's test. The correlation was examined by a least-squares linear regression analysis. A P value of less than 0.05 was considered statistically significant.

Results

There were no postoperative complications.

The durations of CPB, surgery, and anesthesia were 115.6 ± 8.3 min, 343.8 ± 23.4 min, and 425.0 ± 28.4 min, respectively.

Hb and Hct values decreased significantly during surgery, indicating residual hemodilution during CPB immediately after surgery (Hb and Hct values were 13.4 ± $0.5\,g \cdot dl^{-1}$ and 38.5 ± 1.7%, respectively, in pre-op, and 10.7 ± $0.5\,g \cdot dl^{-1}$ and 30.0 ± 1.5%, respectively in post-op). Neutrophils ($cell \cdot ml^{-1}$) increased significantly in the postoperative period (17.3 ± 2.5 and 16.3 ± 1.4 in post-op and ICU, respectively) compared with pre-op (4.0 ± 0.5). Plasma levels of EAC rose above the normal range (21–165 $\mu g \cdot l^{-1}$) from 118.1 ± 13.3 to 1118.1 ± 267.1 and 491.4 ± 55.8 in post-op and ICU, respectively. In post-op, the increase in EAC was significant compared with pre-op and ICU. Consequently, the E/N ratio increased significantly (74.8 ± 19.8 $fg \cdot cell^{-1} \cdot ml^{-1}$) in post-op (Fig. 1).

Postoperative A–aDO_2 and RI were not different from pre-op. A–aDO_2 in post-op (304.8 ± 50.3), how-

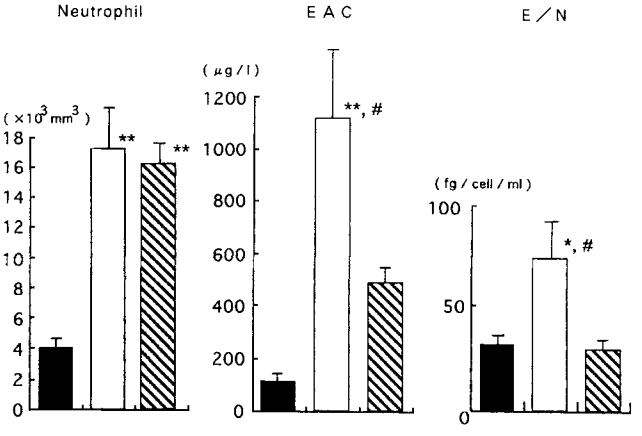

Fig. 1. Effect of cardiopulmonary bypass on perioperative neutrophil, plasma levels of neutrophil elastase in α_1-antitrypsin complex (*EAC*), and the ratio of EAC to neutrophil (*E/N*). Values are mean ± SE. *Solid bars*, pre-op; *open bars*, post-op; *hatched bars*, ICU. *$P < 0.05$ *vs* values before surgery (pre-op); **$P < 0.01$ *vs* values before surgery (pre-op); #$P < 0.05$ *vs* values the following morning in the intensive care unit (*ICU*)

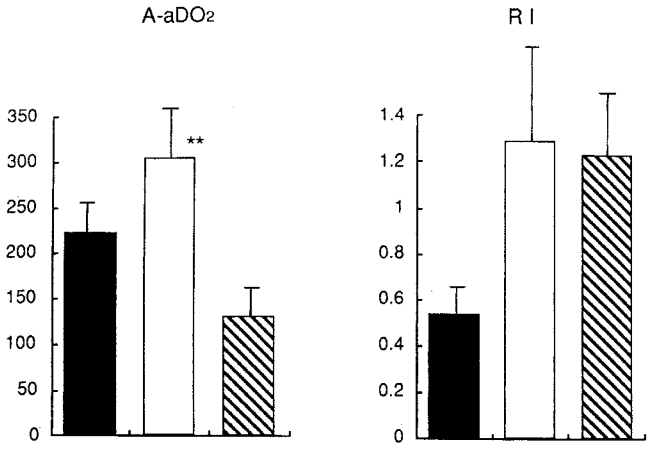

Fig. 2. Effects of cardiopulmonary bypass on perioperative alveolar–arterial oxygen difference ($A\text{–}aDO_2$) and respiratory index (RI: $A\text{–}aDO_2/PaO_2$). $A\text{–}aDO_2$ was calculated as described in Methods when FiO_2 was 1.0. Values are mean ± SE. *Solid bars*, pre-op; *open bars*, post-op; *hatched bars*, ICU. $^{**}P < 0.01$ *vs* values the following morning in the ICU

ever, was significantly higher than that (131.1 ± 25.0) in the ICU (Fig. 2).

