

# Effects of Propofol and Midazolam on the Inflammation of Lungs after Intravenous Endotoxin Administration in Rats

## *Ratlarda Endotoksin Enjeksiyonu Sonrası Akciğerde Oluşan İnflamasyona Propofol ve Midazolamın Etkileri*

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### Abstract

**Objective:** Pulmonary complications are important sepsis (such as ARDS, diffuse pneumonia). Acute respiratory distress syndrome (ARDS) is characterized by the extensive migration of neutrophils into alveoli of the lungs. Propofol and midazolam are the most widely used agents for sedation in intensive care units. Aimed to investigate the effects of anaesthesia with propofol and midazolam on measured hemodynamic variables and neutrophil migration induced by Escherichia Coli endotoxin (ECE) in pulmonary viscera.

**Materials and Methods:** Forty Sprague Dawley male rats were randomly assigned to four groups: Thiopental Sodium 30 mg/kg was administered intraperitoneally to anesthetize the rats. They were ventilated via tracheotomy. Femoral artery was cannulated for the measurement of continuous blood pressure and gases. Group C was the control. After the administration of 1 mL/kg 0.9% NaCl, infusion began at 1 mL/kg/h rate. In Group E 15 mg/kg lipopolysaccharide derived from ECE was administered iv. In Group PE, after a bolus dose of 10 mg/kg propofol and 15 mg/kg ECE, 10 mg/kg/h infusion was applied. In Group ME, after 0.1 mg/kg midazolam bolus dose and 15 mg/kg ECE administration, 0.1 mg/kg/h infusion was administered iv. Rats were sacrificed by iv potassium chloride. The lungs were then removed, fixed in 10% buffered formalin for 3 days and embedded in paraffin. They were graded on a scale of 0-3 according to the aggregation of neutrophils.

**Results:** There was intense neutrophil migration in Group E (grade 2, 3). However, although mild neutrophil migration was obtained in 70% of the rat lungs in Group ME (grade 1, 2), it was recorded in only 30% of Group PE (grade 1).

**Conclusion:** The sepsis model induced by ECE and compared with midazolam, propofol anaesthesia is associated with less neutrophil infiltration. In the light of the literature, propofol attenuate the free-radical-mediated lipid peroxidation and systemic inflammation in patients.

**Keywords:** Sepsis, lung, neutrophil, propofol, midazolam, rat

### Özet

**Amaç:** Sepsiste akciğer komplikasyonları önemli bir yer tutar (ARDS, diffüz pnömoni gibi). Akut respiratuar distres sendromu (ARDS) akciğerde alveollere aşırı nötrofil migrasyonu ile karakterizedir. Propofol ve midazolam yoğun bakımlarda hasta sedasyonu için çok yaygın olarak kullanılan ilaçlardır. Escherichia Coli endotoksini ile oluşturulan sepsis modelinde propofol ve midazolam anestezisi altında akciğerlere nötrofil göçünü ve hemodinamik parametreleri değerlendirmeyi amaçladık.

**Gereç ve Yöntem:** 40 adet rat (Sprague-Dawley cinsi) rastgele dört gruba ayrıldı. 1- Grup E (Endotoksemi grubu) 2- Grup K (Kontrol grubu) 3- Grup PE (Propofol-Endotoksemi grubu) 4- Grup ME (Midazolam-Endotoksemi grubu). Tüm deneklere intraperitoneal 30 mg/kg tiyopental sodyum verildi. Denekler trakeotomi yoluyla ventile edildiler. Devamlı kan basıncının ölçülmesi ve kan örneklerinin alınabilmesi için femoral arter kanüle edildi. Grup E'deki deneklere E.Coli'den elde edilmiş lipopolisakkarit derivesi intravenöz olarak 15 mg/kg uygulandı. Grup K'daki deneklere %0,9 NaCl 1 mL/kg intravenöz uygulandıktan sonra 1 mL/kg/saat infüzyona devam edildi. Grup PE'deki deneklere E. Coli endotoksininden 15 mg/kg intravenöz uygulandıktan sonra 10 mg/kg Propofol intravenöz olarak uygulandı Daha sonra 10 mg/kg/saat propofol infüzyonuna devam edildi. Grup ME'deki deneklere de E.Coli endotoksininden 15 mg/kg intravenöz uygulandıktan sonra 0,1 mg/kg Midazolam enjekte edildi. 0,1 mg/kg/saat midazolam infüzyonu uygulandı. Denekler 5. saat sonunda intravenöz potasyum klorid verilerek sakrifiye edildi. Akciğerleri çıkarıldı. %10 formalin içinde 3-5 gün fikse edildi ve parafin bloklarına gömüldü. Akciğer kesitleri, nötrofil yoğunluğuna göre 0-3 arasında histopatolojik olarak skorlandı.

