ORIGINAL ARTICLE

Efficacy of aprotinin treatment on bilateral blunt chest trauma created in rabbits

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Abstract

Objectives: To investigate the effects of aprotinin, on blood gasses, oxidant-antioxidant status, and lung histopathology in an experimental bilateral blunt chest trauma model.

Methods: Conducted at the Experimental Animal Laboratory of Meram Medical School at Selcuk University, Konya, Turkey, the study comprised 21 New Zealand female albino rabbits who were divided into three groups. Trauma was applied on the sham and aprotinin groups, which was administered intravenous Aprotinin 20.000 U/kg. Arterial blood samples were obtained from all rabbits at hours 0, 3, 24, and 96. At hour 96 after trauma, all rabbits were sacrificed using the decapitation method, and then blood and lung tissue samples were obtained. Blood nitric oxide, malondialdehyde and blood gas measurements were made. Histopathological changes in the lung were examined with a light microscope.

Results: While no positive effect of aprotinin was observed on nitric oxide malondialdehyde and partial pressure of carbon dioxide values, it was seen to have an increasing effect on partial oxygen pressure level. Aprotinin had a partial effect on lung histopathology.

Conclusion: Aprotinin was determined to have a positive effect on PO2 levels. We could not find any positive effects especially on alveolar haemorrhage.

Keywords: Aprotinin, Trauma, Acute lung injury, Pulmonary contusion. (JPMA 63: 32; 2013)

Introduction

In the 1-44 year age group, among the causes of death, the most common reason is trauma.[1] Thorax traumas account for 25% of all trauma-associated deaths. Quick and appropriate initial out-of-hospital intervention and proper transportation to the hospital are expected to reduce these deaths by 30%.[2]

Pulmonary contusion (PC) is the accumulation of blood and other fluids in the lung tissue, as a result of capillary damage due to chest trauma. Depending on the amount of this fluid, gas exchange is impaired and hypoxia occurs. Among PC treatment options, empirical treatments and clinical experiences are still in the forefront.[3] Treatment options generally include oxygen (PCO2) support, cardiopulmonary monitoring, analgesia, and pulmonary hygiene.[4]

The role of oxidant agents on the development of Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS) is understood more by the day, and studies on the development of antioxidant treatments are ever increasing. Oxidative stress and the production of nitric oxide (NO) derivatives easily cause the damage of lung due to its fragile structure. Oxidative stress and NO derivatives play an important role in inflammation with their direct and indirect effects. If antioxidant defense systems are insufficient against the production of reactive oxygen derivatives (superoxide, hydrogen peroxide, hydroxyl radicals) and reactive nitrogen radicals (nitric oxide, peroxynitrite, nitrogen dioxide), ALI occurs.[5]

Pulmonary vascular injury arises along with alveolar haemorrhage secondary to the damage in PC and for this reason, alveoli blood supply is insufficient. Aprotinin (APR) is a serine protease inhibitor; it increases intracellular cyclic guanosine monophosphate (cGMP) levels and reduces cyclic adenosive monophosphate (Camp) levels. Thus, it inhibits the release of lysosomal enzymes and blocks their effects. At the same time, it prevents protein degradation and it is a lysosomal membrane stabilizer.[6] APR prevents the formation of Free Oxygen Radicals (FOR) by preventing the conversion of xanthine dehydrogenase to xanthine oxidase.[7] Additionally, it also prevents NO production by blocking cytokine-dependent NO synthetase.[8]

In this study, the goal was to investigate the efficacy of APR during the initial 96 hours of rabbits with bilateral blunt chest trauma.

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Material and Methods

The study was conducted at the Selçuk University, Meram Medical School, Experimental Animal Laboratory, after the approval of the ethics institutional committee from May to November 2005. In the study, 21 female New Zealand type albino rabbits, weighing between 2650-3450gm (mean: 3000±220gm), were used. The rabbits were divided into 3 (n=7 each) groups: Group 1: Sham Group (underwent trauma + not given treatment); Group 2: Control Group (no trauma + no procedures); Group 3: APR Group (underwent trauma + was administered APR); IV 20.0000 U/kg/day with 12-hour intervals.

All rabbits were put to sleep with Ketamine hydrochloride (HCl) (50mg/kg, IM) and Rompun (xylasine HCl, 15 mg/kg, IM). Additional doses were given as necessary, and respiration was maintained during the experiment. Right after the rabbits were put to sleep, respiration and pulse rates were monitored. The rabbits were not intubated due to inflammatory changes that might have been caused in the lung tissue.

