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Comparison of the Therapeutic Effects of Antibiotic, Steroid, and Vitamin K During Early Sepsis in Laboratory Animals*

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Abstract: The use of corticosteroids alone or with antibiotics in the treatment of sepsis is still a subject of conflict. Besides, coagulation abnormalities in sepsis ranging from bleeding to microvascular thrombosis are needed to be evaluated with respect to Vitamin K (Vit K) dependence. The effects of antibiotic, steroid and Vit K on severe sepsis were investigated to compare therapeutic outcomes in this study. Cecal-ligation-puncture (CLP) was induced by abdominal surgery in rats to produce septic peritonitis. Rats were divided into 7 groups including 12 rats each. Groups were Sham, CLP, CLP+IM (imipenem), CLP+MP (methylprednisolone), CLP+VK (vitamin K_3, menadione), CLP+IM+MP and CLP+IM+VK. Six animals from each group were sacrificed to obtain samples at the 16th h. The remaining ones were observed to record survival times. The highest increases in serum TNF-α, IL-1β and IL-6 levels were observed in Group CLP and CLP+VK. Cytokines did not significantly increase in Group CLP+IM+MP. The platelet count decreased in Group CLP and CLP+MP ($P<0.05$). Imipenem, methylprednisolone and Vit K lead to change for coagulation times in a different manner. No animal survived in the groups CLP, CLP+MP and CLP+VK while 66.7% of them survived in the groups CLP+IM and CLP+IM+MP. Methylprednisolone increased the survival time. Antibiotics have a major protective effect in early stage and steroids may improve this effect. Interestingly, the adjunctive use of Vit K to antibiotic or to steroid deteriorated the protective effects of these drugs. These results suggest that therapeutics should be cautiously used to combat with coagulopathy during sepsis.

Keywords: Cytokine, Imipenem, Menadione, Methylprednisolone, Sepsis.

Antibiyotik, Steroid ve Vitamin K'nın Terapötik Etkilerinin Erken Dönem Sepsis Süresince Laboratuvar Hayvanlarında Karşılaştırılması

Öz: Sepsisin tedavisinde kortikosteroidlerin tek başına ya da antibiyotiklerle birlikte kullanımları halen anlaşılması güç bir konudur. Ayrıca, sepsiste kanamadan mikrovasküler tromboza kadar değişen koagülasyon anormalliklerinin Vitamin K (Vit K) bağımlılığı yönünden araştırılması gerekmektedir. Bu çalışmada antibiyotik, steroid ve Vit K'nın etkilerinin terapötik sonuçları şiddetli sepsiste araştırılmıştır. Sıçanlarda septik peritonitis oluşturmak için abdominal cerrahi ile çekal-bağlama-delme (CLP) yapıldı. Sıçanlar her grupta 12 sıçan olmak üzere 7 gruba ayrıldı. Gruplar Sham, CLP, CLP+IM (imipenem), CLP+MP (metilprednizolon), CLP+VK (vitamin K3, menadione), CLP+IM+MP ve CLP+IM+VK'dir. Her gruptan 6 hayvan 16'ncı saatte örneklerin elde edilmesi için sakrifiye edildi. Kalan hayvanlar yaşam sürelerinin kaydedilebilmesi için gözlemlendi. TNF-α, IL-1β ve IL-6 değerlerindeki en yüksek artış CLP ve CLP+VK gruplarında görüldü. Sitokinler CLP+IM+MP grubunda önemli derecede artmadı. Trombosit sayısı CLP ve CLP+MP gruplarında azaldı ($P<0.05$). İmipenem, metilprednizolon ve Vit K koagülasyon sürelerinde farklı tarzlarda değişikliklere neden oldu. CLP, CLP+MP ve CLP+VK gruplarında hiç hayvan yaşamazken CLP+IM ve CLP+IM+MP gruplarında hayvanların %66.7'si yaşadı. Metilprednizolon yaşam sürelerini uzattı. Antibiyotikler erken dönemde önemli koruyucu etkiye sahiptir ve steroidler bu etkiyi arttırmaktadır. İlginç olarak, Vit K'nın antibiyotiğe ya da steroid'e ilave edilmesi bu ilaçların koruyucu etkilerini kötüleştirmektedir. Bu bulgular, sepsis süresince koagülopati ile mücadelede törapötiklerin kullanılmasında dikkatli olunması gerektiğini göstermektedir.

Anahtar Kelimeler: Imipenem, Menadion, Metilprednizolon, Sepsis, Sitokin.

