P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article

Comparative Efficacy of the Steroids Administered by Inhalation and Parenteral Route in Lambs with Experimentally Induced Endotoxemia

Akin Kirbas,[1]* Emre Karakus [2], Seckin Ozkanlar [3], Semin Gedikli[4], Basak Hanedan[1], Atilla Topcu[5] and Zafer Bayraktutan[6]

[1]Department of Internal Medicine, [2]Department of Pharmacology and Toxicology; [3]Department of Biochemistry; [4]Department of Histology and Embryology, Faculty of Veterinary Medicine, Ataturk University, 25240, Erzurum, Turkey; [5]Department of Pharmacology and Toxicology, Faculty of Medicine, Ataturk University, 25240, Erzurum, Turkey; [6]Department of Biochemistry, Regional Research and Education Hospital, Erzurum, Turkey
***Corresponding author:** akindahiliye55@yahoo.com; akirbas@atauni.edu.tr

Article History: Received: July 08, 2015 Revised: July 24, 2015 Accepted: August 02, 2015

ABSTRACT

The aim of the present investigation was to compare the efficacy of the steroids administered by inhalation and parenteral route in lambs with experimentally induced endotoxemia. In this study, 19 lambs were used at 2 months of age. Seven groups were designed as lipopolysaccharide (LPS) (n=3), LPS+budesonide (BUD) (n=3), LPS+BUD+enrofloxacin (ENR)+trimethoprim-sulfadoxine (TM-SD) (n=3), LPS+dexamethasone (DEX) (n=2), LPS+DEX+ENR+TM-SD (n=2), LPS+flunixine meglumine (FM)+ENR+TM-SD (n=3), and control (n=3). While increase in serum tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) levels in LPS group was found, significant decrease in serum TNF-α in all treatment groups was determined ($P<0.05$). Serum IL-6 level in LPS+BUD and LPS+BUD+ENR+TM-SD groups reached the level of control group compared to that of other groups ($P<0.05$). While lung tissue malondialdehyde level (MDA) in the LPS group increased, MDA level of all treatment groups reached the level of control group ($P<0.05$). Superoxide dismutase activity (SOD) and total glutathione (GSH) level in all treatment groups was significantly increased compared to those of LPS group ($P<0.05$). Histopathological examination showed intra-alveolar haemorrhage, congestion areas, and adhesive leukocyte density of pulmonary veins in the lung sections of LPS group. In LPS+BUD and LPS+BUD+ENR+TM-SD groups, increase in local intra-alveolar haemorrhage, dense congestion areas, and adhesive leukocytes and dilatation of pulmonary capillaries were detected. It was observed that in LPS+DEX, LPS+DEX+ENR+TM-SD and LPS+FM+ENR+TM-SD groups, congestion became dense in specific areas, and intra-alveolar haemorrhage and adhesive leukocyte density was lower. It was concluded that steroids administered by inhalation and parenteral route had similar effects on serum pro-inflammatory cytokine levels and tissue oxidative stress markers in lambs with experimentally induced endotoxemia and that both steroid groups can be beneficial in the treatment of endotoxemia.

Key words: Cytokine, Dexamethasone, Endotoxemia, Inhaler steroid, Lamb, Oxidative stress

INTRODUCTION

Endotoxemia is the presence of lipopolysaccharide (LPS) cell wall components of Gram-negative bacteria in the blood (Smith 2005; Radostits *et al.,* 2007). The endotoxins of several species of Gram-negative bacteria are a major cause of morbidity and mortality in neonatal farm animals, including lambs (Radostits *et al.,* 2007). In neonates, endotoxemia most commonly results from Gram-negative sepsis and can be acquired in utero, during parturition or in the early neonatal period (Moore and Morris, 1992). The endotoxemia leads to the activation of inflammatory cascade, including the release of pro-inflammatory cytokines, the release of acute phase proteins (APPs) and the production of eicosanoids (Barton *et al.,* 2003). Also, the presence of LPS in the blood stream causes fever, hypotension, disseminated intravascular coagulation, multiple organ failure and, in severe cases, septic shock and death (Adams *et al.,* 1990).

There are several therapeutic regimens for the treatment of endotoxemia. The basic approaches to endotoxemia treatment are the prevention of the movement of endotoxin into the bloodstream, neutralization of endotoxins, the prevention of pro-

Cite This Article as: Kirbas A, E Karakus, S Ozkanlar, S Gedikli, B Hanedan, A Topcu and Z Bayraktutan, 2015. Comparative efficacy of the steroids administered by inhalation and parenteral route in lambs with experimentally induced endotoxemia. Inter J Vet Sci, 4(4): 199-205. www.ijvets.com

