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Original article

Comparison of flunixin meglumine and meloxicam influence on postoperative and oxidative stress in ovariohysterectomized bitches

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Abstract

The aim of this study was to compare the effect of flunixin meglumine (FM) and meloxicam (M) on postoperative and oxidative stress in ovariohysterectomized bitches. Twenty four bitches were divided into three groups (n=8 in each) and treated during premedication as follows: FM (2.2 mg/kg, iv, Fluvil, Vilsan, Turkey), M (0.2 mg/kg, sc, Maxicam, Sanovel, Turkey) or 0.9% saline (1 ml, iv, IE, Turkey) – control (C) group. The concentrations of serum cortisol, nitric oxide (NO), malondialdehyde (MDA), antioxidant potential (AOP) and glutation (GSH) were measured in blood samples collected during incision (0 h), closure of incision line (0.5 h) and 1, 2.5, 12 and 24 hours after incision. It was observed that cortisol level was higher at 0.5, 1 and 2.5 h in group C ($p<0.05$), 0.5 h in group FM ($p<0.001$), and 1 and 2.5 h in group M ($p<0.01$), as compared to that determine at 0 h. Group C showed higher cortisol level during 0.5 h ($p<0.05$) than that found in the other groups. Group FM displayed lower levels during 1 h ($p<0.01$) and 2.5 h ($p<0.05$) as compared to those observed in other groups. Concentrations of MDA, AOP and GSH between all the groups did not show any significant differences. MDA level was higher at 0.5 and 1 h in group M ($p<0.05$) than that found in group C and it was the lowest at 2.5 h in group C ($p<0.05$). AOP was higher at 2.5 h in group FM and M ($p<0.05$) than that observed in group C, and at 12 and 24 h in group M than that found in group C and FM. GSH did not show any significant differences between the groups. NO level in group FM after 12 h was higher ($p<0.05$) than that at 0.5, 1 and 24 h. Moreover, NO level was lower at 0.5 ($p<0.01$), 1 ($p<0.05$) and 24 h ($p<0.05$) in group FM than that observed in group C and M. In conclusion, flunixin meglumine decreases cortisol and NO levels more efficiently than meloxicam. Therefore, it is suggested that postoperative stress following ovariohysterectomy may be prevented by flunixin meglumine in bitches.

Key words: NSAID, cyclooxygenase, prostaglandin, nitric oxide, cortisol

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Introduction

Ovariohysterectomy (OHE) is a common surgical contraceptive procedure performed in small animal practice (Devitt et al. 2005, Höglund et al. 2011, Kim et al. 2012). The tissue damage during the surgery leads to surgical stress response and postoperative pain (Firth and Haldane 1999). The stress response to surgery is characterized by the activation of hypothalamic-pituitary-adrenal (HPA) axis (Pacák and Palkovits 2001). The changes in pituitary secretion secondarily lead to hormone secretion from the adrenal cortex (Desborough 2000) that adapts to stress by producing various molecules, such as cytokines, nitric oxide (NO) and prostaglandins which play a role in corticosterone release (John and Buckingham 2003, Ehrhart-Bornstein and Bornstein 2008). Moreover, the trauma caused by surgery may contribute to oxidative stress due to increasing oxidation and lipid peroxidation by releasing free iron and copper from tissues and activation of inflammatory response (Baines and Shenkin 2002).

The pain which commonly occurs during postoperative process is one of the main factors causing postoperative stress. Nonsteroidal anti-inflammatory drugs (NSAIDs) reduce inflammatory pain by the inhibition prostaglandin synthesis via inhibition of the action of cyclooxygenase (COX) (Engelhardt 1996) which converts arachidonic acid into prostanoids (Jones and Budsberg 2000). In the present study, it has been hypothesized that NSAIDs which preferentially inhibit different COX isoenzymes (COX-1 or COX-2) can have various response to prevent probable postoperative and oxidative stress in dogs. Therefore, this study was designed to compare the effect of flunixin meglumine (FM), a nonselective inhibitor of COX-1 and COX-2 (Brideau et al. 2001) and meloxicam (M), a selective COX-2 inhibitor (Kay-Mugford et al. 2000) on cortisol, nitric oxide (NO), malondialdehyde (MDA), antioxidant potential (AOP) and glutation (GSH) concentrations in blood collected at different time intervals during and after the OHE.

