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Effects of drugs used in endotoxic shock on oxidative stress and organ damage markers

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Abstract

The aim of this study was to determine the effects of enrofloxacin (ENR), flunixin meglumine (FM) and dexamethasone (DEX) on antioxidant status and organ damage markers in experimentally-induced endotoxemia. Rats were divided into three groups. To induce endotoxemia, lipopolysaccharide (LPS) was injected into all groups, including the positive control. The two other groups received the following drugs (simultaneously with LPS): ENR + FM + low-dose DEX and ENR + FM + high-dose DEX. After the treatments, blood samples were collected at 0, 1, 2, 4, 6, 8, 12, 24 and 48 h. Oxidative stress parameters were determined by ELISA, while serum organ damage markers were measured by autoanalyser. LSP increased ($p < 0.05$) malondialdehyde, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ and nitric oxide, while LPS reduced vitamin C. These changes were especially inhibited ($p < 0.05$) by ENR + FM + high-dose DEX. LPS increased organ damages markers. Cardiac and hepatic damage was not completely inhibited by any treatment, whereas renal damage was inhibited by two treatments. This study suggested that ENR + FM + high-dose DEX is most effective in the LPS-caused oxidative stress and organ damages.

Keywords: *Oxidative stress, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$, enrofloxacin, flunixin, dexamethasone, organ damage.*

Introduction

Lipopolysaccharide (LPS), outer membrane of Gram (–) bacteria is released during bacterial lysis and causes endotoxic shock. Endotoxic shock is responsible for a high mortality rate in intensive care units. The presence of LPS in the bloodstream causes fever, hypotension, disseminated intravascular coagulation, cytokine production, multiple organ failure and, in severe cases, septic shock and death [1,2].

There is convincing evidence of severe oxidative stress in patients with septic shock. The best-known reactive oxygen species (ROS) generated from oxygen include the superoxide anion, hydroxyl radical, hydrogen peroxide, nitric oxide (NO) and peroxynitrite [3,4]. Under normal physiological conditions, a balance exists between the formation of ROS and antioxidants such as (enzymatic) superoxide dismutase, glutathione peroxidase, catalase, etc. and (non-enzymatic) glutathione and vitamins A, E and C. Oxidative stress occurs when this balance is disrupted by the excessive production of ROS and/or inadequate antioxidative defenses. Both may occur in sepsis [4]. When the antioxidant balance is disrupted, lipid peroxidation occurs. Malondialdehyde (MDA), a very global and crude test of lipid peroxidation occurring under oxidative stress, remains the most useful in clinical settings [5].

Cyclooxygenase 2 is induced by several pro-inflammatory stimuli, leading to the release of

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prostaglandins. 13,14-Dihydro-15-keto-prostaglandin F_{2a} (PGM), a major metabolite of prostaglandin F_{2a}, is increased during the inflammatory response. It can be used as an indicator of *in vivo* lipid peroxidation through the cyclooxygenase (COX) pathways in septic shock [6,7].

Multiple-organ failure is observed in septic patients and organ dysfunction has been reported in intensive-care units at frequencies of cardiovascular 62%, renal 53% and hepatic 24% [8]. It is well known that serum creatine kinase–MB (CK–MB) is an accepted marker of cardiac damage, whereas alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) are indicators of hepatic damage. Serum urea (BUN) and creatinine are indicators of renal damage. Blood lactate levels have also been used as a marker of tissue hypoperfusion during shock [9].

Enrofloxacin (ENR), a flouroquinolone antibiotic, and flunixin meglumine (FM), an NSAID, glucocorticoids (GCs) are recommended for the treatment of endotoxemia or Gram (–) bacterial infections [2,10]. Flouroquinolone antibiotics and FM may effect oxidative status in the endotoxemia or infection [11,12]. GCs are also commonly used in endotoxemia; however there is still some controversy over the dosage, timing and duration of administration of GCs. High-dose GC was the chosen treatment in the 1960s [13], but a meta-analyses found that there was no beneficial effect of high doses used in humans in the 1990s [14]. Nowadays, low-dose GC is preferred in human medicine [13]. To the best of our knowledge, there have been very limited studies of the effects different doses of dexamethasone (DEX) in combination with ENR plus FM on oxidative stress and organ function during endotoxemia.