inflammatory and inflammatory pathways and general supportive care with intravenous fluids, colloids, anti-inflammatory and inotropic agents (Radostits *et al.,* 2007). The treatment of endotoxemia is generally performed in large animals by using non-steroidal anti-inflammatory drugs (NSAIDs) because of their analgesic, anti-inflammatory and antipyretic properties. Flunixin meglumine (FM) is an NSAID and FM potently inhibits cyclooxygenase and the synthesis of eicosanoids, and also modulates the acute hemodynamic changes during endotoxemia (Elmas *et al.,* 2006). Corticosteroids (CSs) are commonly used in the treatment of endotoxemia. However, there is still some controversy over the dosage, timing and duration of the administration of CSs (Keh *et al.,* 2005; Wu, 2006). CSs potently suppress inflammation, and they are the most frequently prescribed classes of drugs in a variety of inflammatory diseases (Barnes, 1998). CSs improve capillary endothelial integrity and tissue perfusion, decrease the activation of complement and the clotting cascade, decrease neutrophil aggregation, stabilize lysosomal membranes, protect hepatic injury and improve survival rate (Radostits *et al.,* 2007). One of the most commonly used glucocorticoids in endotoxic shock is dexamethasone (DEX) (Radostits *et al.,* 2007; Chalmeh *et al.,* 2013). Compared to systemic administration, the inhalation route offers faster onset of action and high in situ drug concentrations. This results in a lower required drug dose and subsequent lower rates of side effects (Pauwels *et al.,* 1997). Budesonide (BUD) is a highly potent synthetic, inhaled, nebulized and non-halogenated corticosteroid. It has high glucocorticoid and weak mineralocorticoid activity (Szefler, 1999). BUD has a high ratio of topical to systemic activity because of high first pass metabolism in the liver and is therefore associated with fewer adverse effects than systemic CSs (Derendorf, 1997; Dye *et al.,* 2012; Pietra *et al.,* 2013). CSs such as BUD have been shown to inhibit interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) production by macrophages (Ek *et al.,* 1998). This drug is one of the widely used corticosteroid in human medicine in a variety of inflammatory and allergic diseases treatment (Szefler, 1999). In addition, nowadays, they have been started to be used in small animal medicine practice of inflammatory diseases such as inflammatory bowel disease (Dye *et al.,* 2012; Pietra *et al.,* 2013). However, there is no experimental evidence demonstrating their efficacy on inhaler steroids in small ruminant medicine including lambs.

Therefore, the aim of this study was to compare the efficacy of inhaler (low dose) and parenteral steroids in lambs with experimentally induced endotoxemia. For this purpose, the measurement of serum cytokine levels, tissue oxidative stress parameters, and histopathological analyses were performed after administration of inhaler and parenteral steroids in endotoxemic lambs.

MATERIALS AND METHODS

Animals and experimental procedures

The study material consisted of 19 Morkaraman lambs, obtained from the region of Erzurum, 8-10 kg body weight, female, 2 months old, and maintained in similar conditions in the farm of University of Ataturk, Faculty of Veterinary Medicine. The present study was approved by Ataturk University's local animal care committee 2014/17.

This study was carried on seven groups comprising LPS, LPS+BUD, LPS+BUD+enrofloxacin (ENR) +trimethoprim-sulfadoxine (TM-SD), LPS+DEX, LPS+DEX+ENR+TM-SD, LPS+FM+ENR+TM-SD and control groups.

Phenol-extracted lipopolysaccharide (LPS) from *E. coli* serotype O55:B5 (Sigma-Aldrich®; Product No. L2880, Germany) at the dose of 10 µg/kg was used to induce endotoxemia in lambs. This endotoxin was diluted in sterile phosphate-buffered saline (PBS) and divided into sixteen equal doses, containing 200 µg of endotoxin, and stored at -80°C until the induction of endotoxemia. After 1 h LPS administration, BUD (Inflacort 400 mcg inhaler®, Bilim Drug Industry, Istanbul, Turkey, via 400 mcg inhalation) was administered to LPS+BUD and LPS+BUD+ENR+TM-SD groups by inhalation via nebuliser (Medic-aid, USA) for 10 min, and additional treatment with ENR (Vetril 10% enj.®, Vetas, Istanbul-Turkey, 2.5 mg/kg i.m.) plus TM-SD (Animar enj.®, Ceva, Istanbul-Turkey, 30 mg/kg i.m.) were administered to LPS+BUD+ENR+TM-SD group. After 1 h LPS administration, DEX (Deksavet 0.4% enj.®, Interhas, Istanbul-Turkey 0.4 mg/kg i.m.) was administered to LPS+DEX group and LPS+DEX+ENR+TM-SD group. ENR+TM-SD combinations were administered to LPS+DEX+ENR+TM-SD group, and after 1 h LPS administration, FM (Fluvil enj.®, Vilsan, Ankara-Turkey, 2.2 mg/kg i.m.) plus ENR+TM-SD combinations were administered to LPS+FM+ENR+TM-SD group.

Blood and tissue samples

Blood samples were taken in vacuum tubes (BD, Vacutainer, UK) from vena jugularis of lambs at 12th h and 24th h after LPS injection. Immediately after the blood collections, sera were separated by centrifugation for 10 min at 3000×*g* and stored at −80 °C until analysis. The lung samples were taken for the measurements of oxidative stress markers and histopathological examination and stored at −80 °C until they were assayed.

Biochemical analyses

IL-6 and TNF-α measurement in serum: The levels of IL-6 and TNF-α (Cusabio Biotech Ltd., China) were measured by high sensitive ELISA kits, specific for sheep cytokines, according to the manufacturer's instruction.

Biochemical analysis of lung tissues: Lipid peroxidation (LPO) in the tissue was determined by estimating the level of malondialdehyde (MDA) using the thiobarbituric acid test (Ohkawa *et al.,* 1979). The amount of total glutathione (GSH) in the tissues was measured according to the method of Sedlak and Lindsay (1968). Superoxide dismutase (SOD) activity measurements were made according to the method of Sun *et al.,*(1988).

