Does aprotinin reduce lung reperfusion damage after cardiopulmonary bypass?☆

Ali Rahmana,*, Bilal Üstündab, Oktay Burman, Ibrahim Haniöff Özerçanc, Ahmet Çekirdekçi, Mustafa Kemal Bayard

aDepartment of Thoracic and Cardiovascular Surgery, Fırat Medical Centre, Fırat (Euphrates) University, Elazığ 23200, Turkey
bDepartment of Biochemistry, Fırat Medical Centre, Fırat University, Elazığ, Turkey
cDepartment of Pathology, Fırat Medical Centre, Fırat University, Elazığ, Turkey
dDepartment of Anaesthesiology and Reanimation, Fırat Medical Centre, Fırat University, Elazığ, Turkey

Received 14 January 2000; received in revised form 13 June 2000; accepted 21 June 2000

Abstract

Objective: The role of aprotinin in the prevention of lung reperfusion injury was investigated in the patients undergoing cardiopulmonary bypass (CPB) for coronary artery bypass grafting (CABG) operations. Methods: The study was planned randomly and prospectively. Two hundred milliliters of physiological saline solution was added to the prime solution of patients in group I (n = 10) whereas, 200 ml aprotinin (Trasylol, Bayer AG) was given to patients in group II (n = 10). In order to measure lung tissue malondialdehyde (MDA) levels, glutathione peroxidase (GSH-Px) activity levels and polymorphonuclear leukocytes (PMNs) numbers, lung tissue samples were taken before CPB and 5 min after removing the cross clamp. In addition, alveolo-arterial oxygen difference (AaDO2) for tissue oxygenation was calculated by obtaining arterial blood gas samples. Results: MDA levels before CPB increased from 41.72 ± 21.00 nmol/g tissue to 66.71 ± 13.44 nmol/g tissue in group I and from 43.44 ± 5.16 nmol MDA/g tissue to 53.22 ± 10.95 nmol MDA/g tissue in group II after cross clamp removal (P < 0.001 and P < 0.001, respectively). The increase in group II was found to be significantly lower than group I (P = 0.048). With the initiation of reperfusion, GSH-Px activity decreased in group I from 3.05 ± 0.97 to 2.31 ± 0.46 U/mg protein (P = 0.015) whereas GSH-Px activity in group II decreased from 3.18 ± 1.01 to 2.74 ± 0.81 U/mg protein (P = 0.055). This decrease in the group II was less than group I (P = 0.049). AaDO2 significantly increased in the group I and II (P = 0.012 and P = 0.020, respectively), but elevation in the group I was significant than in the Group II (P = 0.049). In histopathological examination, it was observed that neutrophil counts in the lung parenchyma rose significantly following removal of cross clamp in both groups (P = 0.001). The increase in group I was significantly larger than in group II (P = 0.050). Conclusion: Results represented in our study indicate that addition of aprotinin (2 million units) into the prime solution during CPB can reduce lung reperfusion injury. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Aprotinin; Cardiopulmonary bypass; Reperfusion injury; Lung

1. Introduction

Biochemical, functional and structural changes occur in the tissues during ischemia and reperfusion [1]. Release of superoxide anion occurs in cardiac operations with cardiopulmonary bypass (CPB) due to both the effect of xanthine oxidase at ischemic vascular endothelium and sequestration of polymorphonuclear leukocytes (PMNs) activated in circulation [2]. Adherent neutrophils are then subject to further activation through the action of chemokines, such as interleukin-8 [3]. Thus, oxygen free radicals and enzymes, such as proteases, (e.g. elastase and metalloproteases) and myeloperoxidase, are generated by the adherent neutrophils, exacerbating damage to endothelial cells and to subendothelial matrix proteins and inducing tissue injury [4]. There is, furthermore, a positive correlation between elastase plasma concentrations after CPB and postoperative respiratory dysfunction, as shown by changes in the respiratory index and increases in the intrapulmonary shunt [5].

Aprotinin is a serine protease inhibitor that is presently widely used in for minimizing perioperative blood loss in cardiac operations [6]. Experimental studies have shown that aprotinin, beyond its antiproteolytic membrane stabilizing property, decreases the release of lysosomal enzymes...
and increases intracellular adenine nucleotides [7–9]. While aprotinin reduces reperfusion injury by suppressing bradykinin [10], it can also inhibit the production of superoxides and peroxides which are originated from of human PMNs [11].

