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Serum YKL-40 as Candidate Biomarker in Sepsis-Induced Lung Injury

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Abstract

Objective: Sepsis is a phenomenon with a higher mortality rate and a higher incidence and its mechanism has not been fully elucidated yet. Oxidative damage is also known to be responsible for damage and mortality in sepsis. In recent years, simvastatin has been reported to be used for the treatment of sepsis and had many pleiotropic effects such as antioxidant and anti-inflammatory. It is demonstrated that chitinase-3-like protein 1 exerts its function in infection. Recent studies have proposed that chitinase-3-like protein 1 may be an indicator of sepsis.

Methods: The effect of simvastatin on chitinase-3-like protein 1 and antioxidant enzymes, glutathione reductase, glutathione peroxidase, superoxide dismutase in rats which formed as control, lipopolysaccharide, simvastatin, and simvastatin + lipopolysaccharide groups was examined.Serum and lung tissue Chitinase-3-like protein 1 levels, which may be an early biomarker in sepsis were investigated. Serum glutathione reductase, glutathione peroxidase, and superoxide dismutase were evaluated as oxidative stress parameters. Also, lung tissues were histologically examined with hematoxylin and eosin staining.

Results: Lung chitinase-3-like protein 1 was improved in the simvastatin+lipopolysaccharide group comparing lipopolysaccharide, meanwhile, serum chitinase-3-like protein 1 was elevated in the lipopolysaccharide group ($p<0.05$), and pretreated of simvastatin was to be reduced YKL-40 levels. In the lipopolysaccharide group, decreased serum glutathione reductase ($P < .01$), glutathione peroxidase ($P < .01$), and superoxide dismutase ($P < .01$) were found to be consumed against oxidative stress, and in the simvastatin + lipopolysaccharide group, increased glutathione reductase and glutathione peroxidase were obtained ($P < .01$). We observed histological damage in the lipopolysaccharide and improved injury in the simvastatin + lipopolysaccharide as similar simvastatin and controls.

Conclusions: Simvastatin may be effective on sepsis and protective against inflammation and oxidative stress. This study proposed that chitinase-3-like protein 1 may be an indicator candidate for sepsis.

Keywords: Lipopolysaccharide, Chitinase-3-like protein 1, Simvastatin, Glutathione peroxidase, Superoxide dismutase, Lung

Introduction

It is reported that chitinase-3-like protein 1 (CHI3L1), also known as YKL-40, is secreted by various cell types such as pro- and anti-inflammatory cells and bronchial epithelial cells.[1,2] Chitinase-3-like protein 1 is thought to have an effect on infection and repairing of tissue. It is found that YKL-40 levels were increased during sepsis.[1,3] There are studies showing YKL-40 relation with chronic diseases like cancer, rheumatoid arthritis, liver fibrosis, and chronic inflammation, but its physiological function is not clear.[4-6] Also, it is proposed YKL-40 appeared as an immune response of tissue to the repairing process of damaged tissue after *Escherichia coli* endotoxin injection in healthy volunteers.[3]

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Sepsis is defined to be involved in increased inflammation and oxidative stress mechanisms of the host organism's response to infectious agents like bacterial toxins in multiple organ failure.[7,8] In sepsis, it is demonstrated that the progression of lipid peroxidation of membrane lipids, proteins, DNA, and RNA macromolecules causes impairment of balance between antioxidant and oxidant molecules. The levels of major affected antioxidant enzymes are important by removing increased superoxide radicals, hydrogen peroxide in multi-organ failure.[7,9]

The lung is one of the first damaged organs. Acute lung injury has the primary symptoms and has the highest frequency. The lipopolysaccharide (LPS)-induced sepsis model is known to cause activation of the inflammatory and the oxidative stress pathways by stimulating cytokine storm and activating macrophages.[7,8]

Simvastatin is one of the members of the statin family and has a lipid-lowering effect in cardiovascular disease.[10] Statins were suggested to be a possible novel therapeutic agent by decreasing levels of inflammatory and immunomodulatory molecules in the development of sepsis.[11]

In this research, we investigated the impacts of simvastatin on YKL-40 levels, oxidative stress, and lung tissue morphology in rats with LPS-induced sepsis.

Methods

Experimental Groups

After getting approval from the Animal Experiments Local Ethics Committee of İstanbul University-Cerrahpaşa (Date: 2012, Number: 138). 32 adult *Wistar albino* male rats with an average weight of 200-250 g were used in this study. Rats were randomly separated into the control, LPS, simvastatin, and simvastatin + LPS groups.

