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İnvestigation of the Efficacy of Aminoguanidine in an Experimental Rat Model with Isolated Bilateral Pulmonary Contusion Due To Blunt Thoracic Trauma

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Abstract

İntroduction: In severe thoracic trauma pulmonary contusions are almost inevitable are associated with high morbidity and mortality. In this study we aimed to evaluate the antioxidant activity of aminoguanidine in pulmonary contusion.

Method: Sixty-three Sprague-male rats were used. Sham and aminoguanidine groups were exposed to isolated blunt thoracic trauma with a force of 1,512 joules. Aminoguanidine was administered intraperitoneally at a dose of 100 mg/kg 3 hours before the trauma and on the 1. and 2. day after the trauma. The contusion group was exposed to blunt thoracic trauma only. In all groups, arterial blood gas analysis and catalase and NO levels were done on the 0th, 1st, 2nd and 3rd days.

Results: PO2 levels were higher in the sham group compared to the contusion group, without statistical significance. On the third day, SaO2 levels were higher in the AG group compared to the contusion group. SaO2 levels were comparable in the AG and sham groups on days 1, 2 and 3. There was no difference between the PaO2 levels of the contusion and sham groups on the 2nd and 3rd days. There was no difference between the PaO2 levels of the AG and sham groups on the 1st, 2nd and 3rd days. We found no difference between the PaCO2 levels of the contusion and sham groups on the 0-3 days. There was no difference between the PaCO2 levels of the AG and sham groups on the 1st, 2nd and 3rd days. No difference was observed between the PaCO2 levels of the AG and contusion groups on the 1st, 2nd and 3rd days. No significant difference was found between the NO levels of the sham and the contusion groups on day 0. There was a significant difference between the sham and contusion groups on the 1st, 2nd and 3rd days. There was no statistically significant difference between the catalase enzyme activities of the sham and AG groups.

Conclusion: In our study, we showed that the use of aminoguanidine did not significantly reduce the severity of pulmonary contusion and the inflammatory reaction induced by thoracic trauma in rats.

Keywords: Blunt Thoracic Trauma,Aminoguanidine,Catalase activity

Introduction

Among all trauma cases, chest trauma is the third most common after head and neck and extremity traumas, respectively. It is the most common cause of death, especially in the first 4 decades of life. It occurs due to traffic accidents, occupational accidents, falls and assaults, and constitutes 25% of trauma-related deaths[1]. The mortality rate of isolated chest trauma is 5,5%, but if an additional organ system is injured, this rate rises to 12-15%, and if there is multiple organ injury, it increases up to 30-35%[2]. Pulmonary contusion is defined as trauma-induced alveolocapillary damage associated with overstretching or even rupture of alveoli, separation of alveoli from bronchioles, intraalveolar bleeding, and interstitial edema. Pulmonary contusion occurring in 30-75% of major thoracic trauma cases is a serious injury with high mortality and morbidity. It may be associated with various serious conditions ranging from simple dyspnea to respiratory failure requiring mechanical ventilation. Acute respiratory distress syndrome and multiple organ dysfunction may develop depending on the extent of the contusion[3]. Because the mortality rate of 11% in an isolated severe contusion increases to 22% in the presence of additional injuries. The incidence of ARDS is 17% in isolated contusions, but up to 78% with additional injuries. Pulmonary embolism and pneumonia are important factors that increase the mortality caused by pulmonary contusion. Pulmonary contusion involves an inflammatory process that occurs due to a mechanical damage, with a mechanism that is not fully known. Inflammatory response, and therefore, neutrophils, which are the major factors in endothelial / epithelial damage, are the most important factors determining prognosis[4]. Therefore, it is an issue open to the development of new treatment methods through further researches. In trauma-induced lung injury, gas exchange in bronchioles and alveoli is impaired, resulting in hypoxemia and hypercarbia. Leukocyte infiltration, production of inflammatory mediators, and free oxygen radi-

