

Protective effect of β -glucan on acute lung injury induced by lipopolysaccharide in rats

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Background/aim: Lipopolysaccharide (LPS)-induced endotoxemia can cause serious organ damage such as acute lung injury and death by triggering the secretion of proinflammatory cytokines and acute-phase reactants. The goal of this study was to evaluate the effects of β -glucan on inflammatory mediator levels and histopathological changes in LPS-induced endotoxemia.

Materials and methods: Forty-seven male Wistar albino rats were randomly allocated into four groups as follows: control group, LPS group (10 mg/kg LPS), LPS + β -glucan group (100 mg/kg β -glucan before LPS administration), and β -glucan group. Twelve hours after LPS administration, lung and serum samples were collected. Concentrations of IL-6, IL-8, C-reactive protein (CRP), and procalcitonin were measured in the serum at hours 0 (basal) and 12. The severity of lung damage was assessed by an appropriate histopathological scoring system.

Results: Serum levels of CRP in the LPS group at 12 h were significantly higher than in the other groups, whereas serum IL-6 levels in the LPS and LPS + β -glucan groups at 12 h were significantly decreased. The mean histopathological damage score of the LPS group was slightly higher than that of the LPS + β -glucan group. Moreover, mortality rate was significantly decreased in the LPS + β -glucan group versus the LPS group.

Conclusion: β -Glucan reduces endotoxemia-induced mortality and might be protective against endotoxemia-induced lung damage.

Key words: Endotoxin, acute lung injury, β -glucan, C-reactive protein, interleukin-6

1. Introduction

Lipopolysaccharide (LPS), an important cell-wall component of gram-negative bacteria and a potent inducer of the host immune system, causes endotoxemia (1). LPS administration induces systemic inflammation characterized by increases in proinflammatory cytokines without bacteremia (2). Several markers and acute-phase reactants have been suggested to provide clues about the severity of endotoxemia. Proinflammatory cytokines such as IL-6 and IL-8, which play critical role in all phases of host defense, are secreted mainly by macrophages (3). Cytokines induce production of acute-phase proteins including C-reactive protein (CRP) and procalcitonin. CRP is a specific late infection marker, whereas cytokines and procalcitonin are early infection markers. Therefore, the use of multiple markers such as CRP, procalcitonin, IL-6, and IL-8 is useful for monitoring the severity of endotoxemia (4). In several

studies, a positive correlation was shown between these mediators and endotoxemia (5,6).

β -Glucans are glucose polymers of the cell walls of yeast, fungi, and cereal plants. They are accepted to be among the most powerful immune response modifiers. Since the beneficial effects of β -glucans on the immune system are devoid of toxic and adverse effects, studies have focused on β -glucan molecules. Several studies reported that β -glucans inhibit tumor development, promote wound healing, protect from ototoxicity, restore hematopoiesis, induce production of cytokines, increase salivary IgA secretion, prolong survival, and enhance defense against bacterial, viral, fungal, and parasitic challenges (7–9).

In the present study, we aimed to investigate the effects of β -glucan on LPS-induced lung injury by histopathological and biochemical analysis. The alterations in plasma concentrations of proinflammatory mediators were determined by measurements at hours 0 (basal) and 12 following LPS administration.

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2. Materials and methods

2.1. Animals and groups

All experimental protocols were approved by the Experimental Animals Ethics Board of Bezmialem Vakıf University. The care and handling of the animals was done in accordance with National Institutes of Health guidelines. Animals were obtained from the Bezmialem Vakıf University Research Center. The rats were kept at a constant temperature (22 ± 1 °C) with 12-h light and 12-h dark cycles and were provided standard rat chow and tap water ad libitum.

Forty-seven male Wistar albino rats, weighing 315 to 350 g, were included in this study. Rats were randomly divided into four experimental groups as follows: control group (n = 7), LPS group (n = 18), LPS + β -glucan group (n = 15), and β -glucan group (n = 7).

2.2. Experimental protocols

All rats were anesthetized with 80 mg/kg ketamine (Ketalar, Pfizer, Turkey) and 5 mg/kg xylazine HCl (Rompun, Bayer, Turkey). Following anesthesia, a p50 polyethylene cannula was inserted into the common carotid artery for the administration of LPS and for taking blood samples before β -glucan and saline pretreatment for basal measuring of inflammatory parameters. β -Glucan groups received 100 mg kg⁻¹ day⁻¹ β -glucan (Sigma, St. Louis, MO, USA) via intragastric gavage for 3 days before LPS administration. The control group received an equal volume of saline. In order to induce acute lung injury, 10 mg/kg LPS (derived from *Escherichia coli* endotoxin; serotype O111:B4, Sigma) dissolved in sterile saline was administered to the rats in LPS groups via a p50 polyethylene cannula over a period of 10 min. Twelve hours after LPS administration, blood and lung tissue samples were collected. An euthanasia procedure was then performed.

