Effect of nitric oxide synthase inhibitors in acute lung injury due to blunt lung trauma in rats

**ABSTRACT**

**Background:** This study aims to investigate the effects of blunt lung trauma performed in experimental rat model on lung tissue and blood as well as proinflammatory cytokines, oxidant-antioxidant enzymes and histopathological parameters after Nγ-nitro-L-arginine methyl ester and N-iminoethyl-L-ornithine administration.

**Methods:** The study included 50 adult male Wistar albino rats (weighing 350 to 400 g). Rats were randomly allocated into four groups. Except in the control, moderate-level pulmonary contusion was created in all other groups. Intraperitoneal saline solution was performed in groups 1 and 2, 25 mg.kg⁻¹ Nγ-nitro-L-arginine methyl ester in group 3, and 20 mg.kg⁻¹ N-iminoethyl-L-ornithine in group 4. Blood and lung tissues were studied biochemically and histopathologically.

**Results:** Best outcomes were recorded statistically significantly in groups as well as proinflammatory cytokines, oxidant-antioxidant enzymes and histopathological parameters after Nγ-nitro-L-arginine methyl ester and N-iminoethyl-L-ornithine administration when malondialdehyde response, mucous and histopathological values were examined. Significant improvement was detected in superoxide dismutase values in the group with administration of Nγ-nitro-L-arginine methyl ester and protective effects were formed with administration of both Nγ-nitro-L-arginine methyl ester and N-iminoethyl-L-ornithine.

**Conclusion:** Free oxygen radicals and lipid peroxidation played a role in pulmonary contusion after blunt lung trauma. According to biochemical and histopathological outcomes, effects of inflammation were decreased and protective effects were formed with administration of both Nγ-nitro-L-arginine methyl ester and N-iminoethyl-L-ornithine.

**Keywords:** Acute lung injury; oxidative stress; trauma.

**ÖZ**

**Amaç:** Bu çalışmada deneySEL çıkan modelli üzerinde uygulanılan kurt akciğer travmasının akciğer dokusu ve kandaki etkileri ile Ngamma-nitro-L-arginin metil ester ve N-iminoetil-L-ornitin uygulamasını takiben proenflamattuvat sitokinler, oksidan-antioksidan enzimler ve histopatolojik parametreler araştırıldı.

**Çalışma planı:** Çalışmaya 50 erişkin erkek Wistar albino çıkan (ağırlık 350-400 g) dahil edildi. Çıkanlar rastgele dört gruba ayrıldı. Kontrol dışındaki diğer tüm gruplarda orta şiddette pulmoner kontüzyon oluşturuldu. Grup 1 ve 2'ye intraperitoneal salın soluyuşu, grup 3'e 25 mg.kg⁻¹ Ngamma-nitro-L-arginin metil ester, grup 4'ye 20 mg.kg⁻¹ N-iminoetil-L-ornitin uygulandı. Kar ve akciğer dokuları biyokimyasal ve histopatolojik açıdan incelendi.


**Sonuç:** Kent akciğer travması sonrası pulmoner kontüzyonda serbest oksijen radikalleri ve lipid peroksidsiyonun rol oynamadı. Biyokimyasal ve histopatolojik sonuçlara göre, enfamasyon etkileri hem Ngamma-nitro-L-argininin metil ester hem N-iminoetil-L-ornitin uygulaması ile azalı ve koruyucu etkiler oluşturdu.

**Anahat sözcükler:** Akut akciğer hasarı; oksidatif stres; travma.
Blunt chest traumas are major clinical problems causing injury at lungs and other structures and known as an independent risk factor for the development of severe acute lung injury (ALI) and acute respiratory distress syndrome (ARDS).[1,2] Both syndromes are characterized by hypoxemia that is resistant to oxygen therapy, decreased lung compliance, increased microvascular permeability, and the presence of diffuse alveolar damage with alveolar edema.[3-5] Although the physiopathology is not clear, pulmonary contusion (PC) is the most frequent diagnosis in thoracic injury (17-25%) related to blunt chest trauma.[6,7] In an experimental study, authors sought to examine the onset of ALI and found that ALI was evident by four hours at early phase of the trauma. The pathogenesis of ALI and ARDS involves multiple mechanisms including oxidant-induced inflammatory damage to the alveolar wall. Pulmonary injury may lead to a variety of pathophysiological alterations; cytokines, reactive oxygen species, and proteolytic enzymes increasing the alveolar-capillary membrane permeability.[8-12]

