Acta Veterinaria (Beograd), Vol. 59, No. 1, 47-51, 2009.

DOI: 10.2298/AVB0901047E UDK 619.615.276

EFFECTS OF DIFFERENT DOSES OF DEXAMETHASONE PLUS FLUNIXIN MEGLUMINE ON SURVIVAL RATE IN LETHAL ENDOTOXEMIA

ER A*, UNEY K*, ALTAN F**, CETIN G*, YAZAR E* and ELMAS M*

*University of Selcuk, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Campus, Konya, Turkey
**University of Dicle, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Campus, Diyarbakir, Turkey

(Received 4. April 2008)

Effects of different doses of dexamethasone plus flunixin meglumine on survival rate were investigated in lethal endotoxemia. A total of 60 Balb/C female mice were divided into 4 equal groups. Lethal endotoxemia (80-100%) was induced by lipopolysaccharide injection (Group 1, 1 mg, intraperinoneally). At 4 hours after the lipopolysaccharide injection; low-dose dexamethasone (0.6 mg/kg, SID, 5 days, intramuscularly) + flunixin meglumine (2 mg/kg, SID, 5 days, subcutaneously), normal-dose dexamethasone (2 mg/kg, SID, 5 days, intramuscularly) + flunixin meglumine (2 mg/kg, SID, 5 days, subcutaneously) and high-dose dexamethasone (10 mg/kg, SID, 5 days, intramuscularly) + flunixin meglumine (2 mg/kg, SID, 5 days, subcutaneously) were injected to Group 2, 3 and 4, respectively. After the injections, survival was monitored at 7 days and 13.3%, 13.3%, 33.3% and 73.3% survival rates were observed in Groups 1, 2, 3 and 4, respectively. As results, high-dose dexamethasone plus flunixin meglumine may be the treatment of choice for endotoxaemia in animals.

Key words: lethal endotoxaemia, dexamethasone, flunixin, survival rate

INTRODUCTION

Septic shock is defined as sepsis-induced persistent hypotension accompanied by hypoperfusion abnormalities or organ dysfunction. The mortality rate is 50-60% in septic shock. Lipopolysaccharides (LPS) play a critical role in the pathophysiology of septic shock (Annane *et al.,* 2004; Sanchez, 2005). When microbes or their products (LPS) are present in the bloodstream, the same mechanisms that are beneficial in resolving a local infection become activated systemically. The initial event is the release of proinflammatory cytokines from activated macrophages; this initiates the cytokine system. Cytokines stimulate neutrophils to produce phospholipid derivates, and reactive oxygen radicals, as

well as activate endothelial cells. Endothelial activation increases production of vasoactive mediators, and disrupts vascular homeostasis. Endothelial activation also leads to thrombosis. These events cause multiorgan dysfunction and cardiac depression (Furr, 2003).

Corticosteroids (CSs) and flunixin meglumine (FM) are generally used in the treatment of septic shock. Dosage, timing and duration of administration of CSs are still debated. In the 1960s, high-dose CS was preferred. However, in the 1990s, there was no beneficial effect found in the meta-analyses (Meduri, 1999; Minneci *et al.,* 2004). Nowadays, low-dose GC is preferred in human medicine (Servansky and Natanson, 2000). Although FM and/or CCs are recommended in the treatment of septic shock, there are few controlled studies that have compared the two classes (nonsteroidal anti-inflammatory drugs and CSs) of drugs on clinical signs of septic shock (Smith, 2005). To our knowledge, there are very limited studies on the effect of combined CS and FM in endotoxemia.

The aim of this study was to determine the effects of different doses of dexamethasone (DEX) plus FM on survival rate in lethal endotoxaemia.

MATERIAL AND METHODS

A total of 60 Balb/C mice (female, 3-4 months of age, 29±6.4 g, Laboratory Animal Unit, Akdeniz University, Antalya, Turkey) were divided into 4 equal groups. The study protocol was approved by The Ethics Committee of Veterinary Faculty. Animals were fed standard pellet diet and tap water *ad libitum*.