Histopathological analyses

Histopathology: The lambs were sacrificed at the end of the experiment. Their lungs were removed, fixed in a 10% formalin solution for 48 h, and embedded in paraffin. The tissues were cut into 5 µm thick sections, and stained with

hematoxylin-eosin (H&E) and Masson Triple stain. The score of lung inflammation was measured as semi-quantitative. Parameters including histopathological changes, such as interstitial inflammation, intra-alveolar haemorrhage, vascular congestion, oedema, adhesive leukocyte were used for scoring.

Immunohistochemistry: NF-kB protein (p65) activity of the lung tissue was determined by immunohistochemical staining. 5 µm thick section of the lung tissue from each animal was stained for NF-kB in VENTANA Bench Mark GX System (Ventana Medical Systems, Inc., Roche, Germany) IHC automated staining instrument using ultraView Universal DAB Detection Kit. After putting the sections on staining instrument, they were deparaffinised. They were treated with citrate buffer. The primary antibody used for NF-kB, and IgG1 class mouse monoclonal directed against the p65 (F-6) relA component of the NF-kB complex (Santa Cruz Biotechnology, cat: sc8008, USA) was used at a dilution of 1:80 for 32 min at 37°C. Then, the sections were stained by ultraView Universal DAB detection kit (Ventana Medical Systems, Inc., Roche, Germany). After staining with DAB, the sections were stained with hematoxylin for contrast staining. Semi-quantitative scoring for NF-kB was made according to the parameters. These were evaluated according to the degree of nuclear and cytoplasmic staining in interalveolar septum and bronchial stromal tissue. Each parameter in both histopathological and immunohistochemical analyses were graded as follows: none (0), minimal (1), mild (2), moderate (3) and severe (4).

Semi-quantitative field analysis: Lung surface area-to-alveolar surface area ratio was determined by stereology software (Stereo Investigator® version 8.0, Micro Bright Field, Colchester, Vermont, USA) as a semi-quantitative analysis with modified Cavalieri's principle volume/area ratio. The lung tissue section was taken from each animal in the groups. 5 randomly selected image areas from each section were evaluated at ×20 magnification. Intra-alveolar area (intra-alveolar area only) and lung tissue area (inter-alveolar septum, vessels, bronchus, and bronchiole) were estimated from each image area (about X20=1000 µm^2).

Statistical analysis

SPSS Statistics 18 computer program package was used for statistical calculations. Data for the serum cytokine levels measured by the ELISA and the oxidant and antioxidant enzymes were subjected to Mann-Whitney *U* test and were considered significant at P<0.05. All data were expressed as means ± standard deviation (SD) in each group.

RESULTS

Biochemical results

The effects of inhaler and parenteral steroids on serum TNF-α level in lambs with experimentally induced endotoxemia are presented in Table 1. While serum TNF-α level significantly increased in the LPS group compared to the control group, serum TNF-α level decreased in all treatment groups (P<0.05). The effects of inhaler and parenteral steroids on serum IL-6 level in lambs with experimentally induced endotoxemia are presented in Table 2. While at 12 h and 24 h after LPS administration, IL-6 level in LPS group was increased (P<0.05), IL-6 level in LPS+BUD and LPS+BUD+ENR+TM-SD groups was decreased to that of the control group (P<0.05). LPS+DEX and LPS+DEX+ENR+TM-SD decreased the IL-6 level to a lesser extent compared to LPS+BUD and LPS+BUD+ENR+TM-SD (P<0.05).

Table 1: Effects of inhaler and parenteral steroids on serum TNF-α levels in lambs with experimentally induced endotoxemia

Groups	N	TNF-α 12th h (pg/ml)	TNF-α 24th h (pg/ml)
CONTROL	3	56.16±2.26	52.90±6.46
LPS	3	125.57±19.64*	145.57±42.21*
LPS+BUD	3	54.03±1.57	72.50±16.13
LPS+BUD+ENR+TM-SD	3	53.83±0.42	68.50±2.27*
LPS+DEX	2	51.84±1.97	69.00±0.88
LPS+DEX+ENR+TM-SD	2	51.84±1.17	74.75±10.52
LPS+FM+ENR+TM-SD	3	55.04±2.93	68.45±1.89*

*: P<0.05

Table 2: Effects of inhaler and parenteral steroids on serum IL-6 levels in lambs with experimentally induced endotoxemia

Groups	N	IL-6 12th h (pg/ml)	IL-6 24th h (pg/ml)
CONTROL	3	46.56±18.12	44.19±12.96
LPS	3	72.00±6.56	77.99±13.77*
LPS+BUD	3	49.80±17.83	43.81±9.24
LPS+BUD+ENR+TM-SD	3	31.49±1.89*	41.29±22.58
LPS+DEX	2	62.10±2.33	83.30±43.57
LPS+DEX+ENR+TM-SD	2	67.13±46.38	56.65±28.25
LPS+FM+ENR+TM-SD	3	60.54±12.32	58.84±25.31

*: P<0.05

Table 3: Effects of inhaler and parenteral steroids on superoxide dismutase (SOD) activity, lipid peroxidation (MDA), and total glutathione (GSH) levels in the lung tissue of lambs with experimentally induced endotoxemia

