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Effects of Enrofloxacin, Flunixin and Dexamethasone on Indicators of Oxidative and Organ Damage in Lipopolysaccharide-Induced Endotoxemia

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Abstract: The aim of this study was to determine the effects of enrofloxacin, flunixin meglumine and dexamethasone on antioxidant status and markers of organ damage in endotoxemia. Rats were divided into four groups. The groups received the following drugs (simultaneously with lipopolysaccharide): enrofloxacin, flunixin meglumine, low-dose dexamethasone or high-dose dexamethasone, respectively. After the treatments, serum and plasma samples were collected at 1, 2, 4, 6, 8, 12, 24 and 48 h. The levels of malondialdehyde, nitric oxide, superoxide dismutase, vitamin C and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ were determined with ELISA. The cardiac, hepatic and renal damage markers were measured with autoanalyzer. Elevated levels of malondialdehyde were relatively inhibited by high-dose dexamethasone. Increases in the levels of nitric oxide were inhibited by low and high-dose dexamethasone while increases in the level of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ were inhibited by all treatments except enrofloxacin. No treatments inhibited the decrease in vitamin C levels. Cardiac and hepatic damage was not inhibited completely whereas renal damage was inhibited by treatment with low or high-dose dexamethasone. The results show that although low-dose dexamethasone had antioxidant activity and protected against organ damage, high-dose dexamethasone may be more beneficial in the treatment of endotoxemia.

Key words: Endotoxemia, oxidative stress, organ damage, enrofloxacin, flunixin, dexamethasone

INTRODUCTION

Lipopolysaccharide (LPS) also called endotoxin is a component of the outer membrane of Gram-negative bacteria. Intravenous administration of LPS results in endotoxemia and causes an acute systemic inflammatory response that mimics the inflammatory response of sepsis. Sepsis is the most common cause of death in hospital intensive care units (Andreasen *et al*., 2008). Severe oxidative damage develops in endotoxemia (Yazar *et al*., 2010a). Superoxide anions, hydroxyl radicals, hydrogen peroxide, Nitric Oxide (NO) and peroxynitrite are well known Reactive Oxygen Species (ROS) that are derived from oxygen (Salvemini and Cuzzocrea, 2002; Macdonald *et al*., 2003). NO is produced by inducible NO Synthase (iNOS) and LPS stimulates the production of NO via upregulation of iNOS (Andreasen *et al*., 2008). Production of a large quantity of NO is a major factor involved in life-threatening hypotension, tissue damage and organ failure and it plays an important role in the pathophysiology of septic shock (Sakaguchi and Furusawa, 2006). Under physiological conditions, there is balance between the generation of ROS and that of antioxidants which include enzymatic forms, such as superoxide dismutase, glutathione peroxidase and catalase and nonenzymatic forms such as glutathione and vitamins A, E and C. Oxidative damage develops when this balance is disrupted.

Both extreme levels of generation of ROS and insufficient antioxidative defenses can be seen in sepsis (Macdonald *et al*., 2003). When the stability of the antioxidant system is disrupted, lipid peroxidation, the oxidative deterioration of polyunsaturated lipids, develops.

Although, many markers of lipid peroxidation exist, Malondialdehyde (MDA) is accepted as the most basic test of lipid peroxidation occurring under oxidative stress in clinical medicine (Berger and Chiolero, 2007).

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The presence of LPS stimulates the synthesis of prostaglandins by macrophages, monocytes and endothelial cells (Andreasen *et al*., 2008). A major metabolite of prostaglandin $F_{2\alpha}$, 13,14-dihydro-15-keto-Prostaglandin $F_{2\alpha}$ (PGM), increases during infection. PGM is accepted as an indicator of *in vivo* lipid peroxidation through the Cyclooxygenase (COX) pathways (Basu and Eriksson, 2000; Basu *et al*., 2000).

Multiple-Organ Failure (MOF) is often detected in patients with sepsis in intensive-care units and dysfunction of the cardiovascular, renal and hepatic organs has been reported in 62, 53 and 24% of cases, respectively (Parke *et al*., 2003). Serum Creatine Kinase MB (CK-MB) is a conventional marker of cardiac damage. Alkaline Phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Gamma Glutamyl Transferase (GGT) are markers of hepatic damage, whereas serum urea (Blood Urea Nitrogen; BUN) and creatinine are indicators of renal damage.

Antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs) and Glucocorticoids (GCs) are used in the treatment of endotoxemia (Elmas *et al*., 2008; Er *et al*., 2009; Yazar *et al*., 2010b). Enrofloxacin (ENR), a fluoroquinolone antibiotic, may also be used in the treatment of endotoxemia (Elmas *et al*., 2006a). Flunixin Meglumine (FM), a NSAID, has anti-inflammatory, analgesic and antipyretic properties and is the NSAID chosen most commonly in the treatment of endotoxemia (Elmas *et al*., 2006b; Yazar *et al*., 2007). GCs are also generally used in the treatment of endotoxemia (Yazar *et al*., 2004a, b). However, the appropriate dosages of GCs are controversial. Although, high-dose GC was the preferred treatment in the 1960s, the use of high-doses of GCs in the treatment of patients with sepsis decreased in the 1980 and 1990s. Nowadays, low-dose GC is generally chosen in human medicine (Minneci *et al*., 2004). Although, FM and/or GCs are recommended for the treatment of endotoxemia, very few controlled studies comparing the effects of NSAIDs and GCs have been reported (Smith, 2005). To the best of the knowledge, there has been very limited study of the effects of ENR, FM and Dexamethasone (DEX) on oxidative stress and organ function during endotoxemia.

The aim of this study was to determine the effects of ENR, FM, low-dose DEX and high-dose DEX on the levels of MDA, NO, SOD, vitamin C (VC) and PGM which are indicators of oxidative damage. The researchers also measured the levels of CK-MB, ALP, ALT, AST, GGT, creatinine and BUN which are indicators of cardiac, hepatic and renal damage in endotoxemia.

MATERIALS AND METHODS

A total of 192 Sprague Dawley rats (6-8 months old; female, n = 96, 190-220 g; male, n = 96, 300-340 g; Laboratory Animal Unit, Akdeniz University, Antalya, Turkey) were used. The study protocol was approved by the Ethics Committee of the Veterinary Faculty. The animals were fed a standard pelleted diet and tap water *ad libitum*.

The rats were divided into four groups. To induce endotoxemia, 4 mg of LPS (*Escherichia coli* 0111:B4; Sigma-Aldrich Chemie, Deisenhofen, Germany) was injected intraperitoneally into the rats of all groups. The groups received the following drug doses (simultaneously with LPS): 10 mg kg^{-1} ENR subcutaneously (Baytril® 10% inj., Bayer Turk Kimya San. Ltd. Sti, Istanbul, Turkey); 2.5 mg kg^{-1} FM subcutaneously (Finadyne® inj., Sol., Dogu Ilac Veteriner Urunleri, Istanbul, Turkey); 0.6 mg kg^{-1} (low-dose) DEX intramuscularly (Dekort® amp., Deva Ilac, Istanbul, Turkey) and 10 mg kg^{-1} (high-dose) DEX intramuscularly, respectively. After administration of the treatments, samples of serum and plasma (per sampling time n = 6) were collected by cardiac puncture under thiopental sodium anesthesia (70 mg kg^{-1} intraperitoneally; Pental® sodium 1 g inj., I. E. Ulagay Ilac Sanayi, Istanbul, Turkey) at 1, 2, 4, 6, 8, 12, 24 and 48 h. Serum levels of MDA (Bioxytech-MDA 586, Oxisresearch, Portland, Oregon, USA), NO (Bioxytech Nitric Oxide Assay, Oxisresearch) and SOD (Sun *et al*., 1988) and plasma levels of vitamin C (Kyaw, 1978) and PGM (13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ EIA kit, Cayman Chemical, Michigan, USA) were determined using an enzyme-linked immunosorbent assay/spectrophotometric reader (MWGt Lambda Scan 200, Bio-Tek Instruments, VT, USA). The levels of CK-MB (Cormay, Lomianki, Polanya), ALP (Cormay), ALT (Cormay), AST (Cormay), GGT (Cormay), creatinine (Cormay) and BUN (Cormay) were measured using an autoanalyzer (Tokyo Boeki Prestige 24i, Japan).