The monitored subjects were placed on their backs and the energy calculated using the E=M*g*L*(1-cos) formula based on Newton's Law, was applied bilaterally to the rib cage. We used Kaya et al's model.[9] This procedure was repeated for each subject except the control group.

Blood samples were obtained, 3cc using biochemistry tubes, from the rabbits at hours 0, 3, 24, and 96, in order to study the arterial blood and NO and serum Malondialdehyde (MDA) levels, and the blood drawn was replaced with IV saline, 3 times the amount of blood obtained.

The rabbits were sacrifised at hour 96 using the decapitation method, and after opening up the rib cage, approximately 1gm of tissue sample was obtained from the left lung. The tissues were preserved in 10% formaline solution. Lung tissue samples were obtained and sent to the pathology laboratory in 10% formaline, for histopathological examination. At the end of the study, the examinations were carried out by a pathologist who was blind to the procedures.

The right lung was removed completely and after measuring its wet weight, it was kept in a 70°C oven for 24 hours. At the end of the 24 hours, its dry weight was measured. The tissue samples obtained were placed in 10% formalin solution and preserved until the study.

Statistical analysis was conducted using SPSS 10.0 software. Intergroup comparisons were made using one-way analysis of variance (ANOVA). Tukey HSD test was used as the Post Hoc test. For the analysis of non-parametric variables, Kruskal-Wallis Chi-Square Test was performed. The mean and standard deviation values of the groups were calculated and $p<0.05$ was considered statistically significant.

Results

The mean NO levels measured on 0, 3, 24 and 96 hours in sham group were 13.78±0.42, 22.83±1.41, 17.67±1.09, 15.31±1.12, respectively. In the APR groups, the NO levels were 13.03±1.09, 31.63±5.98, 25.69±4.84, 20.97±3.16, and in the control group, they were 12.40±1.16, 12.41±1.54, 13.08±1.27, 14.69±1.24, respectively.

NO level increased significantly in sham and APR groups that received trauma. Between the sham group and the control group, there was a statistically significant difference at hour 3 NO values ($p<0.05$). There was also a statistically significant difference between the control and APR groups ($p<0.05$). No statistically significant difference could be determined between other values ($p>0.05$). However, no statistically significant difference could be found between the groups ($p>0.05$). APR treatment could not be determined to have a significant effect on NO levels (Figure-1).

The mean erythrocyte MDA levels measured at 0, 3, 24 and 96 hours in the sham group were 4.52±0.42, 5.32±0.19, 5.24±0.19, and 4.92±0.22, respectively. Furthermore, in the APR group, the erythrocyte MDA levels were 4.50±0.43, 5.23±0.32, 5.62±0.40, and 5.69±0.42, and in the control group they were 4.84±0.14, 4.84±0.19, 4.94±0.23, and 5.16±0.25 respectively.

Erythrocyte MDA levels significantly increased in sham and APR groups that received trauma. However, no statistically significant difference could be found between the groups ($p>0.05$). Based on the hour 24 MDA values, there was a statistically significant difference between the control and

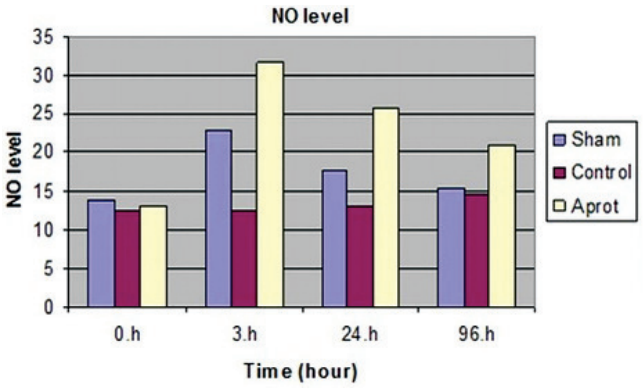

Figure-1: In the blood obtained using tubes with protein filter, nitric oxide measurements were made using IBL brand Roche Nitrite/Nitrate colour indicator kit.

