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EFFECT OF FLUNIXIN MEGLUMIN ON THE ANTIOXIDANT STATUS IN ENDOTOXEMIA

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In this study, the effects of flunixin meglumin on the antioxidant status during endotoxemia were investigated. Thirty Balb/C mice were divided into three equal groups. Mice with no treatment formed the control group (group I). Mice treated with lipopolysaccharide (LPS) and LPS + flunixin meglumin (FM) formed groups II and III, respectively. All mice were sacrificed and tissue samples were collected 6 hours after treatments. Activities of superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), as well as levels of malondialdehyde (MDA) and glutathione (GSH) were determined spectrophotometrically. LPS caused a significant ($p<0.05$) increase in levels of MDA (heart, kidney, spleen) which is an accepted biomarker of oxidative stress, and changed the levels of SOD, GPX, CAT and GSH. When FM was administered simultaneously with LPS, FM inhibited ($p<0.05$) the increase of MDA levels in all tissues. As results, LPS caused MDA levels and oxidative stress to increase. However, FM depressed the LPS induced increase of MDA levels. This antioxidant effect of FM may be beneficial in endotoxemia.

Keywords: flunixin meglumin, antioxidant status, endotoxemia.

INTRODUCTION

LPS, present on the outer membrane of gram negative bacteria, is released during bacterial lysis and causes endotoxic shock. Endotoxic shock is the cause of high mortality in intensive care patients (Cadenas and Cadenas, 2002). There is convincing evidence of severe oxidative stress in sepsis. Oxidative stress occurs when antioxidant balance is disrupted by excessive production of reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical, hydrogen peroxide, singlet oxygen (Cadenas and Cadenas, 2002; Mcdonald *et al.,* 2003). ROS constitute a major part of biologically important free radicals, and current knowledge indicates that free radical damage plays a key role in LPS-induced endotoxic shock (Ben-Shaul *et al.,* 2001; Cadenas and Cadenas, 2002; Salvemini and Cuzzocrea, 2002). SOD catalyses the reaction between two molecules of superoxide into hydrogen peroxide and molecular oxygen. GPX catalyses the reduction of hydrogen peroxide or lipid peroxides with reduced glutathione. CAT

catalyses the breakdown of hydrogen peroxide to molecular water and oxygen. GSH has a very important role in the protection against free radical damage by providing reducing equivalents for several key enzymes. On the other hand, GSH is a scavenger of hydroxyl radicals and singlet oxygen (Diplock, 1994). When antioxidant capacity is not sufficient against ROS, lipid peroxidation occurs in the cell. MDA is formed during lipid peroxidation, and it is accepted as a marker of lipid peroxidation (Mcdonald *et al.,* 2003).

FM belongs to potent nonsteroid anti-inflammatory drugs. These drugs act by inhibiting the synthesis of cyclooxigenase derived eicosanoid inflammatory mediators including prostaglandins. FM has anti-inflammatory, analgesic and antipyretic properties. Thus, FM is commonly used in septic shock cases in veterinary medicine (Elmas *et al.,* 2005a; Elmas *et al.,* 2005b).

In the present study the antioxidant effect of FM in endotoxemia was investigated. For this reason, SOD, GPX, CAT and GSH were measured as antioxidants, and MDA was measured as a biomarker of lipid peroxidation. The liver was selected because it is one of the tissues showing a high rate of free radical generation, and the kidney is the body's most important organ of excretion and homeostasis and it is a frequent target for a variety of toxic agents due to it's metabolic capacity for concentrating toxicants and/or metabolites during the excretory process. The heart is the main organ of the circulatory system and is depressed by beta-endorphins during shock. The spleen is the largest organ in the lymphatic system, and it stores the blood for when the body immediately needs blood as it releases it in the circulatory system.

