Investigation of the Effects of Boric Acid on Preventing Lung Damage in the End of the Lower Extremity Ischemia Reperfusion in Rats

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Summary: In this study, the biochemical and histopathological changes in the lungs due to lower extremity ischemia-reperfusion injury and protective effect of boric acid to prevent them were investigated. The rats were divided into three groups as Group 1 (Sham group, n=9), Group 2 (Ischemia-reperfusion group, I/R, n=9) and Group 3 (Ischemia-reperfusion, (I/R) + boric acid (BA), n=9). Anaesthesia was applied to the first group of rats without any ischemia-reperfusion process. Following a two-hour ischemia, two hours of reperfusion, with the help of left lower extremities tourniquet, were applied to the second group. To the third group, 200 mg/kg (i.p.) boric acid application was performed 10 minutes before the ischemia was initiated and two hours of ischemia and two hours of reperfusion application were carried out. Some antioxidant enzymes in lung tissue (SOD, GSH-Px, CAT) were analyzed. In addition the lung tissue was evaluated histopathologically. Boric acid application was found to significantly enhance the positive effect of antioxidant enzymes. Normal histological structure was better preserved, some of macrophages were non-activated, and a remarkable reduction of neutrophil infiltration was seen after treatment in boric acid given group.

Key words: Antioxidant enzyme, boric acid, histopathology, ischemia/reperfusion, lung damage

Scanned and Alt Ekstremitе İskemi Reperfüzyonу Suonucunda Gelişen Uzak Doku Akciğer Hasarının Önlenmesinde Borik Asidin Etkilerinin İncelenmesi


Anahtar kelimeler: Akciğer hasarı, enzimler, borik asit, histopatoloji, iskemi/reperfüzyon

Introduction

Lower extremity ischemia reperfusion has significant outcomes commonly observed in clinical case studies. After reperfusion followed by ischemia and the impact of inflammatory mediator and free-oxygen radicals, the development of a local and ischemia damage will start (Grace, 1994; Klausner et al., 1989a; Klausner et al., 1988). Despite ischemia is saved through re-start of blood flow, it leads to the development of multiple organ dysfunction and it is especially observed in heart, renal and lungs (Blaisdell, 2002). Lung tissue is the target organ being affected by the lower extremity ischemia reperfusion most and its aetiology is not fully known (Tekeli et al., 2001; Uysal, 2006). Boric acid (BA) occurs naturally in both sea and fresh water sat 1-5 ppm. Its concentrations in the soil are more than waters.
Boric acid and ischemia reperfusion... (Büyükgüzel et al., 2013). Boric acid is abundant in fruits, vegetables and legumes, so it can be obtained in the diet with the consumption of these (Devirian and Volpe, 2003). Found in nature at high concentrations, BA is widely used in the industry, agriculture, cosmetics and other small practices. It is considered that the supplemental use of BA in human and animal food has considerable effects on the physiological and metabolic systems. Acute oral toxicity of inorganic boron-containing compounds is stated as 2660-4000 for rats LD50 (g/kg) (Fail et al., 1998). In some studies it is stated that BA has effects on minerals (Ca and P), vitamin D, hormones (insulin, oestrogen, testosterone, T3,T4), energy substrates (triglyceride, glucose) and reactive oxygen species (Hunt, 1988; Meacham et al., 1994; Hunt, 1996; Armstrong et al., 2001; Eren et al., 2006; Turkez et al., 2007). Nevertheless, the biochemical mechanism of BA is not completely known. In this study, we examined two things biochemically and histopathologically; the lung tissue damage caused by lower extremity ischemia/ reperfusion and the role of boric acid in preventing this damage.

Material and Methods

Experimental animals

Our experimental studies were carried out after the experimental animals were approved by the Bingöl University Ethnic Committee of Animal Research (decision no:2018/02-02/06). The animals were kept in cages in a controlled room at a fixed 20-22°C temperature and a 12-hour shift of light and dark cycle (light 07:00-19.00, dark: 19:00-07:00). They were provided with water and standard food. The experiments started after the rats were rested in their cages for a week in order to provide their adaptation to the environment. Rats were fed with pellet feed obtained from the market and analysis contents given in Table 1.