Materials and Methods

Animals

The study was approved by the animal local ethics committee, Afyon Kocatepe University (approval number 204-13). A total of 24 cross-breed bitches aged between 2-4 years, weighing 21-32 kg were used in the study. The animals were randomly divided into three groups with eight bitches in each group. During premedication, the FM group was treated with flunixin meglumine (2.2 mg/kg, iv, Fluvil, Vilsan, Turkey), the M group was treated with meloxicam (0.2 mg/kg, sc, Maxicam, Sanovel, Turkey) and the control (C) group received 0.9% saline (1 ml, iv, IE, Turkey).

Anaesthesia

The same anaesthetic protocol was used for all the groups. The dogs were fasted 12 hours before the surgery. The animals were premedicated with atropine (0.04 mg/kg, Atropin, Vetas, Turkey) via subcutaneously 30 minutes prior to general anaesthesia. The sedation was achieved with intramuscular injection of 2 mg/kg xylazine HCl (Rompun 2%, Bayer, Germany) and general anaesthesia was induced with intramuscular administration of 15 mg/kg ketamine HCl (Alfamine 10%, Ege Vet, Turkey).

Surgical procedure

Following preparation for aseptic surgery, OHE was performed through a conventionally ventral midline approach. All OHE procedures were performed by the same surgeon to avoid bias between the groups. The surgical procedure lasted within 30 minutes without any complication such as bleeding.

Blood sampling

Blood samples were collected from the *vena cephalica antebrachii* during incision time (0 h), closure of incision line (0.5 h) and 1, 2.5, 12 and 24 hours after incision. The blood samples were centrifuged at 3000 rpm for 10 minutes. Then, the sera were stored at -20°C until the analysis.

Serum cortisol level was measured using a commercial kit (EIA-1887, DRG, USA) utilizing enzyme-linked immunesorbent assay (ELISA) method. The sensitivity of assay was 2.5 ng/ml. The average intra- and interassay coefficients of variation were 8.1% and 6.6%, respectively.

NO level was quantified indirectly by measuring nitrites (NO_2) and nitrates (NO_3), using the Griess method (Miranda et al. 2001). Briefly, 100 µL of sera samples were transfered into 96 well plate and incubated for 30 min in the presence of 50 µL of NEDD [*N*-(1-Naphthyl)ethylenediamine dihydrochloride] (0.1%, w/v), 50 µL of sulfanilamide (2%, w/v) and 100 µL of vanadium (III) chloride (50 mM) at 37°C. After incubation, the absorbance of each

sample was a maximum absorbance at 530 nm. The sensitivity of test was 1.1 U/l.

Table 1. Mean (± SEM) cortisol concentrations (ng/ml) in ovariohysterectomized bitches treated with 0.9% saline (iv; control), flunixin meglumine (2.2 mg/kg b.w. iv) and meloxicam (0.2 mg/kg b.w. sc). Blood sampling was started during the incision time (0 h).

Blood sampling (hour)	Control (n = 8)	Flunixin meglumine (n = 8)	Meloxicam (n = 8)
0	21.92 ± 7.52^{aA}	20.37 ± 3.74^{aA}	22.44 ± 4.41^{aA}
0.5	83.77 ± 21.95^{bA}	41.99 ± 5.68^{bB}	32.84 ± 3.87^{abB}
1	84.64 ± 18.48^{bA}	15.17 ± 2.61^{aB}	59.79 ± 13.57^{bcA}
2.5	78.81 ± 23.54^{bcA}	22.36 ± 5.89^{aB}	80.93 ± 22.52^{cA}
12	26.36 ± 13.10^{acA}	12.13 ± 2.75^{aA}	25.09 ± 3.48^{aA}
24	26.34 ± 3.43^{aA}	18.81 ± 3.81^{aA}	21.40 ± 3.22^{aA}

Small $^{(abc)}$ and capital $^{(AB)}$ letters in superscript indicate significant differences ($p<0.05 - p<0.001$) within the group and between the groups, respectively.

Table 2. Mean (± SEM) concentrations of nitric oxide (μmol/l) in ovariohysterectomized bitches treated with 0.9% saline (iv; control), flunixin meglumine (2.2 mg/kg b.w. iv) and meloxicam (0.2 mg/kg b.w. sc). Blood sampling was started during the incision time (0 h).