MATERIAL AND METHODS

Animals

Thirty clinically healthy Balb/C mice (15 female + 15 male, 2-3 months old, 26-31 g, Kombassan Experimental Medicine Research and Application Center, Konya) were divided into three equal groups, and each group contained 10 mice (5 female + 5 male). Animals were fed standard pellet diet and tap water *ad libitum.*

Study design

Mice with no treatment formed the control group (group I). Mice with LPS (*Escherichia coli* 0111: B4, 250 μg/mouse, intraperitoneally) and LPS (250 μg/mouse, intraperitoneally) + FM (2.5 mg/kg, b.wt., subcutaneously) formed groups II and III, respectively. All mice were sacrificed by cervical dislocation and tissue samples (liver, heart, kidney and spleen) were collected 6 hours after treatments. Samples were stored at –80°C until analyses. Ethical Committee of the Faculty of Veterinary Medicine (report no.2005/014) approved the study protocol.

Table 1. Effect of flunixin meglumin (FM) on malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and glutathione (GSH) levels in healthy and lipopolysaccharide (LPS) induced endotoxemic mice (mean ± SE, n=40)

Biomarkers		Group I (Control)	Group II (LPS)	Group III (LPS+FM)
MDA nmol/mg protein	Heart	0.082±0.007 b	0.337±0.034 a	0.031±0.002 b
	Liver	0.044±0.005 a	0.068±0.005 a	0.048±0.003 a
	Kidney	0.062±0.006 b	0.152±0.011 a	0.069±0.007 b
	Spleen	0.104±0.006 b	0.225±0.011 a	0.078±0.006 b
SOD U/mg protein	Heart	40.02±4.376 b	24.43±1.821 b	118.5±15.34 a
	Liver	39.01±1.264 a	30.64±1.748 b	43.57±2.767 a
	Kidney	76.32±3.847 a	56.11±6.698 b	48.94±9.398 b
	Spleen	28.14±2.238 b	31.42±2.102 b	106.8±16.43 a
GPX U/mg protein	Heart	29.19±1.541 c	63.21±5.547 ab	71.05±5.786 a
	Liver	35.11±1.535 c	66.74±4.038 b	85.86±3.889 a
	Kidney	33.33±2.587 b	69.00±2.933 a	66.33±3.414 a
	Spleen	49.27±4.565 b	73.33±6.244 ab	79.72±7.074 a
CAT U/mg protein	Heart	0.042±0.003 b	0.143±0.011 a	0.120±0.015 a
	Liver	0.240±0.009 c	0.989±0.087 b	2.782±0.347 a
	Kidney	0.160±0.017 b	0.252±0.0238 ab	0.383±0.045 a
	Spleen	0.031±0.002 b	0.042±0.003 b	0.123±0.014 a
GSH μg/mg protein	Heart	43.36±4.997 a	20.37±1.699 b	52.10±5.464 a
	Liver	22.37±1.829 a	10.88±0.712 b	11.77±1.018 b
	Kidney	65.09±2.371 a	20.02±1.739 b	21.19±2.434 b
	Spleen	33.82±4.081 a	22.48±1.126 a	21.07±1.152 a

a, b, c; different letters in the same row are statistically significant ($p<0.05$)

Tissue samples analysis

MDA (Satoh, 1978; Yagi, 1984), CAT (Aebi, 1984) and GSH (Sedlak and Lindsay, 1968) levels were measured by the above reported methods. The results were expressed as nmol/mg, Aebi Unit (AU)/mg and μg/mg tissue protein, respectively. SOD (RANDOX-Ransod) and GPX (RANDOX-Ransel) activities were determined using enzyme essey kits. SOD and GPX activities were expressed as U/mg tissue protein. Tissue protein levels were measured according to Lowry.

Statistical analysis

All values are expressed as mean ± standard error (SE). The results were analyzed by Tukey multiple range test (SPSS for windows). In all cases the probability of error of less than 0.05 was selected as the criterion for statistical significance.

RESULTS

SOD, GPX, CAT activities and MDA and GSH levels of mice in the experimental groups are given in Table 1.