The aim of this study was to determine the effects of low-dose DEX combined with ENR + FM, and high-dose DEX combined with ENR + FM on MDA, NO, SOD, vitamin C (VC) and PGM, which are indicators of oxidative damage and cardiac, hepatic and renal damage markers in endotoxemia.

Material and methods

Animals and experimental design

A total of 150 Sprague Dawley rats (6–8 months; female, $n=75$, 213 ± 20.4 g; male, $n=75$, 348 ± 39.2 g; Laboratory Animal Unit, Akdeniz University, Antalya, Turkey) were used and the study protocol was approved by the Ethics Committee of the Veterinary Faculty. The animals were fed a standard pellet diet and tap water *ad libitum*.

One hundred and forty-four rats were divided into three groups. Six rats were used at a 0 sampling point for all groups. To induce endotoxemia, LPS (4 mg, *Escherichia coli* 0111:B4; Sigma-Aldrich Chemie, Deisenhofen, Germany) was injected intraperitoneally into the rats of all groups. The positive control group received LPS only. The other two groups received the following drugs dose (simultaneously with LPS): ENR (10 mg/kg, subcutaneously, Baytril® 10% inj., Bayer Turk Kimya San. Ltd. Sti, Istanbul, Turkey) + FM (2.5 mg/kg, subcutaneously, Finadyne® inj., Sol., Dogu Ilac Veteriner Urunleri, Istanbul, Turkey) + high-dose DEX (0.6 mg/kg intramuscularly, Dekort® amp., Deva Ilac, Istanbul, Turkey) and ENR (10 mg/kg, subcutaneously) + FM (2.5 mg/kg, subcutaneously) + low-dose DEX (0.6 mg/kg intramuscularly). After the treatments, serum and plasma samples ($n=6$) were collected under thiopental sodium anaesthesia (70 mg/kg, intraperitoneally; Pental® sodium 1 ginj., I. E. Ulagay Ilac Sanayi, Istanbul, Turkey) by cardiac puncture at 0, 1, 2, 4, 6, 8, 12, 24 and 48 h After just bleeding, rats were euthanized. Serum levels of MDA (Bioxytech-MDA 586, Oxisresearch, Portland, Oregon), NO (Bioxytech Nitric Oxide Assay, Oxisresearch), SOD [15], plasma vitamin C [16] and PGM (13,14-dihydro-15-keto-prostaglandin F_{2a} EIA kit, Cayman Chemical, Michigan) were determined with an enzyme-linked immunosorbent assay/spectrophotometric reader (MWGt Lambda Scan 200, Bio-Tek Instruments, VT). Levels of lactate (Spinreact kit, Girona, Spain), CK–MB (Cormay, Lomianki, Polanya), ALP (Cormay, Lomianki, Polanya), AST (Cormay, Lomianki, Polanya), ALT (Cormay, Lomianki, Polanya), GGT (Cormay, Lomianki, Polanya), BUN (BUN; Cormay, Lomianki, Polanya), and creatinine (Cormay, Lomianki, Polanya) were determined with an autoanalyser (Tokyo Boeki Prestige 24i, Japan).

Statistical analysis

Concentrations of MDA, NO, SOD, VC, PGM, lactate, CK–MB, ALP, ALT, AST, GGT, BUN and creatinine in the sampling times were compared by independent *t*-test (SPSS release 10.0). Data are expressed as means ± SE. Significance was accepted at a level of $p < 0.05$.

Results

Oxidative stress

Blood MDA, NO, SOD, VC and PGM levels are given in Tables I and II, respectively. LPS increased MDA levels during 48 hafter treatment (Tables I and II). This increase was completely inhibited ($p < 0.05$) by ENR + FM + high-dose DEX (Table I). NO and PGM levels were increased while plasma VC levels decreased in the LPS group and two combined treatments generally inhibited these changes ($p < 0.05$;

Table I. Effects of lipopolysaccharide (4 mg, intraperitoneally) + enrofloxacin (10 mg/kg, subcutaneously) + flunixin meglumine (2.5 mg/kg, subcutaneously) + high-dose dexamethasone (10 mg/kg, intramuscularly) on serum oxidative status and organ damage markers in endotoxaemia (mean ± SE).