We hypothesized that aprotinin can provide protection against the adverse effects of reperfusion in the lung during cardiopulmonary bypass. In this study we investigated the effects of low dose aprotinin administration on histopathologic neutrophil sequestration, malondialdehyde (MDA) levels, glutathion peroxidase (GSH-Px) (E.C. 1.11.1.9) enzyme activity in lung tissue samples which were obtained before CPB and after aortic clamp releasing. Also, alveoloarterial oxygen difference was calculated as index of tissue oxygenation.

2. Materials and methods

Twenty patients with coronary artery disease who underwent elective aorta-coronary bypass grafting were entered voluntarily in this study. Approval of this study was given by the FIrat University, College of Medicine Ethical Committee on Human Research. The patients who demonstrated no lung pathologies were selected for the study after an evaluation of their respiratory systems. Those having ejection fraction below 30%, left ventricular end diastolic pressure (LVEDP) over 20 mmHg and reoperations were excluded from the study.

The patients were randomly assigned to two groups. Each patient was given morphine sulphate (0.1 mg/kg) and scopolamine (0.2–0.4 mg) intramuscularly before admission to the operating room. A Swan–Ganz catheter via the right internal jugular vein, a radial artery catheter and intravenous lines were placed. Standard anesthetic treatment consisting of fentanyl (Fentanyl, Janssen), midazolam (Dormicum, Roche) and pancuronium (Pavulon, Organon Teknika) were used.

CPB was completed with a roller pump (Sarns 7000, USA), hollow fiber membrane oxygenator (Dideco, Italy) and moderate hypothermia (28°C core temperature). While 200 ml physiologic saline solution was added to the prime solution of ten patients in group I, 200 ml aprotinin (Trasylo, Bayer AG) was given to ten patients in group II. Anticoagulation was obtained by the administration heparin (300 IU/kg, Liquemine, Roche), and activated clotting times (ACTs) were maintained at greater than 480 s with kaolin-Protamine 1000, Roche). Anticoagulation was maintained with 1:1.3 protamine HCl (Protamine 1000, Roche).

To measure lung tissue MDA, GSH-Px activity levels and PMNs count, lung tissue samples were taken before CPB and 5 min after removing the cross clamp. For this purpose, access was provided through left hemithorax which was opened for internal thoracic artery (ITA) removal, and samples (1 × 1 cm) were taken from the lower lobe of the lung. The samples were divided into two equal parts after washing with isotonic solutions. One of the halves was kept at −70°C until the time of biochemical examination. The other half was placed in formol solutions for histologic examination.

2.1. Biochemical measurement

The thiorbituric acid reaction was used to determine lung tissue MDA levels. MDA levels were calculated by measuring absorbance at 532 nm wave length. Results were shown as nmol MDA/g wet tissue.

Lung tissue GSH-Px activity levels were measured based on the oxidation of NADPH with glutathion reductase enzyme. GSH-Px activities were measured in the supernatants obtained by centrifuging the samples for 10 min at 15 000 rev./min at 4°C. Tissue protein levels were measured and results were shown as U/mg protein.

2.2. Histological analysis

Tissue samples were fixed in 10% formol and embedded in paraffin after routine follow-up procedure. Sections with a width of 4 μm were cut from paraffin blocks, and they were colored with hematoxylin and eosin (HE) before examining under the light microscope (Olympus BX 50, Japan). For each sample, PMNs were counted for 20 different magnification areas (×400 magnification). Total number of PMNs were divided by 20 to find the average number of PMNs per area.

2.3. Alveolo-arterial oxygen difference (AaDO2) calculation

The alveolar-arterial oxygen difference for tissue oxygenation was calculated by obtaining arterial blood gas samples before CPB and 5 min after aortic declamping (Radiometer, Copenhagen, Denmark).

2.4. Statistical analysis

The results were evaluated as the mean ± SD. For statistical analysis, the Mann–Whitney U-test was used between groups, non-parametric Wilcoxon signed rank test and Bonferroni-correction methods were used for comparisons within groups. The results were considered statistically significant for P < 0.050.
3. Results

There was no statistically significant difference between two groups when demographic properties, perfusion and X-clamp time and average graft numbers of the patients were compared (Table 1).

3.1. MDA levels

There was no significant difference between groups for lung tissue MDA levels before CPB. MDA levels before CPB of 41.72 ± 21.00 nmol/g tissue rose to 66.71 ± 13.44 nmol/g tissue after release of the aortic clamp, in group I \((P = 0.001)\). In group II also, the MDA levels also increased from 43.44 ± 5.16 to 53.22 ± 10.95 nmol MDA/g tissue after aortic declamping \((P = 0.021)\). The MDA increase in group II after aortic declamping was found to be significantly lower than in group I \((P = 0.048)\) (Fig. 1).