The concentrations of NO, SOD, VC, MDA, PGM, CK-MB, ALP, ALT, AST, GGT, BUN and creatinine were compared using ANOVA and Tukey's test (SPSS 10.0) Data are expressed as means±SE. Significance was accepted at a level of $p<0.05$.

RESULTS AND DISCUSSION

The blood levels of NO, SOD, VC, MDA and PGM are shown in Table 1. The elevated level of MDA was not inhibited completely by any treatment except high-dose

Table 1: Effects of drugs on oxidative stress markers in endotoxemia (mean±SE)

Drugs	1 h	2 h	4 h	6 h	8 h	12 h	24 h	48 h
MDA (μM)								
ENR	2.27±0.09^{bc,A}	3.35±0.48^{b,A}	5.47±0.41^{a,A}	4.51±0.15^{a,AB}	4.84±0.08^{a,A}	2.70±0.07^{bc,A}	3.01±0.23^{bc,A}	2.14±0.14^{c,A}
FM	2.38±0.06^{cd,A}	4.02±0.46^{abc,A}	5.23±0.48^{a,A}	5.24±0.43^{a,A}	4.16±0.34^{ab,A}	2.88±0.43^{bcd,A}	3.54±0.41^{abcd,A}	2.15±0.12^{d,A}
LD	2.35±0.18^{c,A}	2.71±0.35^{c,A}	4.45±0.42^{ab,A}	3.82±0.18^{abc,BC}	4.87±0.37^{a,A}	3.20±0.52^{bc,A}	2.93±0.49^{bc,A}	2.18±0.14^{c,A}
HD	1.85±0.27^{bc,A}	2.20±0.67^{abc,A}	3.40±0.75^{ab,A}	3.30±0.24^{abc,C}	4.09±0.43^{a,A}	1.83±0.21^{bc,A}	1.43±0.12^{c,B}	1.53±0.15^{bc,B}
NO (μM)								
ENR	10.2±0.70^{e,AB}	11.5±0.84^{e,AB}	30.7±3.55^{de,A}	50.3±5.89^{cde,A}	71.4±10.5^{bcd,A}	115±16.2^{a,A}	89.6±5.24^{abc,A}	94.9±15.6^{ab,A}
FM	5.21±1.59^{d,B}	7.11±0.41^{d,B}	41.3±2.69^{c,A}	43.7±7.34^{c,A}	62.6±14.9^{bc,A}	121±4.90^{a,A}	87.5±5.16^{b,A}	84.5±10.2^{b,A}
LD	8.32±2.03^{c,AB}	12.2±1.89^{bc,A}	12.6±1.51^{bc,B}	18.6±1.23^{ab,B}	16.2±1.30^{bc,B}	19.6±1.50^{ab,B}	16.1±2.91^{bc,B}	25.6±2.43^{a,B}
HD	10.9±0.76^{a,A}	11.1±1.06^{a,AB}	14.9±3.30^{a,B}	16.1±1.26^{a,B}	17.4±3.29^{a,B}	17.5±4.14^{a,B}	15.6±1.92^{a,B}	8.40±1.96^{a,B}
SOD (mg dL^{-1})								
ENR	144±9.62^{a,AB}	155±13.1^{a,AB}	224±28.1^{a,A}	218±26.2^{a,A}	137±18.5^{a,B}	193±35.6^{a,A}	193±34.4^{a,A}	142±22.0^{a,A}
FM	178±25.8^{ab,A}	185±26.9^{ab,A}	234±22.9^{ab,A}	269±18.8^{a,A}	254±24.8^{a,A}	200±26.2^{ab,A}	133±16.4^{b,AB}	134±19.3^{b,A}
LD	86.1±10.8^{a,B}	106±8.65^{a,B}	100±13.8^{a,B}	102±11.5^{a,B}	82.4±5.99^{a,B}	78.6±10.2^{a,B}	102±11.4^{a,B}	97.4±13.9^{a,A}
HD	102±10.1^{a,B}	104±16.8^{a,B}	100±13.0^{a,B}	105±7.35^{a,B}	137±15.5^{a,B}	81.0±14.7^{a,B}	112±13.2^{a,AB}	113±12.8^{a,A}
VC (mg dL^{-1})								
ENR	0.81±0.04^{ab,A}	0.58±0.10^{bc,A}	0.79±0.10^{ab,A}	0.61±0.14^{bc,A}	0.34±0.07^{c,A}	0.66±0.10^{bc,A}	0.90±0.06^{ab,A}	1.20±0.09^{a,A}
FM	0.80±0.06^{abc,A}	0.81±0.10^{abc,A}	1.00±0.14^{ab,A}	0.67±0.11^{bc,A}	0.49±0.09^{c,A}	0.60±0.08^{bc,A}	0.83±0.08^{abc,A}	1.20±0.07^{a,A}
LD	0.88±0.14^{abc,A}	0.78±0.12^{abc,A}	0.69±0.13^{bc,A}	0.68±0.06^{bc,A}	0.31±0.14^{c,A}	0.77±0.07^{abc,A}	1.03±0.13^{ab,A}	1.27±0.19^{a,A}
HD	0.81±0.06^{abc,A}	0.91±0.10^{abc,A}	0.95±0.05^{abc,A}	0.80±0.09^{bc,A}	0.53±0.07^{c,A}	0.80±0.10^{bc,A}	0.97±0.15^{ab,A}	1.22±0.08^{a,A}
PGM (mg dL^{-1})								
ENR	469±142^{a,A}	249±40.1^{a,A}	228±110^{a,A}	193±32.0^{a,A}	189±38.0^{a,A}	228±57.3^{a,A}	157±8.57^{a,A}	154±7.70^{a,A}
FM	100±8.65^{a,B}	97.2±3.64^{a,B}	121±16.5^{a,A}	87.6±16.3^{a,B}	110±16.3^{a,AB}	133±31.0^{a,A}	103±10.0^{a,B}	128±32.4^{a,A}
LD	118±24.9^{ab,B}	121±11.0^{a,B}	83.1±7.83^{ab,A}	62.1±10.8^{b,B}	92.5±4.39^{ab,B}	94.7±8.70^{ab,A}	91.4±10.6^{ab,B}	87.6±15.4^{ab,A}
HD	136±52.4^{a,B}	99.0±10.0^{a,B}	93.4±5.38^{a,A}	108±9.99^{a,B}	98.6±9.77^{a,B}	101±25.6^{a,A}	104±12.5^{a,B}	123±11.7^{a,A}