	0 hour	1 hour	2 hours	4 hours	6 hours	8 hours	12 hours	24 hours	48 hours
$MDA_{(LPS)}$ μM	1.61 ± 0.13	3.23 ± 0.35*	4.15 ± 0.44*	5.78 ± 0.69*	6.94 ± 0.74*	7.43 ± 0.65*	3.93 ± 0.38*	4.27 ± 0.30*	2.80 ± 0.11*
$MDA_{(CHD)}$ μM	1.61 ± 0.13	1.93 ± 0.20	2.16 ± 0.49	2.49 ± 0.34	2.51 ± 0.08	2.55 ± 0.11	1.93 ± 0.17	1.59 ± 0.02	1.64 ± 0.09
$NO_{(LPS)}$ μM	8.98 ± 1.01	9.03 ± 1.14	26.8 ± 6.30	30.4 ± 3.29*	65.9 ± 20.6	71.9 ± 7.85*	110 ± 8.82*	104 ± 11.5*	86.4 ± 25.8
$NO_{(CHD)}$ μM	8.98 ± 1.01	6.88 ± 1.74	12.7 ± 1.64	15.0 ± 2.67	20.9 ± 2.23	15.8 ± 2.21	14.7 ± 1.32	14.2 ± 2.01	20.8 ± 2.51
$SOD_{(LPS)}$ mg/dL	163 ± 8.64	134 ± 11.1	137 ± 10.3	137 ± 10.0*	164 ± 16.9*	85.5 ± 5.24	149 ± 22.7*	96.1 ± 12.2	144 ± 19.5
$SOD_{(CHD)}$ mg/dL	163 ± 8.64	127 ± 16.7	125 ± 6.86	79.4 ± 10.4	110 ± 3.79	103 ± 16.2	72.9 ± 3.78	74.0 ± 10.7	120 ± 11.7
$VC_{(LPS)}$ mg/dL	1.04 ± 0.09	0.90 ± 0.07	0.42 ± 0.06	0.51 ± 0.06*	0.50 ± 0.09*	0.64 ± 0.10	0.53 ± 0.06*	0.72 ± 0.06	1.08 ± 0.09
$VC_{(CHD)}$ mg/dL	1.04 ± 0.09	0.96 ± 0.07	0.65 ± 0.07	1.01 ± 0.13	0.89 ± 0.14	0.75 ± 0.09	0.86 ± 0.13	1.04 ± 0.16	1.06 ± 0.03
$PGM_{(LPS)}$ pg/mL	113 ± 4.18	491 ± 87.7*	526 ± 126*	282 ± 52.9*	221 ± 10.3*	285 ± 61.0	227 ± 31.8*	155 ± 10.9*	208 ± 25.7*
$PGM_{(CHD)}$ pg/mL	113 ± 4.18	117 ± 13.3	146 ± 24.8	80.9 ± 8.89	63.3 ± 5.32	112 ± 24.6	43.3 ± 6.48	93.1 ± 10.7	91.5 ± 9.45
$LT_{(LPS)}$ mmol/L	4.82 ± 0.55	3.95 ± 0.26	3.425 ± 0.48	3.94 ± 0.32	3.03 ± 0.93	3.03 ± 0.20	3.71 ± 0.54	3.66 ± 0.50	3.34 ± 0.71
$LT_{(CHD)}$ mmol/L	4.82 ± 0.55	3.62 ± 0.54	3.21 ± 0.26	4.58 ± 1.01	4.42 ± 0.89	4.84 ± 0.98	3.99 ± 0.30	4.58 ± 0.48	7.64 ± 1.97
$CK\text{-}MB_{(LPS)}$ IU/L	759 ± 71.5	3638 ± 439*	3710 ± 272*	3007 ± 206*	1710 ± 80.3	1585 ± 172	1508 ± 131	581 ± 103	504 ± 96.7*
