The effects of selenium and vitamin E on lung tissue in rats with sepsis

Abstract

Purpose: In this study we examined the ability of selenium and vitamin E to prevent sepsis-induced changes in lung tissue.

Methods: Fifty rats were divided into five groups: Group 1: Control group; Group 2: Sepsis group. In this group only cecal ligation and perforation (CLP) was performed. Group 3: Selenium group. An intraperitoneal dose of 100 µg selenium was given for the first two days followed by a daily dose of 40 µg for the next five days. CLP was performed the following day. Group 4: Selenium and vitamin E group. In addition to selenium, vitamin E was given intramuscularly in a dose of 250 mg/kg/day for seven days. CLP was performed the following day. Group 5: Vitamin E group. Vitamin E was given intramuscularly in a dose of 250 mg/kg/day for seven days. CLP was performed the following day.

Results: There were significant differences between Group 2 and all other groups in terms of blood gas values (pH, pCO2, SaO2), and leukocyte, C-reactive protein (CRP) and glutathione peroxidase levels (p<0.005). There was no statistically significant difference between groups 3, 4 and 5 in terms of histopathological changes in lung tissue (p>0.05), but all groups were significantly different compared with Group 2 (p<0.05).

Conclusion: Sepsis-induced lung tissue damage can be reduced or prevented by pretreatment with selenium and/or vitamin E in a rat model.

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The incidence of sepsis is ever increasing. Difficult to cure, with complex pathophysiological effects, it still represents a major challenge in medicine. In spite of aggressive cures and knowledge of the physiopathology of sepsis at a molecular level, factors such as an increase in the number of immunosuppressive patients, infections in intensive care units, radiotherapy, chemotherapy and an increase in the number of resistant microorganisms have all led to a rapid rise in the number of sepsis cases. With recent developments in cell biology, the physiopathology of sepsis has become better understood and the mediators and cytokines involved have been defined along with the effects of these chemicals and resulting metabolic changes in the body [1, 2, 3]. In sepsis, complications related to the lungs often result in a high mortality rate. Selenium, an antioxidant that contributes to the growth and development of cells, exists in the structure of selenoprotein helps to prevent the cellular damage caused by free radicals. Sepsis patients are under severe oxidative stress. Selenium-containing enzymes protect cells against lipid peroxidation and are active in regulating the incidence of inflammation so selenium may help to protect patients against multiple organ failure [4, 5].

Vitamin E has a significant antioxidant function especially for cell membrane lipoproteins [6]. The role of vitamin E in supporting the immune system is enhanced by selenium [7, 8]. Giving vitamin E to newborns with pancreatic cystic fibrosis was found to help alleviate respiratory distress. The antioxidant effects of vitamin E are more prominent at high oxygen concentration [8]. It serves as a natural antioxidant which reduces oxidized cellular components, decomposes reactive oxygen species, and detoxifies toxic oxidation products [9]. Vitamin E also protects erythrocytes against hemolysis in premature infants and reduces DNA damage because of its antioxidant effects [9, 10]. During sepsis, oxidative stress increases while the levels of antioxidants such as selenium and vitamin E decrease. The use of antioxidants in sepsis patients can prevent the increase of oxygen free radicals [10].

In our study, we used an experimental model of sepsis to examine the effects of vitamin E and selenium to prevent sepsis-induced damage to lung tissue.

Materials and Methods
The research was conducted at the Experimental Research Center, Konya University with the approval of the Ethical Committee. Fifty female Sprague-Dawley rats (260-300 g) were separated into five groups of 10:

Group 1: Control group.
Group 2: Sepsis group. Only cecal ligation and perforation (CLP) was performed on this group.

Group 3: Selenium group. An intraperitoneal dose of 100 µg selenium was given for the first two days, followed by a daily dose of 40 µg for the next five days. CLP was performed the following day.

Group 4: Selenium and vitamin E group. An intraperitoneal dose of 100 µg selenium was given for the first two days followed by a daily dose of 40 µg for the next five days. Simultaneously, vitamin E was given intramuscularly in a dose of 250 mg/kg/day for seven days. CLP was performed the following day.