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INTRODUCTION

Sepsis is associated with the abnormal immune function in response to invading pathogens accompanied by the mal-function of the vital organs and excessive activation of inflammatory response (1,2). Furthermore, post-operative peritonitis is a common cause of death in intensive care units in human beings (3). Regulation of disordered inflammatory response is needed for the therapy of sepsis, severe sepsis and septic shock (4).

Pro-inflammatory and anti-inflammatory cytokines interact in a complex manner to influence the immune system and eventually cause multiple end organ effects (5). High levels of TNF-α and IL-6 in serum have been correlated with a prediction of a poor outcome in patients with septic shock (6). The symptoms are manifested through the release of pro-inflammatory cytokines, pro-coagulants, and adhesion molecules from immune cells and/or damaged endothelium (7). An effective treatment protocol is needed to salvage the life-threatening sepsis characterized by haematological derangements and a profound inflammatory response.

The antimicrobial treatment is commonly used through inhibiting various Gram-positive and Gram-negative bacteria. Bacterial invasion of the peritoneal cavity after the abdominal intervention is still a major cause of postoperative morbidity and mortality (8). The use of corticosteroids has become a re-evaluation after the recognition of the adrenal insufficiency during the late stage of polymicrobial sepsis to improve adrenal responsiveness (9). Although corticosteroids have immunosuppressive effects, low-dose corticosteroids might be useful in the patients with septic shock and adrenal insufficiency (10). Moreover, combined immunosuppressive and antibiotic therapy may enhance bacterial clearance (11). Therapies that improve host immunity might increase survival. It is therefore necessary to investigate further evaluation of corticosteroids to reveal the complex influences on immune response and biochemical mechanisms in sepsis.

In sepsis, coagulation abnormalities range from a small decrease in platelet count and prolongation of clotting times to disseminated intravascular coagulopathy (DIC), characterized by simultaneous widespread microvascular thrombosis and profuse bleeding (12). It is obvious that microvascular thrombosis or bleeding from various sites may be seen based on the stage of septic patient. The balance that normally exists between anticoagulant mechanisms and the procoagulant response is altered in sepsis (13). Activated protein C, an endogenous Vit K-dependent anticoagulant, plays a major role in the down-regulation of the procoagulant arm (13). Cytokines such as IL-6 and TNF-alpha can activate the procoagulant arm, contributing to thrombosis and inflammation (13, 14). The effect of Vit K has not been evaluated in inflammatory response of early sepsis.

Therefore, in the present study we aimed to investigate the therapeutic effects of imipenem as a broad spectrum antibiotic, methylprednisolone as a corticosteroid, and menadione sodium bisulfite as Vit K by evaluating cytokines, leukogram values, coagulation tests and surviving rates in septic rats induced by experimental cecal-ligation-puncture (CLP).

MATERIALS and METHODS

The study protocol was approved by the University Ethics Committee for Animal Experiments (Decision number: HADYEK, 2009/111). Eighty-four adult Sprague-Dawley rats (250-300 g body weight) were blocked and fed with regular rat diet and water *ad libitum* under physiological standards (22 ^{o}C temperature, 50-60% humidity and 12 h light period). Rats were divided into 7 groups including 12 rats each (equal amounts of male and female).

Cecal Ligation Puncture (CLP) Technique

Sepsis was induced in the rats according to the CLP technique described previously (15-17). Briefly,

rats were not fasted prior to the procedure and they were anesthetized using 0.5 mg/kg xylazine hydrochlorur (Rompun®, Bayer, Germany) and 2.5 mg/kg ketamine hydrochlorur (Ketasol®, Richter Pharma, Austria) intramuscularly. 2-cm incision in the abdominal wall was made to extrude the cecum from the abdomen under routine surgery procedure. Confirming fecal content inside, the cecum was ligated below the ileocecal valve. The length of ligation site was 30% of total length of cecum from the tip of the ascending cecum to the tip of the descending cecum. The ligated part of cecum was punctured twice using a 16-gauge needle and squeezed to confirm extrusion of the fecal content. Two ml of physiologic saline solution was given inside the abdominal cavity. The muscle and skin were sutured with 2.0 silk. Iodine was applied over the sutured area. In sham group, the surgery procedure was the same without inducing cecal ligation and puncture.

Experimental Protocol

The study protocol in the groups was described in Table 1. Each group had 12 rats. Six of them were sacrificed at the 16th h relative to the CLP induction in order to obtain blood samples. The remaining 6 rats in each group were followed for 7 days (d) to record survival time and rate. The surviving animals were also sacrificed under deep anaesthesia at the end of the study. The treatment protocol in each group was also demonstrated in Table 1.

