Ulus Travma Acil Cerrahi Derg 2010;16 (2):108-112
Deneysel Çalışma

The effect of dexmedetomidine on liver histopathology in a rat sepsis model: an experimental pilot study

Deneysel sepsis geliştirilen sıçanlarda deksmedetomidin'in karaciğer histopatolojisi üzerine etkileri: Deneysel ön çalışma

Atakan SEZER,[1] Dilek MEMİŞ,[2] Ufuk USTA,[3] Necdet SÜT[4]

BACKGROUND

In this pilot study, we aimed to investigate the effect of dexmedetomidine on liver tissues during experimental sepsis by histopathological examination.

METHODS

The animals were allocated randomly to four groups, two of which received endotoxin. In the Sepsis Group (n:10) and Dexmedetomidine/Sepsis Group (n:10), endotoxemia was induced by *E. coli* lipopolysaccharide derived from *E. coli* 0111: B4. Animals in the Control Group (n:10) received an infusion of 0.9% saline (1.0 $mL \cdot kg^{-1} \cdot hr^{-1}$) intravenously. The Dexmedetomidine Group (n:10) and Dexmedetomidine/Sepsis Group received a bolus injection of 0.9% saline (1.0 mL/kg), followed by dexmedetomidine administration (infusion at 5 $\mu g \cdot kg^{-1} \cdot hr^{-1}$). All rats were euthanized at the 8th hour of endotoxin infusion. Histopathological examinations were performed on liver tissues.

RESULTS

In the liver, central venous congestion, congestion and dilation of the hepatic sinusoids and inflammation of the portal tracts were noted in the Sepsis Group. These parameters were seen slightly in the Sepsis/Dexmedetomidine group. There was a statistically significant difference between the Sepsis and Sepsis/Dexmedetomidine Groups ($p<0.001$).

CONCLUSION

Dexmedetomidine has a protective effect on liver tissues during experimental sepsis in the rat. We propose that dexmedetomidine sedation may be useful in the therapy of the liver dysfunction associated with sepsis and in other diseases related to local or systemic inflammation.

Key Words: Dexmedetomidine; experimental sepsis; liver dysfunction.

AMAÇ

Bu çalışmanın amacı, septik sıçanlarda karaciğer işlev bozukluğu üzerine deksmedetomidin koruyucu etkisinin varlığını araştırmaktır.

GEREÇ VE YÖNTEM

Sıçanlar randomize olarak dört gruba ayrıldı. Kontrol grubu (grup I, n=10), herhangi bir tedavi almadı. Deksmedetomidin grubuna (grup II, n=10) ve sepsis/deksmedetomidin grubuna (grup IV, n=10) 5 $\mu g \cdot kg^{-1} \cdot s^{-1}$ deksmedetomidin infüzyonu yapıldı. Sepsis, sepsis grubu (grup III, n=10) ve sepsis/deksmedetomidin grubuna kuyruk veninden *E. Coli* verilerek oluşturuldu. Tüm sıçanlara sepsisin 8. saatinde sakrifiye edildikten sonra orta hat laparotomisi uygulandı. Sıçanların karaciğerleri histopatolojik inceleme için eksize edildi ve doku değişikliklerinin varlığı açısından incelendi.

BULGULAR

Sepsis grubundaki değişiklikler merkezi venöz konjesyon, hepatik sinüzoitlerde konjesyon ve dilatasyon ve portal alanda enflamasyondu. Bu parametreler sepsis/deksmedetomidin grubunda daha az olarak gözlendi. Sepsis ve sepsis/deksmedetomidin grubunda istatistiksel olarak anlamlı farklılık bulundu ($p<0,001$).

SONUÇ

Deksmedetomidin sıçanlarda deneysel olarak geliştirilmiş sepsiste karaciğer organ işlev bozukluğunu engellemektedir. Biz deksmedetomidin sedasyonunun sepsise bağlı karaciğer işlev bozukluğunda ve diğer hastalıkların lokal veya sistemik enflamasyonunda yararlı etkisi olabileceği görüşündeyiz.

Anahtar Sözcükler: Deksmedetomidin; deneysel sepsis; karaciğer işlev bozukluğu.

Departments of [1]General Surgery, [2]Anesthesiology and Reanimation, [3]Pathology, [4]Biostatistics, Trakya University Faculty of Medicine, Edirne, Turkey.