Different letters in the same line (a, b, c, d, e) and column (A, B) are statistically significant

DEX which caused relative inhibition of the increase (Table 1). The increased level of NO was inhibited ($p<0.05$) by low and high-dose DEX, while the augmented level of PGM was inhibited ($p<0.05$) by all treatments except ENR. No treatment had a consistent effect on the level of SOD (Table 1). The decrease in the level of VC was not inhibited by any treatment.

The serum levels of CK-MB, ALP, ALT, AST, GGT creatinine and BUN are given in Table 2. Markers of cardiac, hepatic and renal damage increased. Cardiac damage was not inhibited by any treatment whereas renal damage was inhibited and hepatic damage was partially inhibited after treatment with low and high-dose DEX.

Oxidative stress develops in endotoxemia (Keskin *et al*., 2005; Konyalioglu *et al*., 2007) and ROS may contribute to mortality of endotoxemia (Cadenas and Cadenas, 2002). The increase in MDA, an indicator of lipid peroxidation was relatively inhibited by high-dose DEX (Table 1). High levels of MDA have been reported to occur in endotoxemia (Yazar *et al*., 2004a; Keskin *et al*., 2005) and prednisolone and FM (Yazar *et al*., 2004a; Konyalioglu *et al*., 2007) inhibit lipid peroxidation by reducing the level of MDA in endotoxemia. However, FM did not reduce the increase in the level of MDA seen in this study. The effect of FM on oxidative damage may depend on the dose.