Alveolar macrophages are mobile phagocytic cells located within the alveoli and small airways of the lungs, and produce nitric oxide (NO) via NO synthase (NOS) that is inducible by immunological stimuli like cytokines. Rat alveolar macrophages produce large quantities of NO in response to inflammation and NO formation proceeds along an approximately linear time course during the four-hour incubation period.[13-16] Currently, there is a significant debate over whether the inhibition of NO is beneficial or harmful and, if the excessive amount of NO should be suppressed, what isoform should be targeted. The protective effects of antioxidants such as superoxide dismutase (SOD) have led to the suggestion that toxic metabolites of oxygen may be the key initiators of injury. Blocking NO formation may have significant protective effects against injury in a variety of inflammatory diseases.[17,19] Exogenous arginine treatment was shown to ameliorate the pulmonary function in sheep subjected to lung injury. L-arginine analogues such as N-iminoethyl-L-ornithine (L-NIO), a specific NOS inhibitor, or Ngamma-nitro-L-arginine methyl ester (L-NAME), a competitive NOS inhibitor, may be useful in preventing tissue damage.[20-22] Since L-NIO has recently been described as a highly potent and irreversible inhibitor of NOS, it was tested for its ability to protect against immune complex-induced vascular injury in rats. When compared to the other analogues of L-arginine, more rapid onset in its inhibitory effects occur when added to the phagocytic cells.[23]

Although the standard treatment options for ALI/ARDS remain largely supportive, there is an accumulating support for treating the activation of a secondary inflammatory response.[24-33] We carried out a blunt chest trauma on rats and performed two different antioxidant agents and aimed to seek the effects for both. Therefore, in this study, we aimed to investigate the effects of blunt lung trauma performed in experimental rat model on lung tissue and blood as well as proinflammatory cytokines, oxidant-antioxidant enzymes and histopathological parameters after L-NAME and L-NIO administration.

**MATERIALS AND METHODS**

This study was conducted at Kocaeli University Faculty of Medicine in December, 2012. A total of 50 adult male Wistar albino rats (weighing, 350 to 400 g) were randomly divided into four groups. All animals were housed in the Kocaeli University Experimental Animals Research Unit in climate-controlled rooms (24±1°C). Food and water were available ad libitum. The study protocol was approved by the Kocaeli University Medical School Experiments Ethics Committee. The study was conducted in accordance with the guiding principles for the care and use of laboratory animals (National Institutes of Health publication No. 85-23, revised 1985).[34]

Lung contusion was induced using the model lung contusion described by Yucel et al.[35] for blunt chest trauma. This model was essentially made up from three components: a 90 cm long plastic tube with an inner diameter of 14 mm and outer of 21 mm with tube stabilization part, a support table to fix the rat on its metal plate and make the tube stable in vertical position, and metal cylindrical weights with variable sizes in grams. The rat was placed at left lateral decubitus position on the platform positioned just under the cylindrical tube through which the weight was dropped. Then a cylindrical weight of 500 g was dropped from a definite height (50 cm) towards the right lung; through the vertical stainless steel tube that was positioned on a platform as indicated above. The impact energy created via this mechanism was calculated by using the equation \( E = mgh \) \[E: energy; g: gravity (9.81 m/s^2); h: height form the platform (50 cm); m: mass of the cylindrical weight (0.5 kg)\]. The total energy transferred to the chest wall of the rats was calculated as 2.45 joules.

Animals were randomly allocated into four groups: group 1 (control, n=5), group 2 (sham+contusion, n=15), group 3 (contusion+L-NAME, n=15), and group 4 (contusion+L-NIO, n=15). Rats were anesthetized with
an intraperitoneal injection of 100 mg.kg\(^{-1}\) ketamine hydrochloride (50 mg.mL\(^{-1}\), Ketalar, Pfizer, Istanbul, Turkey) and Xylazine 10 mg.kg\(^{-1}\) (20 mg.mL\(^{-1}\), Rompun, Bayer, Mississauga, Canada). Blunt chest trauma was then performed to groups 2, 3, and 4. Analgesia was provided by morphine sulphate (0.05 mg.kg\(^{-1}\)), administered intraperitoneally. Following the procedure, all subgroups were transferred to their cages. Rats were observed for breathing, respiratory movements and cardiac rhythm. After 25 minutes of observation, animals in groups 1 and 2 received intraperitoneal saline, group 3 was given 25 mg.kg\(^{-1}\) L-NAME, and 20 mg.kg\(^{-1}\) L-NIO was given to group 4. All subgroups were observed for four hours in their cages. Median sternotomy and sacriﬁﬁcation were performed to all rats under anesthetic agents. Because of the right-sided thorax trauma, right lung was resected totally and weighed; a small part (0.21 g–0.46 g) was separated for biochemical studies. Blood samples were collected from the descending aorta, centrifuged and stored at -80°C until further analysis.