Groups	N	SOD (mmol/min/ mg tissue)	GSH (nmol/mg tissue)	LPO (nmol/mg tissue)
CONTROL	3	107.5±10.12	4.33±1.68	2.54±0.56
LPS	3	54.38±7.98*	1.81±0.25*	5.96±0.92*
LPS+BUD	3	135.25±6.11*	4.04±1.33	2.72±0.35
LPS+BUD+ENR+TM-SD	3	132.38±9.80*	3.71±0.77	2.50±0.51
LPS+DEX	2	120.25±3.85	2.99±0.74	3.31±0.36
LPS+DEX+ENR+TM-SD	2	121.25±3.20	3.90±1.91	3.36±0.40
LPS+FM+ENR+TM-SD	3	120.75±3.62*	2.66±0.27*	2.93±0.36

*: P<0.05

Table 4: Results of alveoli-to-lung area by semi-quantitative field analysis in the lungs of lambs with experimentally induced endotoxemia

Groups	N	Alveoli Area (µm^2)	Lungs Area (µm^2)
CONTROL	3	110266±11561*	90133±8982*
LPS	3	83333±2778*	121067±7230*
LPS+BUD	3	97333±4268	108400±1146*
LPS+BUD+ENR+TM-SD	3	134533±7776	78400±2794*
LPS+DEX	2	108533±18209*	99867±17735
LPS+DEX+ENR+TM-SD	2	95867±13464	110400±14076
LPS+FM+ENR+TM-SD	3	111200±10541	93467±14339

*: P<0.05

In the present study, the activity of SOD and the levels of GSH and MDA, which are oxidative markers, were evaluated in lambs with LPS-induced endotoxemia (Table 3). While MDA level of the lung tissues in the LPS group was increased, the lung tissue MDA level in all treatment groups reached that of the control group ($P<0.05$). The activity of SOD and the level of GSH in all treatment groups were significantly increased compared to those of LPS group ($P<0.05$).

Histopathological and immunohistochemical results

In the present study, the LPS group had lowest alveolar area and highest lung tissue area in comparison with the results of semi-quantitative alveoli-lung area in lambs with experimentally induced endotoxemia ($P<0.05$) (Table 4). The lung tissues in the control group had normal histological appearance. Bronchi, bronchial vessels (artery and vein) and inter-alveolar septum (alveolar epithelial cells and pulmonary capillaries) had normal appearance (Fig.1A). Histopathological changes, such as haemorrhage, congestion, inflammatory leukocyte infiltration and vessel injury, were highly dense in the LPS group (Fig.1B). Increase in local intra-alveolar haemorrhage and dense congestion areas were remarkable in the LPS+BUD and LPS+BUD+ENR+TM-SD groups. In either group, adhesive leukocytes and dilated pulmonary capillaries were found (Fig.1C and D). Inter-alveolar septum was thickened in the LPS+BUD group (Fig.1C). In the lung sections belonging to the LPS+DEX, LPS+DEX+ENR+TM-SD and LPS+FM+ENR+TM-SD groups, it was appeared that congestion areas are locally increased, conversely, pathological hallmarks such as intra-alveolar haemorrhage and adhesive leukocyte density were low (Fig.1E, F and G). There was statistically significant difference in the control and LPS groups in comparison with other groups according to histopathological evaluation and lung inflammation scoring in the lungs of lambs with experimentally induced endotoxemia (Fig.1A-G). While the control group had the lowest score, the LPS group with densely lung injury due to acute inflammation had the highest score (Fig.1H).

NF-kB positive cells were in inter-alveolar septum and bronchial stroma. Immunopositivity was minimal in the control group. NF-kB positivity in the lung sections of the LPS group was very dense and diffusive. Both nuclear and cytoplasmic immunoactivity were determined in variable severities in inter-alveolar septum and bronchial stroma cells (Fig.2A-G). In the immunohistochemical examination (NF-kB) of the lung sections, the control group had the lowest score and the LPS group had the highest score for NF-kB activity (Fig.2H).

DISCUSSION

This study, for the first time, investigated the comparative effects of inhaler and parenteral steroids based on serum cytokine levels, histopathological changes, and oxidative stress parameters in the lungs of lambs with experimentally induced endotoxemia. We determined that inhaler and parenteral steroids exert protective effects against LPS-induced endotoxemia, attenuate the lung tissue injury and decrease cytokine response, as confirmed by biochemical assays and histopathological evaluation. This protection is primarily due to the inhibition of oxidative stress, which is one of the important mechanisms of organ injury of LPS-induced endotoxemia and inhibition of the severity of inflammation, as clearly revealed by our findings that treatment with inhaler and parenteral steroids decreased TNF-α and IL-6 levels.

DEX and FM are frequently chosen drugs in the treatment of endotoxemic shock in veterinary medicine (Smith, 2005; Lohman and Baron, 2010). However, there is no data regarding the use of BUD in the treatment of endotoxemia in small ruminant medicine.