Blood sampling (hour)	Control (n = 8)	Flunixin meglumine (n = 8)	Meloxicam (n = 8)
0	31.41 ± 4.28^{aA}	29.58 ± 1.42^{abA}	31.09 ± 3.01^{aA}
0.5	41.09 ± 3.48^{aA}	22.80 ± 2.94^{aB}	32.06 ± 2.97^{aAB}
1	46.93 ± 6.82^{aA}	24.64 ± 3.06^{aB}	36.59 ± 3.90^{aAB}
2.5	38.13 ± 4.20^{aA}	27.55 ± 2.70^{abA}	38.33 ± 4.76^{aA}
12	42.95 ± 7.10^{aA}	36.40 ± 5.02^{bA}	39.70 ± 11.29^{aA}
24	34.58 ± 7.01^{aAB}	23.64 ± 2.71^{aA}	43.69 ± 7.26^{aB}

Small $^{(abc)}$ and capital $^{(AB)}$ letters in superscript indicate significant differences ($p<0.05$) within the group and between groups, respectively.

measured with plate microplate reader (MWGt Lambda Scan 200, Bio-Tek Instruments, USA), with an emission filter set at 545 nm. NO_2/NO_3 concentration was calculated using NO_2 standard curve (0.25-200 mM).

Plasma lipid peroxidation was determined using the procedure described by Yoshoiko et al. (1979), in which MDA, an end product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to form a coloured complex with a maximum absorbance at 530 nm. The sensitivity of test was 0.093 μmol/ml.

The AOPs were measured by the method described by Durak et al. (1998). Briefly, in the reaction medium enriched with fish oil, samples were exposed to a superoxide radical (O^{2-}) produced by the xanthine-xanthine oxidase system for 1 h and then MDA levels were measured as described by Dahle et al. (1962). By using this reaction system, it is possible to obtain more precise information about antioxidant potentials of the sample. After MDA levels were measured in the control and sample studies, the AOP value was assessed from the difference between MDA levels of the control and sample studies, which was proportional to the AOP values of the samples measured with

Serum GSH levels, were determined by the method of Beutler (1975). 100 μL of serum was diluted with 400 μL of distilled water. 500 μL of the diluted serum was taken and to this, 2 mL of phosphate solution and 250 μL of DTNB [5,5-Dithiobis (2nitrobenzoic acid)] reagent were added. Simultaneously, a blank was maintained containing 200 μL of distilled water, 300 μL of precipitating solution, 2 mL of phosphate solution and 250 μL of DTNB. The intensity of yellow colour formed was spectrophotometrically read at 410 nm against blank used. The sensitivity of test was 0.13 g/l.

Statistical analysis

Differences at the concentrations of cortisol, NO, MDA, AOP and GSH detected during measurement times (0, 0.5, 1, 2.5, 12, 24 h) within and between the groups were compared using the analysis of variance (ANOVA) followed by Tukey test (SPSS 13.0). The data were considered to be significantly different at $p<0.05$.