Experimental Procedures

Each animal was provided with a standard diet and water and was housed in cages. All animals were kept at room temperature (22 ± 2°C).

About 20 mg/kg of simvastatin (Sigma Aldrich, Number: S0650000 in 1 mL saline solution was given via p.o. for 5 days every morning. This dose and duration of simvastatin have been reported as the most appropriate curative dose in the literature.[12] Lipopolysaccharide (*E. coli* O127: B8, Sigma Aldrich, Number: L5668) was prepared in saline solution and 1 dose was applied via i.p. as 20 mg/kg for the experimental sepsis model. In the simvastatin + LPS-treated group, LPS was injected 1.5 hours after the fifth day of simvastatin injection. After 4 hours, LPS injection, ketamine–xylazine anesthesia, was administrated (90-10 mg/kg, i.p., respectively).

Under anesthesia, blood samples were collected from the heart by cutting the midline thorax with a vertical incision, and lung tissue samples were dissected in all groups. The tissue samples were rapidly frozen by liquid nitrogen for analysis of YKL-40. The remaining parts of the same tissue samples were separated in 10% formaldehyde for histological examinations. The blood samples were centrifuged, and the separated serum was removed to −20°C for YKL-40, glutathione reductase (GR), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) measurements.

For enzyme-linked immunosorbent assay (ELISA) studies, lung tissues were homogenized with potassium phosphate buffer and centrifuged at 2500 rpm, + 4°C for 10 minutes. Supernatant was collected for determining the levels of total YKL-40 levels.

Enzyme-Linked Immunosorbent Assay Lung Tissue and Serum Chitinase-3-Like Protein 1 Determinations

Chitinase-3-like protein 1 (Shanghai Yehua Biological Technology, China, Lot: YHB20170315843) was determined in serum samples and lung tissue homogenate using ELISA with double antibody technique at 450 nm.

Enzyme-Linked Immunosorbent Assay Serum Glutathione Reductase, Glutathione Peroxidase, and Superoxide Dismutase Determinations

Glutathione reductase (Shangai Yehua Biological Technology, China, Lot: YHB20171106220), GSH-Px (Shangai Yehua Biological Technology, China, Lot: YHB20171106219), and SOD (Shangai Yehua Biological Technology, China, Lot: YHB20171106218) levels were studied in serum samples by ELISA with double antibody technique at 450 nm.

Histological Procedures

Five-micrometer-thick slide sections were prepared and then stained by the hematoxylin and eosin (H&E) staining method. The slides were analyzed with a light microscope, and then they were investigated for the evaluation of inflammatory cell infiltration. The scoring criteria were as follows: "grade 0: absent; grade 1: rare; grade 2 as moderate; and grade 3, as extensive." Photomicrographs were taken using an Olympus BX53F light microscope attached with an Olympus DP27.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) 21.0 Statistical Software (IBM Corp.; Armonk, NY, USA) was chosen for the statistical evaluation. Analysis of variance and Tukey tests were used to compare the data obtained from the study. Data were shown as the mean ± standard deviation. $P < .05$ was reflected as statistically significant.

Results

Enzyme-Linked Immunosorbent Assay Results of Chitinase-3-Like Protein 1, Glutathione Reductase, Glutathione Peroxidase, and Superoxide Dismutase

Serum YKL-40 levels of the LPS groups were incresaed compared to controls ($P < .01$). In lung tissue, the simvastatin-treated LPS group was to higher than those of LPS but there were no statistical differences among groups ($P > .05$) (Figure 1A and B).

Glutathione reductase was found lower in the LPS group than that of the control, simvastatin, and simvastatin + LPS groups in serum samples ($P < .01$, for each group) (Figure 2A).

Glutathione peroxidase was decreased in the LPS compared to the levels in the control and simvastatin groups ($P < .01$) (Figure 2B). In the simvastatin-pretreated LPS group, the levels of GSH-Px were higher than in the LPS group, but the difference was not significant ($P > .05$).

When we examined enzymatic antioxidant molecule SOD enzyme levels in removing superoxide radicals, SOD levels significantly decreased in both of the LPS and simvastatin + LPS groups compared to control "($P < .01$ and $P < .05$, respectively)." Also, SOD levels were higher in the simvastatin group than that in the LPS group ($P < .01$) (Figure 2C).