BUD is a non-halogenated corticosteroid that exhibits potent glucocorticoid activity (Szefler, 1999). BUD has a high relative affinity for the glucocorticoid receptor and widespread anti-inflammatory effects on different cell types (mast cells, lymphocytes, neutrophils and macrophages) and different humoral mediators of non-allergic inflammation (cytokines etc.). There is also evidence that a reduction in the number of inflammatory cells in the bronchial mucosa is correlated with clinical improvements in response to therapy with inhaled CSs (Donnelly and Seale, 2001). DEX is the most commonly used corticosteroid in endotoxemia. It is currently believed that CSs, if they are to be clinically effective, must be given as early as possible to endotoxemic animals (Radostits *et al.,* 2007). Experimentally, beneficial effects of DEX in sheep endotoxemia have been demonstrated (Chalmeh *et al.,* 2013).

Cytokines are small cell-signalling glycoprotein molecules that play important roles in local and systemic inflammatory reactions, including the regulation of the immune system and induction of the host organism's reactions against antigens and microorganisms (Lohman and Baron, 2010). Higher levels of certain cytokines (pro-inflammatory TNF-α, and IL-6) have been associated with poor outcomes, as well as secondary activation of the systemic inflammatory cascade. Cytokines, especially TNF-α and IL-6, have been most strongly associated with the sepsis cascade as pro-inflammatory mediators (Damas *et al.,* 1992). TNF-α is viewed as proximal cytokine, and it is associated with most of the physiological disturbances that are the characteristic of sepsis. TNF-α is a cytokine involved in systemic inflammation and a member of a group of cytokines that stimulate the acute phase response (APR). Later, these cytokines stimulate the production of distal cytokines, such as IL-6. Distal cytokines seem to intensify and perpetuate the inflammatory response, and they are responsible for the modulation of lymphocyte function, activation of coagulation, and induction of hepatic APP synthesis (Balckwell and Christman, 1996).

In the present study, while LPS significantly increased the serum TNF-α and IL-6 levels, all treatment groups decreased the TNF-α to the level of the control group. The LPS+BUD and LPS+BUD+ENR+TM-SD groups inhibited IL-6 levels higher than other treatment groups and the effect maintained in the same level at 24 h. Yazar *et al.,* (2010a) have reported that high and low doses of DEX decrease the serum pro-inflammatory cytokine levels (TNF-α and IL-6). In the present study, TNF-α level of LPS+DEX and LPS+DEX+ENR+TM-SD groups reached that of control group, and serum IL-6 level was not much more affected.

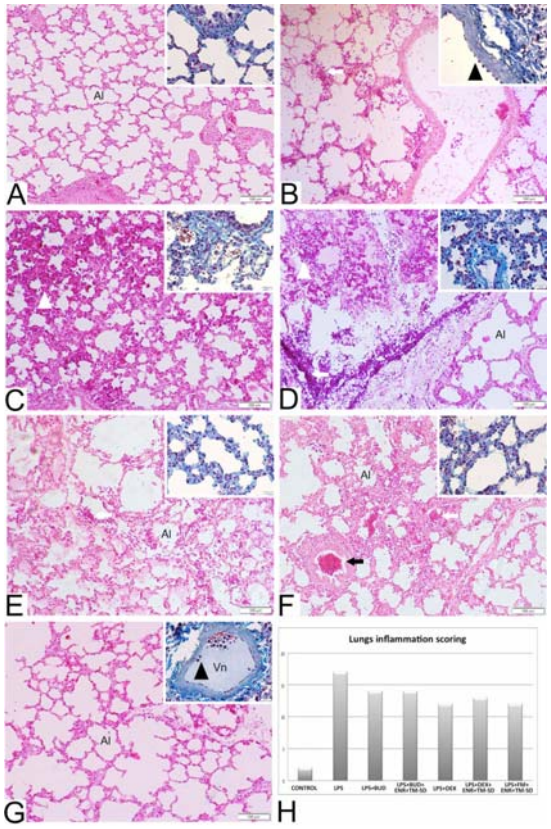

Fig. 1: Histopathological characteristics of lungs in lambs with experimentally induced endotoxemia. **(A)** Histological structure of the lungs belonging to the control group is normal. **(B)** Intense intra-alveolar hemorrhage (white arrow) and adhesive leukocytes (black triangle) are marked in the sections of lung tissue of LPS group. **(C, D)** Local congestion (white triangle) in both LPS+BUD and LPS+BUD+ENR+TM-SD groups is marked. **(E)** Hemorrhage and congestion are less intense in the lung sections of LPS+DEX group. **(E, F, G)** The acute effects of sepsis in LPS+DEX, LPS+DEX+ENR+TM-SD and LPS+FM+ENR+TM-SD groups are appeared to slightly affect the lungs. **(H)** The lung inflammation scoring graphic in lambs with experimentally induced endotoxemia. The lung sections indicated with small magnification were stained with H&E (Bar=100 µm). The high magnification indicated in upper right was stained with Masson Triple stain (Bar=20 µm). (Al: alveolus, Vn: vein, white arrow: intra-alveolar hemorrhage, black arrow: bronchial hemorrhage, white triangle: congestion, black triangle: adhesive leukocytes).