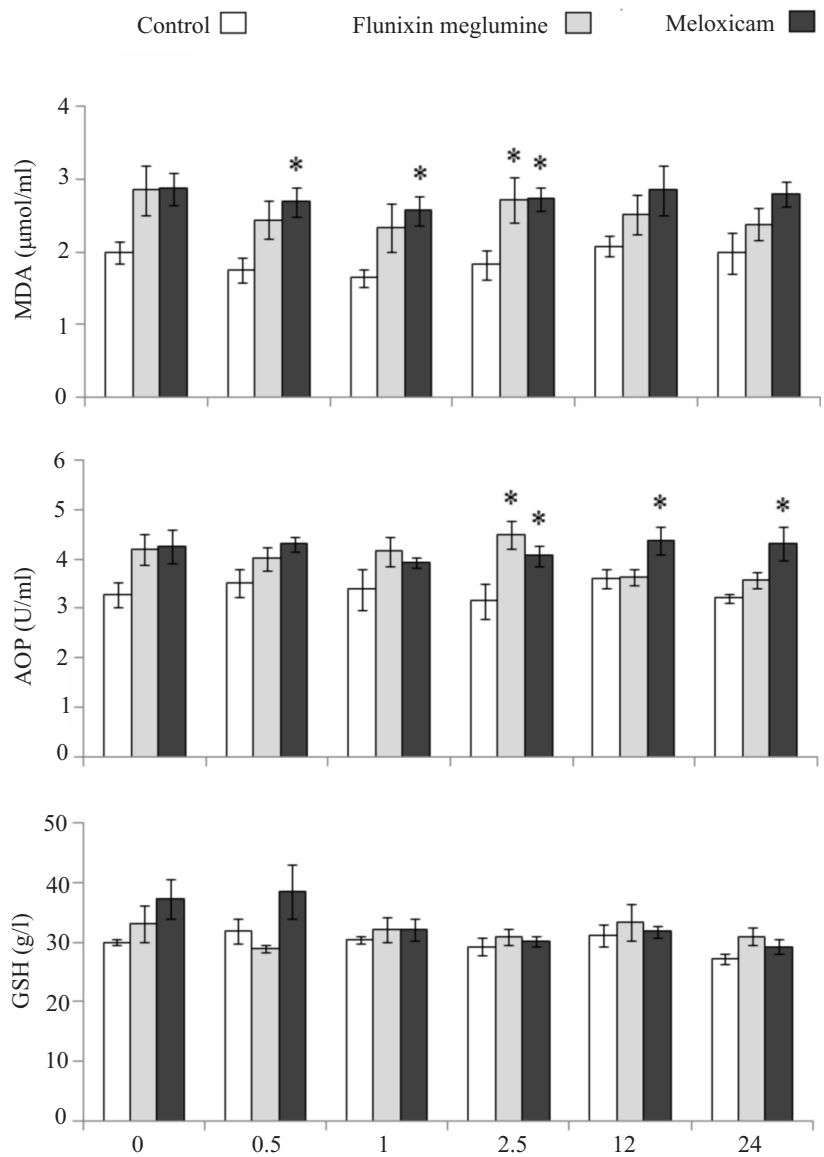

Fig. 1. Mean (± SEM; n = 8 in each group) concentrations of malondialdehyde (MDA), antioxidant potential (AOP) and glutation (GSH) in ovariohysterectomized bitches treated with 0.9% saline (iv; control), flunixin meglumine (2.2 mg/kg b.w. iv) and meloxicam (0.2 mg/kg b.w. sc). Blood sampling was started during the incision time (0 h).
* $p < 0.05$ as compared to the control group.

Results

It was observed that cortisol level was higher at 0.5, 1 and 2.5 h in group C ($p < 0.05$), at 0.5 h in group FM ($p < 0.001$), and at 1 and 2.5 h in the group M ($p < 0.01$), with respect to that found at 0 h (Table 1). Significantly lower cortisol level in group FM and M ($p < 0.05$) was observed at 0.5 h in comparison to that observed in group C. Moreover, in group FM cortisol level was lower at 1 h ($p < 0.01$) and 2.5 h ($p < 0.05$), when compared to that determined in group C and M. There were no significant differences between groups at 0, 12 and 24 h.

The concentration of NO did not differ in group C and M. However, NO level in group FM at 12 h was higher ($p < 0.05$) than that found at 0.5, 1 and 24 h (Table 2). The NO level was lower during 0.5 ($p < 0.01$) and 1 h ($p < 0.05$) in group FM than that observed in group C and lower during 24 h ($p < 0.05$) in group FM than that determined in group M. Concentrations of MDA, AOP and GSH showed no significant differences between all the groups examined (Fig. 1). MDA level was higher at 0.5 and 1 h in group M ($p < 0.05$) and at 2.5 h in group FM and M as compared to that found in group C (Fig. 1). AOP was higher at 2.5 h in group FM and M ($p < 0.05$) than those observed in group C, and at 12 and 24 h in group M than those determined in group C and FM. GSH did not show any significant difference among the groups.

Discussion

In dogs undergoing major abdominal surgery such as OHE, catecholamines, adrenocorticotrophic hormone (ACTH), cortisol and insulin significantly increase in response to surgical manipulation, continue increasing during surgical period and reach the peak near end of the surgery or shortly after the recovery from anaesthesia. Moreover, the stress response to OHE is short lived and returns to preoperative values by 5 h after completion of surgery (Benson et al. 2000), except cortisol which returns baseline values by 24 hours postsurgery (Church et al. 1994, Fox et al. 1994, Benson et al. 2000). Serum cortisol concentrations seem to be a good indicator for evaluating stress response in dogs. Therefore, serum cortisol concentrations were measured to evaluate the postoperative stress response to surgery in the present study. It was found that cortisol level was higher during the first 2.5 hours in group C and returned to baseline 12 hours after the incision time and remained at baseline following blood sampling time. This observation was consistent with previous reports (Church et al. 1994, Fox et al. 1994, Benson et al. 2000, Kim et al. 2012).

