Efficacy of budesonide and interleukin-10 in an experimental rat model with isolated bilateral pulmonary contusion created by blunt thoracic trauma

Kubilay Ocalan⁠, Okan Solak*⁠, Hidir Esme⁠, Mehmet Sirmali⁠, Husniye Dilek⁠, Gurhan Oz⁠, Ayhan Vurmaz⁠, and Ahmet Karaman⁠

INTRODUCTION

Thoracic traumas constitute 25% of trauma cases presenting to the emergency room. Rib fractures and pulmonary contusions develop in a majority of patients with thoracic trauma. The pulmonary contusion occurring in 30–75% of major thoracic traumas is a severe injury with high mortality and morbidity. The mortality rate of 11% in an isolated severe contusion elevates to 22% in the presence of additional injuries [1].

One of the most important factors determining the prognosis after a pulmonary contusion due to thoracic trauma is the inflammatory response and thereby neutrophils, a main factor in the formation of endothelial/epithelial injury [2]. The principal factor ascertaining the development of acute respiratory distress syndrome (ARDS) and acute lung injury (ALI) in patients who developed a lung contusion, is the balance between proinflammatory and anti-inflammatory responses [3]. Today, fluid restriction, steroids, antibiotics, diuretics, oxygen and mucolytics are generally used for ARDS and ALI treatments due to pulmonary contusion. The understanding of the inflammatory mechanisms for the development of ALI and ARDS after a pulmonary contusion is facilitated by studies relevant to anti-inflammatory treatment. In our study, we aimed to study the effects of budesonide (BS), inhaled corticosteroids, interleukin-10 (IL-10) and anti-inflammatory mediators on pulmonary contusion.

OBJECTIVES: In our study, we aimed to investigate the anti-inflammatory mediator effects of budesonide (BS), an inhaled corticosteroid and interleukin-10 (IL-10) on a pulmonary contusion in an experimental rat model in which an isolated bilateral pulmonary contusion was created by blunt thoracic trauma.

METHODS: Fifty-five male Sprague–Dawley rats were used in the study. Sham, control, BS and IL-10 groups were created. A pulmonary contusion was created by performing isolated blunt thoracic trauma in all groups except for the sham group. The trauma’s severity was determined as 1.45 J. BS and IL-10 were administered orogastrically to the respective groups 30 min before trauma, and orogastrically and intraperitoneally, respectively, on the first, second and third days after the trauma. Only the blunt thoracic trauma was performed for the control group. SatO₂, PaO₂ and PaCO₂, blood glutathione, malondialdehyde (MDA) and tumour necrosis factor-α (TNFα) values were recorded on the zeroth, first, second and third days. The histopathological examination and the bronchoalveolar lavage cell count were performed on pulmonary tissues.

RESULTS: Blood gas analysis revealed that SatO₂ and PaO₂ values on the first and second days were significantly lower in the control, BS and IL-10 groups compared with the sham group (P < 0.05). The SatO₂ and PaO₂ values on the third day in the BS and IL-10 groups were higher than in the control group (P < 0.05). The mean MDA in the control group was higher than in the sham, BS and IL-10 groups (P < 0.05). The mean TNFα in the control group was higher than in the sham, BS and IL-10 groups (P < 0.05). Pulmonary pathology scoring in the control group was observed to be higher than in the sham, BS and IL-10 groups (P < 0.05).

CONCLUSION: In this rat experiment model in which an isolated pulmonary contusion was created by blunt trauma, BS and IL-10 were observed to reduce contusion severity in the lung and minimize the inflammatory reaction.

Keywords: Thoracic trauma • Pulmonary contusion • Experimental rat model

© The Author 2012. Published by Oxford University Press on behalf of the European Association for Cardio-Thoracic Surgery. All rights reserved.
MATERIALS AND METHODS
This study was performed in the animal research laboratories and was approved by the Ethics Committee for Animal Experiments. Fifty-five male Sprague-Dawley rats weighing 150–200 g and aged 4–6 months were enrolled. All rats were acclimated for at least a week before the operation to allow them to adjust to the laboratory environment. Throughout the experiment, animals received the standard rat diet and water. They were housed individually in polycarbonate microisolator cages in a controlled environment with a temperature of 20–26°C. They were exposed to a light–dark (L/D) cycle of 24 h (L/D = 12/12 h). The animals were cared for according to the guidelines set out by the Guide for Care and Use of Laboratory Animals.

Rats were randomly assigned to one of the four groups: the sham (n = 5) group included rats without trauma, the control (n = 20) group included rats with trauma and without treatment, the BS (n = 15) group included rats to which BS was administered and trauma was applied and the IL-10 (n = 15) group to which IL-10 was administered and trauma was applied (Table 1).