Trakya Üniversitesi Tıp Fakültesi, [1]Genel Cerrahi Anabilim Dalı, [2]Anesteziyoloji ve Reanimasyon Anabilim Dalı, [3]Patoloji Anabilim Dalı, [4]Biyoistatistik Anabilim Dalı, Edirne.

Correspondence *(İletişim):* Atakan Sezer, M.D. Trakya University Faculty of Medicine, Department of General Surgery, 22030 Edirne, Turkey.
Tel: +90 - 284 - 235 76 41 / 1199 Fax *(Faks)*: +90 - 284 - 235 27 30 e-mail *(e-posta):* atakansezer@hotmail.com

Sepsis ranks as the leading cause of death in intensive care units, and despite intense efforts to improve survival, mortality rates have changed only minimally over recent decades.[1,2] It is a common complex clinical syndrome that results from a harmful host response to infection, in which foreign bacteria and lipopolysaccharide (LPS) are potent activators of different immune cells through stimulation of receptors on their surface.[3,4] Given that Gram-negative bacteria normally colonize the colon, the body has developed strong defensive mechanisms that tightly regulate the entry and processing of LPS.[5,6] LPS (endotoxin) induces extensive damage to a variety of organs, including the liver, due to the increased production of reactive oxygen intermediates and a resultant rise in lipid peroxidation.[7,8]

Dexmedetomidine is a new sedative agent that has received Food and Drug Administration approval for use in intensive care units for sedation.[9] It is a novel lipophilic imidazole derivative with a higher affinity for [alpha] 2-adrenoceptors than the prototype drug clonidine.[10] Other beneficial qualities were its anxiolytic effect and lack of respiratory depression.[11]

Patients who require long-term sedation in the intensive care unit during sepsis especially need organ-preserving anesthetic agents such as dexmedetomidine. However, only a few reports, lacking the evidence of histopathologic verification, have been published about the effects of dexmedetomidine during endotoxemia and endotoxic shock. The purpose of this study was to demonstrate the effect of dexmedetomidine on histopathologic alterations during sepsis in rats.

MATERIALS AND METHODS

Our study protocol was reviewed and approved by the Ethical Committee for Animals of Trakya University (Edirne, Turkey). Female Wistar rats weighing 230-285 g at the age of 6 weeks were obtained from the Institute for Experimental Medicine, Trakya University. All animals were acclimatized for 7 days prior to experimentation in the laboratory of the mentioned institute, which was maintained at 22±2°C and a relative humidity of 55± 10% in a constant 12 h dark/light cycle. The rats were housed in standard wire cages and fed with standard rodent chow and UV sterilized tap water. The approval of the Ethical Review Committee of the Faculty was obtained, and the experimental procedures in this study adhered to the Declaration of Helsinki for the care and use of laboratory animals.

Experimental Protocols

The animals were allocated randomly to one of four groups, two of which received endotoxin.

Sepsis Group (n:10): Endotoxemia was induced by a bolus injection of *Escherichia coli* LPS derived from *E. coli* 0111:B4 (*E. coli* serotype 0111:B4-Sigma), which was injected intravenously at 15 mg/kg over 2 minutes.

Control Group (n:10): The animals in this group received an infusion of 0.9% saline (1.0 $mL \cdot kg^{-1} \cdot hr^{-1}$) intravenously. Dexmedetomidine Group (n:10): This group was not exposed to endotoxin. The animals in this group received a bolus injection of 0.9% saline (1.0 mL/kg), followed by dexmedetomidine administration via the tail vein (infusion at 5 $\mu g \cdot kg^{-1} \cdot hr^{-1}$).

Dexmedetomidine-Sepsis Group (n:10): Endotoxemia was induced as in the Sepsis Group, and dexmedetomidine was administered as in the Dexmedetomidine Group.

Histological Examination

All rats were sacrificed at the 8th hour of endotoxin infusion by overdose of sodium pentothal (200 mg/kg, intramuscularly), and then midline laparotomy was performed.

Resected liver specimens of each rat in all groups were fixed in 10% buffered formaldehyde for 24 hours and embedded into paraffin after 16 h of alcohol process. 5 µm thick sections were obtained from the paraffin blocks and stained with hematoxylin and eosin. Each slide was examined under a light microscope by the same pathologist, who was blinded to the study group allocations. Central venous congestion, congestion and dilation of the hepatic sinusoids and inflammation of the portal tracts were noted and graded from 0 to 3, with "0" indicating no change, "1" slight change, "2" moderate change and "3" severe change. A sum of all grades was regarded as total score, which ranged between 0-9.

