The antioxidant effect of boric acid and CoQ10 on pulmonary fibrosis in bleomycin induced rats

Fatih Çağlar Çelikezen a,*, Gökhan Oto b, Hulya Özdemir b, Ufuk Kömuroğlu c, İbrahim Yörük d, Halit Demir d, Ashli Çilingir Yeltekin d

a Bitlis Eren University, Faculty of Science and Arts, Department of Chemistry, 13000 Bitlis-TURKEY.
b Yüzüncü Yıl University, Faculty of Medicine, Department of Pharmacology, 65080 Van-Turkey.
c Yüzüncü Yıl University, Faculty of Medicine, Department of Biochemistry, 65080 Van-Turkey.
d Bitlis Eren University, Faculty of Science, Department of Chemistry, 13000 Bitlis-TURKEY.

* Corresponding author: celikezen@hotmail.com

Abstract

The purpose of this study is to investigate the antioxidant effects boric acid and CoQ10 has on pulmonary fibrosis in bleomycin induced rats. 32 wistar albino male rats were used in this study. The rats were categorised in four groups; a control group (only given normal saline), a bleomycin (BLM) group, a BLM + boric acid group, and a BLM + boric acid + CoQ10. The study period was adjusted to 30 days. Retinol, vitamin E, vitamin D, catalase, carbonic anhydrase, glucose, total protein, albumin, globulin, and total bilirubin levels were measured at the end of the study. There was a significant increase in the vitamin E levels of all groups (p<0.05). There was a statistically significant increase in the glucose level of all groups (P<0.001). There were significant decreases in albumin and total protein levels of all groups. While the significance level of the decrease in the albumin level was p<0.001, the significance level of the decrease in the total protein level was p<0.05. There was no significant difference in other biochemical parameters.

Keywords: Bleomycin, boric acid, CoQ10, pulmonary fibrosis, rat

1. Introduction

Pulmonary fibrosis (PF) is characterised by an altered cellular composition of the alveolar region with excessive deposition of collagen. However, its etiology is obscure. Lung inflammation is a major underlying component of a wide variety of pulmonary fibro proliferative disorders. In the last decades, it has been suggested that the main agent responsible for lung fibrosis are the reactive oxygen species (ROS), which are also produced under normal physiological conditions of the human body. Many possible treatment protocols for PF have been investigated, but none have succeeded in clinical trials (Tsao et al. 1997).

Bleomycin (BLM) is a mixture of closely related glycopeptide antibiotics isolated from Streptomyces verticillus. It has been used as an antitumor agent against head and neck cancer, squamous cell carcinomas, testicular cancer, and some lymphomas; however, its use is often limited by its toxicity, especially lung inflammation that can lead to fibrosis (Chen & Stubbe 2005).

Boron supplementation in animal and human nutrition may have important effects on various metabolic and physiological systems of an organism. Some studies have demonstrated that boron has effects on the metabolism of minerals (Ca and P) (Meacham et al. 1994), vitamin D (Hunt 1996), energy substrates (triglycerides, glucose) (Eren et al. 2006), and reactive oxygen species (Turkuez et al. 2007).

Coenzyme Q10 (CoQ10), also known as ubiquinone 50, is a fat-soluble molecule produced in the majority of living cells. It plays a key role in the energy metabolism as an integral part of the electron transport system (Ernster & Dallner 1995). CoQ10 is also recognised as a powerful systemic radical scavenger (antioxidant) that blocks oxidative injuries to DNA, lipids, proteins, and other essential molecules, and is also capable of functioning synergistically with other antioxidants (Lass & Sohal 2000).

The aim of this study is to investigate the beneficial effects of boric acid and CoQ10 on pulmonary fibrosis.

2. Material and Method

2.1. Animals

3.5 month old wistar albino rats, weighing 180–200 g, were obtained from the animal laboratory at Yüzüncü Yıl University. All procedures involving animals were approved by the institutional ethics committee (30.06.2011-12). Rats were housed in specific cages. The rats had free access to water and food ad libitum.