Histological Results

There were intensive inflammations in lung sections of the LPS group in H&E staining. Lipopolysaccharide group was detected to have a higher histologic score than the others ($P < .01$). The lung injury was obtained to reduce in the simvastatin + LPS (Figure 3).

The investigation of lung tissue under light microscopy showed damage and immunoreactivity in pneumocytes and bronchial epithelium of lung and leukocyte infiltration in the LPS group. The imaging of the other experimental groups was similar to the controls (Figure 4A, B, C and D).

Discussion

Various inflammatory mediators are released during the developing process from sepsis to septic shock as a result of the immune response of the organism to the microbial threat. Many studies reported that cytokines, one of these mediators, have a major role in the damage of tissue and organs in sepsis.[11] Experimentally, LPS applied to form a sepsis model is known to activate macrophages and neutrophils and induce cytokine release.[11-14] Cytokine release was reported to cause damage to many tissues, especially the lung, and developed oxidative stress and led to multiple organ failure.[8,15,16]

A pro-inflammatory peptide YKL-40 has a critical role in pathological tissue repairing, acute and chronic inflammation,

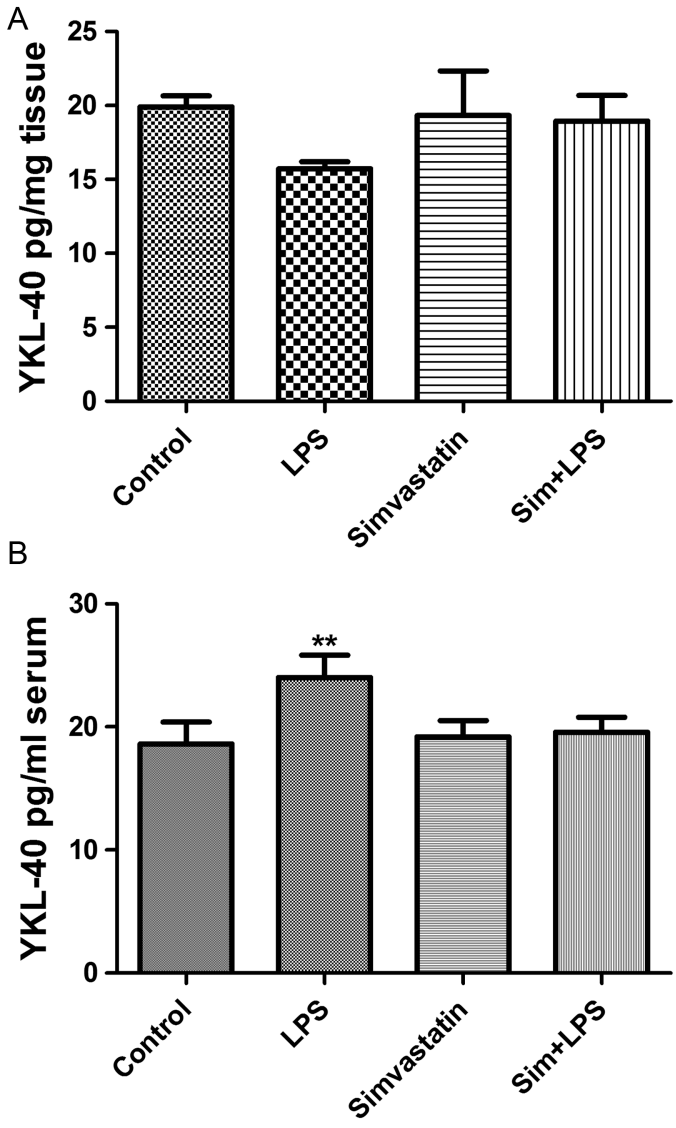

Figure 1. (A) Levels of lung tissue YKL-40 in all experimental groups. (B) Levels of serum YKL-40 in all experimental groups ($^{*}P < .01$; LPS vs. control). LPS, lipopolysaccharide; YKL, chitinase-3-like protein 1.

and oxidative stress.3,6,17 Studies in which inflammation was induced by LPS have been reported to be 31-fold higher in the transcription of YKL-40, a pro-inflammatory peptide, which is suggested to be the induction of monocytes, and a good acute phase reactant.[18]

In clinical and experimental studies, serum YKL-40 levels were reported to increase significantly in individuals with sepsis and experimental animals compared to healthy individuals.[19]

In a sepsis study of pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and YKL-40 levels, TNF-α was reported to induce alveolar macrophages and stimulate YKL-40 secretion in chronic inflammation.[4,19-21]