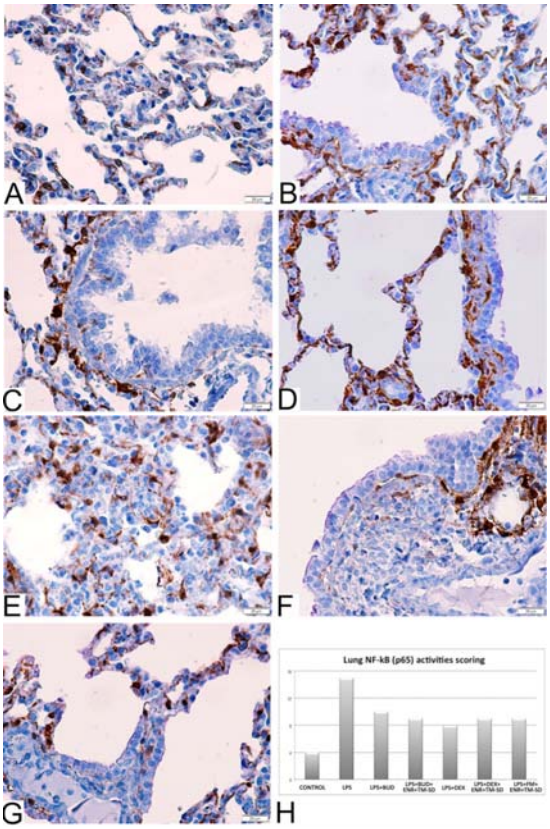

Fig. 2: The activity of NF-kB (p65) in the lungs of lambs with experimentally induced endotoxemia. NF-kB activation was not appeared in both bronchial epithelial cells and type I and type II cells in all groups. NF-kB positive cells were in inter-alveolar septum and bronchial stroma. Immunopositivity was minimal in the control group. NF-kB positivity in the lung sections of the LPS group was very dense and diffusive. Both nuclear and cytoplasmic immunoactivity were determined in variable severities in inter-alveolar septum and bronchial stroma cells. **(H)** Lung NF-kB (p65) protein activity scoring graphic in lambs with experimentally induced endotoxemia.

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are common disorders after the onset of sepsis. ALI is a syndrome of pulmonary inflammation and oedema resulting in acute respiratory failure (De Clue and Cohn, 2007; Saugil and Fargo, 2012). Cytokines and chemokines, including TNF-α and IL-6, are produced and ROS are released (Hashimoto *et al.,* 2004). Alveolar macrophage activation and occurrence of proinflammatory mediators promote neutrophil migration into the pulmonary interstitium and alveolus, further contributing to lung inflammation and injury (Fan *et al.,* 2001; Ozogul *et al.,* 2015). Furthermore, macrophages directly injure alveolar epithelial cells through induction of apoptosis (Takemura *et al.,* 2005). Wang *et al.,* (2008) have reported that low dose DEX suppress pulmonary inflammation and fibrosis in the rats. Jansson *et al.,* (2005) have stated that the therapeutic effect of BUD is dependent on the severity of LPS-induced lung injury in the rats.

According to our findings, the most intense lung inflammation score and NF-kB activity score were found in the LPS group. Inter-alveolar haemorrhage, adhesive leukocyte and injury in pulmonary vessels as ALI indicators were intense in the lung sections of LPS group, decreased in the LPS+BUD and LPS+BUD+ENR+TM-SD groups, and minimum in the LPS+DEX, LPS+DEX+ENR+TM-SD and LPS+FM+ENR+TM-SD groups. These findings may indicate the similar effects of inhaler and parenteral steroids against lung injury in LPS-induced endotoxemia.

Free radical damage plays a key role in endotoxemia (Cadenas and Cadenas, 2002). LPO, as a marker of oxidative damage, can cause changes in membrane fluidity and permeability, thus increasing the rate of protein degradation, which eventually lead to cell lysis (Garcia *et al.,* 1997). When the antioxidant capacity is insufficient, LPO occurs in cell and MDA is formed

(Berger and Chiolero, 2007). The increased generation of ROS from neutrophils has been proposed as a significant mechanism of lung injury during sepsis (Fink, 2002). It has been reported that the anti-oxidative system is severely compromised, and plasma LPO end products are significantly increased, suggesting increased oxidative stress in patients with lung injury (Metnitz *et al.*,1999).

GCs and FM suppress LPO by reducing MDA levels in endotoxemia (Konyalioglu *et al.,* 2007; Yazar *et al.,* 2010b). Yazar *et al.,* (2010b) suggested that ENR+FM+high dose DEX is most effective in the LPS-induced oxidative stress and organ damages. In the present study, LPS-induced LPO was measured as increases in MDA levels and LPS caused a significant increase of LPO levels of the lung tissue ($P<0.05$). In many studies, similar and conflicting results have been reported (Er *et al.,* 2010; Yazar *et al.,* 2010b). In this study, LPS-induced organ oxidative damage (lung) was characterized by increased LPO concentration. These results were consisted with previous studies, which reported increased LPO concentrations in the serum, liver, heart, and kidney (Portoles *et al.,* 1993; Keskin *et al.,* 2005). It is stated that low (2 mg/kg) and high doses (10 mg/kg) of DEX significantly decrease the LPO level in different studies performed on rats (Er et *al.,* 2010; Yazar *et al.,* 2010a). It was found that in this study, the lung LPO levels in the treatment groups are also significantly decreased ($P<0.05$).