Statistical Analysis

The results were expressed as mean±SD. The Kruskal-Wallis test was used to compare differences among groups and then the Student-Newman-Keuls post-hoc test was used when a significant difference was found. A p value <0.05 was considered statistically significant. Statistica 7.0 (StatSoft Inc. Tulsa, OK, USA) statistical software was used for statistical analyses.

RESULTS

No mortality was observed in the Control, Dexmedetomidine and Dexmedetomidine/Sepsis Groups. There were two mortalities in the Sepsis Group. Because the number of rats in each group was small, a statistical comparison was not performed to evaluate mortality.

According to the total scoring of pathological changes in the liver, tissues scores were 0.30±0.67 in the Control Group, 1.80±0.92 in the Dexmedetomidine Group, 5.70±0.95 in the Sepsis Group, and 2.80±1.48 in the Sepsis/Dexmedetomidine Group. According to

Table 1. Changes in central venous congestion, congestion and dilation of the hepatic sinusoids and inflammation of the portal tracts

	Control group (n=10)	Dexmedetomidine group (n=10)	Sepsis group (n=8)	Sepsis/ Dexmedetomidine group (n=10)	p
Central venous congestion	0.20±0.42†	0.80±0.63†‡	2.40±0.52‡	1.00±0.82†‡	<0.001
Congestion and dilation of the hepatic sinusoids	0.10±0.32†#	0.10±0.32†#	1.40±0.52‡	1.00±0.47†‡	<0.001
Inflammation of the portal tracts	0.00±0.00†	0.90±0.32†‡	1.90±0.57‡	0.80±0.63†‡	<0.001
Total score	0.30±0.67†#	1.80±0.92†#‡	5.70±0.95‡	2.80±1.48†‡	<0.001

Central venous congestion, congestion and dilation of the hepatic sinusoids and inflammation of the portal tracts were noted and graded from 0 to 3 with "0" indicating no change, "1" slight change, "2" moderate change and "3" severe change. A sum of all grades was regarded as total score, which ranged between 0-9.

† p<0.001 compared with Sepsis Group; ‡ p<0.001 compared with Control Group; # p<0.001 compared with Sepsis/Dexmedetomidine Group.

the mean of total scoring of tissue alteration, there was a statistically significant difference between all groups (p<0.001). Results of all statistical analyses are shown in Table 1. The tissue alterations consisted of central venous congestion, congestion and dilation of the hepatic sinusoids and inflammation of the portal tracts, and were statistically significant between the Control Group and other groups (p<0.001) (Fig. 1a).

Four rats showed severe central venous congestion while all others showed moderate congestion in the central veins in the Sepsis Group (Fig. 1b). Three rats showed moderate and four rats showed slight venous congestion in the Dexmedetomidine/Sepsis Group (Fig. 1c). There was a statistically significant difference between the Sepsis and the Dexmedetomidine/ Sepsis Groups with respect to the central venous congestion parameter (p<0.001) (Table 1).

Sinusoidal congestion was moderate in four rats and slight in six rats in the Sepsis Group. Nine rats in the Dexmedetomidine/Sepsis Group showed slight