In our study, it was to observed simvastatin was applied as a preventive effect as an anti-inflammatory agent on decreasing serum YKL-40 levels in the simvastatin-treated LPS group. Although serum YKL-40 levels were significantly increased lung tissue YKL-40 levels have not significantly reached an increment in the LPS group compared to the control group. In patients with asthma, liver cirrhosis, Crohn's disease, high serum YKL-40 levels were

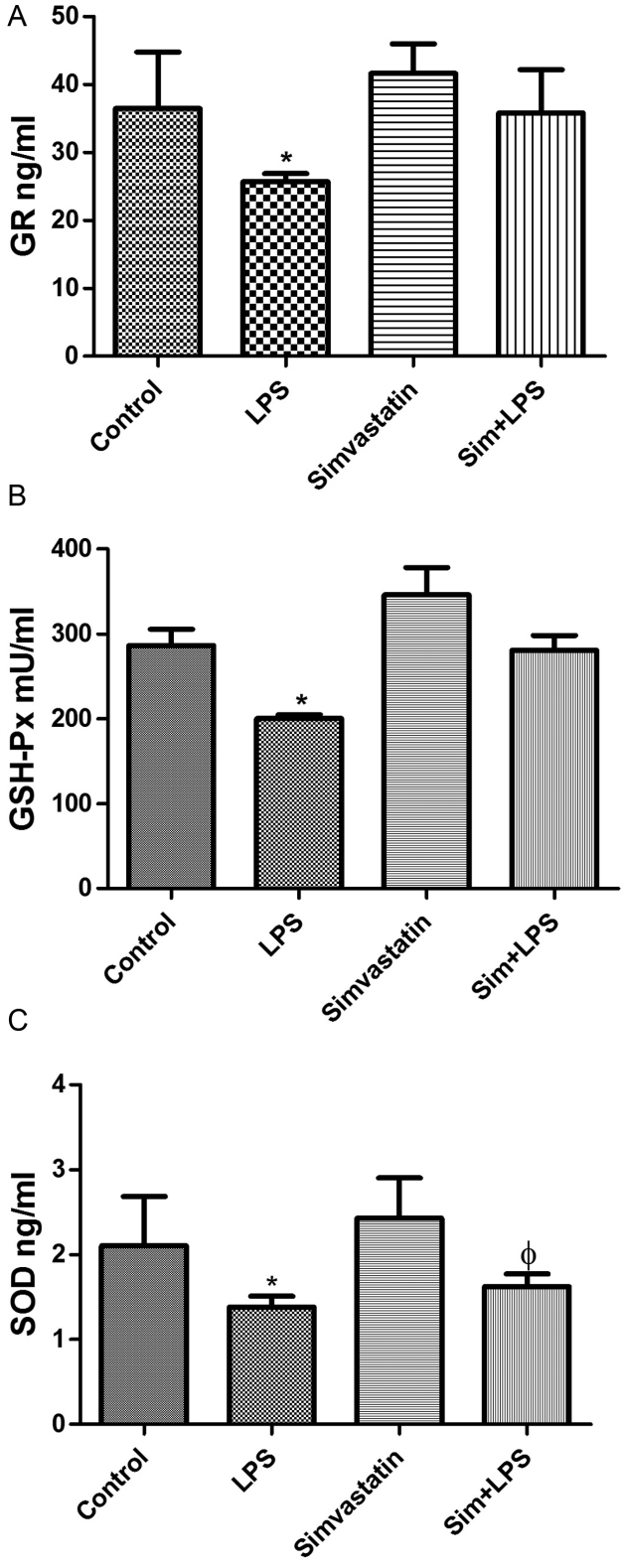

Figure 2. (A-C) Levels of serum antioxidant enzymes. (A) Levels of serum GR in all experimental groups ($^{*}P < 0.01$; LPS vs. control, simvastatin, Sim+LPS). (B) Levels of serum GSH-Px in all experimental groups ($^{*}P < .01$; LPS vs. control, simvastatin). (C) Levels of serum SOD in all experimental groups ($^{*}P < .01$, LPS vs control; $\varphi P < .05$, Sim+LPS vs. Control; $^{**}P < .01$, simvastatin vs. LPS). GR, glutathione reductase; GSH-Px, glutathione peroxidase; LPS, lipopolysaccharide; Sim, simvastatin; SOD, superoxide dismutase.