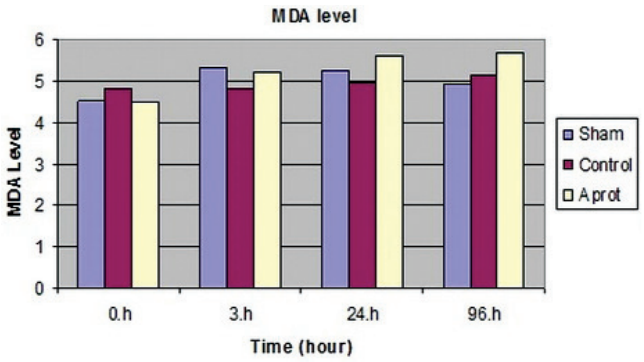

Figure-2: Malondialdehyde levels were determined using the spectrophotometric method with kits appropriate for Erythrocyte OLYMPUS AU 5200 brand apparatus.

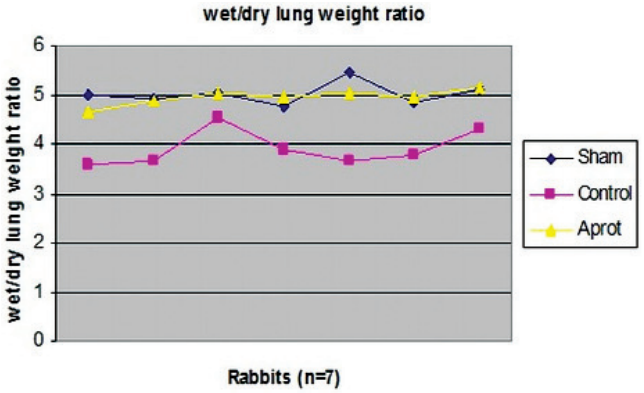

Figure-3: Wet/dry lung weight was noted, and the lungs and liver of the sacrifised rabbits were examined microscopically and macroscopically.

APR groups (p<0.05). Based on other values, no statistically significant difference could be determined between the groups (p>0.05). A significant effect of APR treatment on MDA level could not be determined (Figure-2).

The mean partial pressure carbon dioxide (PCO2) levels measured at 0, 3, 24 and 96 hours in sham group were 25±2.95, 38±3.46, 40.85±4.28, and 40.28±3.39 respectively. In the APR group, the levels were 24.57±2.28, 35.42±3.24, 38.14±3.68, and 36.85±2.17, and in the control group, they were 25±1.38, 28.57±2.79, 26.71±2.12, and 29±3.14 respectively.

PCO2 level significantly increased in sham and APR groups that received trauma. PCO2 values at hours 3, 24, and 96 showed statistically significant difference between the sham and control groups (p<0.05). A statistically significant difference was observed between the control and APR group (p<0.05). There was no statistically significant difference between the groups based on the other values (p>0.05). APR treatment could not be determined to have a significant effect on PCO2 level.

The mean partial pressure of oxygen (PO2) levels measured at 0, 3, 24 and 96 hours in the sham group were 96.28±1.11, 75.42±1.06, 71.28±1.03, and 59.42±0.92 respectively. Furthermore, in the APR group, the PO2 levels were 96±1.04, 83,57±0.95, 83.71±0.76, and 73,85±1.64, and in the control group, they were 95.85±1.22, 95.71±0.83, 96±1.62, and 96±1.87 respectively.

PO2 level was significantly low in sham and APR groups, which underwent trauma. Between the sham and control groups, there was a statistically significant difference in PO2 values at hours 3, 24, and 96 (p<0.05). Between the control and APR groups, there was a statistically significant difference (p<0.05). Between the sham and APR groups, a statistically significant difference at hour 24 PO2 values was noted (p<0.05). There was a statistically significant difference between the control and APR groups at hour 24 (p<0.05). Between sham and APR groups, 96 hour PO2 values were determined to have statistically significant difference (p<0.05). A statistically significant difference was found between the control and APR groups at hour 96 (p<0.05). With regards to other values, no statistically significant difference could be determined between the groups (p>0.05). APR treatment was determined to have an increasing effect on PO2 levels (p>0.05).

The average wet/dry lung weight ratio in sham, APR and control groups was found to be 5.02±0.23, 4.95±0.15, and 3.92±0.35, respectively.

The wet/dry lung weight ratio was determined to be higher in sham and APR groups that underwent trauma than the control group (p >0.05). Based on the wet/dry lung weight ratio, no statistically significant difference could be determined among the three groups (p>0.05). This ratio is important because it indicates the development of oedema in the lung associated with injury. The ratio was found to be lower in the APR group compared to the sham group (Figure-3).