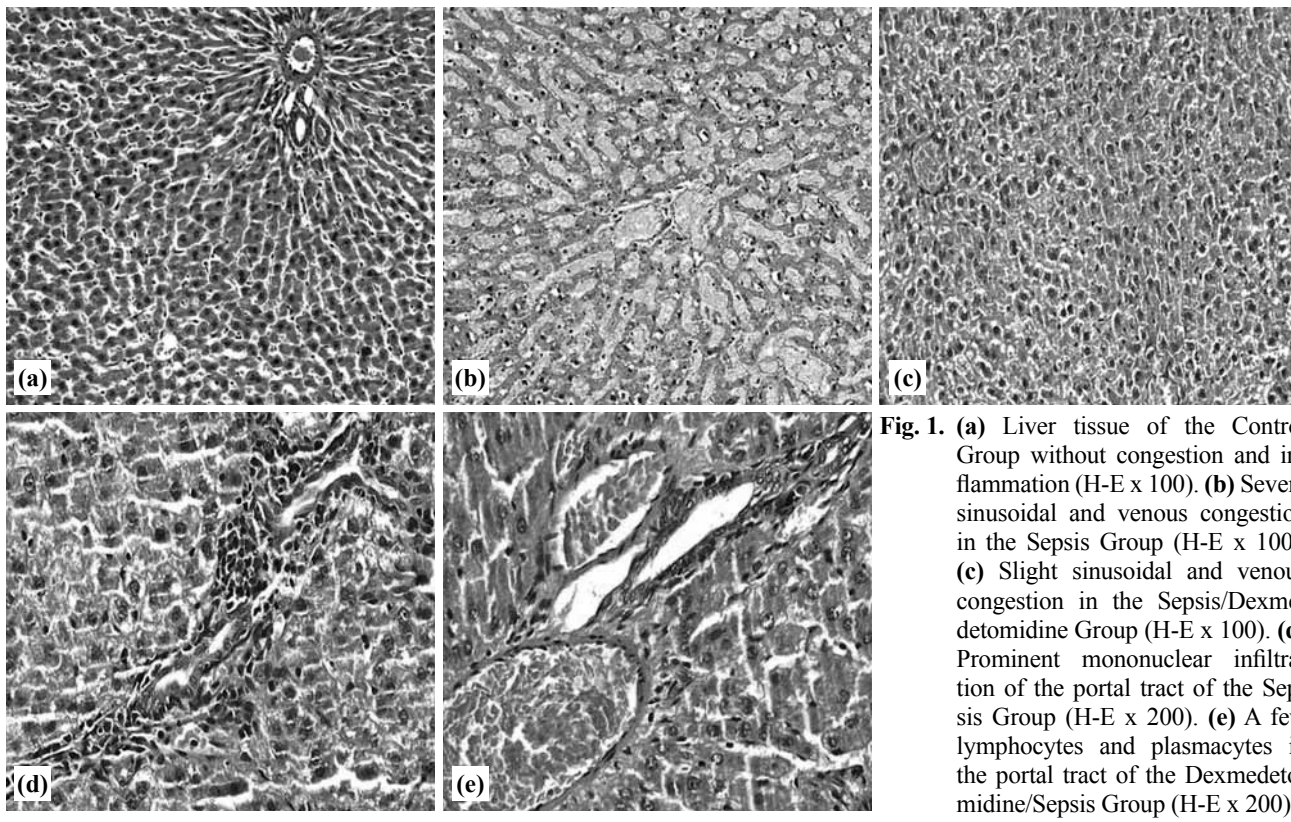

Fig. 1. **(a)** Liver tissue of the Control Group without congestion and inflammation (H-E x 100). **(b)** Severe sinusoidal and venous congestion in the Sepsis Group (H-E x 100). **(c)** Slight sinusoidal and venous congestion in the Sepsis/Dexmedetomidine Group (H-E x 100). **(d)** Prominent mononuclear infiltration of the portal tract of the Sepsis Group (H-E x 200). **(e)** A few lymphocytes and plasmacytes in the portal tract of the Dexmedetomidine/Sepsis Group (H-E x 200).

congestion and one moderate change, and there was a statistically significant difference between these two groups in this parameter ($p<0.001$) (Table 1).

There was slight or moderate portal inflammation in all livers of the Sepsis Group (Fig. 1d). There was only one moderate portal inflammation in the Dexmedetomidine/Sepsis Group (Fig. 1e). There was a statistically significant difference between the Sepsis and the Dexmedetomidine/Sepsis Groups in the portal inflammation parameter ($p<0.001$) (Table 1).

Generally, slight edema accompanied the inflammation in the portal tracts; inflammatory cells were of mixed type, composed of lymphocytes, eosinophil leukocytes and neutrophil leukocytes, in a decreasing order of frequency. Two rats in the Sepsis Group showed zone 3 necrosis and one rat in the Dexmedetomidine/Sepsis Group showed hemorrhage in some portal areas.

DISCUSSION

In our study, we demonstrated the protective effect of dexmedetomidine on liver tissue in septic animals. We hypothesized that dexmedetomidine may attenuate the liver damage associated with sepsis, shock, and other diseases related to local or systemic inflammation.