The study was designed as two steps. First step: lethal endotoxemia was induced by LPS (Group 1, 1 mg, intraperitoneally, *Escherichia coli* 0111:B4, Sigma-Aldrich Chemie, Deisenhofen, Germany) injection. After the LPS injections, survival was assessed 7 days or if necessary, at 6 hours intervals. Approximately at 2-4 hours after the LPS injection, animals were observed to be clinically sick (piloerection, shivering and lethargy). Second step; at 4 hours after the LPS injection, low-dose DEX (0.6 mg/kg, SID, 5 days, intramuscularly, Dekort® amp, Deva Ilac, Istanbul, Turkey) + FM (2 mg/kg, SID, 5 days, subcutaneously, Finadyne® enj. Sol., Dogu Ilac Veteriner Urunleri, Istanbul, Turkey), normal-dose DEX (2 mg/kg, SID, 5 days, intramuscularly) + FM (2 mg/kg, SID, 5 days, subcutaneously), and high-dose DEX (10 mg/kg, SID, 5 days, intramuscularly) + FM (2 mg/kg, SID, 5 days, subcutaneously) were administered in Group 2, 3 and 4, respectively. After the injections, survival was monitored for 7 days. At the end of the study (7 days), live/death ratio was analyzed by the chi-square test. The level of significance was accepted at $P<0.05$.

RESULTS AND DISCUSSION

Survival rates (13.3%, 13.3%, 33.3% and 73.3%) were determined (Figure 1) in Groups 1, 2, 3, and 4, respectively. While survival rates of groups 1, 2 and 3 were statistically similar ($p<0.05$), high-dose DEX+FM significantly ($p<0.05$) reduced (after 48 hours) the death rate in comparison to other 3 groups. There was no

death observed at 48 hours after treatments in any of the studied groups. Surviving animals returned to be clinically healthy within 5 days in all groups.

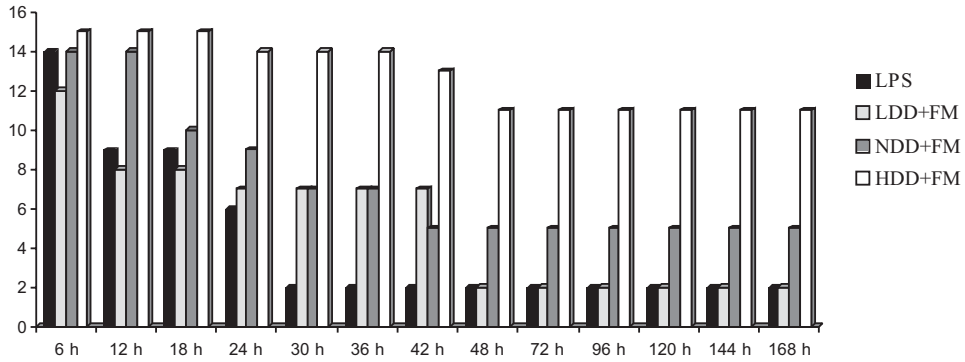

Figure 1. Effects of low-dose DEX (LDD, 0.6 mg/kg, SID, 5 days, IM) + FM (2 mg/kg, SID, 5 days, SC), normal-dose DEX (NDD, 2 mg/kg, SID, 5 days, IM) + FM, and high-dose DEX (HDD, 10 mg/kg, SID, 5 days, IM) + FM on survival in lethal endotoxemia (LPS)

In this study, DEX and FM were selected as anti-septic shock drugs. CSs and FM are frequently chosen drugs in the treatment of septic shock in veterinary medicine (Sanchez, 2005). Although the crucial mechanism of action for CSs remains unclear, it may involve a reduction of blood monocyte nuclear factor kappa B (NF-kB). NF-kB, a transcription factor, appears to have a central role in the pathophsiology of sepsis (Wang *et al.,* 2006; Wu, 2006). Inhibition of NF-kB results in the inhibition of cytokines, inducible nitric oxide synthase and cyclooxygenase-2, cell adhesion molecules, growth factors, oxidative stress and regulation of apoptosis (Keh *et al.,* 2005; Yazar *et al.,* 2004a). FM, a potent nonsteroidal anti-inflammatory drug (NSAID), inhibits the activation of NF-kB (Bryant *et al.,* 2003), oxidative stress (Konyalioglu *et al.,* 2007) and increases of cytokines in endotoxemia (Yazar *et al.,* 2007).