3.2. GSH-Px activity levels

After aortic declamping, with the initiation of reperfusion, GSH-Px activity levels decreased in group I from 3.05 ± 0.97 to 2.31 ± 0.46 U/mg protein \((P = 0.015)\), whereas GSH-Px activity levels in group II insignificantly decreased from 3.18 ± 1.01 to 2.74 ± 0.81 U/mg protein \((P = 0.055)\). The decrease in GSH-Px activity levels in Group II was significantly lower \((P = 0.049)\) than the decrease in group I (Fig. 2).

3.3. PMN count

The PMN count in lung parenchyma was similar in both groups before CPB \((P = 0.091)\). After release of the cross clamp during reperfusion period, the PMN count increased from 3.9 ± 0.7/high power field to 16.9 ± 2.6/high power field in group I \((P = 0.01)\) and from 4.3 ± 0.9/high power field to 12.0 ± 2./high power field in the group II \((P = 0.001)\). After cross clamp releasing, the count was significantly lower in group II than in group I \((P = 0.050)\) (Fig. 3).

3.4. AaDO2

There was no statistically significant \((P = 0.097)\) difference between the AaDO2 values of group I (266.8 ± 44.5) and group II (245.4 ± 24.1) before CPB. These values were measured to be 341.7 ± 10.5 and 280.2 ± 17.7 mmHg in group I and group II, respectively, after cross clamp releasing. The values significantly increased in group I and II \((P = 0.012\) and \(P = 0.020\), respectively) after aortic declamping, but the increase in group II was significantly lower \((P = 0.049)\) than group I (Fig. 4).

4. Discussion

CPB induces organ injuries and a systemic inflammatory response which may lead to postoperative morbidity [12]. PMNs are integrated into the acute inflammatory response to tissue injury which is augmented by reperfusion [3]. Activated and accumulated PMNs can contribute to lung tissue injury by release of oxygen-derived free radicals, proteases and leukotrienes [5]. Leukocyte activation promotes the production of appreciable amounts of cytokines such as tumor necrosis factor (TNF), interleukin-1 (IL-1), interleukin-6 (IL-6) and

![Fig. 1. MDA levels in lung tissue, before CPB and after aortic declamping in both groups. MDA levels before CPB and after aortic declamping were compared (**P = 0.021**). The increase in group I compared to group II was significantly larger (**P = 0.048**), after aortic declamping.](https://academic.oup.com/ejcts/article-abstract/18/5/583/505759)

![Fig. 2. GSH-Px activity levels in lung tissue, before CPB and after aortic declamping in group I and group II. GSH-Px activity levels before CPB and after aortic declamping were compared (**P = 0.015**). The decrease in group II compared to group I was significant (**P = 0.049**) lower after aortic declamping.](https://academic.oup.com/ejcts/article-abstract/18/5/583/505759)
interleukin-8 (IL-8) [13]. Then, these cytokines facilitate accumulating of neutrophils in the lung and myocardium by increasing CD11b [14,15]. Released cytokines such as leukotriene B4 and platelet activating factor (PAF) are agents which also facilitate adhesion of PMNs [3]. In addition to this, proteinases such as elastase are released by PMNs, and these positively-charged proteinases contribute to tissue damage by producing a charge variation on the surface of the cells or increasing direct membrane attachment [16]. Mair et al. reported that there was a significant increase of elastase concentrations during cardiopulmonary bypass in arterial, central venous and coronary sinus blood [17].

Vascular tissue injury occurs when both endothelial injury and neutrophil activation are present [5]. Since circulation is maintained in the peripheral vascular tissue, endothelial control of vascular tonus is preserved and reperfusion injuries do not occur frequently. In the experimental total CPB, but not in partial, however, pulmonary circula-

Fig. 3. The appearance of increase in PMNs in pulmonary parenchyma after aortic declamping: group I (A); group II (B). There was a marked increase in group I compared to group II (HE x400).
tion is completely cut and lung perfusion is maintained with only non-pulsatile bronchial arterial flow. Lung injury seen with restoration of pulmonary artery flow and ventilation may be the result of an inflammatory response after a period relative pulmonary ischemia [5]. In the present study, the aorta was clamped together with the pulmonary artery trunk for total CPB, since we used a two-stage venous cannula.