2.3. Histopathological examination

Tissue samples were placed in 10% neutral formalin and prepared for routine paraffin embedding. Sections of tissues were cut at 5 μ m thick, mounted on slides, and stained with hematoxylin-eosin (H&E) and Masson's trichrome methods. Samples were examined and scored by a blind observer using a Nikon Eclipse i5, a light microscope with a Nikon DS-Fi1c camera, and the Nikon NIS Elements version 4.0 image analysis system (Nikon Instruments Inc., Tokyo, Japan).

The main histopathological lung damage score was calculated as previously described (10), with a minor modification. In brief, histopathological lung damage was assessed by scoring alveolar congestion, hemorrhage, infiltration or aggregation of leukocytes in air spaces/vessel walls, perivascular/interstitial edema, and thickness of the alveolar wall/hyaline membrane formation. The severity for each item was rated on a 4-point scale graded from 0 (minimal) to 3 (maximal), giving a range of total scores from 0 to 15 (most severe).

2.4. Measurement of serum inflammatory factor levels

Blood samples were obtained from all groups at hours 0 and 12 after administration of LPS. The sera were immediately separated by centrifuging at 4000 rpm for 10 min and were stored at -70 °C until analysis. IL-6 and IL-8 levels were measured with enzyme-linked immunosorbent assay (ELISA) kits (Rat IL-6 and IL-8 Platinum ELISA, eBioscience, Bender MedSystems GmbH, Vienna, Austria). The levels of procalcitonin in plasma were quantified using ELISA kits (Rat PCT Elisa Kit, Eastbio Pharm, Hangzhou Eastbiopharm Co., Hangzhou, China). The levels of CRP in plasma were quantified using ELISA kits (Rat CRP Elisa Kit, Assaypro, St. Charles, MO, USA) according to the manufacturer's instructions.

2.5. Statistical analysis

Statistical analyses were carried out using SPSS 19.0. All data are presented as mean $\pm$ standard deviation. Distribution of the groups was analyzed with one-sample Kolmogorov-Smirnov test. The differences among the cytokine measurements of the groups were carried out by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Histopathological damage scores were analyzed by Mann-Whitney U-test. Basal and 12-h cytokine measurements in each group were analyzed by two-way ANOVA with Dunn's multiple comparison tests. Mortality rates were analyzed by chi-square test. $P < 0.05$ was considered significant.

3. Results

3.1. Survival

At 12 h after LPS injection, 9 of 18 rats (50%) in the LPS group and 2 of 13 rats (15.4%) in the LPS + β -glucan group had died. β -Glucan administration before LPS significantly reduced the mortality rate ($P < 0.05$). All rats from the control and β -glucan groups survived until the end of the experimental protocol (Figure 1).

3.2. Inflammatory parameters

Serum concentrations of IL-6, IL-8, procalcitonin, and CRP obtained from animals in each group at hours 0 and 12 were determined. Levels of IL-8, procalcitonin, and

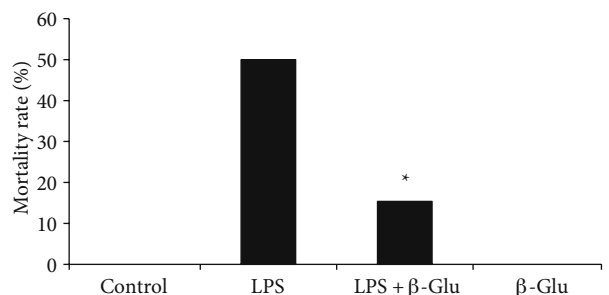


Figure 1. Mortality rates after LPS administration. * $P < 0.05$ vs. the LPS group.

CRP in all groups increased at hour 12 compared to the basal level. These increases may have resulted from the invasive applications, including the insertion of a cannula, applied to all animals. Therefore, these elevations were not marked in the figures. At hour 12, no differences were detected among serum IL-8 and procalcitonin levels of any groups (Figures 2 and 3).