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cals have a significant role in the pathogenesis of this chain of events. Free oxygen radicals cause oxidative damage through lipid peroxidation, thereby disrupting the integrity of the cell membrane and increasing the permeability of the cells. The resulting hypoxia and hypovolemia may cause ischemia at the cellular level, resulting in necrosis. However, the formation of free oxygen radicals during reperfusion usually aggravates the tissue damage. Therefore, it is necessary to reduce the harmful effects of free radicals in patients with pulmonary contusion. In a healthy organism, the balance between the formation of free radicals and their elimination by antioxidants is called the oxidative balance. In animal models, antioxidant therapy has been shown to be beneficial against lung injury. Nitric oxide, which is effective in the inflammatory process, is synthesized from the amino acid L-arginine by the effect of nitric oxide synthetase. Nitric oxide (NO) synthesized from endothelium leads to pulmonary vasodilatation, reducing shunting in well ventilated lung regions, increasing oxygenation and decreasing pulmonary edema[5]. Expression of NOS in acute lung injury and inflammatory lesions of the colon has been shown to lead to overproduction of NO, resulting in the production of superoxide and peroxynitrite. The aminoguanidine, a nucleophilic hydralazine derivative from the biguanide group, inhibits nitric oxide synthase via its hydrazine group. It acts as an antioxidant to prevent the formation of reactive oxygen compounds and lipid peroxidation in cells and tissues, thus performing as a kind of free radical scavenger[6-7]. Catalase is an antioxidant enzyme found in peroxisomes within the cell. It catalyzes the reaction that converts hydrogen peroxide to water and oxygen. It oxidizes organic compounds such as phenol, formaldehyde and alcohol using H2O2, which is formed by the oxidation of glucose molecules during metabolism, especially in liver and kidney cells. Thus, toxic substances from the bloodstream are detoxified. Catalase exhibits its reducing activity on small molecules such as H2O2, methyl and ethyl hydroperoxides. It has no effect on lipid hydroperoxides which have large molecular structure[8]. One way of reducing tissue damage associated with NO and peroxynitrite is to inhibit the overproduction of NO by a specific inhibitor of iNOS, such as AG. The involvement of oxidative stress and inflammatory process in tissue damage induced us to investigate the effects of aminoguanidine on pulmonary contusion in an experimental rat model with isolated bilateral lung contusion due to blunt thoracic trauma. In the light of this information, our study aimed to reduce the endothelial / epithelial damage in lung contusion by suppressing the inflammatory mechanism and to investigate the effects of this reduction on mortality and morbidity.

Methods

The study was approved by the Animal Experiments Ethics Committee (14.05.2011) of the Hospital (2010/19) and used a total of 63 Sprague Dawley rats from Experimental Animal Production Laboratory. Before the experiment, the rats were kept in wire cages for 12 hours at night and 12 hours at daytime, in circadian rhythm at an ambient temperature of 20 to 26°C for 10 days. Twelve hours before the experiment, feeding was stopped, except for water. All rats were cared for in accordance with the principles of Care of Experimental Animals formed by the National Society for Medical Research (NSMR) and with the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institute of Health. The groups were formed by randomly assigning 7 rats to each group (Table 1). Group 1 (Sham) (n = 7), subjected to blunt thoracic trauma and not given aminoguanidine. Group 2 (Control) (n = 7), not subjected to blunt thoracic trauma but given aminoguanidine. Group 3 (Contusion) (n = 28), subjected to pulmonary contusion by blunt thoracic trauma and given saline via intraperitoneal route. A total of four subgroups were created for the 0th, 1st, 2nd and 3rd days. Group 4 (Aminoguanidine) (n = 21),

Table 1. SatO2, PaO2 (Blood gas) values between groups

Day	Sham	Contusion	Aminoguanidine	Aminoguanidine + control
SatO2				
0	94.85 (91.0-96.6)	65.03 (31-90)	-	90.51(84-96.6)
1	94.85 (91.0-96.6)	65 (32-89.1)	87.3 (78.1-95)	90.51(84-96.6)
2	94.85 (91.0-96.6)	77.27 (64.3-86.1)	81.48 (75-86.4)	90.51(84-96.6)
3	94.85 (91.0-96.6)	80.7 (71.8-83)	89.78 (84.5-96.8)	90.51(84-96.6)
PaO2				
0	81.98 (64.7-94.1)	47.03 (27.3-70.2)	-	75.17(53.7-85.7)
1	81.98 (64.7-94.1)	48.03 (27.3-71.2)	79.3 (57-104)	75.17(53.7-85.7)
2	81.98 (64.7-94.1)	67.17 (57.3-78.7)	61.94(55.2-77.4)	75.17(53.7-85.7)
3	81.98 (64.7-94.1)	65.25 (52-87)	72.88 (58-92.4)	75.17(53.7-85.7)