Table 1 The study protocol in the groups.
Tablo 1. Gruplardaki çalışma protokolü.

Groups	Applications / Drug Administrations
Sham	-
CLP	CLP
CLP+IM	CLP + Imipenem (7.1 mg/kg/12h s.c.)
CLP+MP	CLP + Methylprednisolone (0.5 mg/kg/12h i.m.)
CLP+VK	CLP + Vitamin K_3, Menadione (0.22 mg/kg/12h i.m.)
CLP+IM+MP	CLP + Imipenem (7.1 mg/kg/12h s.c.) + Methylprednisolone (0.5 mg/kg/12h i.m.)
CLP+IM+VK	CLP + Imipenem (7.1 mg/kg/12h s.c.) + Vitamin K_3, Menadione (0.22 mg/kg/12h i.m.)

Sham Group (Negative Control) and CLP Group (Positive Control) had no medication. Groups CLP+IM, CLP+MP, CLP+VK, CLP+IM+MP and CLP+IM+VK were administrated drugs for 3 d with 12 h interval starting from one hour after inducing CLP procedure. Group CLP+IM received 7.1 mg/kg/12h s.c. imipenem (Tienam, Merck Sharp & Dohme, France). Group CLP+MP received 0.5 mg/kg/12h i.m. methylprednisolone (Prednol-L, Mustafa Nevzat, Turkey). Group CLP+VK received 0.22 mg/kg/12h i.m. menadione sodium bisulfite (vitamin K_3, Libavit K, Liba, Turkey). Group CLP+IM+MP was given same amount of imipenem plus methylprednisolone. Group CLP+IM+VK received same amount of imipenem plus menadione sodium bisulfite.

Biochemical and Hematologic Analyses

At least 10 ml of whole blood sample was drawn directly from the right ventricle of the rats in each treatment groups. The tube with cloth activator (SST II Advance, Becton Dickinson Co. UK) was used to obtain sera samples for cytokines analyses. Blood samples were centrifuged at 1,500 *g* and +4 ^{o}C for 10 min and sera were kept at -80 ^{o}C until analysis. Serum concentrations of TNF-α (Invitrogen, Cat.No: KRC3012), IL-6 (RayBio Cat.No: ELR-IL6-001) and IL-1β (Invitrogen Cat.No: KRC0012) were analysed by sandwich ELISA method (BIO-TEK µQuant, USA) according to the directions of the manufacturers.

The tube with anticoagulant (K2 EDTA, Becton Dickinson Co. UK) was used to collect samples for leukogram and platelet analyses (Abacus Junior Vet5, Diatron, Hungary). The tube with sodium citrate (Hema&Lab Saglik Co. Turkey) was used to collect samples for coagulation tests (Instrumentation Laboratory ACL-TOP 700, USA). A pediatric blood culture tube was used to collect samples for microbiologic analysis (BacT/ALERT, BioMerieux, France).

Statistical Analysis

Comparisons were made for each parameter among groups. Statistical significance was determined by One-Way ANOVA with Tukey's multiple comparison post hoc test (SPSS Statistics, Version 22, IBM Corp., Armonk, NY, USA). Chi-Square test was used for the comparison of survival rates. A P value <0.05 was defined as significant. Data were presented as mean ± standard error of the mean (SEM).