CRP levels in the LPS group were significantly increased compared with the control and β -glucan groups at hour 12 after LPS injection ($P < 0.05$). LPS-induced increase in CRP level was significantly reduced with the application of β -glucan before LPS ($P < 0.05$; Figure 3).

At hour 12, IL-6 levels of the LPS and LPS + β -glucan groups were significantly lower than those of the control and β -glucan groups ($P < 0.05$). However, there was no significant difference between IL-6 levels of the LPS and LPS + β -glucan groups. Furthermore, IL-6 levels of the LPS and LPS + β -glucan groups at hour 12 were significantly lower than the basal levels. Additionally, IL-6 level at hour 12 in the control group was increased. However, this elevation was attenuated by using only β -glucan (Figure 2).

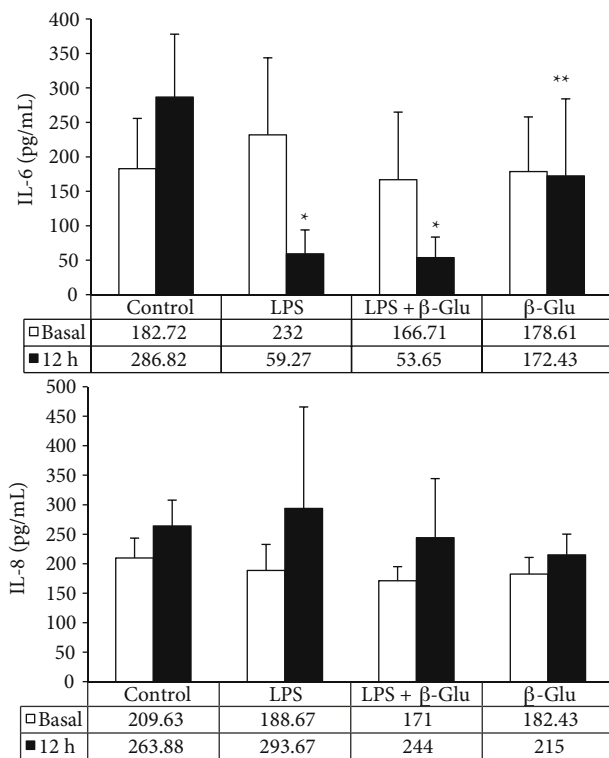


Figure 2. Serum IL-6 and IL-8 levels at hour 0 (basal) and hour 12 for all groups. IL-8 levels were significantly elevated in all groups at hour 12 versus basal levels in each group (not labeled). IL-6 was significantly elevated in the control group at hour 12 versus the basal level (not labeled). * $P < 0.05$ vs. basal level of the same groups and 12-h levels of the other groups; ** $P < 0.05$ vs. 12-h levels of the other groups.

3.3. Histopathological findings

Samples from the control and β -glucan groups revealed normal lung histology (Figures 4A and 4B, respectively). However, advanced inflammation of the alveolar wall, leukocyte (mainly neutrophil and lymphocyte) infiltration and aggregation of leukocytes in air spaces or vessel wall, congestion, perivascular and interstitial edema, prominent thickness of alveolar wall, and alveolar collapse were observed in the LPS group (Figures 4C and 4D). In the LPS + β -glucan group, the findings indicating lung injuries were somewhat improved (Figures 4E and 4F). The main histopathological lung damage scores were 1.0 ± 1.07 in the control group, 9.7 ± 1.8 in the LPS group, 6.3 ± 1.93 in the LPS + β -glucan group, and 0.7 ± 0.76 in the β -glucan group. LPS administration significantly increased lung injury score in the LPS and LPS + β -glucan groups versus the control and β -glucan groups ($P < 0.05$). β -Glucan pretreatment attenuated the LPS-induced increase in the lung injury score (Figure 5). In accordance with the lung injury scores, cell infiltration rates were 0.38 ± 0.52 in the control group, 2.67 ± 0.5 in the LPS group, 2.22 ± 0.83 in