subjected to pulmonary contusion by blunt thoracic trauma and given aminoguanidine. A total of three subgroups were created for the 1st, 2nd and 3rd days. In the groups given aminoguanidine, the administration was performed by intraperitoneal route at a dose of 100 mg / kg 3 hours before the trauma. In the control and aminoguanidine groups, 100 mg / kg dose of aminoguanidine was continued on the 2nd and 3rd days. Rats which were fasted 12 hours prior to the procedure were anesthetized with 100 mg / kg xylazine and 10 mg / kg ketamine. The lexon platform was used to create pulmonary contusion through the effect transmitted to the thoracic wall by saving the sternum and heart. The rats were exposed to blunt thoracic trauma with a force of 1,512 joules, except for sham and control groups. After the trauma, 2 cc blood samples were taken from the abdominal aorta of 7 rats in contusion and sham groups under anesthesia. In these blood samples, it was aimed to determine the levels of PaO2, SaO2 and PCO2, and of catalase and malondialdehyde among oxidative stress parameters. On day 1, the sham group, 0th and 1st day subgroups of the contusion group and 1st day subgroup of the AG group were sacrificed. There are a total of 4 rats died on the 1st, 2nd and 3rd days. On day 2, administration of the drug continued in the remaining rats (not sacrificed). On the 2nd day, the 2nd day sub-groups of the contusion group and AG groups were subjected to the procedures performed in the sham group and the 1st day subgroup of the AG group. On the 3rd day, the 3rd day subgroups of the contusion group and AG groups were subjected to the procedures performed in the sham group and the 1st day subgroup of the AG group. The exploration revealed that the causes of death in the rats were pneumothorax, hemothorax, and cardiac tamponade (Figure 1, 2).

Blood samples were taken into EDTA-K3 anticoagulant tubes and centrifuged at 3000 rpm for 10 minutes. After centrifugation, the supernatant plasma was transferred to Eppendorf tubes and stored at -80 ° C until analysis. Plasma levels of NO and catalase were studied (Figure 3).

Statistical Analysis

Data were recorded in pre-prepared forms. The recorded data was numbered and transferred to the computer. Statistical analysis were performed using SPSS for Windows version 15.0. Median, minimum and maximum values of the groups were calculated. Intergroup comparison was performed by Kruskal-Wallis variance analysis. Chi-Square Test was used for intergroup comparison of categorical data. A p value of <0.05 was considered significant.

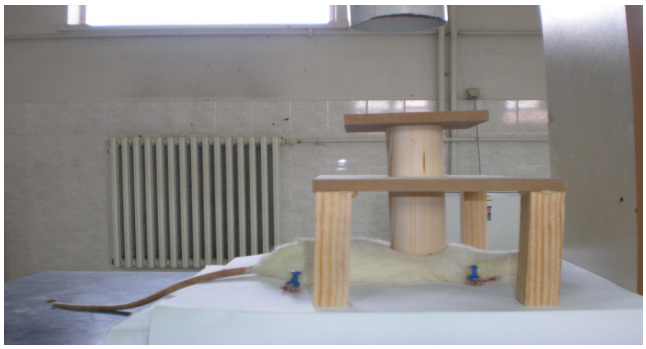

Figure 1. Mechanism used for blunt thoracic trauma

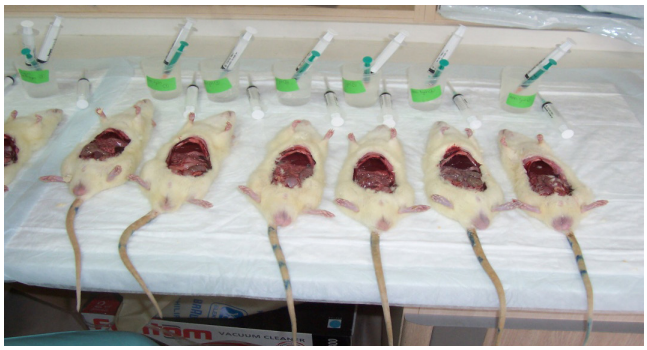

Figure 2. System for collection of blood samples for serial blood gas and other studies in rats which were under anesthesia and underwent trauma.