**Bulgular:** Grup E deki deneklerin akciğer dokularında yoğun nötrofil göçü mevcuttu (grade 2, 3). Grup ME deki denek akciğerlerinde orta derecede nötrofil göçü mevcutken (grade 1, 2) Grup PE de sadece %30 denekte hafif nötrofil göçüne rastlandı (grade 1).

**Sonuç:** Endotoksinle indüklenen sepsis modelinde propofol infüzyonunun midazolamla karşılaştırıldığında akciğerlere daha az nötrofil göçü ile ilişkili olduğu ve literatürün de ışığında serbest radikal aracılı lipid peroksidasyonunu ve hastaların sistemik inflamasyonu hafifletebileceği sonucuna vardık.

**Anahtar Kelimeler:** Sepsis, akciğer, propofol, midazolam, rat

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## Introduction

Sepsis is a condition related to acute respiratory distress syndrome that causes morbidity and mortality in intensive care units [1]. Lipopolysaccharide (LPS, endotoxin) is a component of the outer membrane in gram-negative bacteria. It mediates sepsis-related toxic situations including inflammation and oxidative stress [2, 3].

LPS-induced endotoxemia causes fever, multiple organ insufficiency, septic shock, and death [4]. Following the administration of the LPS, several alterations occur in the organisms. Increased cytokine synthesis, alterations in the blood count (hemogram), organ damage, and changes in lipid metabolism may occur [5].

Anaesthetic agents are commonly used in intensive care units for sedation. The most commonly used agents are propofol and midazolam. Being aware of their effects on immune system is important when treating severe patients [6]. Several *in vivo* and *in vitro* studies have demonstrated their strong immunosuppressive effects [7].

The primary aim of this study was to explore the effects of propofol and midazolam on neutrophil migration in a sepsis model that was created with *E. Coli* endotoxin (ECE). The second aim of the study was to evaluate the potential effects of these two agents on the hemodynamic parameters and blood gases.

## Materials and Methods

Following the ethical committee approval, we included 40 Sprague-Dawley rats that weighed between 250 and 300 gr.

To obtain reliable results, we followed the below mentioned criteria when including the rats:

1. The rats should not have been used in another study previously and should not be exposed to any medication,
2. The rats had to be free of any disease history or incident diseases during our experiments.

Thiopental Sodium 30 mg/kg (Pental® Sodyum I.E.; Ulagay, Istanbul, Turkey) was administered intraperitoneally to all subjects. The neck region was shaved and the surgical area was sterilized for ventilation when the subjects were in the supine position. Head extension was provided and a midline pre-tracheal surgical incision was made onto reach trachea. Tracheostomy was then performed. Femoral artery cannulation was performed in order to measure the blood pressure and to obtain blood samples. Blood pressure was measured invasively and monitored continuously with Nihon Kohden Life Scope 9 (Nihon; Kohden Corporation, Tokyo 161, Japan). Ventilation was provided with a breath frequency of 40 and at an inspiration/expiration ratio of 1/1 with 100% oxygen (Siemens 900D; Siemens-Elcoma, AB Sweden). Anaesthesia

was maintained with 1-2% sevoflurane to all subjects. The heart rate, systolic blood pressure and oxygen saturation (Bayer Rapidlab 865) were recorded at the baseline (T0).

The subjects were randomly categorized into 4 groups:

- Group E: Endotoxemia group (n=10)
- Group C: Controls (n=10)
- Group PE: Propofol- Endotoxemia group (n=10)
- Group ME: Midazolam- Endotoxemia group (n=10)