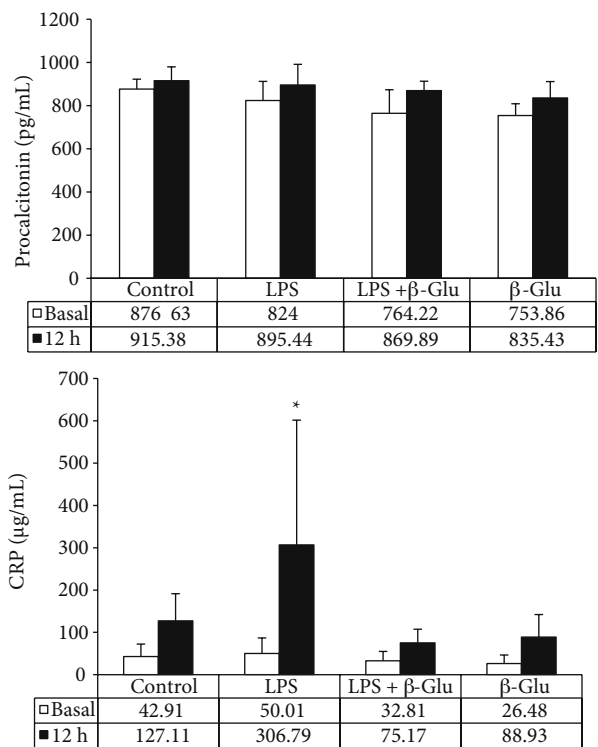


Figure 3. Serum CRP and procalcitonin levels of all groups. CRP and procalcitonin levels were significantly elevated in all groups at hour 12 versus basal levels in each group (not labeled). * $P < 0.05$ vs. the other groups.

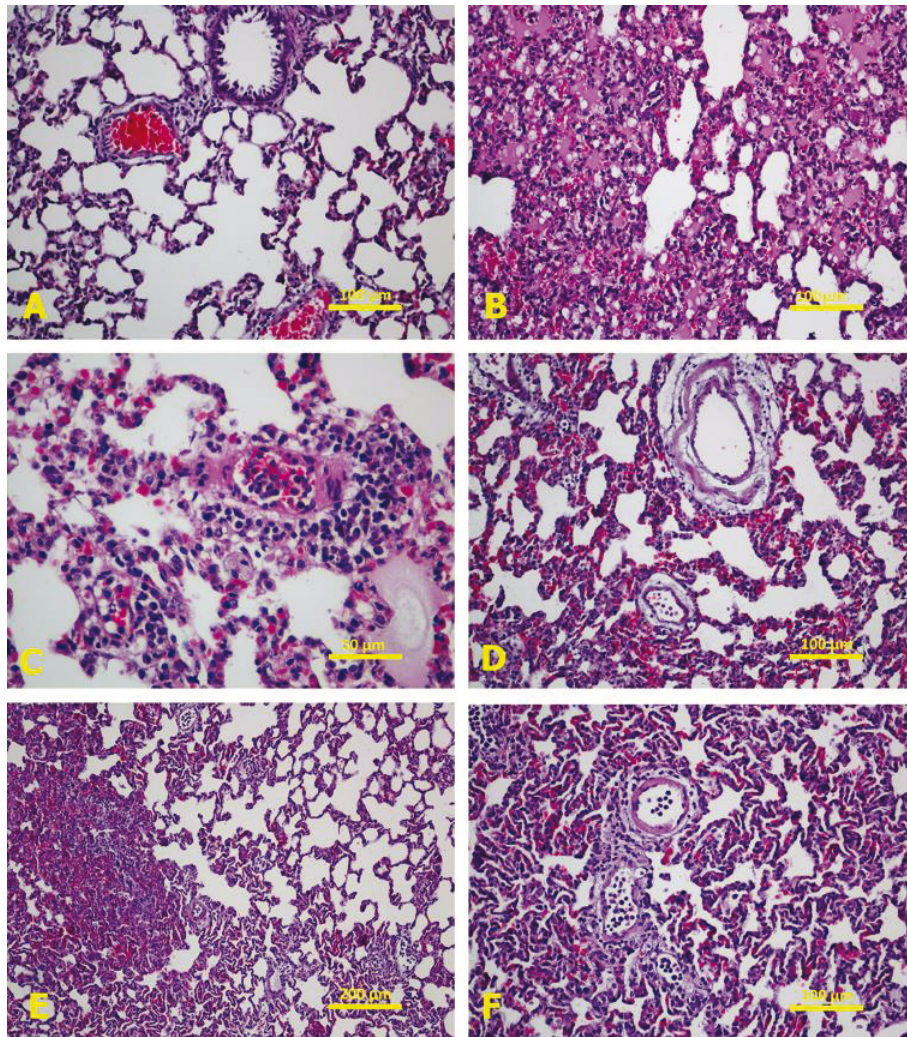


Figure 4. Histopathological features of the lung tissue samples in all groups. (A) Section from control group reveals normal lung histology, H&E, 20×. (B–D) Sections obtained from LPS group: (B) interstitial edema, congestion, inflammatory cell infiltration, alveolar collapse, and thickening of alveolar wall are observed, H&E, 20×; (C) infiltration and aggregation of leukocytes in air spaces or vessel wall are prominent, H&E, 40×; (D) congestion and obvious perivascular edema are observed, H&E, 20×. (E, F) Sections obtained from the LPS + β-glucan group: (E) congestion, inflammatory cell infiltration, alveolar collapse, and thickening of alveolar wall are observed to the left, whereas the right side seems normal, H&E, 10×; (F) mild congestion and perivascular edema are observed, and numerous leukocytes are seen within the lumen of the vessels, H&E, 20×.