Biochemical parameters were studied in blood and lung tissues; tissue samples were washed in 0.9% sodium chloride and kept at -80°C until analyzing. Tissue samples were homogenized with cold 1.15% sodium chloride and kept at -80°C until analyzing. Tissue lipid peroxide levels, expressed in terms of MDA, were determined according to the method of Buege and Aust\(^{[36]}\) as nmol/100 mg protein. The protein concentrations were determined by the method of Lowry et al.\(^{[40]}\) Blood samples after centrifuging and storing were analyzed for tumor necrosis factor-alpha (TNF-\(\alpha\)), interleukin (IL)-1, IL-6, IL-10, C-reactive protein (CRP) levels, measured with enzyme-linked immunosorbent assay method using microarray device.

More of the lung tissue samples were immediately fixed in 10% formaldehyde after removal, dehydrated in graded concentrations of ethanol, cleared in xylene. Horizontal sections taken from all lobes were sampled and embedded in paraffin blocks. At least eight tissue sections of 5-μm thickness were obtained and then stained with hematoxylin eosin and examined under a light-microscope by a specific pathologist in a blinded manner for 14 parameters. All histopathological changes were documented in each lung tissue, including atelectasis, emphysema, intra-alveolar hemorrhage, mucus, bronchial injury, septal hyperemia, alveolar edema and congestion, eosinophil, macrophage, bronchial macrophage, leukocyte infiltration, lymphocytic infiltration, alveolar leukocyte and bronchial neutrophil degrees.

Atelectasis, emphysema, intra-alveolar hemorrhage, alveolar edema, and septal hyperemia were scored on scale from 0 to 3 where 0: absence of pathology (pathology <5%), 1: mild, limited at one lobe at a small focus (<10%), 2: moderate, diffused at same lobe or multiple foci (10-20%) (or hemosiderin included macrophage deposits at interstitium for macrophages), and 3: severe, diffuse at multiple foci (>20%). Bronchial injury was scored as normal: 0, erosion: 1 and ulceration: 2, and mucus as 0: none, 1: localized, 2: diffuse. Leukocyte infiltration was also evaluated to determine the severity of expected inflammation occurred due to the contusion. Each section was divided into 10 subsections, and leukocytic infiltration was examined in each of the subsections at a magnification of 400× with the following scale; 0: no extravascular leukocytes; 1: <10 leukocytes; 2: 10-45 leukocytes; 3: >45 leukocytes. The average of the numbers was used for comparison.\(^{[41]}\)

**Statistical analysis**

The results were displayed as mean ± standard error. All data were tested for normal distribution. Kruskal-Wallis test or analysis of variance (post hoc Tukey’s multiple comparison test) was used to evaluate the data. The GraphPad Prism 3.0 program (GraphPad Software, San Diego, CA, USA) was used for all the statistical analyses.

**RESULTS**

None of the rats died during the experiment including the five in the control group and 45 subjected to unilateral blunt chest trauma and all contused rats exhibited similar behavior concerning mobilization and activity during the follow up period. Four hours after the trauma, median sternotomy grossly indicated the effects of blunt trauma on the affected lung; showed contused areas with heterogeneous pattern differing from the other side.

Biochemical analysis of the lung tissue in blunt trauma model was assessed after sacriﬁﬁcation and results of MDA, SOD, GSH, NO, and protein are shown in Table 1.

When compared with control (group 1) and treatment groups (groups 3 and 4), tissue MDA levels...
in group 2 (trauma) showed statistically significant increase according to all groups (p<0.001).

Tissue SOD levels in group 2 showed the most significant decrease (p<0.05) compared to all other groups (groups 1, 3 and 4). In group 2, lung contusion due to blunt trauma caused SOD activity to decrease in rat lung tissue. Levels of SOD were elevated in groups 3 and 4 compared to group 2, with a significant difference between groups 2 and 3 (p<0.01). There was no statistically significant difference between groups 3, 4 and 1.