After recording the basal levels, Group E (n=10) received intravenously 15 mg/kg of lipopolysaccharide derivate that had been obtained from *E. Coli* (ECE) (ATCC 35150 *E. Coli* 0157: H7 American Type Culture Collection 12301 Parklown Drive Rockville, MD USA). Group C (n=10) received 1 mL/kg iv bolus dose of 0.9% NaCl (Biofleks®; Biosel Medicine, Istanbul, Turkey) and then 1 mL/kg/hour of 0.9% NaCl iv infusion. Group PE (n=10) first received 15 mg/kg iv ECE and iv bolus dose of 10 mg/kg Propofol (Propofol 1% Fresenius®; Fresenius Kabi Medicine, Istanbul, Turkey). They kept receiving 10 mg/kg/hour of Propofol infusion. Group ME received 15 mg/kg iv ECE and iv bolus dose of 0.1 mg/kg of midazolam (Dormicum®; Roche, Istanbul, Turkey). Midazolam infusion was continued at a dose of 0.1 mg/kg/hour. During the experiment, the systolic blood pressure, pulse rate and the levels of blood gases were recorded at the 1<sup>st</sup> (T1), 2<sup>nd</sup> (T2), 3<sup>rd</sup> (T3), 4<sup>th</sup> (T4) and the 5<sup>th</sup> (T5) hours after the application of endotoxin.

Rectal temperature was monitored using an invasive probe and monitor. Temperature was stabilized between 36-38 °C with a heating blanket. All subjects were sacrificed at the end of the 5th hour with iv potassium chloride.

**Histopathological examination:** Following the scarification of the subjects, their lungs were removed, held in 10% Formalin for 3-5 days and then stored in paraffin blocks. Four-micron sections were obtained and stained with Hematoxylin-Eosin. All samples were scored by a pathologist who was blinded to the administered agent during the experiment. He scored all samples for eosinophil density on the lung vessel walls and alveolar space with an Olympus BH-2 microscope.

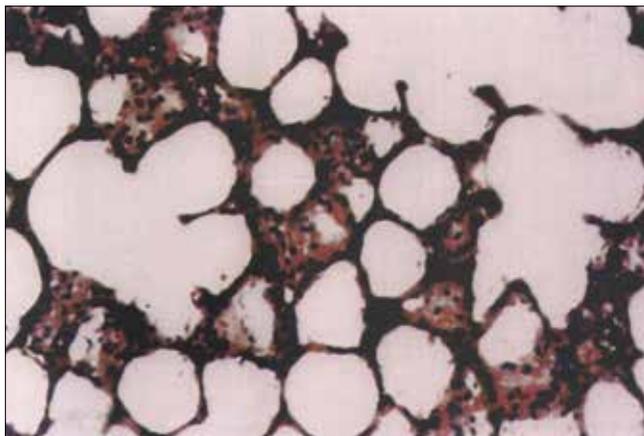
Score 0: No neutrophil migration to the lungs. (HE\*20) (Figure 1) Score 1: Minimal neutrophil migration to the lungs. (HE\*40) (Figure 2)

Score 2: Moderate neutrophil migration to the lungs. (HE\*40) (Figure 3)

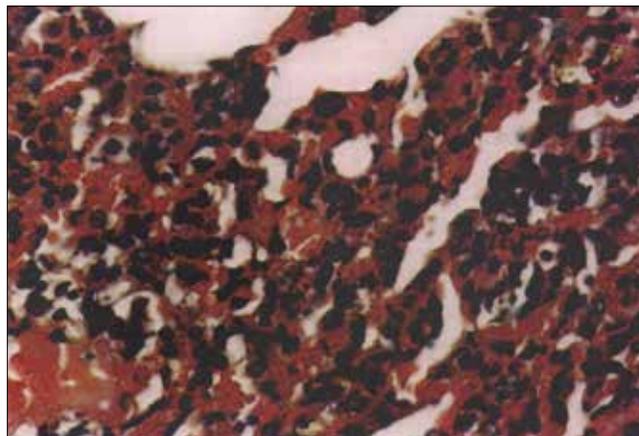
Score 3: Severe neutrophil migration to the lungs. (HE\*20) (Figure 4)

## Statistical Analysis

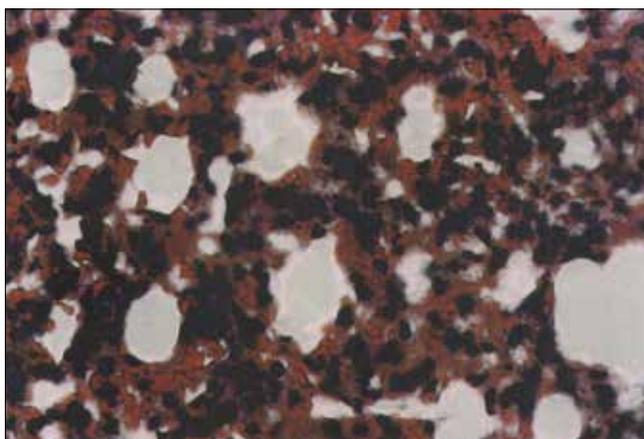
The SPSS 10.0 (SPSS Inc.; Chicago, IL, USA) computer program package was used for statistical calculations. To compare the histopathological lesion scores, we used a chi-square test. The Analysis of Variance (ANOVA) test was used