Group 5: Vitamin E group. Vitamin E was given intramuscularly in a dose of 250 mg/kg/day for seven days. CLP was performed the following day.

Sepsis model
Sepsis was induced using the CLP model; a commonly used experimental animal model of sepsis [11, 12]. After anesthesia with ketamine hydrochloride (60 mg/kg), the animals were restrained in the supine position, shaved, and a 2 cm midline incision was made under sterile conditions. The cecum was isolated and tied with 4-0 silk, and ligated just below the ileocecal valve without interrupting the continuation of the small bowel and colon. The cecum was then punctured twice with a 22-gauge needle and a small amount of bowel content was extruded through the puncture holes. The ligated and punctured cecum was returned to the peritoneal cavity and the abdominal cavity was closed with 4-0 silk sutures. Normal saline (20 mg/kg body weight) was then administered subcutaneously to replace insensible losses.

CLP was not performed on Group 1 (control), only the cecum was explored. The animals were then grouped in to cages in rooms in which humidity, light and heat (22°C) were controlled. Twelve hours later the rats were fed standard rat food and fresh water. After 24 hours, the rats were anesthetized and blood was taken by intracardiac puncture to measure arterial blood gas and glutathione peroxidase, leukocytes and C-reactive protein (CRP) levels. Lung tissue was removed by sternotomy. Glutathione peroxidase was checked and a pathological evaluation of the lungs for edema, congestion, emphysema and inflammation was performed.

The precise dose of vitamin E was based on previously published studies [13]. The precise dosage of Na selenite was also based on previous studies, particularly that of Forceville et al. [14]. An intraperitoneal dose of 100 µg/day for 2 days was given, which was then reduced to 40 µg/day for 5 days. During the experiment, no toxicity or other side effects were observed.
**Pathological examinations**

Blind pathological examinations were then conducted and pathological changes were graded according to the degree of edema, congestion, emphysema and inflammation features in the lung tissue samples. The changes are expressed as follows: Grade 0: No pathological changes in lung tissue, Grade 1: minor changes, Grade 2: moderate changes, Grade 3: distinct changes.

**Glutathione peroxidase (GPx) measurements**

Blood and left lung tissue was kept at -80°C in the freezer and used for measurements of glutathione peroxidase. Blood glutathione peroxidase activity was measured with Paglia and Valentine's spectrophotometric kinetic method using a commercial kit (Ransel-Randox kit). After weighing, the tissue was homogenized with a Teflon homogenizer in cold 50 mM Tris-HCl (pH=7.4) 33% weight/volume. Samples were sonicated three times in 30 second intervals, and then centrifuged at 5000 rpm at 4°C for 30 minutes. The protein was measured using the Ransel-Randox kit and then measured with a Beckman LX 20 autoanalyzer using a Beckman marker kit. The results were given as (U/g protein) [15].

**Statistical methods**

Variance analysis (ANOVA) was used for comparison between groups. The Tukey HSD was used as a posthoc test and the Kruskal-Wallis variance analysis was used for variables not conforming to normal distribution. The Bonferroni-corrected Mann-Whitney-U test was used for paired comparison, and p<0.05 was accepted as the level of significance.

**Results**

Table 1 summarizes the blood gases and biochemical data (mean ± SD) for all groups. pH, pCO2, SaO2 and HCO3 values in the arterial blood gas of Group 2 were significantly different in comparison with the other groups (p<0.05). pH values showed no statistically significant difference between Groups 3, 4 and 5 (p>0.05)

pCO2 values in Group 4 (selenium + vitamin E) were significantly different in comparison with Group 5 (p<0.05). SaO2 values showed a statistically significant difference between Group 3 and Group 4 (p>0.05), but the SaO2 values in Group 4 were significantly higher than in Group 5 (p<0.05).