Anaesthesia and treatment administration
Rats were left hungry 12 h before the process. After anaesthesia with intramuscular sodium thiopental (pentothal sodium, Abbot, USA) of 40 mg/kg was achieved in traumatic rats, they were bound to the assembly from the extremities for the application of blunt trauma. BS of 0.5 mg/ml and IL-10 of 2 μg/kg were administered orogastrically and intraperitoneally, respectively, 30 min before and on the first and second days after the administration of blunt thoracic trauma [4, 5]. No treatment was administered for the control and sham groups.

The trauma apparatus and the administration of blunt thoracic trauma
A pipe system conveying a specified weight to the piston by free fall and a stand apparatus preventing the impact of the weight to the head and abdominal space of the rat were designed. In the piston conveying the impact force to the isolated thoracic wall, a space was generated to protect the sternum and heart. Impact energy (E) was calculated by $E = mgh$, where $m$ is the mass (kg), $g$ the gravitational acceleration (9.8 m/s²) and $h$ the height where the weight is left (m). Frictional force was ignored. Blunt thoracic traumas by a 1.45-J force was applied to the rats, except for the zeroth day, when the head and abdominal space of the rat were designed. In the trauma apparatus and the administration of blunt trauma [4, 5].

Table 1: Numbers of rats sacrificed each day from each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Zeroth day (n)</th>
<th>First day (n)</th>
<th>Second day (n)</th>
<th>Third day (n)</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham (n = 5)</td>
<td>5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Control (n = 20)</td>
<td>5</td>
<td>4*</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>BS (n = 15)</td>
<td>–</td>
<td>5</td>
<td>4*</td>
<td>4*</td>
<td>2</td>
</tr>
<tr>
<td>Interleukin (n = 15)</td>
<td>–</td>
<td>5</td>
<td>4*</td>
<td>4*</td>
<td>2</td>
</tr>
</tbody>
</table>

*One rat died before being sacrificed.

Laboratory analysis
Two cc of intra-cardiac blood was taken from five rats from each of the control and sham groups after trauma when under anaesthesia. The levels of PaO2, SatO2, pCO2, glutathione (GSH), tumour necrosis factor-α (TNFα) and malondialdehyde (MDA) from oxidative stress parameters of the taken blood were measured. The chest space of the rats was opened by the median sternotomy. Both the lungs were excised from the thoracic space by dissecting the trachea. In traumatic rats, diffuse contusion areas were observed in the lungs. In the sham group, the lungs were seen to be healthy. Bronchoalveolar lavage (BAL) was performed by administering 2 cc of isotonic (0.9% NaCl) fluid to the lungs through the trachea. The pulmonary surface was irrigated with normal saline. The left lung was reserved for histopathological examination and placed in a 10% formaldehyde solution. The right lung was reserved to detect the wet/dry pulmonary weight rate. The same processes were performed on the first, second and third days for five rats from each group in the control, BS and IL-10 groups.

Tissue specimens were stained with HE stain for histopathological examination. Smears, interstitial pulmonary tissue and inflammatory cells in alveoli were examined under a light microscope (Nikon UFX-IIA, Japan) by a pathologist who was blind to the groups. The extent of the injury that occurred in the lungs was detected with a modified scoring system used by Tassiopoulos et al. [7]. Evaluation was done as 0 point = no change (score 0), 1 point = focal mild changes (score 1), 2 points = multifocal moderate changes (score 2), 3 points = multifocal pronounced changes (score 3) and 4 points = diffuse pronounced changes (score 4).

The bronchoalveolar lavage (BAL) fluid was centrifuged at 3500 rpm. Staining was made with a direct PAP stain. The cell count was performed by examining prepared smears under a light microscope (Nikon UFX-IIA, Japan) by a pathologist who was blind to the groups. When cells were evaluated on the basis of BAL, inflammatory cell, blood components, the cells were scored as 0 if they were under 5%, as 1 if they were between 5 and 50% and as 2 if they were over 50%.

The measurement of wet/dry pulmonary weight
The right lung was weighed after excising from the main bronchus, and the wet pulmonary weight was obtained. Immediately after, the dry pulmonary weight was obtained as mg by drying in an autoclave at +80°C for 24 h and the wet/dry lung rates were calculated. Pulmonary oedema was evaluated using a wet/dry weight ratio.

Statistical analysis was performed using the SPSS 15.0 statistical package program. The median, minimum and maximum values of the groups were calculated. The Kolmogorov–Smirnov test and the ANOVA test were used. The significance level was accepted as $P < 0.05$.