A significant positive correlation was found between RI and the E/N ratio during the perioperative period ($r = 0.67$, $P < 0.01$) with the exception of 2 points in post-op (because of extremely abnormal values in EAC, possibly caused by hemolysis during CPB) (Fig. 3).

The hydrogen peroxide content of expired breath condensate did not increase in the postoperative period compared with pre-op, and all values were below 2.5 μmol·l^{-1} (Table 1).

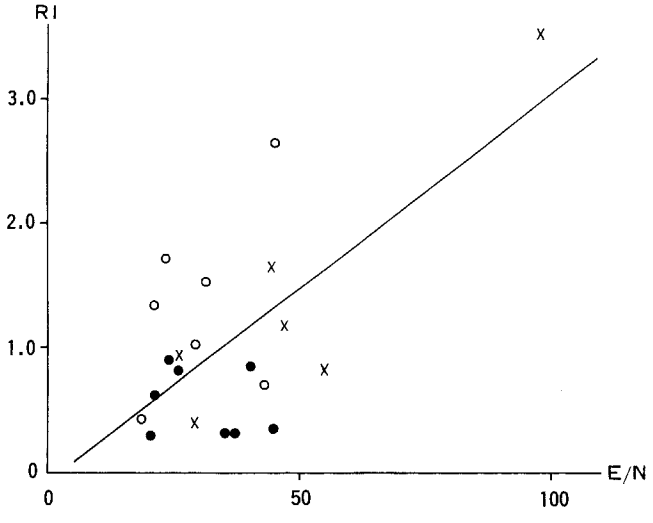

Fig. 3. Correlation between perioperative RI and the ratio of neutrophil elastase in α_1-antitrypsin complex to neutrophil (E/N) in cardiopulmonary bypass. *Solid circle*, pre-op; *cross*, post-op; *open circle*, ICU. Linear regression analysis for all the perioperative values (except 2 points in post-op) revealed a significant positive correlation ($r = 0.67$, $P < 0.01$) with a regression line of $Y = 0.031\ X - 0.096$

Table 1. Hydrogen peroxide concentration of expired breath condensate (μmol·l^{-1})

Case	Pre-op	Post-op	ICU
32M	2.4	1.5	—
62M	—	—	—
62M	1.3	—	—
57M	2.0	1.5	—
58M	1.3	1.2	—
44M	0.9	—	—
60M	1.1	0.9	0.9
70F	—	1.1	1.0

—, less than the measurable limit.

Discussion

In our study, immediately after cardiac surgery with CPB, neutrophil, EAC, and E/N ratio were significantly elevated (Fig. 1). $A\text{–}aDO_2$ and respiratory index (RI) did not increase significantly compared with their preoperative level (Fig. 2) and hydrogen peroxide concentration of expired breath condensate also did not increase postoperatively (Table 1). However, there is a significant correlation between RI and E/N ratio ($r = 0.67$, $P < 0.01$) (Fig. 3).

During extracorporeal circulation such as hemodialysis and cardiac surgery with CPB, blood is subjected to mechanical trauma [5]. As a result, PMN lysosomal enzymes are released and complements are activated during CPB [13]. Complement-exposed PMNs are stimulated both to adhere to other surfaces and to aggregate. Complement conversion and pulmonary leukocytes sequestration were also observed during CPB [14,15]. Furthermore, stimulated PMNs liberate a variety of highly reactive reduced oxygen species almost at the same time as the release of elastase [3]. Thus activated PMNs may play a causative or aggravating role in one of the most common side effects, postoperative pulmonary dysfunction known as post-perfusion lung, which is thought to fall under the category of ARDS. Therefore we measured PMNs, neutrophil EAC, RI, and hydrogen peroxide concentration in the expired breath, as an index of reactive reduced oxygen species released by sequestered pulmonary PMNs, in order to evaluate the effects of CPB on postoperative pulmonary function. The effect of EAC was evaluated as the E/N ratio to eliminate the effect of hemodilution during CPB.