Many animal models of septic shock have a limited application in clinical practice. For example, anti-endotoxaemic drugs were administered simultaneously and/or before initiation of septic shock (Otto, 2007; Yazar *et al.,* 2004b). In clinical practice, this is not possible. Our study model is more realistic, because treatments (DEX and FM) were started just before the first death and when all animals were observed clinically ill.

In this study, high-dose DEX + FM reduced ($p<0.05$) the death ratio in lethal endotoxemia. However, high-dose CS showed no significant improvement in survival rates (Servansky and Natanson, 2000), long courses of low-dose CS is preferred in human medicine (Annane *et al.,* 2004). On the contrary to this, a recent study showed that hydrocortisone did not improve survival in septic humans (Vincent, 2008). In fact, many studies have been done to evaluate CS in

sepsis, the results have been mixed (Kumar and Mann, 2004). In human medicine, generally CS is used alone. However, in this study, DEX and FM were used in combination. Hence, FM may potentiate the effects of DEX. In addition, there is disagreement amongst practitioners as to whether CSs or NSAID are more effective. Although there are few controlled studies that have compared these two classes of drugs (Smith, 2005), it is stated that a combination of DEX and FM has been beneficial clinically in treating horses and calves with septic shock (Ewert *et al.,* 1985; Margolis *et al.,* 1987). However, the effectiveness of different doses of DEX were not investigated in these studies.

As results, at least a short course (2-3 days) of high-dose DEX plus FM may be preferred in the treatment of the septic animals, but normal and low-dose DEX plus FM seems to be not effective in the endotoxemia.

Address for correspondence:
Dr. Enver YAZAR
Selcuk University
Veterinary Faculty
Dept of Pharmacology, 42031, Campus
Konya, Turkey
e-mail: eyazar@selcuk.edu.tr

REFERENCES

1. *Annane D, Bellissant E, Bollaert PE, Briegel J, Keh D, Kupfer Y,* 2004, Corticosteroids for severe sepsis and septic shock: a systemic review and meta-analysis, *BMJ*, 329, 480-4.
2. *Bryant CE, Farnfield BA, Janicke HJ,* 2003, Evaluation of the ability of carprofen and flunixin meglumine to inhibit activation of nuclear factor kappa B, *Am J Vet Res*, 64, 211-5.
3. *Ewert KM, Fessler JF, Templeton CB, Bottoms GD, Latshaw HS, Jhonson MA,* 1985, Endotoxin-induced hematologic and blood chemical changes in ponies: Effects of flunixin meglumine, dexamethasone and prednisolone, *Am J Vet Res*, 46, 24-30.
4. *Furr M,* 2003, Systemic inflammatory response syndrome, sepsis and antimicrobial therapy, *Clin Tech Equin Pract,* 2, 3-8.
5. *Keh D, Feldheiser A, Ahlers O,* 2005, Current state of corticosteroid therapy in patients with septic shock, *Clinical Intensive Care*, 16, 151-61.
6. *Konyalioglu S, Er A, Uney K, Elmas M, Yazar E,* 2007, Effect of flunixin meglumine on the antioxidant status in endotoxemia, *Acta Vet Beograd*, 57, 241-6.
7. *Kumar A, Mann HJ,* 2004, Appraisal of four novel approaches to the prevention and treatments of sepsis, *Am J Vet Res,* 61, 765-76.
8. *Margolis JH, Bottoms GD, Fessler JF,* 1987, The efficacy of dexamethasone and flunixin meglumine in treating endotoxin-induced changes in calves, *Vet Res Commun*, 11, 479-91.
9. *Meduri GU,* 1999, An historical review of glucocorticoid treatment in sepsis. Disease pathophysiology and the design of treatment investigation, *Sepsis,* 3, 21-38.
10. *Minneci PC, Deans KJ, Banks SM, Eichacker PQ, Natanson C,* 2004, Meta-analysis: The effect of steroids on survival and shock during sepsis depends on the dose, *Ann Intern Med*, 141, 47-56.
11. *Otto CM*, 2007, Clinical trials in spontaneous disease in dogs: a new paradigm for investigations of sepsis, *J Vet Emerg Clin Care,* 17, 359-67.
12. *Sanchez LC*, 2005, Equine neonatal sepsis, *Vet Clin Equine,* 21, 273-93.
13. *Servansky J, Natanson C,* 2000, Clinical trials in sepsis: an update. *Curr Opin Anaesthesiol*, 13, 125-9.
14. *Smith GW,* 2005, Supportive therapy of the toxic cow, *Vet Clin Food Anim*, 21, 595-614.