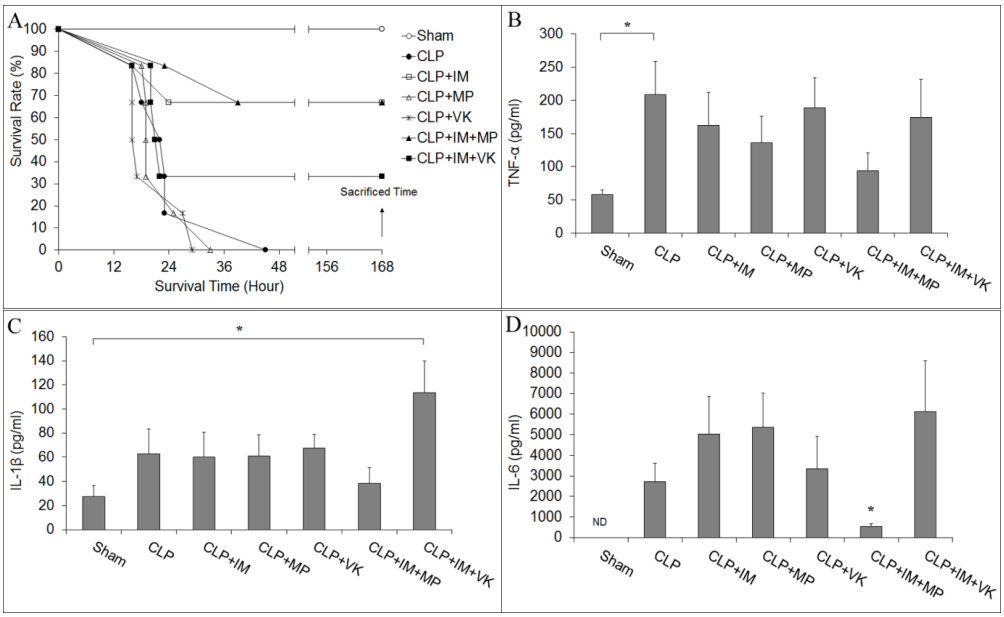
RESULTS

Survival Rates

In order to observe survival rates, 6 of the animals were blocked from each group at the initiation of the experimental course. The relationship between survival rate and survival times for each animal in each group was showed in Figure 1A. During the course, all animals lived in Sham Group and all animals died in CLP Group. Imipenem with/without methylprednisolone administration prevented most of mortalities caused by sepsis in Group CLP+IM and CLP+IM+MP ($P<0.05$). Addition of methylprednisolone to the antibiotic prolonged the survival times of dying animals (20±4 vs. 31±8 hrs.) in Group CLP+IM+MP. However, Vit K administration and methylprednisolone did not prevent mortalities in groups CLP+MP and CLP+VK. Moreover, Vit K administration worsened the healing effects of the antibiotic administration in Group CLP+IM+VK.

Figure 1. The relationship between survival rate and survival times for each animal in each group (A) and mean values of TNF-α (B), IL-1β (C) and IL-6 (D). Blood samples for cytokine analyses were obtained at the 16th hour of sepsis in 6 of 12 animals in each group. The resting 6 animals were followed for surviving rate. Living animals at the end of the 7th day were sacrificed. The data are presented as mean±SEM. *: P < 0.05. ND: Not detected.

Şekil 1. Gruplardaki hayvanların yaşam oranları ve yaşam süreleri arasındaki ilişki (A) ve ortalama TNF-α (B), IL-1β (C) ve IL-6 (D) değerleri. Her gruptaki 12 hayvanın 6'sından alınan kan örneklerindeki sitokin analizleri sepsisin 16'ncı saatinde yapıldı. Geriye kalan 6 hayvan yaşam oranlarını belirlemek için takip edildi. 7'nci günün sonunda yaşayan hayvanlar sakrifiye edildi. Veriler Ortalama±SEM olarak sunuldu. *: P<0,05. ND: Tespit edilmedi.



Cytokines

Mean TNF-α value in CLP Group increased significantly compared to Sham Group (P<0.05) (Figure 1B). Among the treatment groups, TNF-α value was tended to decrease in Group CLP+IM+MP given imipenem plus methylprednisolone. IL-1β values slightly increased in the septic groups (Figure 1C). The highest value of IL-1β was in Group CLP+IM+VK given imipenem plus Vit K compared to Sham Group (P<0.05). IL-1β value in Group CLP+IM+MP tended to decrease and it was very close to Sham group. Presence of IL-6 was not detected in sera samples of Sham Group because it was below the detectable limits (Figure 1D). Among the treatment groups, IL-6 value was the lowest in Group CLP+IM+MP (P<0.05).

Leucocyte and Platelet Values

The mean leukocyte values were presented in Table 2. Mean WBC values in the CLP Group and Group CLP+IM+VK decreased significantly compared to sham Group (P<0.05). Antibiotic alone and antibiotic plus methylprednisolone prevented the decrease in WBC count. Antibiotic plus Vit K did not prevent this decrease (P<0.05). Severe decreases in lymphocyte counts were observed in all septic groups (P<0.05). Neutrophil counts in Group CLP+IM (P<0.05) and Group CLP+MP (P<0.01) increased significantly compared to both CLP and Sham groups. Treatment with antibiotic and antibiotic plus methylprednisolone of septic rats prevented the decreases in eosinophil and basophil counts. Vit K administration with or without antibiotic did not prevent these decreases.

Table 2 The mean leukocytes and platelet values.
Tablo 2. Ortalama lökosit ve trombosit değerleri.