When all groups were compared to each other, tissue GSH levels in group 2 showed the most significant decrease. This decrease was statistically significant between groups 1 and 2 (p<0.01). Comparison of groups 3, 4 and the control group revealed that the difference in GSH levels was not statistically significant (Table 1).

When compared with all other groups (groups 1, 3, and 4), NO levels in group 2 showed the most significant increase. Increase was obvious in all trauma-applied groups (groups 2, 3, and 4), but mostly in L-NAME group. There was a slight decrease in L-NIO applied group compared to group 2, while without significance (Table 1).

Protein levels were mostly elevated in group 2 compared to group 1 and treatment groups (groups 3 and 4). This elevation was statistically significant between groups 1 and 2 (p<0.05). There was no statistically significant difference between other groups.

When we evaluated the blood sample results shown in Table 2; TNF-α, IL-1, IL-6 and IL-10 levels were increased with trauma in group 2 and decreased with L-NIO in group 4. L-NAME administration in group 3 showed no difference in TNF-α, IL-1, IL-6, or IL-10

### Table 1. Comparison of biochemical parameters of tissue analysis results between control, sham and drug-administered groups, obtained four hours after contusion

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n=5)</th>
<th>Trauma Group (n=15)</th>
<th>Trauma + L-NAME Group (n=15)</th>
<th>Trauma + L-NIO Group (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>MDA</td>
<td>0.230±0.03</td>
<td>0.825±0.11</td>
<td>0.145±0.01</td>
<td>0.148±0.02</td>
</tr>
<tr>
<td>SOD</td>
<td>13.86±1.33</td>
<td>7.804±0.54</td>
<td>14.84±2.01</td>
<td>10.95±1.10</td>
</tr>
<tr>
<td>GSH</td>
<td>50.17±10.41</td>
<td>17.16±7.02</td>
<td>37.64±6.95</td>
<td>38.25±4.77</td>
</tr>
<tr>
<td>NO</td>
<td>0.017±0.008</td>
<td>0.059±0.009</td>
<td>0.094±0.02</td>
<td>0.057±0.01</td>
</tr>
<tr>
<td>Protein</td>
<td>108.5±3.48</td>
<td>174±9.83</td>
<td>139.6±11.76</td>
<td>142.2±15.20</td>
</tr>
</tbody>
</table>

L-NAME: Ngamma-nitro-L-arginine methyl ester; L-NIO: N-iminoethyl-L-ornithine; SD: Standard deviation; MDA: Malondialdehyde; SOD: Superoxide dismutase; GSH: Glutation peroxidase; NO: Nitric oxide; * Group 1 vs. group 2; † Group 2 vs. group 3 and group 4; ‡ Group 2 vs. group 3; § Group 1 vs. group 2.

### Table 2. Comparison of biochemical parameters of blood analysis results between control, sham and drug-administered groups, obtained four hours after contusion

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n=5)</th>
<th>Trauma Group (n=15)</th>
<th>Trauma + L-NAME Group (n=15)</th>
<th>Trauma + L-NIO Group (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>TNF-α</td>
<td>44.21±3.47</td>
<td>161.6±48.26</td>
<td>168.6±46.57</td>
<td>70.32±25.27</td>
</tr>
<tr>
<td>IL-1</td>
<td>58.47±15.32</td>
<td>214.5±52.16</td>
<td>243.0±51.02</td>
<td>150.10±67.97</td>
</tr>
<tr>
<td>IL-6</td>
<td>38.88±3.14</td>
<td>129.3±40.47</td>
<td>130.3±36.18</td>
<td>60.45±23.78</td>
</tr>
<tr>
<td>IL-10</td>
<td>120.10±63.95</td>
<td>198.2±86.48</td>
<td>210.2±75.90</td>
<td>79.57±39.05</td>
</tr>
<tr>
<td>CRP</td>
<td>40.96±23.05</td>
<td>115.50±10.20</td>
<td>74.09±15.64</td>
<td>61.07±13.53</td>
</tr>
</tbody>
</table>

L-NAME: Ngamma-nitro-L-arginine methyl ester; L-NIO: N-iminoethyl-L-ornithine; SD: Standard deviation; TNF-α: Tumor necrosis factor alpha; IL: Interleukin; CRP: C-reactive protein; * Group 1 vs. group 2.
levels. There was no statistically significant difference between any groups.