Table 1. Nutrient composition of pellets

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>87.2%</td>
</tr>
<tr>
<td>Crude protein</td>
<td>23.0%</td>
</tr>
<tr>
<td>Crude fat</td>
<td>3.7%</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>7.7%</td>
</tr>
<tr>
<td>Ash</td>
<td>5.8%</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.6%</td>
</tr>
<tr>
<td>Mn</td>
<td>96 mg/kg</td>
</tr>
<tr>
<td>Fe</td>
<td>31 mg/kg</td>
</tr>
<tr>
<td>Zn</td>
<td>960 mg/kg</td>
</tr>
<tr>
<td>Co</td>
<td>0.60 mg/kg</td>
</tr>
<tr>
<td>S</td>
<td>0.30 mg/kg</td>
</tr>
<tr>
<td>I</td>
<td>2.28 mg/kg</td>
</tr>
</tbody>
</table>

The rats were fasted for 12 hours before the experiments, with free access to water. A total of 27 male Wistar albino rats weighing between 200 and 300 grams each were randomly divided into three groups. Group 1 (control/sham group, n: 9); these rats were only given anesthesia. Group 2 (ischemia/reperfusion, I/R, n: 9); Group 3 (ischemia reperfusion (I/R) + BA, n: 9) where 10 minutes before ischemia, a 200 mg/kg dosage of BA was applied intraperitoneally as described Başbuğ et al. (2015). All the rats were exposed to ischemia for two hours through the bilateral lower extremity tourniquet method. The loss of arterial flow was determined through the inability to detect Doppler signals through Doppler USG. Two hours later, the tourniquets was opened, and reperfusion was performed for two hours (Klausner et al., 1989b). The control of arterial flow was again checked by Doppler USG. At the end of the fourth hour, the rats were sacrificed through injection of a high dosage of 60 mg/kg (i.p.) ketamine hydrochloride (Ketalar, Parke-Davis) and 10 mg/kg Xylazine (i.p.). Their lung tissues were taken out through mediastinotomy, and biochemical and histopathological analyses (SOD, GSH-Px, CAT levels) of the tissues were carried out.

Biochemical assays

Superoxide dismutase activity (SOD) was performed according to the method Sun et al. (1988). Tissue glutathione peroxidase’s activity (GSH-Px) Paglia et al. (1967) and Catalase activity (CAT) were performed according to the method Aebi (1984).

Anaesthesia, necropsy and histopathologic examination

The main materials of this study were 10-week-old 27 Wistar albino rats in 3 groups (n:9). Firstly, all rats were anesthetized with intraperitoneal injection of 10 mg/kg xylazine and 60 mg/kg ketamine, and euthanasia was applied by decapitation under deep anaesthesia (Tranquilli et al., 2013). For light microscopy, the lungs were inflated via the trachea with 10% phosphate-buffered formaldehyde after sacrificing the animal as described above (Fiette and Slaouni, 2011). The lungs were then removed and placed into 10% phosphate-buffered formaldehyde until ready for sectioning and treated as routine histopathological
protocol of dehydrated samples in ascending grades of ethanol (50%, 70%, 96% and 100%) cleared in xylene and then embedded in paraffin (Bancroft et al., 2008). The paraffin-embedded tissue samples were sectioned at 5 µm thicknesses by a rotary microtome (Leica, RM2125). The slides were stained with haematoxylin & eosin (H&E) for histopathological analyses (Fisher et al., 2008). Finally, the slides were examined and photographed by using a light microscope with an imaging system (Leica, DM2500/DFC295) for detection of degenerative changes in the lung tissues.

**Statistical analysis**

The results are expressed as mean ± the standard error of the mean (SEM). The comparisons between the groups were done with Kruskal Wallis and Benferroni corrected Mann Whitney U test. The statistical analysis was performed utilizing SPSS®, v23 statistical software (SPSS Inc. Chicago Illinois).

**Results**

In this study, which evaluated the remote tissue damage that occurred in the lungs in connection with rat lower extremity ischemia reperfusion damage, antioxidant defence parameters such as SOD, CAT and GSH-Px enzyme activities were determined. The results of the SOD, CAT and GSH-Px values for each group are shown in Table 2.

### Table 2. Distribution of SOD, CAT and GSH-Px values based on the groups

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control (sham)</th>
<th>Group 1 (I/R)</th>
<th>Group 2 (I/R+BA)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td>2828.68±2.45</td>
<td>2809.73±1.86</td>
<td>2815.64±2.83</td>
<td>***</td>
</tr>
<tr>
<td>CAT</td>
<td>34.96±1.87</td>
<td>17.07±1.50</td>
<td>32.31±1.79</td>
<td>***</td>
</tr>
<tr>
<td>GSH-Px</td>
<td>1.66±0.057</td>
<td>0.8513±0.073</td>
<td>1.301±0.046</td>
<td>***</td>
</tr>
</tbody>
</table>

***: P<0.001; a,b,c: different letters, significant differences between control (Sham), Group 1 (I/R), Group 2 (I/R+BA) groups

**Figure 1.** Super oxide dismutase levels in the lung tissues of the control (Sham), Group 1 (I/R), Group 2 (I/R+BA) groups (SOD) (EU/g)

**Figure 2.** Catalase levels in the lung tissues of the control (Sham), Group 1 (I/R), Group 2 (I/R+BA) groups (EU/g)

In this study, which evaluated the remote tissue damage that occurred in the lungs in connection with rat lower extremity ischemia reperfusion damage, antioxidant defence parameters such as SOD, CAT and GSH-Px enzyme activities were determined. The results of the SOD, CAT and GSH-Px values for each group are shown in Table 2.