Under normal physiological conditions, a balance exists between the formation of ROS and antioxidants such as SOD and GSH. Oxidative stress occurs when this balance is disrupted by the excessive production of ROS and/or inadequate antioxidative defences (Cadenas and Cadenas, 2002). SOD and GSH are the main enzymes involved in the anti-oxidative system, limiting the cytotoxic effects of toxic free radicals (Fang *et al.,* 2002). SOD is the key antioxidant enzyme because superoxide is one of the main ROS in the cell (Bauer and Bauer, 1999). GSH is the most important cellular antioxidant and plays a major role in protecting cells against oxidative stress caused by ROS. If the GSH level decreases, the tissue cannot protect itself against ROS damage, and oxidative damage occurs (Fang *et al.,* 2002).

The effects of FM on the antioxidant status during endotoxemia have been investigated. When FM was administered simultaneously with LPS, FM significantly inhibited the increase of MDA levels in all tissues (Konyalioglu *et al.,* 2007). In the present study, the lung tissue SOD activity and GSH level in all treatment groups significantly increased compared to those of the LPS group ($P<0.05$).

In conclusion, this study, for the first time, showed the comparative effects of inhaler and parenteral steroids in lambs with experimentally induced endotoxemia. As a result, it was concluded that steroids administered by inhalation and parenteral route had similar effects on serum pro-inflammatory cytokine levels and tissue oxidative stress markers, and both steroid groups can be beneficial in the treatment of endotoxemia.

Acknowledgments

We acknowledge Prof. Dr. Zekai HALICI for scientific contributions.

REFERENCES

Adams JL, SD Semrad and CJ Czuprynski, 1990. Administitration of bacterial lipopolysaccharide elicits circulating tumor necrosis factor-alpha in neonatal calves. J Clin Microbiol, 28: 998-1001.

Barnes PJ, 1998. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. Clin Sci, 94: 557-572.

Barton MH, L Williamson, S Jacks and N Norton, 2003. Effects on plasma endotoxin and eicosanoid concentrations and serum cytokine activities in horses competing in a 48-, 83-, or 159-km endurance ride under similar terrain and weather conditions. Am J Vet Res, 64: 754-761.

Bauer V and F Bauer, 1999. Reactive oxygen species as mediators of tissue protection and injury. Gen Physiol Bioph,18: 7-14.

Berger MM and RL Chiolero, 2007. Antioxidant supplementation in sepsis and systemic inflammatory response syndrome. Crit Care Med, 35: 584-590.

Blackwell TS and JW Christman, 1996. Sepsis and cytokines: current status. Br J Anaesth, 77: 110-117.

Cadenas S and AM Cadenas, 2002. Fighting the stranger-antioxidant protection against endotoxin toxicity. Toxicol, 180: 45-63.

Chalmeh A, KH Badiei, M Pourjafar and S Nazifi, 2013. Acute phase response in experimentally Escherichia coli serotype O55:B5 induced endotoxemia and its comparative treatment with dexamethasone and flunixin meglumine in Iranian fat-tailed sheep. Vet Arhiv, 83: 301-312.

Damas P, D Ledoux, M Nys, Y Vrindts, D De Groote, P Franchimont and M Lamy, 1992. Cytokine serum level during severe sepsis in human IL-6 as a marker of severity. Ann Surg, 215: 356-362.

De Clue AE and LA Cohn, 2007. Acute respiratory distress syndrome in dogs and cats: a review of clinical findings and pathophysiology. J Vet Emerg Crit Care, 17: 340-347.

Derendorf H, 1997. Pharmacokinetic and pharmacodynamic properties of inhaled corticosteroids in relation to efficacy and safety. Respir Med, 91: S22-28.

Donnelly R and JP Seale, 2001. Clinical pharmacokinetics of inhaled budesonide. Clin Pharmacokinet, 40: 427-440.

Dye TL, KJ Diehl, SL Wheeler and DS Westfall, 2012. Randomized, controlled trial of budesonide and prednisone for the treatment of idiopathic inflammatory bowel disease in dogs. J Vet Intern Med, 27: 1385-1391.

Ek A, K Larsson, S Siljerud and L Palmberg, 1998. Fluticasone and budesonide inhibit cytokine release in human lung epithelial cells and alveolar macrophages. Allergy, 54: 691-699.

Elmas M, E Yazar, K Uney and A Karabacak, 2006. Pharmacokinetics of flunixin after intravenous administration in healthy and endotoxaemic rabbits. Vet Res Commun, 30: 73-81.

Er A, F Altan, G Cetin, K Uney, B Tras, M Elmas and E Yazar, 2010. Effects of enrofloxacin, flunixin and dexamethasone on indicators of oxidative and organ

damage in lipopolysaccharide-induced endotoxemia. J Anim Vet Adv, 9: 1495-1500.

Fan J, RD Ye and AB Malik, 2001. Transcriptional mechanisms of acute lung injury. Am J Physiol Lung Cell Mol Physiol, 281: L1037-L1050.

Fang YZ, S Yang and GY Wu, 2002. Free radicals, antioxidants, and nutrition. Nutrition, 18: 872-879.

Fink MP, 2002. Reactive oxygen species as mediators of organ dysfunction caused by sepsis, acute respiratory distress syndrome, or hemorrhagic shock: potential benefits of resuscitation with Ringer's ethyl pyruvate solution. Curr Opin Clin Nutr Metab Care, 5: 167-174.