Lipopolysaccharide (LPS) is able to stimulate the synthesis or release of interleukin (IL)-1, IL-6, or tumor necrosis factor-alpha (TNF-α).[12,13] Other lines of evidence have shown that nuclear factor-κB (NF-κB) plays an important role in inflammatory responses through the regulation of genes encoding proinflammatory cytokines and inducible enzymes such as inducible nitric oxide synthesis and cyclooxygenase.[13-15] The synthesis of cytokines is mediated by NF-κB, which is an appropriate target for the treatment of septic shock because NF-κB-activated gene products play an important role in the pathogenesis of sepsis.[13] The activation of the NF-κB transcription family plays an important role in inflammation through its ability to induce a transcription of proinflammatory genes.[13] This pathway is activated on appropriate cellular stimulation, most often by signals related to pathogens or stress.

The inhibitory effect of dexmedetomidine on the production of TNF-α and IL-6 after endotoxin injection is very interesting. Circulating endotoxin induces the release of cytokines such as TNF-α and IL-6, which can produce hypotension and metabolic acidosis.[16-18] Several investigators have published reports on the effects of dexmedetomidine and [alpha] 2-adrenergic receptor agonists on cytokines.[19-22] *In vitro*, paminoclonidine, an [alpha] 2-adrenoceptor agonist, suppressed IL-6 production,[19] and clonidine suppressed TNF-α production of monocytes.[20] Moreover, [alpha] 2-adrenoceptor agonists modulated LPS-induced TNF-α production on macrophages.[21] Taniguchi and colleagues[23] demonstrated that dexmedetomidine has an inhibitory effect on cytokine responses to endotoxemia. Memis et al.[24] found that the dexmedetomidine infusion decreased cytokine production in sepsis.

Sepsis, as a leading problem of mortality in intensive care units and emergency departments, must be treated urgently. The main causes of mortality and morbidity in sepsis are multiple organ dysfunction syndrome (MODS) with renal and hepatic damage. Liver dysfunction has a primary and progressive effect on MODS due to its being both the source of inflammatory mediators and the target organ affected by inflammatory mediators. In our study, we investigated whether or not dexmedetomidine prevents liver damage in septic animals. We found that dexmedetomidine decreases central venous congestion, congestion and dilation of the hepatic sinusoids and inflammation of the portal tracts. Our findings demonstrated that, even in an *in vivo* experiment, dexmedetomidine protected against liver damage in endotoxemia. These findings suggest that one of mechanisms of the anti-inflammatory effects of dexmedetomidine may be the modulation of cytokine production by macrophages and monocytes.

The aim of this study was to demonstrate the effect of dexmedetomidine on liver histopathology during experimental sepsis in rats. We propose that dexmedetomidine may have a protective effect on liver damage in sepsis, shock, and other diseases associated with local or systemic inflammation. The liver damage during sepsis and treatment modalities have been studied numerous times in experimental models. While previous studies have shown the antagonist effect of dexmedetomidine on inflammatory mediators, this pilot work provides additional information on the effect of dexmedetomidine on liver histopathology during endotoxemia. Nonetheless, randomized studies with larger numbers of animals, different dosages of medications, and measurement of biochemical markers of liver function and cytokines are needed to better clarify the role of dexmedetomidine in sepsis.

REFERENCES

1. Abe Y, Hashimoto S, Horie T. Curcumin inhibition of inflammatory cytokine production by human peripheral blood monocytes and alveolar macrophages. Pharmacol Res 1999;39:41-7.
2. Diekema DJ, Pfaller MA, Jones RN, Doern GV, Winokur PL, Gales AC, et al. Survey of bloodstream infections due to gram-negative bacilli: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, and Latin America for the SENTRY Antimicrobial Surveillance Program, 1997. Clin Infect Dis 1999;29:595-607.
3. Wenzel RP, Pinsky MR, Ulevitch RJ, Young L. Current un-