2.2. Experimental protocol

32 male Wistar albino rats were used in this study. The planned study time was 30 days. The rats were divided into 4 groups. Control group (n=8); Sterile saline solution was given to these, administrated intraperitoneally. BLM applied group (n=8); Bleomycin (Nippon Kayaku, Tokyo, Japan) was dissolved in 250 μL of phosphate-buffered saline (PBS) solution and instilled into the animals at a dose of 7.5 mg/kg body weight intratracheally under
chloroform anaesthesia. The animals were shaken to facilitate distribution of bleomycin and saline (Ozyurt et al. 2004). BLM+boric acid group (n=8); in this group, boric acid was induced at a dose ratio of 10 mg/kg/body weight/day orally during 30 days, after BLM administration. BLM+boric acid+CoQ10 (n=8); CoQ10 administrated intraperytomally the ratio of 4 mg/kg/body weight/day in addition to BLM and boric acid. Animals were euthanized 30 days after the instillation. Blood samples were collected for analysis.

2.3. Determination of vitamin levels in the serum
Vitamin A, D, and E (retinol, tocopherol and vitamin D3) levels were determined in accordance with the HPLC (high performance liquid chromatography, Agilent 1100, Germany) method (Zaspel & Csallany 1983; Reynolds & Judd 1984; Miller & Yang 1985).

2.4. Determination of catalase and carbonyl hydrolase activity
A method described by (Aebi 1994) was used to conduct biochemical analysis of CAT activity in erythrocytes. The CA activity was assayed by hydrating CO2. The hydration of CO2 was measured using the method of Rickli and Wilbur-Ander, with bromothymol blue as the indicator (Rickli et al. 1964).

2.5. Determination of the total protein, albumin, globulin, glucose, and total bilirubin levels in the serum
The total protein, albumin, globulin, glucose, and total bilirubin levels were determined by a Roche Modular PP auto analyser using commercial Roche Kits.

2.6. Statistical Analysis
A statistical package for the social sciences (SPSS 17) package programme was used to conduct statistical analysis together with the one way ANOVA test.

3. Result and Discussion
BLM is known to generate reactive oxygen metabolites, including superoxide and hydroxyl radicals. Generation of the ROS in the lung tissue results in DNA injury, lipid peroxidation, alteration in lung prostaglandin synthesis and degradation, and an increase in lung collagen synthesis. As a result of the injury, inflammation and cytokine dysregulation occur after BLM administration, fibroblasts are activated, and collagen production is stimulated, while collagen degradation is inhibited (Stieffer 2001). In addition, several studies have demonstrated that bleomycin administration in rats decreases the antioxiditive capacity, while increasing oxidative stress in the lung tissue (Mata et al. 2003).

There are three major enzymes that are in charge of reactive oxygen species: superoxide dismutase, glutatime peroxidase and catalase (Krinisky 1992). The main enzymatic defence is provided by albumin, uric acid, bilirubin, sistein, glutatone, beta-caroten, dihydroloapat, ubiquinone, ceruloplasmin, transferrine, zinc, manganese, selenium, vitamin A, vitamin C and vitamin E (Frei et al. 1988). One of the most important of these is albumin (Şahin 2006).

Vitamin A and its active metabolite credits are important factors in supporting epithelial differentiation and normal respiratory growth (Georgieff et al. 1991). Retinoids also have anti-fibrotic and anti-inflammatory properties (Tabata et al. 2006). Retinoic acid (RA) stimulates the cell proliferation (Nabeyrat et al. 1998) and affects the polyamine transport and synthesis in cultured type II pneumocytes. Polyamine maybe important during the lung cell repair process (Heger & Bayburt 1999). Tabata et al. (2006) investigated the preventive effect that All-trans-retinoic acid (ATRA) had on the progression of the lung fibrosis in irradiated and BLM-treated rats. They concluded that ATRA improved the fibrosis induced by BLM in the lung tissues of rats; their data may provide a basis to explore the clinical use of ATRA in order to prevent fibrosis induced by pathologic radiation of the lung implying pulmonary fibrosis. Mert et al. (2009) reported that there was a decrease in the retinyl ester level in the pulmonary fibrosis lung tissue, produced by giving bleomycin to rats. There was a decrease in the retinol level for all groups addressed in study (p<0.05) as seen in Table 1.