15. *Wang Z, Kang J, Li Y, Yuan Z, Liu S, Sun L,* 2006, The effects of dexamethasone on rat brain cortical nuclear factor kappa B (NF-kB) in endotoxic shock, *Toxicol Appl Pharm*, 214, 263-9.
16. *Wu CC*, 2006, Possible therapies of septic shock: Based on animal studies and clinical trials, *Curr Pharm Design*, 12, 3535-41.
17. *Vincent JL*, 2008, Steroids in sepsis: another swing of the pendulum in our clinical trials, *Crit Care*, 12, 1-2.
18. *Yazar E, Konyalioglu S, Col R, Birdane YO, Bas AL, Elmas M,* 2004a, Effects of vitamin E and prednisolone on some oxidative stress markers in endotoxemic rabbits, *Revue Med Vet*, 155, 538-42.
19. *Yazar E, Col R, Konyalioglu S, Birdane YO, Elmas M, Bas AL,* 2004b, Effects of vitamin E and prednisolone on biochemical and haematological parameters in endotoxaemic New Zealand White rabbits, *Bull Vet Inst Pulawy*, 48, 105-8.
20. *Yazar E, Er A, Uney K, Altunok V, Elmas M*, 2007, Effect of flunixin meglumine on cytokine levels in experimental endotoxemia in mice, *J Vet Med A,* 54, 352-5.

UTICAJ RAZLIČITIH DOZA DEKSAMETAZONA I FLUNIKSIN MEGLUMINA NA STEPEN PREŽIVLJAVANJA PRI LETALNOJ ENDOTOKSEMIJI

ER A, UNEY K, ALTAN F, CETIN G, YAZAR E i ELMAS M

SADRŽAJ

U ovom radu su izneti rezultati proučavanja uticaja različitih doza deksametazona i fluniksin meglumina na stepen preživljavanja miševa pri letalnoj endotoksemiji. U studiju je bilo uključeno ukupno 60 ženki miševa soja Balb/C, a letalna endotoksemija (80-100 %) je bila izazivana intraperitonealnom aplikacijom 1 mg lipopolisaharida (grupa 1). Četiri sata nakon ove injekcije, miševima grupe 2 bilo je intramuskularno aplikovano 0,6 mg/kg deksametazona (niska doza) i 2 mg/kg fluniksin meglumina subkutano, tokom pet dana. Treća i četvrta grupa miševa bile su tretirane na isti način sa 2 mg/kg i 10 mg/kg deksametazona (srednja i visoka doza) uz istu dozu fluniksin meglumina. Stepen preživljavanja je određivan posle 7 dana i iznosio je po grupama: 13,3 %, 13,3 %, 33,3 % i 73,3 % respektivno. Ove razlike su bile statistički značajne između četvrte i svih ostalih oglednih grupa ($p<0,05$). Na osnovu ovih rezultata, može se zaključiti da visoka doza deksametazona, zajedno sa fluniksin megluminom, može da bude od koristi u tretmanu endotoksemije životinja.