$CK\text{-}MB_{(CHD)}$ IU/L	759 ± 71.5	2372 ± 262	2579 ± 140	2152 ± 203	1822 ± 74.4	1604 ± 235	1380 ± 180	707 ± 64.7	706 ± 97.9
$ALP_{(LPS)}$ IU/L	52.3 ± 11.8	217 ± 22.1	192 ± 20.6	306 ± 89.2	204 ± 20.6	278 ± 37.3*	239 ± 48.6	338 ± 46.6	270 ± 26.2
$ALP_{(CHD)}$ IU/L	52.3 ± 11.8	211 ± 7.36	229 ± 28.3	184 ± 16.1	194 ± 21.1	183 ± 23.6	175 ± 19.2	197 ± 17.8	290 ± 12.3
$ALT_{(LPS)}$ IU/L	68.6 ± 9.67	65.6 ± 13.7	74.1 ± 11.4	180 ± 39.1*	455 ± 54.7*	501 ± 44.6*	214 ± 51.6*	318 ± 100	114 ± 33.6
$ALT_{(CHD))}$ IU/L	68.6 ± 9.67	60.1 ± 7.50	53.3 ± 3.46	55.8 ± 4.11	75.0 ± 11.0	99.1 ± 9.21	91.3 ± 15.3	183 ± 38.4	96.0 ± 17.8
$AST_{(LPS)}$ IU/L	153 ± 15.7	291 ± 64.6	292 ± 51.8	384 ± 35.8*	485 ± 34.6*	447 ± 25.1*	247 ± 58.8	246 ± 45.7	227 ± 33.5
$AST_{(CHD)}$ IU/L	153 ± 15.7	186 ± 14.5	184 ± 12.9	212 ± 16.8	218 ± 24.1	302 ± 23.4	315 ± 33.1	316 ± 80.2	251 ± 9.17
$GGT_{(LPS)}$ IU/L	0.71 ± 0.28	2.00 ± 0.44	2.50 ± 0.83	4.50 ± 1.02*	5.50 ± 0.22*	8.50 ± 0.56*	7.00 ± 1.61*	4.50 ± 1.17	3.83 ± 0.41
$GGT_{(CHD)}$ IU/L	0.71 ± 0.28	1.83 ± 0.30	2.33 ± 0.33	1.6 ± 0.21	2.33 ± 0.55	2.16 ± 0.16	2.00 ± 0.44	2.50 ± 0.34	2.16 ± 0.54
$CR_{(LPS)}$ mg/Dl	0.66 ± 0.02	0.66 ± 0.02*	0.63 ± 0.02*	0.68 ± 0.06	0.74 ± 0.02	0.80 ± 0.03	0.73 ± 0.02	0.63 ± 0.02*	0.68 ± 0.03
$CR_{(CHD)}$ mg/dL	0.66 ± 0.02	1.20 ± 0.05	0.86 ± 0.02	0.81 ± 0.03	0.74 ± 0.06	0.71 ± 0.02	0.73 ± 0.02	0.78 ± 0.04	0.76 ± 0.02
$BUN_{(LPS)}$ mg/dL	33.8 ± 2.39	40.1 ± 1.72*	46.1 ± 1.72*	59.8 ± 4.78*	99.3 ± 4.31*	90.0 ± 4.61*	145 ± 15.6*	61.8 ± 10.7	58.5 ± 4.70
$BUN_{(CHD)}$ mg/dL	33.8 ± 2.39	36.9 ± 1.47	39.3 ± 0.72	41.8 ± 1.32	40.9 ± 1.81	43.1 ± 0.83	46.5 ± 1.17	51.4 ± 2.75	57.5 ± 1.93