The serum cortisol concentration in group FM and M did not differ during 0.5 h but was lower as compared to group C. Prostaglandins are important signal transducers during basal and stress conditions (Bugajski et al. 2004, Rettori et al. 2009). They stimulate not only the secretion of corticotropin releasing hormone (CRH), vasopressin and ACTH (Gadek-Michalska et al. 2005) but also steroidogenesis and the release of corticosterone, by acting directly in the adrenal gland (Wang et al. 2000, Mohn et al. 2005). It is possible that the initiation of decreasing serum cortisol level is caused by the inhibition of prostaglandin synthesis via COX inhibition. On the other hand, group FM presented lower cortisol levels during 1 and 2.5 h as compared to the other groups, whereas group M and C did not show any significant difference. It is well known that flunixin meglumine preferentially inhibits COX1 and is indicated for acute and surgical pain (Dow et al. 1990), however meloxicam has been used in dogs for medium to long term treatment of pain and inflammation and has selectivity against COX2 versus COX1 (Distel et al. 1996). In the present study, it was clearly demonstrated that OHE procedure caused higher cortisol levels throughout 2.5 hours as detected in group C. Therefore, it is suggested that flunixin meglumine inhibits prostaglandin synthesis more efficiently than meloxicam because it faster reaches maximum plasma concentration and thus more rapidly evokes the inhibitory effect. Nitric oxide is synthesized by nitric oxide synthase (NOS) isoenzymes, which utilizes the semiessential amino acid L-arginine as a substrate (Palmer et al. 1988). Nitric oxide synthase is an enzyme group and has inducible (iNOS) and constitutive (cNOS), which includes neuronal NOS (nNOS) and endothelial NOS (eNOS), isoforms (Forstermann et al. 1991, 1998). Each isoform has a specific distribution in the body including the adrenal gland (Kishimoto et al. 1996, Cymeryng et al. 2002, Lai et al. 2005). Nitric oxide, similarly to prostaglandins, can modulate the release of stress hormones such as CRH, vasopressin, ACTH and corticosterone (Bugajski et al. 2004, Rettori et al. 2009). NOS activity is increased during stress and infection (Gadek-Michalska et al. 2005, Monau et al. 2009). Nitric oxide can regulate cortical and medullary adrenal gland functions such as the secretion of aldosterone (Sainz et al. 2004) and corticosterone (Cymeryng et al. 1998). In the present study, similar to cortisol concentration in group C, NO increased during 0.5 and 1 h but this increment was not statistically significant. It was reported that NO concentration was increased 30 minutes after the initiation of xylazine-ketamine anaesthesia but it did not differ 60 minutes after the induction of anaesthesia (Alva et al. 2006). It has been shown elsewhere that COX activation modulates L-arginine-NO pathway and COX inhibition decreases NOS activity in human platelets (Chen et al. 1997) thus, COX enzymes are clearly important receptor targets for the action of NO (Mollace et al. 2005). The present study revealed that NO concentration decreased during 0.5 and 1 h in group FM compared to that found in group C. Therefore, it is suggested that rapid COX inhibition by FM may result from decreasing NO concentrations.

The trauma of a surgical procedure is known to support a prooxidative state, due to ischaemia or reperfusion processes (Halliwell 1994). It was reported that MDA concentration increased and GSH concentration decreased 24 h after OHE which occurred in dogs anaesthetized by xylazine and ketamine combination in approximately 60 minutes (Serin et al. 2008). Similar data were also reported in dogs anaesthetized by xylazine and ketamine, however no duration of surgery was given (Gunay et al. 2011). MDA increased 30 minutes after but did not change 2 and 24 h after exposure of enflurane anaesthesia for 2 hours in dogs, whereas GSH concentration displayed no significant difference (Naziroglu and Gunay 1999). Moreover, Alva et al. (2006) reported that one hour exposure of xylazine and ketamine or pentobarbital-induced anaesthesia which was given every 30 minutes for maintenance of anaesthesia did not alter MDA levels at 15 and 60 minutes, while GSH decreased. In the present study, it was expected that the concentration of MDA, a marker for lipid peroxidation (Haliwell and Chirico 1993), would be increased during and end of the sur-

gery or shortly after the recovery from anaesthesia. Nevertheless, in our study, the concentrations of MDA, AOP and GSH within all the groups did not show any significant difference throughout the sampling times. The discrepancies between our data and the results presented in the above mentioned studies may be related to duration of surgery or anaesthesia process. Indeed, the surgery and anaesthesia procedures in this study were accomplished in 30 minutes and did not require any further anaesthetics. However, in the previous studies, the duration and maintenance of surgery or anaesthesia were longer than those in our study.

Although MDA, AOP and GSH did not show any significant difference within groups, the concentrations of MDA and AOP displayed significant differences between the groups. It is suggested that these statistically significant differences found in the present study is likely due to accidentally baseline levels of MDA, AOP or GSH in the groups. Moreover, it is suggested that neither FM nor M are capable to alter MDA, AOP or GSH.

In conclusion, the present study has demonstrated that OHE causes elevation in cortisol concentration which is maintained for 2.5 hours. It seems that flunixin meglumine decreases cortisol and NO levels more efficiently than meloxicam. Thus, postoperative stress following OHE in bitches may be prevented by flunixin meglumine administration prior to the surgery.

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