the LPS + β-glucan group, and 0.29 ± 0.49 in the β-glucan group. LPS administration significantly elevated cell infiltration in the LPS and LPS + β-glucan groups versus the control and β-glucan groups ($P < 0.05$). However, no significant difference was detected between the LPS and LPS + β-glucan groups (Figure 5).

4. Discussion

Endotoxins can cause serious organ damage and death by triggering activation of inflammatory cells (11) and a

sequence of events including blood vessel dilation, increased blood flow, and leukocyte infiltration (12). Massive accumulation of neutrophils, interstitial pulmonary edema, and increased expression of proinflammatory mediators are the major characteristics of acute pulmonary injury (13). Neutrophil adhesion plays an important role in the development of chronic pulmonary inflammation (14,15). In LPS-induced endotoxemia, enhanced immune response is accomplished by an increased production of the proinflammatory cytokines.

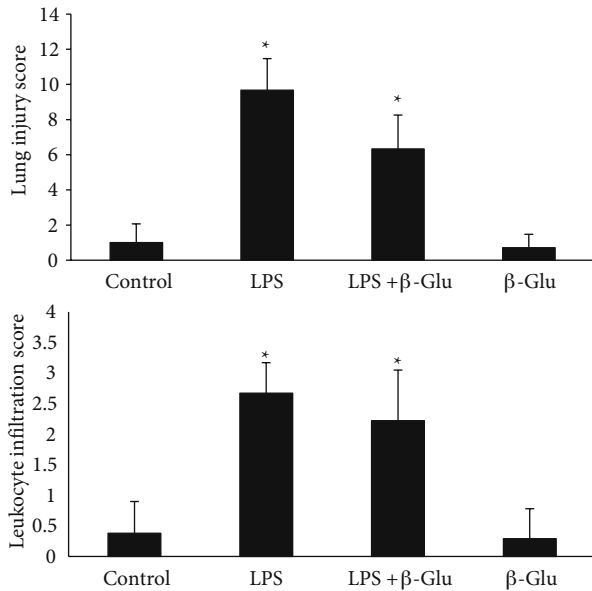


Figure 5. Lung injury and leukocyte infiltration scores of all groups. * $P < 0.05$ vs. the control and β -glucan groups.

β -Glucans, accepted to have no toxic or adverse effects, are known to be beneficial for immune system functions (3). β -Glucans are potent immunostimulants of both natural and adaptive immunity. Several experimental studies have reported antibacterial, antifungal, antiviral, and antioxidant activities of β -glucans (14,16–19). Babayigit et al. (20) reported that β -glucan is effective in decreasing leukocyte infiltration in lung damage and improving survival. β -Glucan has also been reported to decrease mortality rates in experimental sepsis models (21–23). Das et al. (24) demonstrated that β -glucan suspension increases the immunity of *A. testudineus* spawns against *S. parasitica* and reduce mortality. In the present study, the effect of β -glucan on lung injury was assessed by biochemical and histopathological analysis. β -Glucan derived from barley diminished the LPS-induced mortality rate in rats. Additionally, histopathological evaluation showed that LPS caused prominent inflammation in the alveolar wall, leukocyte (mainly neutrophil) infiltration and aggregation of leukocytes in air spaces or vessel wall, and pulmonary congestion. β -Glucan slightly reduced these findings. In accordance with our study, several other studies reported that β -glucan attenuated histopathological changes associated with lung injury in experimental rat models (8,19,23). The protective effect of β -glucan against endotoxemia may be due to a reduction in proinflammatory cytokines. On the other hand, neutrophils assume an important role in the defense system. In fact, neutrophils cause tissue damage by carrying potent compounds including toxic oxygen radicals and granular proteins. Suppression of neutrophils by β -glucan may play

a protective role against experimental lung injury.