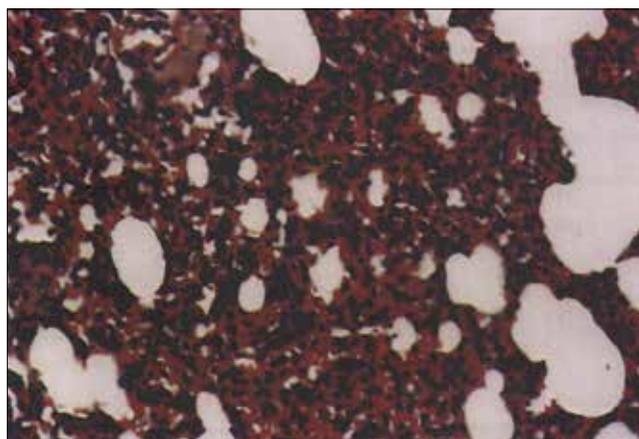
**Figure 1.** No neutrophil migration to the lungs (Score 0).



**Figure 3.** Moderate neutrophil migration to the lungs (Score 2).



**Figure 2.** Minimal neutrophil migration to the lungs (Score 1).



**Figure 4.** Severe neutrophil migration to the lungs (Score 3).

to compare the physiological parameters. Pairwise comparisons of physiological parameters were performed using the Scheffe method. A p-value threshold of 0.05 was used for the statistical significance.

## Results

The descriptive results of the physiological parameters including weight, heart rate (HR), pH, partial pressure of oxygen (pO<sub>2</sub>), partial pressure of carbon dioxide (pCO<sub>2</sub>), and systolic arterial pressure (SAP) have been presented in Table 1.

There were no significant differences between the two groups in terms of weight ( $p > 0.05$ ).

There was a statistically significant difference in terms of pulse rates at T4 and T5 (Hour 4 and 5 following the endotoxin injection) between Group C and the other groups ( $p < 0.001$ ). The differences between Group PE and Group E and Group ME at T4 and T5 were also statistically significant

in terms of the pulse rate ( $p < 0.01$ ). There were no significant differences determined between Group E and Group ME at T4 and T5 ( $p > 0.05$ ). The measurements at the other time points did not differ among the other groups ( $p > 0.05$ ).

The measurements of pH at T3, T4 and T5 (Hour 3, 4, and 5 following the endotoxin injection) differed significantly between Group C and the other groups ( $p < 0.001$ ). Furthermore, there was a significant difference in terms of the pH level among Group PE and Group E and Group ME at T5 ( $p < 0.01$ ).

The systolic arterial pressure measurements at T3, T4, and T5 differed significantly between Group C and the other groups ( $p < 0.001$ ). At T4 and T5, the differences among Group PE, Group E and Group ME were statistically significant ( $p < 0.001$ ). At these time points, we did not find any significant difference in terms of the systolic arterial pressure between Group E and Group ME ( $p > 0.05$ ).

There were no significant differences in terms of pCO<sub>2</sub> and pO<sub>2</sub> measurements at any time points ( $p > 0.05$ )