CHD; lipopolysaccharide (4 mg, intraperitoneally) + enrofloxacin (10 mg/kg, subcutaneously) + flunixin meglumine (2.5 mg/kg, subcutaneously) + high-dose dexamethasone (10 mg/kg, intramuscularly), LPS; lipopolysaccharide, MDA; malondialdehyde, NO; nitric oxide, SOD; superoxide dismutase, VC; vitamin C, PGM; 13, 14-dihydro-15-keto-prostaglandin F_{2a}, LT; lactate, CK-MB; creatine kinase-MB, ALP; alkaline phosphatase, ALT; alanine aminotransferase, AST; aspartate aminotransferase, GGT; gamma glutamyl aminotransferase, CR; creatinine, BUN; blood urea nitrogen.

*Statistically significant for each values between CDH and LPS sampling times ($p < 0.05$).

Table II. Effects of lipopolysaccharide (4 mg, intraperitoneally) + enrofloxacin (10 mg/kg, subcutaneously) + flunixin meglumine (2.5 mg/kg, subcutaneously) + low-dose dexamethasone (0.6 mg/kg, intramuscularly) on serum oxidative status and organ damage markers in endotoxaemia (mean ± SE).

	0 hour	1 hour	2 hours	4 hours	6 hours	8 hours	12 hours	24 hours	48 hours
$MDA_{(LPS)}$ μM	1.61 ± 0.13	3.23 ± 0.35*	4.15 ± 0.44*	5.78 ± 0.69*	6.94 ± 0.74*	7.43 ± 0.65*	3.93 ± 0.38*	4.27 ± 0.30*	2.80 ± 0.11*
$MDA_{(CLD)}$ μM	1.61 ± 0.13	2.05 ± 0.18	1.82 ± 0.27	2.55 ± 0.29	2.74 ± 0.24	3.16 ± 0.18	2.28 ± 0.18	1.69 ± 0.10	1.71 ± 0.09
$NO_{(LPS)}$ μM	8.98 ± 1.01	9.03 ± 1.14	26.8 ± 6.30*	30.4 ± 3.29*	65.9 ± 20.6	71.9 ± 7.85*	110 ± 8.82*	104 ± 11.5*	86.4 ± 25.8*
$NO_{(CLD)}$D μM	8.98 ± 1.01	6.68 ± 1.06	10.3 ± 1.51	14.2 ± 1.48	30.8 ± 5.82	16.0 ± 2.35	15.9 ± 1.21	20.8 ± 4.11	21.6 ± 3.35
$SOD_{(LPS)}$ mg/dL	163 ± 8.64	134 ± 11.1	137 ± 10.3	137 ± 10.0*	164 ± 16.9*	85.5 ± 5.24	149 ± 22.7*	96.1 ± 12.2	144 ± 19.5*
$SOD_{(CLD)}$ mg/dL	163 ± 8.64	125 ± 8.28	108 ± 12.6	85.2 ± 3.97	97.7 ± 11.3	100 ± 8.51	80.9 ± 5.16	84.4 ± 7.48	95.3 ± 8.27
$VC_{(LPS)}$ mg/dL	1.04 ± 0.09	0.90 ± 0.07	0.42 ± 0.06*	0.51 ± 0.06*	0.50 ± 0.09	0.64 ± 0.10*	0.53 ± 0.06*	0.72 ± 0.06	1.08 ± 0.09
$VC_{(CLD)}$ mg/dL	1.04 ± 0.09	1.01 ± 0.05	0.94 ± 0.03	0.98 ± 0.06	0.67 ± 0.07	1.10 ± 0.16	0.86 ± 0.14	0.92 ± 0.10	1.17 ± 0.06
$PGM_{(LPS)}$ pg/mL	113 ± 4.18	491 ± 87.7*	526 ± 126*	282 ± 52.9*	221 ± 10.3*	285 ± 61.0*	227 ± 31.8*	155 ± 10.9*	208 ± 25.7*
$PGM_{(CLD)}$ pg/mL	113 ± 4.18	89.3 ± 9.62	62.4 ± 4.71	68.3 ± 5.97	80.2 ± 27.4	76.6 ± 8.55	82.9 ± 5.50	83.4 ± 5.52	88.5 ± 10.5
$LT_{(LPS)}$ mmol/L	4.82 ± 0.55	3.95 ± 0.26	3.43 ± 0.48	3.94 ± 0.32*	3.03 ± 0.93*	3.03 ± 0.20	3.71 ± 0.54	3.66 ± 0.50*	3.34 ± 0.71