Experiments carried out with sheep have demonstrated an increase in the left atrium tromboxane level, pulmonary vascular resistance, lung lymph flow, lymph protein clearance, lung water content, pulmonary leukosequestration, and platelet sequestration [14]. It was also shown that, PMN accumulation was increased in the lungs of the patients who had undergone CPB. Leukocyte depletion recently has been introduced for cardiac surgical patients to attenuate leukocyte-mediated inflammation and organ reperfusion injury. Some studies suggested that lung injury during total CPB can be reduced by leukocyte depletion [18]. On the other hand, Gu et al. reported that they did not observe any improvement in the lung functions even though leukocyte depletion administered 10 min before reperfusion decreased circulation leukocytes [19]. It was shown that, aprotinin decreased IL-6 release, TNF release and CD11 b production in CPB patients. Hill et al. found that, in the presence of aprotinin in CPB, the accumulation of IL-8 dependent leukocytes in the broncho-alveolar lavage material was decreased [20]. This finding is consistent with our findings. We also detected a clear increase in the number of lung neutrophils after aortic declamping in both groups (group I: from 3.9 ± 0.7 to 16.9 ± 2.6 and group II: from 4.3 ± 0.9 to 12.0 ± 2.1). But, this increase in group II which is treated with aprotinin was significantly lower (P = 0.049).

Mc Goven et al. demonstrated that superoxide radical activity is detectable by chemiluminescence and a 15-fold increase in chemiluminescence activity in specimens of human right atrium during the reperfusion phase of routine cardiac operations. This increase peaked 1 min after aortic declamping and declined in the 30th min [21]. In another study with undergoing CABG patients, when the right ventricle samples were examined, it was seen that chemiluminescence values after declamping were doubled compared to baseline values [22].

Davies et al. observed that there was a significant increase in free radical indices in arterial and mixed venous bloods of 15 patients undergoing elective coronary bypass surgery [23]. On the other hand, they reported that small increase in coronary venous blood was not statistically significant. Based on these observations, they conclude that distribution pattern between the different sampling sites suggest that much of the observed increase in peroxidized lipids originates from tissues other than myocardium. To the best of our knowledge, there is no reference in literature investigation of radical indicators in the lung tissues of CPB patients. Our findings, however, show that there is an increase in lipid peroxydation after reperfusion in lung tissue as well as in the myocardium.

Superoxide dismutase (SOD) activities in the patients underwent CPB were reduced significantly [24]. In the same study it was also observed that GSH levels were slightly increased. These results indicate that oxygen free radical generation exceed the intracellular antioxidant capacity. Another a clinical study shown that serum levels of MDA increased, catalase activity decreased spectophometrically during reperfusion of CPB, and administration of aprotinin significantly reduced this oxidative stress [25]. In our study, we also observed a decrease in lung tissue GSH-Px levels after cross clamp releasing in both groups. The decrease was significant in group I but not in group II. However, we can not evaluate this result accurately since relevant literature data do not exist based on our knowledge.

There are several studies in dogs that aprotinin improves myocardial preservation [7,8]. In these studies, decreased levels of cyclic guanosine monophosphate and increased levels of cyclic adenosine monophosphate have been shown to inhibit release of lysosomal enzyme. In the experimental studies, an increase in myocardial performance was noted in isolated rat hearts treated with aprotinin [1]. The protective effect of aprotinin might be induced by myocardial protease inhibition and by protecting myocardial membranes from these protease attacks in addition to well-known anti-inflammatory properties of aprotinin.

An increase in the endothelial cell viability at the hypoxic preservation period was demonstrated by Sunomori et al. [7]. Aprotinin addition to organ preservation solutions was reported to improve lung function by a decrease in alveolar-arterial oxygen difference, an increase oxygen tension, a decrease capillary filtration coefficient and an increase compliance [4]. We also noticed in our study that, in group II there was a decrease in lung tissue leukocyte sequestration, radical production and alveolo-arterial oxygen difference.
As a result, the findings obtained with aprotinin in present study may be related with decreased leukocyte activation, elastase-like protease release and agents like cytokines inducing inflammatory response. However, we believe that on effect of aprotinin in the lung tissue during CPB needs to be further evaluated. We used a hemo statically recommended minimum standard low dose in our study. Therefore, further experiments with lower and/or higher doses of aprotinin needs to be performed to obtain more reliable results.

References