Increased levels of NO were inhibited by low and high-dose DEX in the current study (Table 1). The presence of LPS causes the release of large amounts of NO via upregulation of iNOS (Andreasen *et al*., 2008) and the NO level correlates with the severity of septic shock and mortality (Keh *et al*., 2005). The LPS increases the activation of nuclear factor kappaB (NF-κB), a ubiquitous transcription factor. Overactivation of NF-κB causes overexpression of inflammatory mediators including cytokines, iNOS and cycloxygenase 2. These mediators may be associated with the harmful effects seen in sepsis (Macdonald *et al*., 2003). It has been reported that GCs (Booke and Westphal, 2003) and FM (Bryant *et al*., 2003) inhibit the activation of NF-κB. However, FM had no effect on the generation of NO in this study (Table 1). The effect of FM on iNOS expression may be dose dependent. The inhibition of NO production might be beneficial in cases of life-threatening hypotension in endotoxemia.

The administered drugs had no consistent effect on the level of SOD (Table 1). SOD protects the cell or tissue against superoxide radicals (Sakaguchi and Furusawa, 2006). It has been reported that LPS and ENR change the levels of antioxidants such as SOD, glutathione peroxidase, catalase and reduced glutathione (Yazar *et al*., 2004a; Keskin *et al*., 2005; Yazar and Tras, 2001). The results of studies in which only antioxidant enzymes are evaluated are contradictory; however, when MDA is evaluated as an indicator of oxidative damage, reliable findings have been reported (Konyalioglu *et al*., 2007; Yazar *et al*., 2010a) which are supported by the results of the current study.

The decreased plasma levels of VC seen in endotoxemia were not inhibited by any treatment applied (Table 1). Low levels of VC have been reported in

Table 2: Effects of drugs on organ damage markers in endotoxaemia (mean±SE)