IL-6 is an important cytokine of the early host response against the infection. Its concentration increases sharply after exposure to bacterial products and precedes the increase in CRP (20,24). However, in our study, serum levels of IL-6 after LPS injection were significantly decreased compared with the basal levels in the LPS and LPS + β -glucan groups. Serum levels of IL-6 were also significantly decreased in the β -glucan group versus the control group, but no significant difference was detected in level of IL-6 between the basal measurement and at 12 h in the β -glucan group. Similarly, Bedirli et al. (23) reported that administration of β -glucan completely inhibited the elevation of IL-6. Therefore, we suggest that β -glucan may reduce the level of IL-6. The diminished level of IL-6 after LPS administration may be due to its very short half-life (5,6) and also the presence of excessive antiinflammatory response. In the late phase of sepsis, the amount of produced proinflammatory cytokines of monocytes and lymphocytes may be severely impaired due to lower expression of MHC class II and decreased lymphocyte proliferation and activation (25–27). The impairment of production of proinflammatory cytokines is known as ‘immunoparalysis’. We could only measure serum values of IL-6 at hours 0 and 12. Nevertheless, we realized that β -glucan had no significant effect on IL-6 levels of LPS-administrated groups at hour 12.

During inflammation, IL-6 concentration increases and returns to normal values more quickly than that of CRP (28). CRP, an acute-phase reactant, is synthesized by the liver in the early phase of infection and tissue injury. IL-6 is the major inducer of CRP production. It is synthesized within 6 to 8 h of exposure to an infective agent or tissue damage, peaks at about 24 to 48 h, and then diminishes over time as the inflammation resolves (5,29). In the present study, serum levels of IL-6 and CRP were assessed after the injection of LPS. CRP levels were increased at 12 h after LPS injection as compared to the basal levels. Samuelsen et al. (30) reported that increased intake of barley β -glucan decreased levels of CRP and proinflammatory cytokines. Similarly, we found a significant decrease in the level of CRP following β -glucan treatment. However, some studies reported that consumption of β -glucan did not affect plasma level of CRP in hypercholesterolemic subjects (31,32). Pionnier et al. (33) showed that oral administration of β -glucan for 14 days stimulated serum CRP levels during an *Aeromonas salmonicida* infection in an animal study. Since a few studies confirmed the effect of β -glucan on serum CRP levels, we suggest that further studies need to be performed.

Procalcitonin, another acute-phase marker, is synthesized by the liver and induced by similar means as CRP. Although its level is undetectable in the blood of

healthy people, its concentration increases in relation to the intensity of inflammatory reaction due to infection in septic patients. Serum concentrations of procalcitonin begin to rise 4 h after exposure to the bacterial endotoxin, peak at 6–8 h, and remain elevated for at least 24 h in humans. It has been shown that a great amount of procalcitonin is produced by human liver cells following IL-6 stimulation. The procalcitonin level remains continuously high despite a decrease in IL-6 levels in parallel with the severity of the ongoing infection (5). Elevations in procalcitonin are generally observed before CRP rises and they decline in a much shorter time. Additionally, when patients respond to therapy, procalcitonin levels return to normal much more quickly than those of CRP (29). Accordingly, we found that at hour 12 CRP was increased after LPS administration, and pretreatment with β -glucan returned its level to the level of the control group. However, we did not observe any change in procalcitonin levels among groups at 12 hours. We suggest that it may have increased to its peak levels and returned to normal during the first 12-h period.

In the present study, systemic administration of LPS caused no significant change in plasma levels of IL-8 among groups at hour 12. Theuwissen et al. (31) reported that consumption of β -glucan in hypercholesterolemic

subjects did not change plasma concentrations of inflammatory parameters, including IL-6, IL-8, and CRP. Similarly, Samuelsen et al. (30) reported that a mixed-linked β -glucan isolated from barley showed no significant effect on IL-8 secretion. In contrast to these studies, human whole blood incubated with soluble yeast β -glucan showed enhanced production of IL-6 and IL-8. Moreover, when LPS was added together with this β -glucan, IL-8 concentration was strongly increased, whereas no further increase in IL-6 concentration was observed (25).

In conclusion, β -glucan treatment decreased mortality rate. Moreover, it decreased lung damage according to histopathological data in the present experimental endotoxemia study. Decreases in leukocyte infiltration and CRP levels by β -glucan may be useful in prevention of acute lung injury. We suggest that β -glucan may support conventional therapy. However, further studies are required to evaluate the potential of β -glucan as an option for protecting lungs from LPS-induced endotoxemia.

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