Groups (n=6)	WBC (10^3/μl)	LYM (10^3/μl)	NEU (10^3/μl)	MON (10^3/μl)	EOS (10^3/μl)	BAS (10^3/μl)	PLT (10^3/μl)
Sham	4.4±0.5^{b}	3.4±0.5^{b}	0.6±0.1^{a}	0.10±0.04	0.14±0.01^{b}	0.07±0.01^{b}	785±67^{b}
CLP	2.1±0.5^{a}	1±0.2^{a}	0.9±0.3^{a}	0.12±0.03	0.06±0.01^{a}	0.02±0^{a}	526±71^{a}
CLP+IM	3.9±0.4^{b}	1.6±0.3^{a}	2±0.2^{b}	0.12±0.04	0.12±0.05^{ab}	0.05±0.01^{b}	661±94^{b}
CLP+MP	4.8±1^{b}	1.4±0.2^{a}	3.1±0.8^{b}	0.16±0.04	0.15±0.03^{b}	0.06±0.01^{b}	429±77^{a}
CLP+VK	2.5±0.1^{ab}	1.2±0.1^{a}	1.1±0.3^{a}	0.13±0.02	0.05±0.01^{a}	0.02±0^{a}	624±71^{b}
CLP+IM+MP	4±0.7^{b}	1.2±0.2^{a}	2.5±0.5^{b}	0.14±0.02	0.12±0.05^{ab}	0.05±0.01^{b}	683±70^{b}
CLP+IM+VK	2.2±0.2^{a}	0.9±0.1^{a}	1.1±0.3^{a}	0.12±0.01	0.04±0.01^{a}	0.02±0^{a}	654±68^{b}

The data are presented as mean±SEM. Different letters in the same column indicate a statistical difference. A P value <0.05 was defined as significant. WBC: white blood cells, LYM: lymphocyte, NEU: neutrophil, MON: monocyte, BAS: basophil, PLT: platelet.

Coagulation Tests

Coagulation test results were presented in Table 3. Mean values of PT test in all septic groups prolonged significantly compared to Sham Group (P<0.001). The mean PT value in groups CLP+MP, CLP+VK, CLP+IM+MP and CLP+IM+VK were lower than CLP Group (P<0.05). The findings of INR were similar that of PT values. The aPPT value prolonged in CLP Group (P<0.05). The prolongations of aPPT values were not statistically significant in the treatment groups. Imipenem plus methylprednisolone and Vit K prevented, to some extent, the shortened ACT values in the groups CLP+IM+MP and CLP+IM+VK compared to CLP group (P<0.05).

Table 3 The mean coagulation values.
Tablo 3. Ortalama koagülasyon değerleri.

Groups (n=6)	PT (sec)	INR (PT_{test}/PT_{normal})	aPPT (sec)	ACT (sec)
Sham	8.7±0.1^{a}	0.8±0.01^{a}	15.6±0.3^{a}	175.5±3.3^{c}
CLP	13.5±0.7^{c}	1.3±0.06^{c}	23.4±2.3^{b}	79.8±6.3^{a}
CLP+IM	12.5±0.4^{bc}	1.2±0.03^{bc}	18.8±1^{ab}	89.9±5.4^{ab}
CLP+MP	12±0.3^{b}	1.1±0^{b}	19.9±0.8^{ab}	95.1±4.2^{ab}
CLP+VK	12±0.3^{b}	1.1±0^{b}	21.1±2.2^{ab}	95.9±5.2^{ab}
CLP+IM+MP	11.4±0.7^{b}	1.1±0.06^{b}	21.4±1.8^{ab}	107.3±12.3^{b}
CLP+IM+VK	11.5±0.4^{b}	1±0^{b}	20±0.7^{ab}	102±5.3^{b}

The data are presented as mean±SEM. Different letters in the same column indicate a statistical difference. A P value <0.05 was defined as significant. PT: Prothrombin time, INR: international normalized ratio, aPPT: activated partial prothrombin time and ACT: activated clothing time.

Microorganisms

The microorganism species isolated in blood culture tubes were presented in Table 4. One of the 6 tubes in the Sham group produced two microorganisms. All tubes in the CLP group produced bacterial isolations. *E. coli* and Enterococcus spp. are the most common organisms isolated from the blood culture tubes. Antibiotic enhanced the bacterial clearance in groups CLP+IM, CLP+IM+MP and CLP+IM+VK.