Previously, a tremendous elevation in PMN and PMN-E was reported in patients who underwent cardiac surgery with CPB. There is also a positive correlation between levels of PMN or PMN-E and respiratory functions such as $A\text{–}aDO_2$ [7,8], repiratory index [9], and oxygenation index [10]. Comparing our results, the temporal elevation of PMNs and EAC after CPB in our study would not contribute to postoperative pulmonary

disorder such as is seen in ARDS, because the PMN and PMN-E in bronchoalveolar lavage fluid showed a significantly positive correlation to A–aDO_2 only in patients with ARDS [7,8].

Hydrogen peroxide concentrations of expired breath condensate in our cases were below 2.5 $\mu mol \cdot l^{-1}$, which was the highest normal range reported [4,16]. This result also indicates that pulmonary leukocyte sequestration during CPB in this study would be temporal. Elastases released by inflammatory stimuli such as endotoxin, complements, or immunocomplex are proteinases that can degrade almost all components of the extracellular matrix and cleave a variety of key plasma proteins and even attack intact cells [1]. However, plasma and interstitial fluid contain a series of powerful antiproteinases including α_1-proteinase (α_1-antitrypsin), which irreversibly inhibits PMN-E by forming an enzyme-inhibitor complex; consequently, PMN-E levels have been measured in the form of the PMN elastase–α_1-antitrypsin complex (EAC) by an enzyme-linked immunoassay such as we applied in this study. Thus measured EAC is inactive elastase. EAC itself, however, is a neutrophil chemoattractant [17], and therefore pulmonary leukocyte sequestration, which has been observed during CPB, is suggested as contributing to the pathogenesis of post-perfusion lung [6]. Activated PMNs release reduced oxygen species such as superoxide and hydrogen peroxide, but increased levels of hydrogen peroxide were only detected in the expired breath of ICU patients with focal lung infiltration and in ARDS patients [4,16]. We also reported an increase in hydrogen peroxide concentration in the expired breath of one patient who had an excessive plasma EAC level after intraoperative blood transfusion [18]. Thus pulmonary PMN sequestration during CPB in this study is temporal and not sufficient to inactivate all α_1-antitrypsin and cause postoperative pulmonary dysfunction. Because the increase in PMN and PMN-E during CPB related to perfusion time [6], the relatively short time of CPB in our cases would affect our results.

The excess release of PMN-E, especially under conditions that compromise the function of their regulatory inhibitors, can lead to tissue damage in a broad spectrum of diseases such as endotoxin shock, septicemia, ARDS, and other inflammatory diseases [1]. By oxidatively inactivating a series of key proteinase inhibitors such as α_1-antitrypsin [19] and simultaneously activating latent proteinase, activated PMN can create an environment in which elastase, collagenase and gelatinase are able to exert destructive effects more effectively and with greater specificity than could even enormous doses of oxidants [3]. In this study, there is a significant correlation between RI and E/N ratio ($r = 0.67$, $P < 0.01$). Therefore the excessive elevation of PMN-E during cardiac surgery with prolonged CPB or in the patients with preoperative lung disease may be a factor of postoperative pulmonary disorder.