Between sham and APR groups, statistically significant difference was determined based on lung histopathology, atelectasis, emphysema, oedema, septum damage, septum thickening, septum bleeding, septal hyperaemia, lymphocyte infiltration, neutrophil prevalence, neutrophil infiltration, and bronchial macrophage levels (p<0.05).

APR was found to have a positive effect on the recovery of emphysema, oedema, septum damage, septum bleeding, septal hyperaemia, lymphocyte infiltration, and bronchial macrophage values. However, this effect was not statistically significant (p>0.05). APR could not be determined to have a positive or negative effect on

atelectasis, bronchial damage, macrophage, neutrophil prevalence, septum thickening, neutrophil accumulation, alveolar neutrophil, bronchial neutrophil, mucus, apoptosis and eosinophils.

Discussion

When APR is at a concentration of the kallikrein inhibitor, its haemostatic and anti-inflammatory effects rise. In addition to inhibiting coagulation and fibrinolysis, APR also inhibits the release of vasoactive peptides and bradykinin. When kallikrein is inhibited, the production of high molecule weight kininogen, a direct precursor of bradykinin, is blocked. Based on the reports by Nagahiro et al, with bradykinin that increases during the ischaemia-reperfusion period, its repression by APR decreases the reperfusion damage.[10]

In their study where they created mild blunt chest trauma on rats, Türüt et al. determined an obvious drop in NO and MDA levels, in the group that was administered APR. However, this was not statistically significant.[11] Soejima et al. have determined that during events such as sepsis and multiple injuries accompanied with ARDS formation, NO plays and important role, and NO's fixed product, plasma nitrite (NI) increases 2 to 3.5 fold.[12]

In lung damage, dependent on ischaemia-reperfusion establishment, Ischiropoulos et al. have shown increased NO production.[13] In our study, contrary to other studies, the lack of any significant difference between the groups based on NO levels ($p>0.05$), suggests that the use of APR at a dose of 20.000 U/kg, does not have a significant effect on decreasing NO level in ALI.

MDA level, which is the final product of lipid peroxidation, is a good indicator for tissue oxidation. Giyasettin B. et al. have determined a significant decrease in plasma MDA levels after the administration of antioxidant agents.[14] In their study where Riise et al. examined antioxidant status and oxidative stress in patients who received lung transplant, they showed MDA, a final product of fat peroxidation in the plasma and bronchial fluid, to be elevated and it stayed elevated up to one year after the operation.[15]

Rahman et al. have shown decreased MDA levels in the lung tissue, as a result of adding APR to the protection fluid during cardiopulmonary bypass.[16] In addition, Eren et al. have determined a significant decrease in tissue MDA, in the 2 hour in situ normothermic ischaemia lung model, by adding APR to the lung protection solution.[17]

In our study, APR was not observed to have a positive effect on lowering serum MDA levels. The reason for this may be due to our application of APR intravenously as opposed to the APR added to the protection solution in the study by Eren et al.[17] This difference may also be caused by us looking at the serum MDA levels instead of tissue MDA levels. By having a longer experimental period, it might be possible to determine the time when maximum MDA levels end, thus clarifying when APR starts to affect MDA and what level it brings MDA in how much time. This situation may be related to the APR dose administered. In addition to this, in lung transplant cases, which we can be taken as injury, when plasma MDA levels that stay elevated for up to one year is taken into account, more long-term studies would provide a better understanding as to how APR influences plasma MDA levels.

Additionally, the measurement of elevated MDA levels could be considered an expected situation due to the lung with greater amount of damage.

One of the aspects that differentiate our study from the others is MDA levels being measured in the serum, instead of the lung tissue. If MDA level in the lung tissue was examined, the values may have been found to be different.

Türüt et al. determined lowered partial pressure of oxygen in arterial blood (PaO_2) levels in contused groups, and PaO_2 levels were significantly higher 6 hours after the contusion. They observed APR to facilitate significant improvement in the oxygenation capacity of the lungs.[11]

Similarly, in our study, serious hypoxia was observed in the rabbits right after trauma. In contused rabbits, PO_2 levels remained low throughout the study. PO_2 values of the APR group was significantly lower compared to the values of the control group ($p<0.05$). PO_2 values of the APR group showed improvement compared to the sham group values, starting from hour 24, until hour 96 ($p<0.05$). In the group which received APR, the decrease of PO_2 values at hour 96 suggests that APR does not have a significant effect on PO_2. The increase of damage, oedema and widespread bleeding in the lung tissue observed starting at hour 96, might have limited the effect of APR by negatively influencing gas exchange. This result suggests that APR may have a positive effect on PO_2. However, this effect disappears in the late period.