Table 4 Microorganisms isolated from blood culture tubes.
Tablo 4. Kan kültürü tüplerinden izole edilen mikroorganizmalar.

Groups	No. of Blood Tubes*	Microorganisms					
		E. coli	Enterococus spp.	*Sphingomonas paucimobilis*	*Klebsiella pneumonia*	Klostridium spp.	*Proteus vulgaris*
Sham	1	-	1	-	1	-	-
CLP	6	4	3	-	-	-	-
CLP+IM	5	3	2	1	-	1	-
CLP+MP	6	6	-	-	-	-	-
CLP+VK	6	6	3	-	-	-	1
CLP+IM+MP	4	4	1	2	-	-	-
CLP+IM+VK	4	4	3	-	-	-	-

*: Number of blood tubes having at least one bacterial production in each group (n=6). Please note that some of the tubes showed more than one bacterial production for the representation of microorganisms.

DISCUSSION and CONCLUSION

The surgical CLP technique to induce peritonitis has been developed and standardized in the rat for basic model of sepsis research (1,16,17). Basically, after puncture of the cecum fecal content extrudes from the abdominal cavity causing the peritoneal contamination and bacteremia resulting in systemic inflammatory response and multi-organ failure. In the present study, cytokine response, leukogram changes, platelet count, coagulation tests and survival rates have revealed the significance of pathophysiologic adaptation mechanisms and antibiotic treatment with low dose corticosteroid in the sepsis.

The changes of cytokine response have important roles in the pathogenesis and treatment procedures of sepsis-induced immune suppression and inflammatory response (18,19). TNF-α, IL-1β and IL-6 values have been found high at the 16^{th} h in the septic rats, representing natural septic peritonitis in CLP Group. In the previous studies, the increases in TNF-α and IL-6 were determined after the 6^{th} h of CLP-induced sepsis (15). Furthermore, high levels of TNF-α, IL-6 and IL-10 were confirmed at the CLP-induced sepsis and colon ascendens stent peritonitis (20). Prevention of inflammatory cytokines is important to prevent consecutive effects (21). CLP-induced sepsis caused a marked increase in TNF-α value and imipenem plus methylprednisolone tended to prevent this effect (209±50 vs. 94±28 pg/ml). IL-1β value was depressively increased by the administration of antibiotic plus Vit K and mostly prevented by the imipenem plus methylprednisolone. The results clearly indicate that the addition of methylprednisolone to antibiotic is beneficial at the initial phase of inflammatory process. Surprisingly, Vit K administration deteriorates the healing effects of antibiotic with respect to cytokine response. Exhaustion of platelets and coagulation proteins may be expected along with severe haemorrhage during sepsis (22). The survival rates in this study design support this idea regarding that survival rates are higher in imipenem given groups (66.7%). For the dying animals, addition of methylprednisolone to antibiotic prolongs the mortality time (from 20±4 h to 31±8 h). This prolongation may be an important contribution in some critical cases to have more time for clinical intervention.

Leukopenia, lymphopenia, neutrophilia, and increase in neutrophil/lymphocyte ratio have been reported in the CLP-induced sepsis (23). Incidences of neutrophilia and lymphopenia are significant responses of the body against sepsis. This outcome was accompanied with stress leukogram since the most increased values of neutrophil counts were seen in the groups given methylprednisolone for the treatment of CLP-induced septic rats. Therapeutic strategies include inhibiting excessive lymphocyte apoptosis caused by sepsis (2,4). Methylprednisolone prevented the decrease of neutrophil count in the bloodstream regarding inhibition of the immigration consequently.

Recent investigations have focused on coagulation abnormalities and on the link between coagulation and inflammation. The decrease in platelet counts along with the increases in PT, aPTT and fibrinogen levels have been reported in septic rats (24). The prolongation of PT time and INR value compared to Sham control group may be caused by platelet aggregation resulting in an absolute decrease in the number of platelet cells associated with septic peritonitis in these rats. The complex response of a body against inflammation and coagulation that are regulated through a common pathway are mediated by protein C (25). It should be noted herein that the activated protein C as an anti-coagulant drug is used to treat severe sepsis under certain circumstances of INR value and platelet counts (26,27). The inflammation and coagulation pathways are regulated by systemic inflammatory response mechanisms. The increased inflammatory cytokines of TNF-α, IL-1β and IL-6 in septic rats used herein activate coagulation and inhibit fibrinolysis along with stimulation of inflammatory pathways by procoagulant thrombin (28-30). Coagulation and haemorrhagic diathesis may follow each other consecutively during coagulation abnormalities regarding the stage of the septic patient. The administration of Vit K in the early stage of the sepsis in the CLP-induced septic peritonitis model increased the mortality rate. The most isolated microorganism in this study was E. coli as a Gram-negative bacterium. Imipenem is commonly used in clinics as a broad-spectrum antibiotic. Addition of low dose methylprednisolone increased the survival time of imipenem although the addition of Vit K deteriorated the survival rate.