**Table 1. Hemodynamic parameters and blood gas analyses**

Groups	Weight (gr)	Values	T0	T1	T2	T3	T4	T5
Group E (n=10)	280±10	HR/min	329±2	335±1	335±2	320±2	273±3 <sup>*,#</sup>	225±2 <sup>*,#</sup>
		SAP (mmHg)	137±3	130±4	114±5	105±5*	84±4 <sup>*,##</sup>	60±4 <sup>*,##</sup>
		PH	7.46±0.30	7.46±0.36	7.34±0.13	7.25±0.15*	7.21±0.20*	7.10±0.17 <sup>*,#</sup>
		pO <sub>2</sub>	513±6	530±4	527±7	523±10	523±7	523±7
		pCO <sub>2</sub>	37±2	33±2	36±2	34±2	30±2	30±3
Group K (n=10)	282±12	HR/min	327±3	335±1	328±3	320±2	321±2	309±2
		SAP (mmHg)	139±3	135±3	137±2	129±2	128±3	129±2
		pH	7.49±0.21	7.51±0.23	7.49±0.19	7.44 ±0.22	7.40±0.14	7.37±0.18
		pO <sub>2</sub>	517±4	519±3	528±7	526±7	526±5	521±7
		pCO <sub>2</sub>	35.4±2	36±2.	36±2	35±2	36±2	35±2
Group PE (n=10)	284±11	HR/min	333±5	335±2	334±2	317±3	299±3*	278±4*
		SAP (mmHg)	138±3	131±3	116±2	105±4*	105±4*	69±4*
		pH	7.45±0.21	7.49±0.23	7.40±0.21	7.35±0.22*	7.32±0.16*	7.25±0.12*
		pO <sub>2</sub>	526±6	531±9	543±6	534±4	528±7	527±5
		pCO <sub>2</sub>	35±2	34±2	33±2	34±2	32±2	34±2
Group ME (n=10)	283±12	HR/min	335±4	336±2	334±2	324±3	275±4 <sup>*,#</sup>	230±4 <sup>##</sup>
		SAP (mmHg)	140±3	129±4	116±3	100±4*	88±3 <sup>*,##</sup>	66±2 <sup>*,##</sup>
		pH	7.46±0.25	7.47±0.29	7.34±0.11	7.25±0.21*	7.22±0.23*	7.12±0.20 <sup>*,#</sup>
		pO <sub>2</sub>	516±5	534±5	525±7	525±4	524±7	524±9
		pCO <sub>2</sub>	36±2	34±2	36±2	34±2	32±1	30±3

T0: Basal values; T1: One hours after the application of endotoxin; T2: Two hours after the application of endotoxin; T3: Three hours after the application of endotoxin; T4: Four hours after the application of endotoxin; T5: Five hours after the application of endotoxin; pO<sub>2</sub>: partial oxygen pressure; pCO<sub>2</sub>: partial pressure of carbon dioxide; HR: heart rate; SAP: systolic arterial pressure. \*According to Group C p<0.00L #According to Group PE p<0.00L ## According to Group PE p<0.00L

**Table 2. Histopathological examination**

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
GROUP E <sup>*,#</sup>			3	7
GROUP C	10			
GROUP PE	7	3		
GROUP ME <sup>*,##</sup>		7	3	

\*According to Group C p<0.001 ## According to Group PE p<0.01 # According to Group PE p<0.001  
Score 0: No neutrophil migration to the lungs; Score 1: Minimal neutrophil migration to the lungs  
Score 2: Moderate neutrophil migration to the lungs; Score 3: Severe neutrophil migration to the lungs

None of the subjects in Group C (n=10) demonstrated neutrophil migration to the lungs (Score 0) (Figure 1), whereas 3 of the subjects in Group E demonstrated moderate (Score 2) (Figure 3) and 7 demonstrated severe (Score 3) (Figure 4) neutrophil migration to the lungs. In total, 7

subjects in Group ME (n=10) displayed minimal neutrophil migration (Score 1) (Figure 2) and 3 displayed moderate neutrophil migration to the lungs (Score 2). In Group PE (n=10), 7 subjects showed no neutrophil migration to the lungs (Score 0) and 3 subjects showed minimal neutrophil migration (Score 1). There was a significant difference among Group C, E and ME (p<0.001). Group C and Group PE did not differ significantly (p>0.05). The differences among Group PE and Group E and Group ME were statistically significant in terms of histopathological classification (p<0.001 and p<0.01, respectively) as shown in Table 2.

## Discussion

For many decades, researchers and clinicians have thought that anaesthetics have negative effects on the immune system. This opinion is related to the high frequency of post-operative infections [8] and bone marrow suppression in the patients exposed to the anaesthetics for long time [9].

This has helped the researchers understand the potential side effects of anaesthetics in patients with immune deficiency.

Immune system functions are depressed following the anaesthesia and surgery. Most of the immune alterations following the surgery are not generally related to anaesthesia exposure only. The adverse effects of medications, the endocrine response (increased ACTH, catecholamines, and corticosteroids) and surgery trauma are responsible for the altered immune functions [10-12].

Neutrophils play an important role in the perioperative period. Neutrophils are considered as principal members of the host immune system [13]. The neutrophil response to microbial invasions can be classified into different phases; chemotaxis, adherence, phagocytosis, and intracellular killing. Neutrophils move towards the extravascular area (for phagocytosis and to kill the organism) by means of chemotactic agents. It is also thought that neutrophils play an important role on the pathogenesis of ischemia-reperfusion injury such as SIRS. Therefore, it is important to control the neutrophil functions during the perioperative period [14]. Moudgil, Krunholz, Weiss and Skoultelia found that the phagocytosis and chemotaxis functions of neutrophils were suppressed when exposed to *in vitro* anaesthetics. As a result of decreased production of oxygen radicals, the intracellular killing process of bacteria is inhibited [15-19].