**Table 1.** The retinol, vitamin E and D levels in control, BLM, BLM+boric acid, BLM+boric acid+CoQ10 groups (p<0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Retinol</th>
<th>Vitamin E</th>
<th>Vitamin D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.63±0.01</td>
<td>1.42±0.19</td>
<td>0.29±0.01</td>
</tr>
<tr>
<td>BLM</td>
<td>0.56±0.04</td>
<td>2.25±0.30</td>
<td>0.03±0.00</td>
</tr>
<tr>
<td>BLM+boric Acid</td>
<td>0.55±0.02</td>
<td>2.40±0.29</td>
<td>0.02±0.00</td>
</tr>
<tr>
<td>BLM+boric Acid+CoQ10</td>
<td>0.61±0.05</td>
<td>2.14±0.21</td>
<td>0.02±0.00</td>
</tr>
<tr>
<td>BLM+Bleomycin</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Vitamin E is a major antioxidant in biological systems acting as a powerful chain-breaking agent through the scavenging of peroxyl radicals (Shi et al. 1999). Vitamin E terminates the chain reaction of lipid peroxidation in membranes and lipoproteins. Thus, a number of studies have been carried out to determine the protective effects of vitamin E in different biological models of injury (Chen & Tappel 1995). Değer et al. (2007) proved that the pulmonary formation of fibrosis prevents vitamin E supplementation. Kato et al. (1990) noted that the concentration of vitamin E in the lung tissue increased significantly after intraterecheal administration of BLM. In this study, significant increase was determined in the vitamin E values of all the serum groups (p<0.05) as seen in Table 1.

The benefits of vitamin D were originally thought to be associated only with bone health based on increasing calcium and phosphorus absorption; several studies have extended the benefits to many noncalcemic effects. These effects include reduced risk of many forms of cancer (Garland 2006), bacterial infections (Bikle 2008), viral infections (Cannell 200), autoimmune diseases (Munger et al. 2006), and cardiovascular disease (Wang et al. 2008). Mert et al. (2009) reported that there was a significant decrease in the vitamin D5 level in the pulmonary fibrosis lung tissue, produced by giving rats bleomycin (p<0.001). For this study, while there was only an increase in the vitamin D levels of the BLM group, decreases were observed in all other groups (p<0.05) as shown in Table 1.

Carbonic anhydrase (CA) is present in nearly all organisms and catalyzes reversible hydration of CO2 to HCO3 and H+. Tashian et al. 1991. In addition to its role in the regulating of pH homeostasis, CA activity facilitates biosynthetic processes, which involve an early carboxylation step requiring bicarbonate. CA (IX) was first recognised as a novel tumor-associated antigen in
several human carcinomas (Liao et al. 1994). Simi et al. (2006) found that high levels of CA were found to be an independent prognostic feature in some cancer types. Ertelkin et al. (2007) reported a significant increase in CA activity in lung fibrosis created using bleomycin in rats. Beydemir et al. (2002) reported that aminoglycoside antibiotics inhibited the activity of carbonic anhydrase in low concentrations in vitro, but activated it at high concentrations in vitro. In this study, there was a decrease in the CA activity of all groups (p>0.05) as seen in Table 2.

Table 2. The catalase (CAT) and CA levels in control, BLM, BLM + boric acid, BLM + boric acid+ CoQ10 groups (P>0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>BLM</th>
<th>BLM + Boric Acid</th>
<th>BLM + Boric Acid+ CoQ10</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLM: Bleomycin, CA: Carbonic anhydrase, CAT: Catalase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Catalase (CAT) is essential to protect aerobic organisms from the toxic effects of H₂O₂. Catalase was also utilized in an enzymatic oxidation reaction to deactivate the relevant enzyme and/or the side reaction due to H₂O₂ that was produced (Yoshimoto et al. 2005). Ozurt et al. (2004) reported the significant decrease in CAT and SOD activities in lung tissue in bleomycin-induced lung fibrosis in rats. Yen et al. (2005) identified the markedly lower MnSOD and CAT activities in VA13 cells, which were observed after the after bleomycin treatment. In this study, there was an increase in the catalase level of all groups (P<0.05) as seen in Table 2.