RESULTS
In the blood gas analysis, SatO2 and PaO2 values in the first and second days were significantly lower in the control, BS and IL-10.
groups compared with the sham group ($P < 0.05$). SatO$_2$ and PaO$_2$ values on the third day were higher in the BS and IL-10 groups compared with the control group ($P > 0.05$). No difference was observed in the SatO$_2$ and PaO$_2$ values on the third day in the BS and IL-10 groups compared with the sham group ($P > 0.05$) (Fig. 1). In rats administered for trauma, hypoxia eventuated.

In the blood gas analysis, PaCO$_2$ values on the first, second and third days were observed to be high in the control group compared with the sham group ($P < 0.05$). No difference was observed between the BS, IL-10 and control groups in the PaCO$_2$ values on the first, second and third days ($P > 0.05$). In rats administered for trauma, hypercapnia developed only on the first day of trauma. However, elevation in PaCO$_2$ was not different on the following days between groups in which treatment was administered and not administered.

In the MDA analysis, MDA values on the first, second and third days were observed to be high in the control group relative to the sham, BS and IL-10 groups ($P < 0.05$). No difference was observed in the MDA value on the third day between BS and IL-10 groups relative to the sham group ($P > 0.05$). The MDA value on the third day was observed to be high in the BS group relative to the IL-10 group ($P < 0.05$) (Fig. 2). We observed with the MDA results that BS and IL-10 groups administered for treatment are effective on pulmonary contusions. Also, we can say that BS is more effective than IL-10.

In the TNF$\alpha$ analysis, TNF$\alpha$ values on the first, second and third days in the control group were observed to be high relative to the sham, BS and IL-10 groups ($P < 0.05$). TNF$\alpha$ values on the first, second and third days in the BS group were observed to be high relative to the sham group ($P < 0.05$). No difference was observed in the TNF$\alpha$ value on the third day in the IL-10 group relative to the sham group ($P > 0.05$) (Fig. 3). We observed with the TNF$\alpha$ results that BS and IL-10 groups are effective treatments for contusions.

In the GSH analysis, GSH values on the first, second and third days in the BS and IL-10 groups were observed to be low relative to the control group ($P < 0.05$). GSH values on the first, second and third days in the control and BS groups were observed to be low relative to the sham group ($P < 0.05$). No difference was observed in the GSH value on the first and second days in the BS group ($P > 0.05$), and the GSH value on the third day in the IL-10 group was observed to be high relative to the BS group ($P < 0.05$). No difference was observed in the GSH value on the third day between the IL-10 and sham groups ($P < 0.05$) (Fig. 4). The high levels of GSH in the BS and IL-10 groups compared with the sham group are consistent with the BS and IL-10 groups administered for treatment are effective on pulmonary contusions.
groups relative to the control group were evaluated as the success of treatment.

In the BAL scoring analysis, BAL scoring on the first, second and third days in the control group was observed to be high relative to the sham, BS and IL-10 groups. BAL scoring on the first, second and third days in the BS group was observed to be high relative to the sham group (P < 0.05). No difference was observed in the BAL scoring on the first day between the BS and IL-10 groups (P > 0.05). BAL scoring on the second and third days in the BS group was observed to be high relative to the IL-10 group (P < 0.05). No difference was observed in the BAL scoring on the second and third days between the BS and sham groups (P > 0.05). In the identification of trauma finding and treatment period efficiency, in consequence of the BAL scoring analysis, the BAL score was found to be low in the BS and IL-10 groups relative to the control group.

On pulmonary histopathological examination, pathology scoring on the first, second and third days in the control group was observed to be high relative to the sham, BS and IL-10 groups (P < 0.05). Pathology scoring on the first, second and third days in the BS and IL-10 groups was observed to be high relative to the sham group (P < 0.05). Pathology scoring on the first day in the BS group was observed to be high relative to the IL-10 group (P < 0.05), and not different on the second and third days (P > 0.05) (Fig. 5). We observed with the histopathological scoring performed for the evaluation of pulmonary injury that BS and IL-10 groups, used for treatment, are effective. However, no priority over each other was observed between BS and IL-10.

In the wet/dry pulmonary weight rate analysis, no difference was observed in the wet/dry pulmonary weight rate on the first, second and third days in the sham, control, BS and IL-10 groups (P > 0.05). The lack of difference in the wet/dry pulmonary weight rate was attributed to pulmonary oedema that occurred within the first 3 days, which is a quantity that creates no difference between groups.