$LT_{(CLD)}$ mmol/L	4.82 ± 0.55	3.97 ± 0.77	4.25 ± 0.31	6.23 ± 0.68	6.21 ± 1.01	4.32 ± 0.63	5.73 ± 1.11	6.51 ± 0.78	4.14 ± 0.43
$CK\text{-}MB_{(LPS)}$ IU/L	759 ± 71.5	3638 ± 439	3710 ± 272	3007 ± 206	1710 ± 80.3	1585 ± 172	1508 ± 131	581 ± 103	504 ± 96.7
$CK\text{-}MB_{(CLD)}$ IU/L	759 ± 71.5	3601 ± 511	3431 ± 560	2572 ± 503	2363 ± 559	1914 ± 335	2065 ± 315	1264 ± 460	420 ± 137
$ALP_{(LPS)}$ IU/L	52.3 ± 11.8	217 ± 22.1	192 ± 20.6	306 ± 89.2	204 ± 20.6	278 ± 37.3	239 ± 48.6	338 ± 46.6*	270 ± 26.2
$ALP_{(CLD)}$ IU/L	52.3 ± 11.8	228 ± 29.9	227 ± 51.8	256 ± 39.1	166 ± 15.4	200 ± 12.6	201 ± 7.11	212 ± 12.7	257 ± 18.1
$ALT_{(LPS)}$ IU/L	68.6 ± 9.67	65.6 ± 13.7	74.1 ± 11.4	180 ± 39.1*	455 ± 54.7*	501 ± 44.6*	214 ± 51.6	318 ± 100	114 ± 33.6
$ALT_{(CLD)}$ IU/L	68.6 ± 9.67	46.3 ± 5.85	56.3 ± 7.29	74.1 ± 5.44	90.5 ± 9.31	87.1 ± 13.4	182 ± 40.2	229 ± 38.1	175 ± 33.3
$AST_{(LPS)}$ IU/L	153 ± 15.7	291 ± 64.6	292 ± 51.8*	384 ± 35.8*	485 ± 34.6*	447 ± 25.1*	247 ± 58.8	246 ± 45.7	227 ± 33.5
$AST_{(CLD)}$ IU/L	153 ± 15.7	145 ± 14.5	167 ± 20.8	225 ± 13.9	242 ± 22.9	285 ± 22.3	345 ± 28.5	296 ± 52.5	282 ± 19.4
$GGT_{(LPS)}$ IU/L	0.71 ± 0.28	2.00 ± 0.44	2.50 ± 0.83	4.50 ± 1.02	5.50 ± 0.22*	8.50 ± 0.56*	7.00 ± 1.61	4.50 ± 1.17	3.83 ± 0.41
$GGT_{(CLD)}$ IU/L	0.71 ± 0.28	2.16 ± 0.16	3.00 ± 0.25	2.83 ± 0.40	2.16 ± 0.47	2.50 ± 0.42	3.33 ± 0.42	3.50 ± 0.43	2.50 ± 0.67
$CR_{(LPS)}$ mg/Dl	0.66 ± 0.02	0.66 ± 0.02*	0.63 ± 0.02	0.68 ± 0.06	0.74 ± 0.02	0.80 ± 0.03*	0.73 ± 0.02	0.63 ± 0.02	0.68 ± 0.03
$CR_{(CLD)}$ mg/dL	0.66 ± 0.02	0.53 ± 0.03	0.61 ± 0.03	0.55 ± 0.05	0.56 ± 0.04	0.63 ± 0.02	0.68 ± 0.03	0.63 ± 0.04	0.63 ± 0.04
$BUN_{(LPS)}$ mg/dL	33.8 ± 2.39	40.1 ± 1.72*	46.1 ± 1.72*	59.8 ± 4.78*	99.3 ± 4.31*	90.0 ± 4.61*	145 ± 15.6	61.8 ± 10.7	58.5 ± 4.70
$BUN_{(CLD)}$ mg/dL	33.8 ± 2.39	33.5 ± 1.53	35.2 ± 1.31	33.1 ± 1.91	38.8 ± 2.45	43.1 ± 1.58	63.1 ± 3.94	44.4 ± 3.67	50.6 ± 2.22

CLD; lipopolysaccharide (4 mg, intraperitoneally) + enrofloxacin (10 mg/kg, subcutaneously) + flunixin meglumine (2.5 mg/kg, subcutaneously) + low-dose dexamethasone (0.6 mg/kg, intramuscularly), LPS; lipopolysaccharide, MDA; malondialdehyde, NO; nitric oxide, SOD; superoxide dismutase, VC; vitamin C, PGM; 13, 14-dihydro-15-keto-prostaglandin $F_{2\alpha}$, LT; lactate, CK-MB; creatine kinase-MB, ALP; alkaline phosphatase, ALT; alanine aminotransferase, AST; aspartate aminotransferase, GGT; gamma glutamyl aminotransferase, CR; creatinine, BUN; blood urea nitrogen.
*Statistically significant for each values between CLH and LPS sampling times ($p < 0.05$).