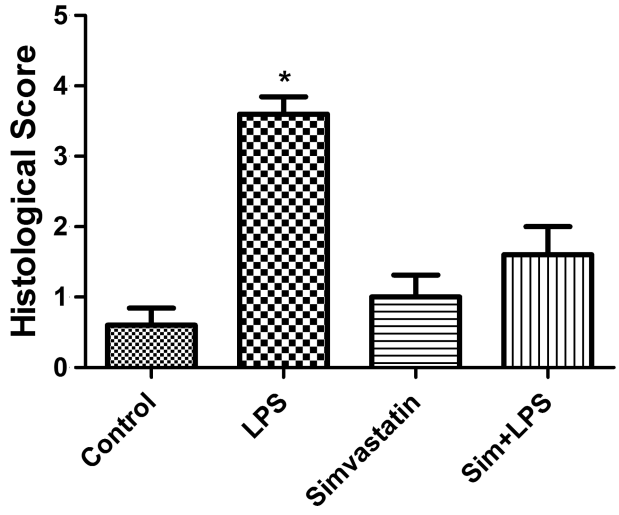

Figure 3. Histological scores of all experimental groups (*$P < .01$; LPS vs. control, simvastatin, Sim + LPS). LPS, lipopolysaccharide; Sim, simvastatin.

detected in Broncoalveolar Lavage Fluid (BALF) and alveolar epithelial cells as well as high YKL-40; these high YKL-40 levels have been associated with fibrotic lesions in patients.[3,21,22] In this study, the failure of lung YKL-40 levels to reach high levels according to serum YKL-40 levels in the LPS group can be associated with the fact that the histological change in the lung tissue is not at a level to increase YKL-40 levels. It was thought that the decapitation of animals 4 hours after LPS administration may be due to the lack of sufficient time for YKL-40 levels to remain in the lung tissue.[1,23]

There were studies about increased plasma YKL-40 being associated with disease severity, mortality, and outcome of disease in patients with bacterial pneumonia, sepsis, and septic shock.[24] Kjaergaard et al[25] reported that YKL-40 is a marker for infectious diseases and its increased level was related to poor survival after infectious diseases.

A recent study reports pro-inflammatory cytokine levels are related to pathologic conditions and that their levels increase in sepsis. In another study, the levels of pro-inflammatory cytokines started to lower after hours of continuous blood purification application showing the decrement as YKL-40.[26]

It was shown that YKL-40 has diagnostic and prognostic indicators for pulmonary illness and also has an important role in the progression and survival of pneumonitis.[27-29]

In our previous study, when pretreated simvastatin was used in LPS-induced septic group, we demonstrated that pro-inflammatory cytokine TNF-α was reduced and anti-inflammatory cytokine interleukin-10 levels were increased.[15] In another study, inhaled LPS to humans were to show anti-inflammatory effect of simvastatin reduced TNF-α levels in Broncoalveolar Lavage Fluid (BALF).[30]

Simvastatin was indicated to have anti-inflammatory and antioxidant impacts in the studies. Wang et al[31] reported using the protective effect of simvastatin by downregulating inflammatory cascade proteins in lung tissue in the sepsis group. Simvastatin has an effect on lung injury by blocking TLR4/NfkB signaling pathway.

There are various enzyme systems, such as SOD, catalase, GSH-Px, GR, which provide enzymatic protection against reactive oxygen species and oxidative stress, which is defined as the imbalance between the antioxidant defense and free radical production of the body.[31,32]

It was shown that GR, GSH-Px, and SOD antioxidant enzyme levels decreased in lung tissue of LPS-induced sepsis and approached their values of control and simvastatin groups with simvastatin treated sepsis group.10 Altintas et al[33] in their study investigating pretreatment of simvastatin demonstrated decreased malondialdehyde (MDA) levels in the endotoxemic group while increased glutathione levels and also improved histopathology in mice. In a previous study, it was demonstrated that the MDA levels were higher in the septic group as an end product of lipid peroxidation while in simvastatin pretreated LPS group, there were lower MDA levels.[8]

The level of the GR enzyme is responsible for regenerating the intracellular non-enzymatic antioxidant glutathione from