Drugs	1 h	2 h	4 h	6 h	8 h	12 h	24 h	48 h
CK-MB (IU L^{-1})								
ENR	3653±216^{a,A}	3051±145^{ab,A}	2195±131^{bc,AB}	2116±170^{c,A}	1926±377^{c,A}	1771±185^{cd,A}	967±165^{d,A}	932±144^{d,A}
FM	2865±461^{a,A}	2738±370^{a,A}	2021±221^{ab,B}	1932±130^{ab,A}	1714±357^{ab,A}	1736±383^{ab,A}	1473±276^{ab,A}	741±91.6^{b,A}
LD	2849±199^{a,A}	2420±229^{ab,A}	2480±194^{ab,AB}	2191±239^{abc,A}	2019±278^{abc,A}	1884±232^{bc,A}	1435±108^{cd,A}	728±65.1^{d,A}
HD	3674±326^{a,A}	2968±420^{ab,A}	2861±268^{ab,A}	2089±261^{bc,A}	1810±213^{bcd,A}	2120±369^{bc,A}	1171±220^{cd,A}	610±72.8^{d,A}
ALP (IU L^{-1})								
ENR	208±25.1^{b,A}	216±40.1^{ab,A}	175±28.9^{b,A}	243±15.5^{ab,A}	294±38.4^{ab,A}	357±41.4^{a,AB}	228±19.7^{ab,AB}	181±30.1^{b,A}
FM	283±43.5^{a,A}	293±67.1^{a,A}	302±70.2^{a,A}	335±57.4^{a,A}	265±30.2^{a,A}	441±77.1^{a,A}	456±89.7^{a,A}	266±41.6^{a,A}
LD	251±37.1^{a,A}	144±17.8^{b,A}	237±26.3^{ab,A}	218±22.4^{ab,A}	226±30.2^{ab,A}	173±16.3^{ab,C}	234±13.5^{ab,B}	201±14.2^{ab,A}
HD	185±24.1^{a,A}	262±41.9^{a,A}	183±26.2^{a,A}	212±18.1^{a,A}	181±29.3^{a,A}	188±25.5^{a,BC}	278±30.9^{a,B}	275±30.5^{a,A}
ALT (IU L^{-1})								
ENR	78.3±16.3^{b,A}	87.0±16.6^{b,A}	97.3±19.2^{b,A}	153±45.9^{ab,A}	198±38.6^{ab,A}	324±71.9^{a,A}	136±48.4^{ab,A}	114±51.8^{b,A}
FM	75.3±20.9^{ab,A}	45.1±3.70^{b,B}	94.6±15.5^{ab,A}	275±50.5^{a,A}	225±85.1^{ab,A}	133±42.5^{ab,B}	166±50.4^{ab,A}	182±50.3^{ab,A}
LD	60.3±5.83^{b,A}	44.0±7.07^{b,B}	86.6±13.3^{ab,A}	85.3±18.7^{ab,B}	72.1±12.5^{ab,A}	142±36.4^{a,AB}	84.0±21.6^{ab,A}	68.5±7.38^{ab,A}
HD	63.1±8.73^{b,A}	58.0±9.91^{b,AB}	87.6±14.2^{ab,A}	80.5±9.92^{ab,B}	67.3±6.78^{ab,A}	141±26.0^{a,AB}	113±25.9^{ab,A}	84.1±20.2^{ab,A}
AST (IU L^{-1})								
ENR	303±49.6^{a,A}	417±36.1^{a,A}	303±56.3^{a,A}	327±61.4^{a,A}	312±53.5^{a,A}	372±55.2^{a,AB}	256±35.4^{a,A}	204±38.7^{a,A}
FM	199±26.4^{ab,AB}	176±17.6^{b,B}	188±26.2^{ab,A}	362±66.7^{a,A}	183±16.3^{ab,B}	171±50.5^{b,B}	220±49.3^{ab,A}	235±43.1^{ab,A}
LD	173±10.5^{bc,B}	158±14.9^{c,B}	248±33.1^{bc,A}	274±52.8^{abc,A}	223±23.1^{bc,AB}	406±57.5^{a,A}	321±25.6^{ab,A}	193±5.01^{bc,A}
HD	181±16.1^{c,B}	165±16.0^{c,B}	261±28.1^{abc,A}	229±18.4^{bc,A}	251±10.4^{abc,AB}	361±37.1^{ab,AB}	390±62.4^{a,A}	300±54.3^{abc,A}
GGT (IU L^{-1})								
ENR	1.50±0.34^{c,AB}	2.83±0.30^{bc,AB}	2.50±0.22^{bc,B}	3.33±0.42^{abc,AB}	4.33±1.11^{ab,AB}	6.00±0.73^{a,AB}	4.66±0.80^{ab,A}	2.83±0.47^{bc,B}
FM	2.33±0.21^{b,A}	3.66±0.55^{ab,A}	3.83±0.40^{ab,A}	6.66±1.99^{ab,A}	5.50±0.56^{ab,A}	9.33±2.45^{a,A}	7.83±0.02^{ab,A}	5.66±1.05^{ab,A}
LD	2.00±0.36^{b,AB}	2.16±0.16^{b,B}	2.50±0.34^{ab,B}	2.83±0.30^{ab,AB}	2.16±0.16^{b,B}	2.16±0.31^{b,B}	3.50±0.22^{a,A}	2.33±0.21^{ab,B}
HD	1.16±0.16^{b,B}	1.83±0.16^{b,B}	2.00±0.25^{b,B}	2.50±0.22^{ab,B}	2.00±0.63^{b,B}	2.00±0.36^{b,B}	3.66±0.21^{a,A}	2.33±0.49^{ab,B}
CR (mg dL^{-1})								
ENR	0.65±0.02^{bcd,B}	0.61±0.01^{cd,B}	0.58±0.01^{d,B}	0.62±0.02^{cd,A}	0.70±0.02^{bc,A}	0.85±0.02^{a,A}	0.71±0.04^{bc,A}	0.75±0.02^{ab,A}
FM	0.68±0.03^{ab,B}	0.55±0.02^{b,B}	0.66±0.04^{ab,AB}	0.66±0.02^{ab,A}	0.68±0.03^{ab,A}	0.80±0.07^{a,A}	0.70±0.02^{ab,A}	0.70±0.04^{ab,A}
LD	0.65±0.02^{ab,B}	0.56±0.04^{b,B}	0.68±0.03^{ab,AB}	0.68±0.03^{ab,A}	0.70±0.02^{ab,A}	0.73±0.02^{a,A}	0.75±0.03^{a,A}	0.78±0.02^{a,A}
HD	1.20±0.06^{a,A}	0.81±0.04^{b,A}	0.71±0.02^{b,A}	0.72±0.03^{b,A}	0.73±0.02^{b,A}	0.75±0.02^{b,A}	0.75±0.03^{b,A}	0.80±0.03^{b,A}
BUN (mg dL^{-1})								
ENR	46.2±1.77^{c,A}	56.8±3.54^{bc,A}	66.9±4.56^{abc,A}	67.0±6.18^{abc,A}	82.8±7.06^{ab,A}	90.6±9.31^{a,A}	46.7±9.25^{c,A}	56.5±2.31^{bc,A}
FM	46.8±2.29^{b,A}	47.6±2.92^{b,AB}	54.7±2.34^{b,B}	57.9±3.67^{b,AB}	71.4±8.07^{b,A}	99.3±9.17^{a,A}	62.8±6.60^{b,A}	73.7±8.26^{ab,A}
LD	44.4±1.43^{ab,A}	47.0±2.78^{ab,AB}	41.6±0.94^{ab,C}	43.6±1.02^{ab,BC}	37.5±1.37^{c,B}	50.6±4.18^{b,B}	50.1±3.08^{b,A}	74.2±2.11^{a,A}
HD	41.3±1.19^{b,A}	41.8±1.35^{b,B}	38.4±0.79^{b,C}	41.9±1.74^{b,C}	39.5±1.45^{b,B}	51.1±1.54^{a,B}	51.6±2.64^{a,A}	56.7±3.41^{a,A}