Tables I and II). No treatment had a consistent effect on SOD levels (Tables I and II).

Organ damage

Serum lactate, CK–MB, ALP, ALT, AST, GGT, BUN and creatinine levels are given in Tables I and II, respectively. Cardiac, hepatic and renal damage marker increased after LPS treatment. Cardiac and hepatic damage was not completely inhibited by any treatment, whereas renal damage was inhibited by two treatments. \

Discussion

Endotoxic shock has an unacceptably high mortality rate, although many new therapeutic agents are available to treat it. ROS-induced oxidative damage plays a key role in septic shock and ROS may contribute to mortality in Gram-negative bacterial sepsis [17]. Increased ROS production and higher MDA levels occur in septic patients [18,19]. LPS-induced lipid peroxidation was measured as increases in MDA levels in this study (Tables I and II). Although the two combined treatments inhibited ($p < 0.05$) this lipid peroxidation, they had variable effects on SOD levels (Tables I and II). The results of antioxidant studies in which only antioxidant enzymes are evaluated are conflicting. However, when MDA is evaluated as a biomarker of oxidative stress, consistent results have been reported [12]. GCs and FM suppress lipid peroxidation by reducing MDA levels in endotoxemia [12,20]. It has been reported elsewhere that antioxidants have therapeutic potential in preventing multiple-organ failure and in treating septic shock [21].

LPS increased serum NO levels and the combined treatments usually inhibited this increase ($p < 0.05$; Tables I and II). Mitochondria play a central role in the intracellular events associated with septic shock because they are a target of NO [22]. LPS induces iNOS-derived NO and the excessive formation of NO is a major factor in the pathological vasodilatation, tissue damage and organ failure of septic shock [23,24] and the amount of NO produced correlates with the severity of septic shock and mortality [25]. The expression of nuclear factor kappaB (NF-κB), a ubiquitous transcription factor, is increased by LPS and the resulting over-expression of inflammatory mediators (cytokines, iNOS, COX2) may account for the deleterious effects seen in sepsis [4]. DEX and FM inhibit iNOS expression by blocking NF-κB [26,27] and the inhibition of iNOS (the 'NO effect') might be useful in overcoming the life-threatening hypotension observed in septic shock [23].

LPS reduced plasma VC levels and two combined treatments generally inhibited this reduction ($p < 0.05$; Tables I and II). VC may be used in the body during septic shock, because ascorbate inhibits the excessive production of NO [28] and endothelial NADPH oxidases, which produce ROS, which can cause endothelial dysfunction in sepsis [29]. It has been proposed that the administration of VC might be a useful adjunct to conventional approaches to the management of septic shock [30].

In this study, LPS increased PGM, an indicator of *in vivo* lipid peroxidation through the COX pathways and it reached its peak concentration after 2 h Plasma PGM levels are related to the degree of infections [31,32]. Increase of PGM inhibited by two combined treatments (Tables I and II) might be attributable to the potent inhibitor effect of FM on the synthesis of prostaglandins.

LPS increased cardiac, hepatic and renal damage markers and damages were not completely inhibited at all sampling times by any treatment (Tables I and II). Myocardial contractility depression is a common finding in septic patients. The cause of myocardial dysfunction in sepsis is not entirely clear, but the production of cytokines and ROS, fluid–electrolyte imbalance and the release of intracellular mediators are likely to play a role. Hence, damaged myocytes release CK–MB [33]. Hepatic and renal damages have been reported in LPS-induced endotoxemia [34–38]. It seems that high-dose DEX with ENR + FM was more effective in the inhibition of organ damages than low-dose DEX with ENR + FM.

This study results indicate that high-dose DEX with ENR + FM has the most antioxidant and preservative effect to organ damage in the endotoxemia.

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