The immune functions of neutrophils are like a double-edged sword [14]. It is still debated whether disturbed neutrophil functions are an advantage or disadvantage. A dysfunction in neutrophil functions may result in bacterial infections. Several anaesthetics including intravenous agents have negative effects on neutrophil functions [20]. In an *in vitro* study, it was demonstrated that propofol may inhibit neutrophil functions [21].

Mikawa et al. [22] reported that propofol inhibits phagocytosis and the production of reactive oxygen radicals in neutrophils.

Intravenous agents may not only cause perioperative immunosuppression. They are used also for sedation in intensive care patients and this may cause a substantial problem as they inhibit the host defence. On the other hand, suppression of neutrophil functions by anaesthetics may help prevent the organ dysfunctions related to autologous tissue injury in which excess neutrophil accumulation plays a role [23].

Neutrophils may accumulate in tissues as a response to chemotactic agents and may cause organ damage. One of these clinical entities is called ARDS. Weiland et al. [24] found dense neutrophil accumulation in bronchoalveolar lavage (BAL) samples in patients with ARDS.

Rinaldo et al. [25] found a dense infiltration of neutrophils in histopathological examination of the lung samples in patients with ARDS.

Donnelly et al. [26] carried out a study of ARDS on 29 patients and found high levels of IL-8 in BAL samples. They concluded that IL-8, as a strong chemotactic agent, causes increased neutrophil migration to the lungs and neutrophils play an important role on the pathophysiology of the ARDS. In our study, we created an immune system activation via intravenous endotoxin administration and explored the histopathological changes in the lungs. In this experimental sepsis model, we found that lung damage is related to neutrophil migration to the lungs.

Neutrophil polarization indicates the role of neutrophils in the immune response. Donnel et al. [27] explored the inhibitor effects of midazolam, propofol and thiopental on neutrophil polarization. Propofol and thiopental in normal plasma concentrations inhibited the neutrophil polarization by approximately 15%. Midazolam, on the other hand, inhibited neutrophil polarization only by 15%, even at high doses. They also found that propofol in high concentrations inhibited the neutrophil polarization by more than 90%. They concluded that chemotaxis of neutrophils via polarization is inhibited to a higher extent with propofol than other agents tested. Galley et al. [28] compared the effects of propofol and midazolam on IL-8 release in ARDS and found that both agents decreased the IL-8 release in lipopolysaccharide-induced neutrophils. Moreover, they reported that propofol suppressed the neutrophil chemotaxis and polarization. However, midazolam did not affect the neutrophil polarization and chemotaxis towards respiratory organs, even in clinical concentrations. In the current study, we also found that propofol suppressed the neutrophil migration to the lungs to a higher extent than midazolam.

Taniguchi et al. [29] reported a decrease in the cytokine response (TNF-alpha and IL-8) and in neutrophil infiltration in the lungs with propofol infusion (10 mg/kg/hour) in rats in which an endotoxemia model had been created by the administration of 15 mg/kg of endotoxin.

We found that propofol prevented the decrease in pH in endotoxemia model. It has been shown in a study that propofol may inhibit an inflammatory response and prevent the development of metabolic acidosis during endotoxemia [29].

The present study showed no difference in PaO<sub>2</sub> among the groups by the fifth hour after lung injury, although propofol attenuated the increases in infiltration or aggregation of neutrophils in the lungs. The probable reasons for this finding are that 100% oxygen was used during the experiment and that the observation period was too short.

They also found that hemodynamic parameters are affected to a lower extent with propofol infusion when compared to subjects who had hemodynamic endotoxemia.

In conclusion, we found that the lung histopathology in rats with sepsis model that received propofol was similar to

those control subjects. Propofol stabilized the hemodynamic parameters to a higher extent than midazolam in rats with endotoxemia and it may attenuate free-radical-mediated lipid peroxidation and systemic inflammation in patients.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Ataturk University (26.03.2002 No: 8/3).

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept - M.G.C.; Design - A.S., T.S.; Supervision - A.A., M.A.; Materials - H.K.; Data Collection and/or Processing - A.D.; Literature Review - I.I.; Writing - M.G.C.; Critical Review - H.K., M.G.C.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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