When the enzyme activities of SOD, CAT and GSH-Px are compared to the control group, it is clear that, while the values decreased in the ischemia reperfusion group. According to the I/R group, the SOD, CAT, GSH-Px enzyme activities increased in the group which received boric acid treatment compared to the I/R group. Moreover, in comparison to the control group, a significant decrease in the GSH-Px enzyme activity level was observed in the group that received boric acid treatment (Table 2 and Figures 1, 2 and 3). In the control (sham) group (Group 1), no significant histopathologic lesions were observed in the investigated lung tissues, and the alveolus, bronchioles and pneumocytes displayed a normal histologic structure (Figure 4-A). In the group that received I/R (ischemia/reperfusion) treatment (Group 2) in the lung tissues, interstitial and perivascular oedema, neutrophil leukocyte accumulation, a small amount of fibrin deposits, and erythrocyte permeation in alveolus swelling and desquamation of bronchiolar and alveolar epithelial cells were determined (Figure 4-B). In addition to this, in the compared lung tissues of the boric acid treatment group (Group 3), the histopathological findings were seen to be significantly decreased. Only limited swelling of bronchiolar and alveolar epithelial cells and remarkable reduction of neutrophil infiltration were observed after treatment in the group that was given boric acid (Figure IV-C).
Discussion and Conclusion

Türkez et al. (2007) reported that boron compounds (as boric acid) caused significant increases in antioxidant enzyme activities. However, the defence of boric acid against reactive oxygen species (ROS) has not been adequately described yet. The activities of antioxidant enzymes (SOD, CAT, GSH-Px) have important roles in cell defence (Tapiero et al., 2004). Superoxide dismutase has a central role against oxidative damage. This enzyme catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen (Kakarla et al., 2005). Catalase activity protects lipids and proteins in erythrocyte membranes against peroxide radicals. Glutathione peroxidase is associated with increases in the quantity of glutathione. Glutathione is a tripeptide that is very important for erythrocytes. It has a key role in antioxidant enzyme activities (Türkez et al. 2007). Hunt et al. (1996) stated that boron compounds have a protective effect on oxidative damage to human health by showing their effects on SOD, CAT and GSH. It is considered that the effects occur because they stimulate the immune system and lead to secretion of Vitamin D metabolism or insulin. It is stated that lung damage occurred in the reperfusion period after lower extremity ischemia in connection with free oxygen radicals. In the same study, it was also found that melatonin, an antioxidant compound, played a protective role against I/R damage. In this study, it was also determined that boric acid applications used against radicals which are caused by extremity ischemia led to a significant increase in the protective effect of antioxidant enzymes. In comparison to SOD and GSH-Px, the enzyme CAT, which is the main subject of this study, was also affected more positively. The late phase of reperfusion injury is known to be neutrophil-dependent (Uysal, 2006). Although there are important studies on the effects of remote organ ischemia-reperfusion cases that lead to lung injury (e.g., hind-limb, ischemia reperfusion) (Seekamp et al., 1993), much fewer studies have been conducted to focus on the lungs as the direct aim of ischemia and reperfusion injury. In this study, using an in vivo rat lung ischemia-reperfusion model, we characterized a model of injury in the lung during the early and delayed reperfusion periods by determining the protective effects of boric acid and its relationships in inflammatory injury. In similar studies, oedema was mentioned in the alveolar cells (Den-Hengst et al., 2010; Ferrari et al., 2015). Macrophages and neutrophils are determinants for the improvement of lung ischemia-reperfusion injury (Den-Hengst et al., 2010). Alveolar type II cells appear to be quite sensitive to the effects of ROS and RNS. These cells are responsible for the release of inflammatory mediators and cytokines (Sharma et al., 2007). The light microscopy examinations observed considerable perivascular oedema in the lungs undergoing ischemia and 2 hours of reperfusion in comparison to the lungs of the sham-operated rats. At 2 hours of reperfusion, there were also significant interstitial and perivascular oedema, deposition of fibrin in the alveolar spaces, intra-alveolar haemorrhage and leukocyte accumulation. The normal histological structure was better preserved, some macrophages were non-activated, and a remarkable reduction of neutrophil infiltration was seen after treatment in the group that was given boric acid (Beckers et al., 2017).

Damage to the lung tissue of the rats exposed to ischemia-reperfusion was detected. This damage was observed as a decrease in enzyme activities and histopathological deterioration. After the application of boric acid, the increase in enzyme activity (SOD, CAT, GSH-Px) in lung tissue and histopathology were determined as normalization. According to these results, boric acid has therapeutic effects in lung damage after ischemia reperfusion.
References