LPS caused a statistically significant ($p<0.05$) increases in MDA (heart, kidney and spleen), GPX (heart, liver and kidney) and CAT (heart and liver) levels and statistically significant ($p<0.05$) decreases in SOD (liver and kidney) and GSH (heart, liver and kidney) levels. FM inhibited the increase of MDA (heart, kidney and spleen) when administered with LPS. SOD (heart and spleen), GPX and CAT (heart, liver, kidney and spleen) activities were significantly ($p<0.05$) elevated in the LPS+FM group, in comparison to the control group.

DISCUSSION

Endotoxic shock causes high mortality, and free radical damage plays a key role in endotoxemia. The body is protected against oxidative damage by enzymatic and non-enzymatic mechanisms such as SOD, GPX, CAT and GSH. In the present study, LPS caused a significant ($p<0.05$) increase of GPX (heart, liver and kidney) and CAT (heart and liver) levels and a decrease ($p<0.05$) of SOD (liver and kidney) and GSH (heart, liver and kidney) levels. In many studies, similar and conflicting results have been reported. It was stated by a number of authors that SOD, GPX, CAT and GSH levels in the plasma, heart, liver and kidney increased after LPS administration (Portoles *et al.,* 1996; Yazar *et al.,* 2004; Keskin *et al.,* 2005). On the contrary it was stated that SOD, GPX, CAT and GSH levels of liver decreased after LPS and alive E. coli K99 treatments (Portoles *et al.,* 1993; Portoles *et al.,* 1996; Watson *et al.,* 1999; Yazar and Tras, 2001, Keskin *et al.,* 2005). When the antioxidant capacity is insufficient against ROS, lipid peroxidation occurs and MDA is formed. In the present study, LPS induced multiple organ oxidative damages (heart, kidney, spleen) characterized by increased MDA concentration. These results were in agreement with previous studies, which reported increased MDA concentrations in the serum, liver, kidney,

heart and diaphragmatic muscle (Portoles *et al.,* 1993; Zheng *et al.,* 2000; Ozdulger *et al.,* 2002; Keskin *et al.,* 2005). In the studies associated with antioxidants such as SOD, GPX, CAT and GSH, when only antioxidants are evaluated, conflicting results were reported. However, when MDA is evaluated as a biomarker of oxidative stress, similar results are reported. In generall, MDA level increases in the acute phase (within first 5-10 h) of endotoxemia. In the present study, when LPS and FM were simultaneously administered, increased MDA levels were depressed ($p<0.05$) by FM. To our knowledge, there isnt any published literature data about the effect of FM on the antioxidant status of sick or healthy animals.

It can be concluded that LPS caused oxidative stress and increased MDA level in animals. However, FM depressed the LPS induced MDA levels. This antioxidant effect of FM may be beneficial in endotoxemia.

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EFEKTI FLUNIXIN MEGLUMINA NA ANTIOKSIDATIVNI STATUS MIŠEVA SA ENDOTOKSEMIJOM

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SADRŽAJ

U ovom radu ispitivani su efekti flunixin meglumina na antioksidativni status miševa sa endotoksemijom. U ogled je bilo uključeno 30 Balb/C miševa: kontrolnu grupu sačinjavale su životinje bez tretmana; u grupi II bili su miševi tretirani sa lipopolisaharidom, a u grupi III jedinke tretirane kombinacijom lipopolisaharida i flunixin meglumina. Sve životinje su žrtvovane radi uzimanja uzoraka tkiva, 6 sati nakon tretmana. Aktivnost superoxid dismutaze, glutation peroksidaze i katalaze, kao i koncentracije malonilaldehida i glutationa su određivani spektrofotometrijski. Aplikacija lipopolisaharida imala je za posledicu povećanje koncentracije malonil aldehida u srcu, bubrezima i slezini kao i promene u aktivnosti ispitivanih enzima. Simultana aplikacija flunixin meglumina sa lipopolisaharidom nije rezultirala povećanjem koncentracije malonildialdehida. Autori smatraju da flunixin meglumin ispoljava korisne antioksidativne efekte kod miševa sa endotoksemijom.