**DISCUSSION**

Traumas are the most common cause of death in humans under 40 years old. Twenty-five percent of deaths due to trauma depend on thoracic trauma [8]. Overextension and rupture in alveoli, disconnection of alveoli from bronchioles, intra-alveolar bleeding, interstitial oedema and thus consequential alveolo-capillary injury occurring in the lungs after trauma are defined as pulmonary contusions. The pulmonary contusion resultant in 30–75% of the major thoracic traumas is a serious injury with high mortality and morbidity [1]. Mortality given as 11% in an isolated severe contusion rises up to 22% in the presence of additional injuries. Also in our study, mortality in rats in which we created a traumatic isolated pulmonary contusion was observed as 10%. ALI is a very common clinical state seen in 30–75% of blunt thoracic traumas [9]. While the ARDS development rate is 17% in an isolated contusion, it rises up to 78% with additional injuries. ARDS mortality is over 50%. In pulmonary contusion pathogenesis developing after trauma, leucocyte infiltration, production of inflammatory mediators and free oxygen radicals play an important role.

In experimental pulmonary injuries, the long-term administration of steroids has been effective in a reduction in pulmonary collagen and oedema formation. This positive effect rapidly reduced after the cessation of steroids [10–13]. In an experimental model, the increase in collagen aggregation was demonstrated by limiting corticosteroid treatment in the first 6 days of acute pulmonary injury and by discontinuing treatment [14]. These observations explain the difference between the short-term use of steroids in early ARDS and the useful results in the long-term use in the late fibro-proliferative phase. Additionally, the duration of the pathological period is also important for changes in results in ARDS. Meduri et al. took open pulmonary biopsies from 12 patients with late ARDS before methylprednisolone treatment. In the group giving response to treatment, pulmonary histological analyses showed mixed cellular fibrosis, protected alveolar structure and arterial subintimal fibroproliferation was shown. In the group without response to treatment, poor fibrosis in intensive cells, impaired alveolar structure and arterial subintimal proliferation were seen [15]. Although steroids have opened a therapeutic window into fibroproliferative ARDS treatment, the treatment will not be effective when the final stage fibrosis develops. In the clinical study by Lee et al. [16], related to the steroid use at the early stage of ARDS, once a diagnosis was established in cases where ARDS developed after thoracic surgery, maintenance treatment of 2 mg/kg/day following a loading dose of intravenous methylprednisolone of 2 mg/kg was administered, it was demonstrated that respiratory parameters recovered in the group and mortality reduced at a significant rate. Jansson et al. defined severe airway function loss and pulmonary injury in the fourth hour following intratracheal lipopolysaccharide administration in horses. They used BS in their study and reported the prevention of the inflammatory mediators such as TNFα, IL-1, IL-6 and MCP-1. Jansson et al. reported leucocyte/neutrophil elevation in BAL fluid and oedema in both lungs after intratracheal lipopolysaccharide administration of 0.5 mg/ml/kg in the fourth hour in rats. Treatment with BS completely prevented pulmonary injury and inflammation after lipopolysaccharide at a low dose; its partial, but notable, effect on the administration of lipopolysaccharide at a high dose was reported. In the study, cytokine quantities in BAL fluid were reported to be low in the BS group relative to the control group [4].

In our study, we administered early term BS and IL-10 after trauma in rats where an isolated bilateral lung contusion was created by blunt thoracic trauma. In blood gas evaluation, PaO2 and SatO2 values were observed to recover significantly in the BS group relative to the control group. MDA and TNFα levels...
were observed to be significantly low in the BS group relative to the control group. The GSH level was observed to be significantly high in the BS group relative to the control group. BAL and histopathological lung scoring were seen to be significantly low in the BS group relative to the control group. As a result, our study demonstrates that BS may have protective effects on traumatic pulmonary injury.

IL-10 is the most important anti-inflammatory cytokine in human immune response. IL-10 shows its anti-inflammatory activity by inhibiting inflammation mediators such as IL-1β, TNFα, IL-8, interferon-γ, IL-6 and prostaglandin metabolites [17]. It also blocks the production of IL-2 and interferon-γ from T helper-1 cells and antigen-specific T cell activation. It inhibits cytokine production from monocytes and macrophages [18]. In our study, in the light of this information, we administered early term IL-10 before and after the trauma in rats in which lung contusion was created by blunt thoracic trauma and observed its effects.

To our knowledge, there is no study in the literature relevant to use of IL-10 for ARDS and ALI in lung contusion after trauma. However, in the study on rats by Yurumez et al. [5], they histopathologically showed that IL-10 use decreases lung injury due to intoxication. Results obtained from the IL-10 group are similar to the BS group and showed in consequence of data obtained that IL-10 may have protective effects on lung injury mechanisms.

In conclusion, our study set an experimental model for studies on lung contusion, demonstrating that isolated lung contusion may be created in rats with the apparatus that we prepared. Second, BS and IL-10 use in rats in which isolated lung contusion was created with blunt trauma was observed to decrease the severity of the contusion that occurs in the lung and to minimize the inflammatory reaction.

Conflict of interest: none declared.

REFERENCES