In a study by Ortolani et al., 180 minutes after blunt chest trauma, hypoxemia and CO_2 gas increase in the arterial blood were observed.[18] In our study, in APR and sham groups that underwent trauma, PCO_2 started to increase at hour 3 and continued to increase until hour 96. Between APR and sham groups, no statistical difference could be determined based on the hour 96 blood PCO_2 values. In

our study, no positive effect of APR could be determined on blood PCO_2 evaluation of the groups ($p>0.05$).

In one study, after the administration of APR, improvement in oxygenation, decrease in oedema formation and significant increase in compliance was noted.[19] In rats that were given APR, Türüt et al. have determined a significant improvement in alveolar haemorrhage and oedema score when compared to contused rats.[11] In our study, possibly to its antifibrinolytic effects, APR was observed to have positive effects on alveolar oedema. However, different from other studies, a significant effect of APR on alveolar haemorrhage could not be observed. More advanced and comprehensive studies are necessary to investigate this issue. In our study, in rabbits that were given APR, improvement in oxygenation and decrease in oedema formation were determined.

Tranbaugh et al. measured the amount of water in the lungs of 16 trauma patients who were in shock. In four patients with PC, lung fluid increased with resuscitation. However, in those without lung injury, shock associated with bleeding could not be found.[20] In our study, an increase in the lung's wet/dry weight ratio was determined in contused groups ($p>0.05$). Türüt et al. determined that APR significantly improved pulmonary haemorrhage and oedema score. In our study, however, a statistical difference was not found between the groups, based on wet/dry weight ratio of the lung. In contused groups, an increase was observed in lung's wet/dry weight ratio when compared to the control group. This supports the formation of oedema and bleeding in the lung associated with trauma. Compared to the sham group, the wet/dry lung weight ratio was determined to be lower in the APR group. This may indicate the effect of APR in decreasing lung oedema, even if it is minimal. This situation may be related to APR decreasing bleeding and edema formation due to trauma. However, in our study, APR had minimal effect on decreasing pulmonary oedema, but no significant effect could be determined on alveolar haemorrhage.

The importance of alveolar macrophages in ischaemia/repurfusion (I/R) associated lung injuries are understood even better recently. During lung transplantation, alveolar macrophages are activated in the early phase of the reperfusion.[21] The secreted proinflammatory cytokines promote neutrophil activation. In this case, neutrophils are attached to the vascular endothelial cells and following its activation, alveolar interstitial space is invaded, and at the end, it causes the production of FOR that leads to parenchyma damage in the lung. In various studies, during mesenteric microcirculation or cariopulmonary bypass, neutrophil extravasation associated with FOR production, was shown to be inhibited by APR.[22] Although the exact mechanism of action is not known, these results indicate APR to reduce lung reperfusion injury.[23]

In sheep with created ALI, an excessive amount of neutrophils were found in lung lymphatics and airways.[24] The oxygen radical released from the affected neutrophils lead to elastase tissue damage.[25] Also, in our study, an increase in neutrophils was observed in groups that underwent trauma. A significant effect of APR on neutrophil levels in the lung tissue could not be determined.

In our study, all rabbits that got ALI using the bilateral blunt chest trauma model, lived until hour 96 when they were sacrifised with the decapitation method. For this reason, a statistical comparison could not be made between the survival lengths of the groups. The effectiveness of aprotinin on the subjects that underwent blunt chest trauma enabled the rabbits to live until the end of the experiment, thus allowing us enough time to evaluate the lungs and examine pathological data. The bilateral blunt chest trauma model we developed can be an appropriate model for evaluating the effectiveness of drugs used in the treatment of ALI.

Conclusion

With the passage of time, a better understanding of the role of oxidant agents in the development of ALI and ARDS associated with blunt chest trauma brings forth antioxidant agents among treatment options. In the bilateral blunt chest trauma model, APR administration has partially positive effect on FOR plasma levels. Additionally, it has positive effects on the blood gas PO_2. It did not have a significant effect on PCO_2 levels. Contrary to what is known regarding haemorrhaging, a significant effect could not be determined. It was partially effective on lung histopathology. New and long-term studies on the use of different APR doses will facilitate a better understanding of drug effects on blood gases, biochemical values and lung tissue damage.

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