C-reactive protein, another acute phase reactant mostly used in clinical laboratory conditions, was increased in group 2 statistically significantly \((p<0.05)\) compared to group 1. Administration of both L-NAME and L-NIO (groups 3 and 4) caused a decrease compared to group 2, although without statistical significance. Difference between groups 1, 3, and 4 was also not significant (Table 2).

Histopathological assessment of lung tissue results is shown in Table 3 as scores, and as microphotograph in Figure 1. Scores of atelectasis, emphysema, hemorrhage, mucus, septal hyperemia, alveolar edema and congestion, eosinophil, leukocyte infiltration, and lymphocyte infiltration were significantly higher in group 2 compared to group 1 \((p<0.05)\). Bronchial injury, alveolar leukocyte and bronchial neutrophil scores were also higher in group 2, while without significance. Mucus was the only parameter that showed a statistically significant decrease when comparing group 2 with groups 3 and 4 \((p<0.01)\). Eosinophile levels were increased in all trauma groups (groups 2, 3, and 4) \((p<0.01)\). Although not statistically significant, a serious decrease was detected in scores of atelectasis, hemorrhage, bronchial injury, alveolar edema and congestion, leukocyte infiltration, alveolar leukocyte, and bronchial neutrophil in groups 3 and 4 compared to group 2.

**DISCUSSION**

In this study, we carried out a unilaterally blunt chest trauma on rats, then performed L-NAME or L-NIO as two different antioxidant agents and aimed to seek for both the effects of the trauma and therapeutic efficiency of the drugs by evaluating lung tissue histopathology, oxidative system parameters and serum cytokine levels. Nitric oxide synthase is an enzyme that catalyzes the production of NO from L-arginine. L-NIO and L-NAME are NOS inhibitors and cause concentration-dependent inhibition of the

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**Table 3. Comparison of histopathological parameters of tissue analysis results between control, sham and drug-administered groups, obtained four hours after contusion**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Group ((n=5))</th>
<th>Trauma Group ((n=15))</th>
<th>Trauma + L-NAME Group ((n=15))</th>
<th>Trauma + L-NIO Group ((n=15))</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atelectasis</td>
<td>0</td>
<td>1.267±0.18</td>
<td>0.533±0.19</td>
<td>1.00±0.22</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Emphysema</td>
<td>0.800±0.20</td>
<td>2.00±0.17</td>
<td>2.200±0.22</td>
<td>1.800±0.20</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>0</td>
<td>1.267±0.15</td>
<td>1.000±0.24</td>
<td>1.133±0.21</td>
<td>&lt;0.05***</td>
</tr>
<tr>
<td>Mucus</td>
<td>0</td>
<td>1.000±0.19</td>
<td>0.067±0.07</td>
<td>0.133±0.09</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Bronchial injury</td>
<td>0.400±0.24</td>
<td>1.400±0.25</td>
<td>1.200±0.28</td>
<td>1.200±0.24</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Septal hyperemia</td>
<td>0.200±0.20</td>
<td>1.667±0.16</td>
<td>1.667±0.13</td>
<td>1.267±0.18</td>
<td>&lt;0.01‡</td>
</tr>
<tr>
<td>Alveolar edema-congestion</td>
<td>0.200±0.20</td>
<td>1.800±0.20</td>
<td>1.467±0.13</td>
<td>1.333±0.16</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Eosinophile</td>
<td>0.400±0.40</td>
<td>1.800±0.17</td>
<td>1.933±0.18</td>
<td>1.733±0.18</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Macrophage</td>
<td>1.000±0.32</td>
<td>1.000±0.19</td>
<td>1.200±0.24</td>
<td>1.267±0.18</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Bronchial macrophage</td>
<td>0.400±0.24</td>
<td>0.400±0.13</td>
<td>0.733±0.18</td>
<td>0.600±0.23</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Leukocyte infiltration</td>
<td>0</td>
<td>1.200±0.22</td>
<td>0.667±0.19</td>
<td>0.400±0.19</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Lymphocytic infiltration</td>
<td>0.800±0.20</td>
<td>2.200±0.20</td>
<td>2.467±0.19</td>
<td>2.133±0.19</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Alveolar leukocyte</td>
<td>0.200±0.20</td>
<td>0.867±0.19</td>
<td>0.333±0.13</td>
<td>0.400±0.13</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Bronchial neutrophil</td>
<td>0.600±0.24</td>
<td>1.267±0.21</td>
<td>1.067±0.25</td>
<td>0.733±0.25</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