Different letters in the same line (a, b, c, d) and column (A, B, C) are statistically significant

endotoxemia (Macdonald *et al*., 2003). The body may use VC during septic shock because ascorbate inhibits excessive production of NO (Wu *et al*., 2004). Therefore, VC may be a useful addition to the conservative management of endotoxemia.

An increased level of PGM, which is a marker of lipid peroxidation through the cyclooxygenase pathways, was inhibited by all treatments except ENR. Elevated levels of PGM have been reported in cases of infection (Hagman *et al*., 2006; Jana *et al*., 2007). FM inhibits PGM synthesis, as do low and high dose-DEX because of its potent inhibitory effect on the synthesis of prostaglandins.

Antioxidant therapy may be beneficial in preventing multiple-organ failure in septic shock (Biesalski and McGregor, 2007). In the present study, increased markers of heart and liver damage in endotoxemia were not inhibited completely by any treatment (Table 2). The level of CK-MB, an indicator of cardiac damage, increased and no treatment inhibited this increase. The production of cytokines and NO may cause depression of myocardial contractility in patients with sepsis. Hence, CK-MB may be released by damaged myocytes (Makwana and Baines, 2005). In contrast, increases in the markers of renal and hepatic damage were relatively inhibited by both low and high-dose DEX, although high-dose DEX was more effective in the current study (Table 2). Increased levels of markers of hepatic and renal damage have been reported previously in endotoxemia (Yazar *et al*., 2004c; Elmas *et al*., 2006a, b, 2008) and administration of prednisolone or DEX has been shown to inhibit the increase in indicators of hepatic and renal damage during endotoxemia (Yazar *et al*., 2004b; Ruetten and Thiemermann, 1997; Chatterjee *et al*., 2007). In sepsis, ROS cause hepatic injury via induction of macrophages. Stimulated macrophages induce tumor necrosis factor alpha which is responsible for many pathophysiological responses in the liver and for hepatic lipid peroxidation (Sakaguchi and Furusawa, 2006). In endotoxemia, acute renal failure may result from decreased renal blood flow (Wan *et al*., 2003). In addition to these effects, multiple-organ damage may develop in endotoxemia as a result of disseminated intravascular coagulation caused by LPS. In patients with sepsis, the incidence of disseminated

intravascular coagulation is 25-50% and it may be an important predictor of mortality (Zeerleder *et al*., 2005). It has been reported that administration of DEX inhibits the progression of disseminated intravascular coagulation in endotoxemia (Elmas *et al*., 2009).

CONCLUSION

The results of this study show that ENR has no negative effect on markers of oxidative and organ damage in endotoxemia and it may be chosen as an antibiotic for the treatment of suitable patients. The use of FM and low-dose DEX may be beneficial but their actions seem to be insufficient. High-dose DEX had the greatest effect in protection against oxidative and organ damage in endotoxemia.

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