The systemic response to severe septic shock is regulated by inflammatory and compensation mechanisms such as high cytokine level, lymphopenia, thrombocytopenia, neutrophilia and prolongation of coagulation tests. The pathophysiological events of inflammation, immunosuppression, coagulopathy and homeostasis abnormalities directly cause death in sepsis. Imipenem administration has life-saving potentials and low dose methylprednisolone adjunction prolongs surviving time while Vit K administration in the early stage deteriorates the effects of the sepsis. In this study, we found that the use of steroids, a subject of discord in the sepsis, may have a protective effect in early stage and this effect was increased by the antibiotic treatment. Interestingly the coagulopathy, a key responsible for the septic damage, was not ameliorated by Vit K administration. Moreover, the adjunctive use of Vit K to steroid and antibiotic deteriorated the protective effects of these drugs. These results suggest that therapeutics should be used cautiously to combat with coagulopathy during sepsis.

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REFERENCES

1. Aziz M., Jacob A., Yang WL., Matsuda A., Wang P., 2013. Current trends in inflammatory and immunomodulatory mediators in sepsis. Journal of Leukocyte Biology, 93, 329-342.
2. Chen F., Fan XH., Wu YP., Zhu JL., Wang F., Bo LL., Li JB., Bao R., Deng XM., 2014. Resolvin D1

improves survival in experimental sepsis through reducing bacterial load and preventing excessive activation of inflammatory response. European Journal of Clinical Microbiology, 33, 457-464.

3. Khamphommala L., Parc Y., Bennis M., Ollivier JM., Dehni N., Tiret E., Parc R., 2008. Results of an aggressive surgical approach in the management of postoperative peritonitis. Australian and New Zealand Journal of Surgery, 78, 881-888.
4. Delsesto D., Opal SM., 2011. Future perspectives on regulating pro-and anti-inflammatory responses in sepsis. Contributions to Microbiology, 17: 137-156.
5. Jaffer U., Wade RG., Gourlay T., 2010. Cytokines in the systemic inflammatory response syndrome: a review. HSR Proceedings in Intensive Care δ Cardiovascular Anesthesia, 2, 161-175.
6. Pinsky MR., Vincent JL., Deviere J., Alegre M., Kahn RJ., Dupont E., 1993. Serum cytokine levels in human septic shock. Relation to multiple-system organ failure and mortality. Chest, 103, 565-575.
7. Cunneen J., Cartwright M., 2004. The puzzle of sepsis: fitting the pieces of the inflammatory response with treatment. American Association of Critical- Care Nurses, AACN Clinical Issues 15, 18-44.
8. Emmanuel K., Weighardt H., Bartels H., Siewert JR., Holzmann B., 2005. Current and future concepts of abdominal sepsis. World Journal of Surgery, 29, 3-9.
9. Koo DJ., Jackman D., Chaudry IH., Wang P., 2001. Adrenal insufficiency during the late stage of polymicrobial sepsis. Critical Care Medicine, 29, 618-622.
10. Burry LD., Wax RS., 2004. Role of corticosteroids in septic shock. The Annals of Pharmacotherapy, 38, 464-472.
11. Assfalg V., Huser N., Reim D., Kaiser-Moore S., Rossmann-Bloeck T., Weighardt H., Novotny AR., Stangl MJ., Holzmann B., Emmanuel KL., 2010. Combined immunosuppressive and antibiotic therapy improves bacterial clearance and survival of polymicrobial septic peritonitis. Shock, 33, 155-161.
12. Levi M., Schultz M., van der Poll T., 2013. Sepsis and thrombosis. Seminars in Thrombosis and Hemostasis, 39, 559-566.
13. Satran R., Almog Y., 2003. The coagulopathy of sepsis: pathophysiology and management. The Israel Medical Association Journal, 5, 516-520.
14. Esmon CT., 2004. Crosstalk between inflammation and thrombosis. Maturitas, 47, 305-314.
15. Singleton KD., Wischmeyer PE., 2003. Distance of cecum ligated influences mortality, tumor necrosis factor-alpha and interleukin-6 expression following cecal ligation and puncture in the rat. European Surgical Research, 35, 486-491.
16. Rim KP., Kim K., Jo YH., Lee JH., Rhee JE., Kang KW., Suh GJ., Kwon WY., Lee MJ., Lee HS., 2012. Effect of therapeutic hypothermia according to severity of sepsis in a septic rat model. Cytokine 60, 755-761.
17. Akpinar E., Halici Z., Cadirci E., Bayir Y., Karakus E., Calik M., Topcu A., Polat B., 2014. What is the role of renin inhibition during rat septic conditions: preventive effect of aliskiren on sepsis-induced lung injury. Naunyn-Schmiedeberg's Archives of Pharmacology, 387, 969-978.
18. Fijen JW., Kobold AC., de Boer P., Jones CR., van der Werf TS., Tervaert JW., Zijlstra JG., Tulleken JE., 2000. Leukocyte activation and cytokine production during experimental human endotoxemia. European Journal of Internal Medicine, 11, 89-95.
19. Cadirci E., Altunkaynak BZ., Halici Z., Odabasoglu F., Uyanik MH., Gundogdu C., Suleyman H., Halici M., Albayrak M., Unal B., 2010. Alpha-lipoic acid as a potential target for the treatment of lung injury caused by cecal ligation and puncture-induced sepsis model in rats. Shock, 33, 479-484.