L-NAME: N\(\gamma\)-nitro-L-arginine methyl ester; L-NIO: N-iminoethyl-L-ornithine; SD: Standard deviation; * Group 1 vs. group 2; ** Group 1 vs. group 3; *** Group 1 vs. group 2 and group 4; † Group 2 vs. group 3 and group 4; ‡ Group 1 vs. group 2 and group 3; § Group 1 vs. group 2 and group 4; ¶ Group 1 vs. group 3; § Group 1 vs. group 3 and group 4.
Ca²⁺-dependent NOS. The L-arginine derivative L-NAME decreases alveolar epithelium damage and NO production in lung injury in rats and has been widely used to inhibit constitutive NOS in different biological systems.\cite{32,33,42,43} Clinical or experimental studies about L-NIO have not provided definitive explanations.

Various blunt thorax trauma models have been previously established. The pathophysiology of PC and lung injury with different treatment strategies have been studied.\cite{11,12,25,27,28,44-47} Pulmonary contusion is associated with a progressive inflammatory response mediated by local and systemic immunological alterations and with a leukocyte-mediated secondary inflammatory response leading to capillary leaking, interstitial edema and protein extravasation, proteolytic and lipolytic enzymes, and reactive oxygen species, macrophages and neutrophils.\cite{11,12,25-28,48,49}

Trauma, in general, is the most frequent cause of mortality after atherosclerosis and cancer at all ages, and is the leader below 40 years of age. Thoracic trauma composes 25% of all deaths and PC is the most frequently diagnosed injury due to blunt chest trauma.\cite{7,31,48-51} Despite the increasing cases of ALI due to blunt chest trauma, there is no exact treatment strategy. Today; fluid restriction, steroids, antibiotics, diuretics,
oxygen and mucolitics are generally used for treatment in ALI occurring after blunt chest trauma.[52] There are complex autocrine and paracrine interrelationships of cytokines, as well as proinflammatory mediators that initiate and amplify the inflammatory response in ALI. The cellular responses include the endothelial adhesion molecules’ expression, as well as the margination and migration of polymorphonuclear cells. There are also humoral responses of the cells such as cytokines, lipid mediators, proteases, oxidants, growth factors, NO, and neuropeptides.

Formation from ALI to more severe syndromes includes the activation of inflammation, chemoattractants which result in endothelial changes and the release of proinflammatory cytokines such as TNF, IL-1 and IL-6.[2] Cytokines, proteolytic enzymes and oxygen metabolites are released by both leukocytes and macrophages. Alveolar levels of pro-inflammatory mediators like interleukins and TNF have been shown to rise precipitously after blunt lung injury.[31] In our study, proinflammatory mediators like IL-1, IL-6, IL-10 and TNF were increased in blood after trauma as compatible with the literature.

Parenchymal changes in the contused lungs were characterized by extensive hemorrhage with disruption of the alveoli, congestion, and alveolar edema as well as increased leukocyte infiltration in the alveolar space.[31] Hemorrhage, alveolar edema and congestion, septal hyperemia, lymphocyte, leukocyte infiltration, lymphocyte infiltration, mucus, eosinophil, atelectasis, and emphysema scores were significantly affected with trauma in our study. Biochemical and histopathological findings compatible with early phase of lung injury were detected in trauma-applied rats. Humoral and cellular responses were obviously improved in treatment groups in which NOS inhibitors were applied after trauma; macrophage and bronchial macrophages were increased, which was thought to indicate the removal of degenerated elements earlier and contribute to a more rapid cure. Particularly in L-NIO group, proinflammatory cytokines were found to be obviously depressed. Also, depression of alveolar leukocytes and bronchial neutrophils while eosinophils not being affected could be explained by limited inflammatory reactions. CRP, which plays an important role in diagnosis and clinical course of inflammation, was obviously decreased with treatment particularly with L-NIO. Despite the few numbers of reports we obtained from the literature about L-NIO, this supported our hypothesis for using L-NIO in limiting lung injury.