References

1. Janoff A (1985) Elastase in tissue injury. Annu Rev Med 36:207–216
2. Ogawa M (1989) Neutrophil elastase and the lung, with special reference to the pathogenesis of ARDS (in Japanese with English abstract). Kokyu to Junkan (Respir Circ) 37:1258–1269
3. Weiss SJ (1989) Tissue destruction by neutrophils. N Engl J Med 320:365–376
4. Baldwin SR, Simon RH, Grum CM, Ketai LH, Boxer LA, Devall LJ (1986) Oxidant activity in expired breath of patients with adult respiratory distress syndrome. Lancet 1:11–14
5. Hörl WH, Jochum M, Heidland A, Fritz H (1983) Release of granulocyte proteinases during hemodialysis. Am J Nephrol 3:213–217
6. Ratliff NB, Young WG Jr, Hackel DB, Mikat E, Wilson JW (1973) Pulmonary injury secondary to extracorporeal circulation. An ultrastructural study. J Thorac Cardiovasc Surg 65:425–432
7. Idell S, Kucich U, Fein A, Kueppers F, James HL, Walsh PN, Weinbaum G, Colman RW, Cohen AB (1985) Neutrophil elastase-releasing factors in bronchoalveolar lavage from patients with adult respiratory distress syndrome. Am Rev Respir Dis 132:1098–1105
8. Weiland JE, Davis WB, Holter JF, Mohammed JR, Dorinsky PM, Gadek JE (1986) Lung neutrophils in the adult respiratory distress syndrome: Clinical and pathophysiologic significance. Am Rev Respir Dis 133:218–225
9. Shimanuki K, Sakurabayashi I, Kanazawa K (1989) Perioperative fluctuation in plasma levels of granulocyte elastase and alpha-1-antitrypsin: The influence of the severity of surgical intervention and their effect on the respiratory index. Jpn J Surg 19:410–417
10. Mishima A, Takeuti Y, Usami S, Kotani H, Suzuki Y, Yura J (1990) Effects of ulinastatin on plasma polymorphonuclear leukocyte elastase activity and respiratory function in patients undergoing cardiopulmonary bypass (in Japanese with English abstract). Nihon Kyobu Geka Gakkai Zasshi (J Jpn Assoc Thorac Surg) 38:607–612
11. Neumann S, Gunzer G, Hennrich N, Lang H (1984) "PMN-elastase assay": Enzyme immunoassay for human polymorphonuclear elastase complexed with α_1-proteinase inhibitor. J Clin Chem Clin Biochem 22:693–697
12. Gallati VH, Pracht I (1985) Peroxidase aus meerrettich: Kinetishe studien und optimierung der peroxidase-aktivitatsbestimmung mit den substraten H_2O_2 und 3,3′,5,5′-tetramethylbenzidin. J Clin Chem Clin Biochem 23:453–460
13. Antonsen S, Brandslund I, Clemensen S, Søfeldt S, Madsen T, Alstrup P (1987) Neutrophil lysosomal enzyme release and complement activation during cardiopulmonary bypass. Scand J Thorac Cardiovasc Surg 21:47–52
14. Hammerschmidt DE, Stroncek DF, Bowers TK, Lammi-Keefe CJ, Kurth DM, Ozalins A, Nicoloff DM, Lillehei RC, Craddock PR, Jacob HS (1981) Complement activation and neutropenia occurring during cardiopulmonary bypass. J Thorac Cardiovasc Surg 81:370–377
15. Utoh J, Yamamoto T, Kambara T, Goto H, Miyauchi Y (1988) Complement conversion and leukocyte kinetics in open heart surgery. Jpn J Surg 18:259–267
16. Sznajder JI, Fraiman A, Hall JB, Sanders W, Schmidt G,Crawford G, Nahum A, Factor P, Wood LDH (1989) Increased hydrogen peroxide in the expired breath of patients with acute hypoxemic respiratory failure. Chest 96:606–612
17. Banda MJ, Rice AG, Griffin GL, Senior RM (1988) The inhibitory complex of human α_1-proteinase inhibitor and human leuko-

cyte elastase is a neutrophil chemoattractant. J Exp Med 167: 1608–1615
18. Yusa T, Nohara A, Matayoshi K, Zukeran H (1994) Effect of intraoperative stored blood transfusion on plasma neutrophil elastase and its modification by ulinastatin (in Japanese with English abstract). Masui (Jpn J Anesthesiol) 43:1486–1492
19. Matheson NR, Wong PS, Travis J (1979) Enzymatic inactivation of human alpha-1-proteinase inhibitor by neutrophil myeloperoxidase. Biochem Biophys Res Commun 88:402–409