Garcia JJ, RJ Reiter, JM Guerrero, G Escames, BP Yu, CS Oh and A Munoz-Hoyos, 1997. Melatonin prevents changes in microsomal membrane fluidity during induced lipid peroxidation. FEBS lett, 408: 297-300.

Hashimoto N, T Kawabe, K Imaizumi, T Hara, M Okomato, K Kojima, K Shimokata and Y Hasegawa, 2004. CD40 plays a crucial role in lipopolysaccharide-induced acute lung injury. Am J Respir Cell Mol Biol, 30: 808-815.

Keh D, A Feldheiser and O Ahlers, 2005. Current state of corticosteroid therapy in patients with septic shock. Clin Intensive Care, 16: 151-161.

Keskin E, E Menevse, R Col, A Sivrikaya, K Uney and E. Yazar, 2005. Effect of pentoxifylline on antioxidant status of healthy and endotoxemic New Zealand white rabbits. Acta Vet Brno, 74: 17-21.

Konyalioglu S, A Er, K Uney, M Elmas and E Yazar, 2007. Effect of flunixin meglumine on the antioxidant status in endotoxemia. Acta Vet Beograd, 57: 241-246.

Lohman KL and MH Baron, 2010. Endotoxemia. In: Reed SM, WM Bayly and DC Sellon, (eds.), Equine Internal Medicine, 3nd edition, Saunders, Elsevier, USA, pp: 807-822.

Jansson AH, C Eriksson and X Wang, 2005. Effects of budesonide and N-acetylcysteine on acute lung hyperinflation, inflammation and injury in rats. Vascular Pharmacol, 43: 101-111.

Metnitz PG, C Bartens, M Fischer, P Fridrich, H Steltzer and W Druml, 1999. Antioxidant status in patients with acute respiratory distress syndrome. Intensive Care Med, 25: 180-185.

Moore JN and DD Morris, 1992. Endotoxemia and septicemia in horses: experimental and clinical correlates. J Am Vet Med Assoc, 200: 1903-1914.

Ohkawa H, N Ohishi and K Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95: 351-358.

Ozogul B, Z Halici E Cadirci, E Karagoz, Z Bayraktutan, M Yayla, E Akpinar, SS Atamanalp, D Unal and M Karamese, 2015. Comparative study on effects of nebulized and oral salbutamol on a cecal ligation and puncture-induced sepsis model in rats. Drug Res, 65: 192-198.

Pauwels R, S Newman and L Borgstrom, 1997. Airway deposition and airway effects of antiasthma drugs delivered from metered-dose inhalers. Eur Respir J, 10: 2127-2138.

Pietra M, F Fracassi, A Diana, T Gazzotti, G Bettini, A Peli, M Morini, G Pagliuca and P Roncada, 2013. Plasma concentrations and therapeutic effects of budesonide in dogs with inflammatory bowel disease. Am J Vet Res, 74: 78-83.

Portoles MT, MJ Ainaga and R Pagani, 1993. The induction of lipid peroxidation by *E. coli* lipopolysaccharide on rat hepatocytes as an important factor in the etiology of endotoxic liver damage. Comp Biochem Physiol B, 1158: 287-292.

Radostits OM, CC Gay, KW Hinckliff and PD Constable, 2007. Veterinary Medicine.10th edition, Saunders, Edinburgh, London, New York, Oxford, Philadelphia, St. Louis, Sydney, Toronto.

Saguil A and M Fargo, 2012. Acute respiratory distress syndrome: diagnosis and management. Am Fam Physician, 85: 352-358.

Sedlak J and RH Lindsay, 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem, 25: 192-205.

Smith GW, 2005. Supportive therapy of the toxic cow. Vet Clin Food Anim Pract, 21: 595-614.

Sun Y, LW Oberley and Y Li, 1988. A simple method for clinical assay of superoxide dismutase. Clin Chem, 34: 497-500.

Szefler SJ, 1999. Pharmacodynamics and pharmacokinetics of budesonide: A new nebulized corticosteroid. J Allergy Clin Immunol, 104: S175-183.

Takemura Y, Y Iwasaki, K Nagata, I Yokomurs, S Tando, S Fushiki and H Matsubara, 2005. Influence of depletion of alveolar macrophages on apoptosis in Candida-induced acute lung injury. Exp Lung Res, 31: 307-321.

Wang XQ, X Zhou, Y Zhou, L Rong, L Gao and W Xu, 2008. Low-dose dexamethasone alleviates lipopolysaccharide-induced acute lung injury in rats and upregulates pulmonary glucocorticoid receptors. Respirology, 13: 772-780.

Wu CC, 2006. Possible therapies of septic shock: Based on animal studies and clinical trials. Curr Pharm Des, 12: 3535-3541.

Yazar E, A Er, K Uney, A Bulbul, GE Avci, M Elmas and B Tras, 2010a. Effects of drugs used in endotoxic shock on oxidative stress and organ damage markers. Free Radic Res, 44: 397-402.

Yazar E, A Bulbul, GE Avci, A Er, K Uney, M Elmas and B Tras, 2010b. Effects of enrofloxacin, flunixin meglumine and dexamethasone on disseminated intravascular coagulation, cytokine levels and adenosine deaminase activity in endotoxaemia in rats. Acta Vet Hung, 58: 357-367.