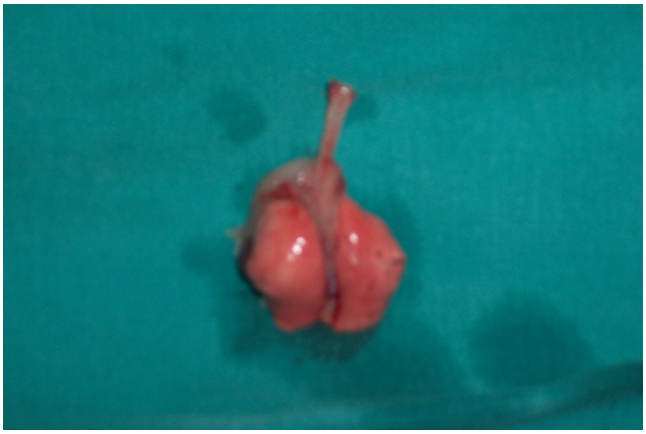

Figure 3. Lung taken from the sham group after sacrification

Results

Evaluation of the SaO2 and PaO2 levels showed no difference between the Sham and AG groups on the 1st, 2nd and 3rd days, whereas a significant difference between sham and contusion groups on the 2nd and 3rd days ($p < 0.05$). There was no significant difference in the SaO2 levels of AG and contusion groups on the 1st and 2nd days ($p > 0.05$). The SaO2 levels of the AG group on day 3 were significantly different compared to the contusion group ($p < 0.05$). There was no statistically significant difference between paO2 levels of AG and contusion groups. No significant difference was found between the groups Sham and AG in terms of PaCO2 levels on the 1st, 2nd and 3rd days ($p > 0.05$). No significant difference was found between the sham and contusion groups in terms of PaCO2 levels on 0th and 1st days. Significant differences were found in paCO2 levels between the sham and contusion groups on the 2nd and 3rd days ($p < 0.05$) (Table 1). No significant difference was found between the AG and the contusion groups in terms of paCO2 levels ($p=0.05$) (Table 2). No significant difference was found between the NO levels of the sham and the contusion groups on day 0. There was a significant difference between the sham and contusion groups on the 1st, 2nd and 3rd days ($p < 0.05$) (Table 2). We found a significant difference between the groups Sham and AG on the 1st and 2nd days, with no significant difference on the 3rd day ($p > 0.05$). No significant difference was found between the Sham and AG control groups on the 1st, 2nd and 3rd days ($p > 0.05$). Comparison of AG and contusion groups showed a decrease in NO levels in AG group, with no statistically significant difference ($p > 0.05$). No significant difference was found between the AG and AG control groups in terms of NO levels ($p > 0.05$) (Table 3). No significant difference was found between the catalase enzyme activities of sham and contusion groups on all days ($p > 0.05$) (Table 3). There was no statistically significant difference between Sham and AG groups ($p > 0.05$). No significant difference was found between Sham and AG control groups ($p > 0.05$). The comparison of AG and contusion groups showed a significant increase in catalase enzyme activity on days 1 and 2 in AG group ($p < 0.05$), without significant difference on day 3 ($p > 0.05$). No significant difference was found between the AG and AG control groups in terms of catalase enzyme activity ($p > 0.05$) (Figure 4).

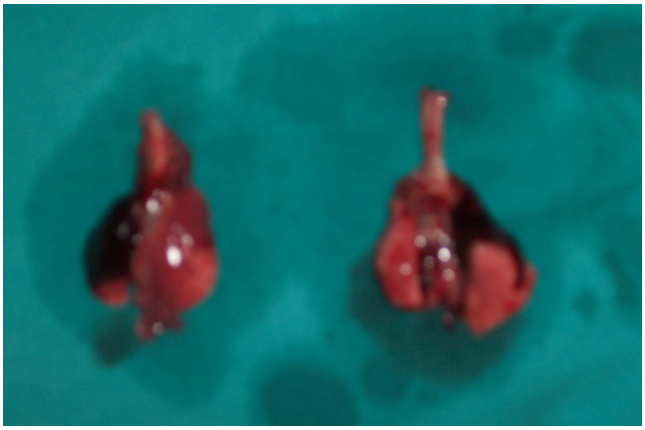

Figure 4. Hemothorax in rats died after contusion

Table 2. Nitric oxide values between groups

Day	Sham	Contusion	Aminoguanidine + contusion	Aminoguanidine + control
0.	3.7(1.68-6.76)	8.58 (1.72-20.05)	-	3.06(0.35-4.6)
1.	3.7(1.68-6.76)	10.3(4.64-16,6)	5.25(1,6-10.68)	3.06(0.35-4.6)
2.	3.7(1.68-6.76)	9.77(2.6-11.6)	7.26(4.38-9.54)	3.06(0.35-4.6)
3.	3.7(1.68-6.76)	9.78(2.6-13.5)	4.32 (3.37-5.5)	3.06(0.35-4.6)