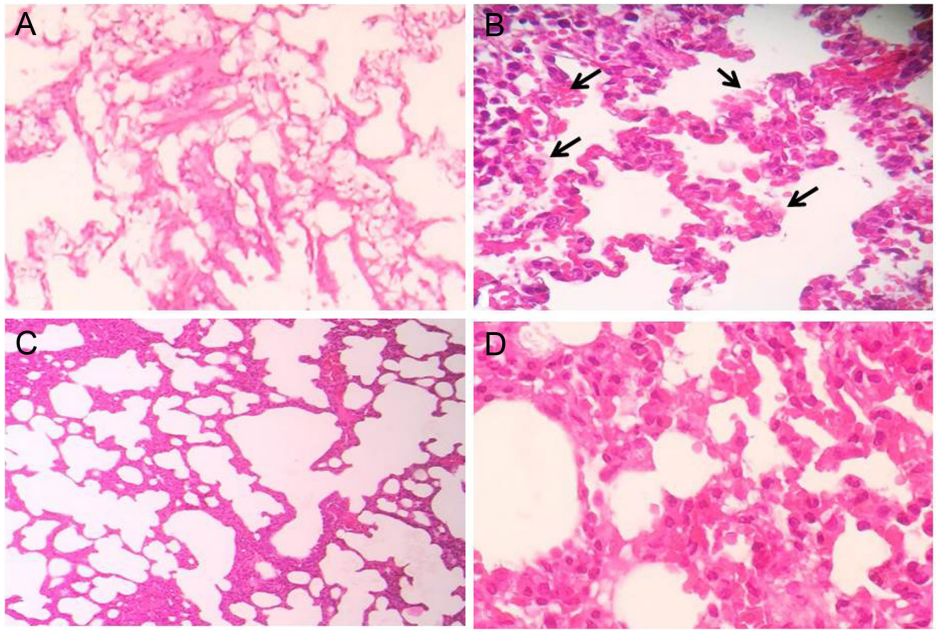

Figure 4. Sections of lung tissue stained with hematoxylin and eosin. 40× magnification. (A) Control group, (B) LPS group, (C) simvastatin group, and (D) simvastatin + LPS group. LPS, lipopolysaccharide.

glutathione oxidized (GSSG) to GSH under oxidative conditions, in the presence of NADPH, is of great importance. In the present study, GR was significantly decreased in the LPS group, compared to other experimental groups. Glutathione peroxidase was significantly lower in the LPS group than in simvastatin-treated LPS and control groups. Glutathione peroxidase preserves cells against oxidative injury caused by metabolizing H_2O_2 using reduced GSH as the electron source in cells' cytoplasm. The SOD levels were lower in the simvastatin-treated LPS group than those of the control group; This may be due to excessive amounts of superoxide radicals consumed during oxidative damage conditions. A decrease in enzymes in the antioxidant enzyme chain was shown in other studies that produced sepsis with LPS.[12,29,31,32] On the other hand, it was observed that SOD levels increased in the simvastatin group compared to the control group. As reported in much literature, many pleiotropic effects of simvastatin are known. It has been noticed that the antioxidant effect is one of them. The increase in SOD levels in the simvastatin group is thought to be due to the antioxidant effect of simvastatin.[31-35]

In histological lung tissue evaluation, we found similar results in both LPS and simvastatin-treated LPS groups compatible with other literature findings.[31,32] The investigation of lung tissue under light microscopy after H&E staining showed damage and immunoreactivity in pneumocytes and bronchial epithelium in the LPS group while the protective impact of simvastatin was to improve tissue injury in the sepsis induced LPS.

Simvastatin was reported to have many pleiotropic effects in the studies. We observed in the sepsis model induced with LPS that the increased YKL-40 was reduced by simvastatin treatment. Simvastatin may have improving effects on sepsis. We proposed that YKL-40 may be an indicator candidate for lung injury in sepsis.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of İstanbul University-Cerrahpaşa (Date: 2012, Number: 138).

Informed Consent: Our study is an experimental sepsis model in rats, so there was no need the written/verbal informed consent was obtained from the patients.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – G.A., S.T., E.O., H.Y.; Design – G.A., S.T., E.O., H.Y.; Supervision – S.T., E.O.; Resources – G.A., S.T., E.O.; Materials – G.A., H.Y.; Data Collection and/or Processing – G.A., E.O.; Analysis and/or Interpretation – G.A., S.T., E.O., H.Y.; Literature Search – G.A.; Writing Manuscript – G.A., S.T., E.O.; Critical Review – G.A., E.O., S.T., H.Y.; Other – G.A., S.T., E.O., H.Y.

Declaration of Interests: The authors have no conflict of interest to declare.

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