Severely injured patients requiring admission to intensive care unit suffer from oxidative stress due to the cytokine release and systemic inflammation triggered by reactive oxygen and nitrogen-oxygen species. Some organs might be protected from the damaging effects of the oxidant species through antioxidant defenses including enzymes such as SOD or catalase.[53-55] Malondialdehyde is a good indicator of free radical formation, and its elevation indicates oxidative stress with increased lipid peroxidation. Free radicals damage cells and tissues.[56,57] In our study, MDA levels showed a significant increase after contusion. Activity of SOD was demonstrated to be decreased, GSH levels were significantly decreased and protein accumulation was significantly increased with trauma in group 2. These results suggest that oxidants and antioxidants participate in the mechanism of lung injury induced by PC.

Beneficial effects of antioxidant molecules in experimental ALI, blunt trauma and PC models have been established in previous studies.[28,31,58-61] According to our results, L-NIO-applied group (group 4) was superior to L-NAME-applied group (group 3) when compared in terms of affecting serum cytokine levels.

In trauma group, which could be considered as ALI, results may indicate that inhibition of NO formation selectively takes a more effective role in increasing levels of antiinflammatory components. Antioxidants inhibit lipid peroxidation by preventing a peroxidation chain reaction and/or picking up reactive oxygen derivatives. Endogenous antioxidants include mitochondrial cytochrome oxidase, SOD, catalase, glutathione peroxidase, glutathione S-transferase, hydroperoxidase and coenzyme Q. In treatment added trauma groups, histopathology showed an obvious improvement in parameters reflecting oxidant injury.[1,62] And this may clinically play an important role for therapeutic efficiency in ALI.

In trauma, it is known that the level of NO affects the degree of the inflammation and protects the tissues from ischemia-reperfusion damage due to its vasodilator role while increasing airway hyperemia and edema. Inhibition of NO also induces the repair of ischemia-reperfusion. It has dose related effects, and has an important role in occurring clinically symptoms of inflammatory reactions and in pathogenesis of serious conditions like airway obstruction. Such data indicate that NO has both toxic and protective abilities; neutralizing the mediators and free radicals, repairing neutrophil functions, and reducing capillary permeability.
Nitric oxide is a double-edged sword. We have seen NO levels were also elevated in the traumatic lung tissue. Some other reports show that NO appears in traumatic tissue and joins to tissue damage, but probably affects perfusion in a different pathway while playing a role in releasing many varied mediators. In our study, NO was decreased in L-NIO group. Although not statistically significant, effects of inflammation due to trauma were decreased and limited, but not terminated, or cured with NOS inhibitors; L-NAME and L-NIO. Signs of healing were shown, but not an exact remission. Structural changes in histopathological findings indicate the activation of defensive mechanisms against trauma; however, cytokines like TNF and ILs that play role as indicators of metabolic changes in tissue seem to occur more slowly.

Plasma and structural changes in trauma gave an early response to trauma effects; however, we needed more time to detect the defensive signs as metabolic changes in tissue. Thus, the limited dosage and treatment period may be considered as limitations of our study. Due to the fact that trauma is at an earlier stage, efficiency of treatment agents might be still unclear and discussed. Nevertheless, these results support the idea that early treatment with NOS inhibitors may yield favorable effects on pulmonary pathophysiologic parameters of blunt thorax trauma patients.

In conclusion, we have demonstrated that the best outcome in a rat model was recorded with administration of both Ngamma-nitro-L-arginine methyl ester and N-iminoethyl-L-ornithine with respect to malondialdehyde levels, mucus and histopathological outcomes, which were statistically significant. Also, compatible nitric oxide synthase inhibitor Ngamma-nitro-L-arginine methyl ester improved superoxide dismutase scores significantly. After blunt thorax trauma, it appears that free oxygen radicals and lipid peroxidation play a role in pulmonary contusion, while Ngamma-nitro-L-arginine methyl ester and N-iminoethyl-L-ornithine had protective effects on biochemical and histopathological outcomes of pulmonary contusion. In this study, we aimed to observe the acute effects of blunt lung trauma and examine the early treatment choices. Our results should be considered accordingly. Animal models in laboratory cannot imitate exactly the characteristics as in humans. However, unfortunately, there is no alternative. Impact of Ngamma-nitro-L-arginine methyl ester and N-iminoethyl-L-ornithine in lung injury after blunt chest trauma may be further clarified with variable dosages, administration times, animal models, larger groups, and more detailed studies with longer observation periods.

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