Table 3. Nitric oxide values between groups

Day	Sham	Contusion	Aminoguanidine + Contusion	Aminoguanidine + control
0.	12.51(7.5-18.9)	12.16 (7.7-14.3)	-	11.4(6.8-15)
1.	12.51(7.5-18.9)	9.8 (7.42-10.8)	12.7 (9.5-14.7)	11.4(6.8-15)
2.	12.51(7.5-18.9)	9.28 (6.1-11.7)	11.6 (9.7-13.2)	11.4(6.8-15)
3.	12.51(7.5-18.9)	8.76 (5.6-11.6)	10.8 (9.6-13)	11.4(6.8-15)

Discussion

Trauma is one of the most common causes of death in the young adult population. Chest traumas, which usually occur as a result of traffic accidents, occupational accidents, assaults and falls, are the third most common after head and limb traumas.[9] Pulmonary contusion can be seen in approximately 50% of patients with chest trauma, which is one of the most important factors that increase the mortality by adversely affecting the clinical course. It is important to investigate the preventable causes of death to reduce mortality from chest trauma seen predominantly in young adults. Secondary damage following a trauma causing pulmonary contusion involves oxidative stress, lipid peroxidation, and inflammatory response.[10, 11] Oxidative stress is the deterioration of the physiological balance between the formation of free radicals and their elimination by antioxidants. Lipid peroxidation is the process of degradation of polyunsaturated fatty acids by oxidation. After this process, the resulting products can adversely affect the surrounding tissues. The effects of lipid peroxidation on the cell membrane are irreversible.[12] The presence of superoxide radicals (SORs) in the environment leads to the synthesis of NO and peroxynitrite and triggers lipid peroxidation. These released SORs can cause oxidative damage through lipid peroxidation.[13, 14] Aminoguanidine is a compound that inhibits selectively and competitively inducible nitric oxide synthase and thereby causes reduced nitric oxide formation. This is an effective antioxidant and free radical scavenger. It prevents the formation of lipid peroxidation in cells and tissues.[15] There are studies which have proven that aminoguanidine reduces both the inflammatory response and the degree of pulmonary injury.[16] In a study, O. Soy et al. showed that initiation of aminoguanidine treatment (100mg / kg) immediately after the trauma prevented both nitric oxide production and lipid peroxidation and improved the functional status of animals.[17] The administration of systemic antioxidant agents in intensive care patients with mechanical ventilator support reduced the plasma levels of lipid peroxidation products and the amount of mucus in the respiratory tract, resulting in better clinical results. In the present study, NO levels in the contusion group were significantly higher than the sham group. In addition, NO levels were lower in the aminoguanidine group compared to the contusion group. These elevated levels were attributed to trauma-induced alveolar capillary damage and subsequent ventilation/perfusion mismatch. No statistically significant difference was found between NO levels of aminoguanidine and contusion groups. In addition, there was no significant difference between NO levels of the sham and aminoguanidine control groups. In our study, it was found that CAT enzyme levels increased significantly in the contusion group compared to the Sham group. No significant difference was found between CAT enzyme activities of the contusion and AG groups ($p > 0.05$). In addition, there was no significant difference between CAT enzyme activities of the sham and AG control groups. The data obtained in our study were consistent with the literature and it was shown that aminoguanidine at a dose of 100 mg / kg might have protective effects against pulmonary damage mechanisms. We believe that it may be useful to carry out further studies with different doses to reveal the maximum efficacy of AG.[18]

In our study, we observed that the use of aminoguanidine in rats exposed to isolated pulmonary contusion by blunt trauma reduces the severity of pulmonary contusion and minimizes the inflammatory reaction.

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Conflict of interest: None declared.

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