derstanding of sepsis. Clin Infect Dis 1996;22:407-12.
4. Bochud PY, Calandra T. Pathogenesis of sepsis: new concepts and implications for future treatment. BMJ 2003;326:262-6.
5. Su GL. Lipopolysaccharides in liver injury: molecular mechanisms of Kupffer cell activation. Am J Physiol Gastrointest Liver Physiol 2002;283:G256-65.
6. Kaur G, Tirkey N, Bharrhan S, Chanana V, Rishi P, Chopra K. Inhibition of oxidative stress and cytokine activity by curcumin in amelioration of endotoxin-induced experimental hepatoxicity in rodents. Clin Exp Immunol 2006;145:313-21.
7. Kono H, Asakawa M, Fujii H, Maki A, Amemiya H, Yamamoto M, et al. Edaravone, a novel free radical scavenger, prevents liver injury and mortality in rats administered endotoxin. J Pharmacol Exp Ther 2003;307:74-82.
8. Matsuda H, Ishikado A, Nishida N, Ninomiya K, Fujiwara H, Kobayashi Y, et al. Hepatoprotective, superoxide scavenging, and antioxidative activities of aromatic constituents from the bark of Betula platyphylla var. japonica. Bioorg Med Chem Lett 1998;8:2939-44.
9. Venn RM, Bradshaw CJ, Spencer R, Brealey D, Caudwell E, Naughton C, et al. Preliminary UK experience of dexmedetomidine, a novel agent for postoperative sedation in the intensive care unit. Anaesthesia 1999;54:1136-42.
10. Virtanen R, Savola JM, Saano V, Nyman L. Characterization of the selectivity, specificity and potency of medetomidine as an alpha 2-adrenoceptor agonist. Eur J Pharmacol 1988;150:9-14.
11. Triltsch AE, Welte M, von Homeyer P, Grosse J, Genähr A, Moshirzadeh M, et al. Bispectral index-guided sedation with dexmedetomidine in intensive care: a prospective, randomized, double blind, placebo-controlled phase II study. Crit Care Med 2002;30:1007-14.
12. Lee JJ, Huang WT, Shao DZ, Liao JF, Lin MT. Blocking NF-kappaB activation may be an effective strategy in the fever therapy. Jpn J Physiol 2003;53:367-75.
13. Jobin C, Bradham CA, Russo MP, Juma B, Narula AS, Brenner DA, et al. Curcumin blocks cytokine-mediated NF-kappa B activation and proinflammatory gene expression by inhibiting inhibitory factor I-kappa B kinase activity. J Immunol 1999;163:3474-83.
14. Feng SH, Lo SC. Lipid extract of Mycoplasma penetrans proteinase K-digested lipid-associated membrane proteins rapidly activates NF-kappaB and activator protein 1. Infect Immun 1999;67:2951-6.
15. Franzoso G, Bours V, Park S, Tomita-Yamaguchi M, Kelly K, Siebenlist U. The candidate oncoprotein Bcl-3 is an antagonist of p50/NF-kappa B-mediated inhibition. Nature 1992;359:339-42.
16. Wintroub BU. Inflammation and mediators. Int J Dermatol 1980;19:436-42.
17. Till GO, Johnson KJ, Kunkel R, Ward PA. Intravascular activation of complement and acute lung injury. Dependency on neutrophils and toxic oxygen metabolites. J Clin Invest. 1982;69:1126-35.
18. Tracey KJ, Beutler B, Lowry SF, Merryweather J, Wolpe S, Milsark IW, et al. Shock and tissue injury induced by recombinant human cachectin. Science 1986;234:470-4.
19. Straub RH, Herrmann M, Berkmiller G, Frauenholz T, Lang B, Schölmerich J, et al. Neuronal regulation of interleukin 6 secretion in murine spleen: adrenergic and opioidergic control. J Neurochem 1997;68:1633-9.
20. Maes M, Lin A, Kenis G, Egyed B, Bosmans E. The effects of noradrenaline and alpha-2 adrenoceptor agents on the production of monocytic products. Psychiatry Res 2000;96:245-53.
21. Szelényi J, Kiss JP, Vizi ES. Differential involvement of sympathetic nervous system and immune system in the modulation of TNF-alpha production by alpha2- and beta-adrenoceptors in mice. J Neuroimmunol 2000;103:34-40.
22. Venn RM, Bryant A, Hall GM, Grounds RM. Effects of dexmedetomidine on adrenocortical function, and the cardiovascular, endocrine and inflammatory responses in post-operative patients needing sedation in the intensive care unit. Br J Anaesth 2001;86:650-6.
23. Taniguchi T, Kidani Y, Kanakura H, Takemoto Y, Yamamoto K. Effects of dexmedetomidine on mortality rate and inflammatory responses to endotoxin-induced shock in rats. Crit Care Med 2004;32:1322-6.
24. Memiş D, Hekimoğlu S, Vatan I, Yandim T, Yüksel M, Süt N. Effects of midazolam and dexmedetomidine on inflammatory responses and gastric intramucosal pH to sepsis, in critically ill patients. Br J Anaesth 2007;98:550-2.