Garcia-Gonzalez et al. (1991) suggested that boron deficiency increased the concentration of superoxide dismutase (SOD), catalase (CAT), and peroxidase in Anabaena PCC 7119 heterocysts. In addition, Turkez et al. (2007) observed that at low doses (15 mg/L) boron compounds increased both SOD and CAT activities in the erythrocytes when compared to the control group, while at high doses (500 mg/L) these decreased both SOD and CAT activities in erythrocytes. In our study, boric acid may have been the reason behind the increase in CAT levels, and the decrease in CA activity. No studies were found in literature that addressed the relationship between CA and boric acid. We believe this is the only study that addresses the same relationship.

Responses to oxidative stress can be affected by several factors in the cellular environment such as glucose. Glucose deprivation results in a loss of substrate for ATP production via glycolysis. Oxidative phosphorylation would then be the alternative pathway to produce energy. Evidence suggests that glucose deprivation causes mesenchymal cell death, which increases the generation of superoxide and hydrogen peroxide during mitochondrial respiration (Blackburn et al. 1999). It was seen that the glucose metabolism was directly related to cellular sensitivity to hydrogen peroxide (Averill-Bates & Przybylowski 1994).

Baynes set forth the relationship between diabetes and oxidative stress, and reported that the increase in lipid peroxidation caused hyperglycemia (Baynes 1991). Giri et al. (1985) reported bleomycin-treated animals were hyperglycemic in comparison to nutritionally comparable pair-fed animals, and had plasma glucose levels similar to those of control-fed animals. There was a significant increase in the glucose level of all groups (p<0.001) as seen in Table 3.

Table 3. The glucose, T. protein, albumin, globulin and T.Billuribin levels in control, BLM, BLM+ boric acid, BLM+boric acid+CoQ10 groups (p>0.05, p<0.05, p<0.001).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tissue</th>
<th>T. Protein</th>
<th>Albumin</th>
<th>Globulin</th>
<th>T. Billuribin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>149.4286±1.5562</td>
<td>7.4029±0.1629</td>
<td>4.5614±0.0606</td>
<td>2.8286±0.2408</td>
<td>0.0971±0.1294</td>
</tr>
<tr>
<td>BLM</td>
<td>160.5714±2.2768</td>
<td>7.0529±0.1747</td>
<td>3.9243±0.0633</td>
<td>3.0000±0.2204</td>
<td>0.0743±0.0149</td>
</tr>
<tr>
<td>BLM + Boric Acid</td>
<td>192.4286±2.6445</td>
<td>6.9857±0.1327</td>
<td>6.1014±0.1283</td>
<td>3.0857±0.1993</td>
<td>0.1286±0.0163</td>
</tr>
<tr>
<td>BLM + Boric Acid+ CoQ10</td>
<td>172.2857±2.9416</td>
<td>7.3986±0.1606</td>
<td>4.4443±0.0767</td>
<td>3.0714±0.1358</td>
<td>0.1371±0.0180</td>
</tr>
</tbody>
</table>

BLM: bleomycin, CA: carbonic anhydrase, CAT: catalase
to protect cells (Dudnik et al. 2001) against lipid peroxidation, and contribute to the antioxidant capacity of the jaundiced newborn infants (Bélanger et al. 1997).

There are no studies available in literature regarding the relationship between BLM-induced pulmonary fibrosis and bilirubin. We believe our study is the first. In our study, while there were significant decreases in the bilirubin levels of the BLM group, there were significant increases in the bilirubin levels of all the other groups (p<0.05) as seen in Table 3.

Results concluded that boric acid and CoQ10 had an antioxidant effect against bleomycin-induced pulmonary fibrosis.

References


Giri NS, Nakashima JM, Curry DL (1985). Effects of intratracheal administration of bleomycin or saline in pair-fed and control-fed hamsters on daily food intake and on plasma levels of glucose, cortisol, and insulin, and lung levels of calmodulin, calcium, and collagen. Exp Mol Pathol 42, 206-219.