HCO3 values showed statistically significant differences between Group 1 and the other groups (p<0.05). No significant differences were found between Groups 2, 3, 4, and 5 (p<0.05). Leukocyte values for Group 2 were significantly higher than all the other groups (p<0.05). Values for Group 5 were significantly higher than Group 4 (p<0.05), but there was no significant difference between Group 3 and Group 4 (p>0.05). CRP levels for Group 2 were significantly higher than all the other groups (p<0.05). There were no significant differences between Groups 3, 4, and 5 (p>0.05). Glutathione peroxidase levels in the blood samples were not significantly different between Groups 3, 4, and 5 (p>0.05), but the glutathione peroxidase values of Group 2 were significantly lower than the other groups (p<0.05).

Glutathione peroxidase values in lung tissue were not significantly different between Groups 3, 4 and 5 (p>0.05) but were significantly lower in Group 2 (p<0.05). In terms of histopathological changes in lung tissue, there were no significant differences between Groups 3, 4, and 5 but Group 2 (p<0.05) values were significantly different. (Table 2)

**TABLE 1. Blood gases and biochemical data for all five groups.**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (mean±SD)</th>
<th>Group 2 (mean±SD)</th>
<th>Group 3 (mean±SD)</th>
<th>Group 4 (mean±SD)</th>
<th>Group 5 (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCO2</td>
<td>34.22±2.2</td>
<td>51.01±3.8**</td>
<td>47.1±4.7</td>
<td>39.2±1.9*</td>
<td>42.1±4.9</td>
</tr>
<tr>
<td>SaO2</td>
<td>92.5±3.9</td>
<td>61.9±7.7**</td>
<td>79.3±2.2</td>
<td>82.9±3.7*</td>
<td>76.1±2.4</td>
</tr>
<tr>
<td>pH</td>
<td>7.4±2.3</td>
<td>7.3±2.4**</td>
<td>7.3±1.6</td>
<td>7.3±1.4</td>
<td>7.3±2.7</td>
</tr>
<tr>
<td>Leucocytes (106/L)</td>
<td>9197±503</td>
<td>2763±522**</td>
<td>4310±457</td>
<td>4983±710*</td>
<td>4029±710</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>6.4±9.7</td>
<td>3.2±2.9**</td>
<td>0.92±2.01</td>
<td>0.7±1.4</td>
<td>0.8±1.9</td>
</tr>
<tr>
<td>Blood GPx (U/g)</td>
<td>652±430</td>
<td>1526±1565**</td>
<td>731±370</td>
<td>622±271</td>
<td>1189±1635</td>
</tr>
<tr>
<td>Tissue GPx (U/g)</td>
<td>70±25</td>
<td>78±43**</td>
<td>58±34</td>
<td>72±46</td>
<td>94±41</td>
</tr>
</tbody>
</table>

* Significant (p<0.05) with one- way ANOVA, and TUKEY HSD with pairwise comparison vs Group 5
** Significant (p<0.05) with one- way ANOVA, and TUKEY HSD with pairwise comparisons vs Groups 1, 3, 4 and 5

GPx - glutathione peroxidase
CRP - C-reactive protein

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Histopathological views of right lung tissue were obtained post mortem. Figure 1 shows images of specimens of right lung tissue from each group.

**Discussion**

Sepsis has always been a major health problem because it is difficult to treat and is associated with high mortality and morbidity; thus, early diagnosis and treatment of sepsis are important. In sepsis, bacterial toxins cause the release of cytokines and the activation of mediator systems. Endotoxin release is a common feature of all gram-negative bacteria with Lipid A structure. These substances activate the complement and coagulation cascade that results in the biochemical changes leading to the clinical picture of sepsis [3].

Epidemiological studies show that selenium helps to prevent carcinogenesis. It was discovered that selenium support decreases the frequency of liver, colon, prostate and lung cancers; however, the response to selenium is not identical for all individuals [16]. The report of the Selenium and Vitamin E Cancer Prevention Trial (SELECT) found no reduction in risk of prostate cancer with either selenium or vitamin E supplements but a minor increase in prostate cancer risk with vitamin E. Longer follow-up and more prostate cancer events would
provide further insight into the relationship of vitamin E and prostate cancer [17]. Because immune response is extremely complicated in the sepsis process, antimicrobial treatment alone is not sufficient to increase the possibility of survival of patients. It is now thought that the use of antioxidants may have a role in the treatment of sepsis.