20. Maier S., Traeger T., Entleutner M., Westerholt A., Kleist B., Huser N., Holzmann B., Stier A., Pfeffer K., Heidecke CD., 2004. Cecal ligation and puncture versus colon ascendens stent peritonitis: two distinct animal models for polymicrobial sepsis. Shock, 21, 505-511.
21. Orsal E., Halici Z., Bayir Y., Cadirci E., Bilen H., Ferah I., Aydin A., Ozkanlar S., Ayan AK., Seven B., Ozaltin S., 2013. The role of carnitine on ovariectomy and inflammation-induced osteoporosis in rats. Experimental Biology and Medicine, 238, 1406-1412.
22. Col R., Durgun Z., 2007. Sepsis, lökositler, sitokinler ve disseminant intravasküler koagulasyon. Veteriner Bilimleri Dergisi, 23, 97-106.
23. Brooks HF., Osabutey CK., Moss RF., Andrews PL., Davies DC., 2007. Caecal ligation and puncture in the rat mimics the pathophysiological changes in human sepsis and causes multi-organ dysfunction. Metabolic Brain Disease, 22, 353-373.
24. de Oliveira, LM., Pires MG., Magrisso AB., Munhoz TP., Roesler R., de Oliveira JR., 2010. Fructose-1,6-bisphosphate inhibits in vitro and ex vivo platelet aggregation induced by ADP and ameliorates coagulation alterations in experimental sepsis in rats. Journal of Thrombosis and Thrombolysis, 29, 387-394.
25. Esmon CT., Taylor FB. Jr., Snow TR., 1991. Inflammation and coagulation: linked processes potentially regulated through a common pathway mediated by protein C. Thrombosis and Haemostasis, 66, 160-165.
26. Bernard GR., Vincent JL., Laterre PF., LaRosa SP., Dhainaut JF., Lopez-Rodriguez A., Steingrub JS., Garber GE., Helterbrand JD., Ely EW., Fisher CJ. Jr., 2001. Efficacy and safety of recombinant human activated protein C for severe sepsis. The New England Journal of Medicine, 344, 699-709.
27. Riedemann NC., Guo RF., Ward PA., 2003. Novel strategies for the treatment of sepsis. Nature Medicine, 9, 517-524.
28. Bevilacqua MP., Pober JS., Majeau GR., Fiers W., Cotran RS., Gimbrone MA. Jr., 1986. Recombinant tumor necrosis factor induces procoagulant activity in cultured human vascular endothelium: characterization and comparison with the actions of interleukin 1. Proceedings of The National Academy of Sciences of the United States of America, 83, 4533-4537.
29. Conkling PR., Greenberg CS., Weinberg JB., 1988. Tumor necrosis factor induces tissue factor-like activity in human leukemia cell line U937 and peripheral blood monocytes. Blood, 72, 128-133.
30. Stouthard JM., Levi M., Hack CE., Veenhof CH., Romijn HA., Sauerwein HP., van der Poll T., 1996. Interleukin-6 stimulates coagulation, not fibrinolysis, in humans. Thrombosis and Haemostasis, 76, 738-742.