In oxidative cases like sepsis, the release of free radicals causes tissue damage by lipid peroxidation. Antioxidants like vitamin E, selenium and glutathione peroxidase inhibit lipid peroxidation. Studies have shown that the use of antioxidants improve patient survival. Glutathione peroxidase protects erythrocytes and cell membranes against the effects of oxidation [4,8,13]. The primary aim of this study was to examine the ability of selenium to reduce respiratory problems in rats with sepsis. Vitamin E was added to the study because it increases the efficiency of selenium but was not the main focus.

The removal of oxygen free radicals from the body is carried out by the antioxidant defense system. When there is an increase in oxygen free radicals, the body may be susceptible to several diseases. A decrease in glutathione peroxidase, an antioxidant able remove free radicals, is associated with a decrease in selenium levels. In severe sepsis cases, selenium concentration and glutathione peroxidase activity were both found to be low and an increase in glutathione peroxidase activity can be promoted by the replacement of selenium. In intensive care cases, a low serum selenium level indicates low antioxidant defense system. When there is an onset of infection and decreases with the initiation of treatment and regression of inflammation and this series of events is used to monitor the efficacy of treatment [26]. In our study, the CRP level of the induced sepsis group was significantly higher than all other groups (p<0.05), but there were no statistically significant differences between other groups (groups 3, 4 and 5) (p>0.05). The leukocyte level was significantly higher in Group 2 compared with the other groups (p<0.05). Leukocyte values of the groups given selenium and vitamin E were lower than in the group given only vitamin E. (p<0.05).

In sepsis, free radicals increase the synthesis of cytokines. In previous studies, it has been shown that alpha tocopherol analogs from antioxidant agents, which are applied to neutralize the effects of the oxygen free radicals that occur in sepsis, may extend survival of patients [13]. Recently, the role of antioxidant agents in the physiopathology of sepsis has become better understood and it is thought that they may be an effective treatment for sepsis. It has been shown that selenium replacement increases natural killer cell activity, immunoglobulin synthesis and phagocytosis [27,28]. Araujo et al. showed that the risk of infection in newborns was higher in those with a selenium and vitamin E deficiency [29]. In septic rats, a short-term, high-dose enteral supplementation of vitamin E modulated the monocyte and macrophage response to endotoxins. In patients with major burns, Vitamin E levels were seen to decrease, while serum lipid peroxides increased. In patients treated with vitamin E, serum levels increased and lipid peroxides decreased to the levels of the healthy control group [30].
Many studies show the potential benefits of selenium and vitamin E in sepsis; however, to date, no conclusive study based on histopathological findings describing the therapeutic ability of selenium and vitamin E to decrease respiratory problems of the lung in sepsis has been published.

Decreased serum selenium levels have been observed in acute and chronic inflammatory disease with high CRP values. In the present study, the CRP values for Group 2 were significantly higher than for all other groups (p<0.05). In critically ill patients, especially those affected by sepsis, low selenium levels have been found and have been associated with more extensive tissue damage and organ failure [31]. Selenium supplementation resulted in increased activity of GPx, reduced oxidative damage and an improved clinical outcome including a significant reduction in the rate of secondary infection and reduced mortality. Hawker et al. reported that in rats, induced oxygen toxicity decreased after the administration of selenium. They also found an increase in glutathione peroxidase levels in blood and tissue (heart, liver, lung and spleen) of rats given selenium and reported that pulmonary edema was more severe in the group that was not given selenium [32]. In our study, glutathione peroxidase levels showed no statistically significant difference between the groups given selenium and vitamin E (p>0.05)

This study indicates that the sepsis-induced changes in lung tissue can be reduced or prevented by pre-treatment with selenium – either with or without the addition of vitamin E.

References


