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Research Article

Effects of Butorphanol, Meloxicam and Butorphanol-Meloxicam Combination on Wound Healing after Ovariohysterectomy in Dogs

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ABSTRACT

This was a randomized controlled clinical trial conducted to evaluate the effects of butorphanol, meloxicam and butorphanol-meloxicam combination on wound healing in dogs after ovariohysterectomy. Forty-eight healthy client-owned dogs scheduled for ovariohysterectomy were randomly assigned to four treatment groups of twelve animals each. The treatment groups were designated as B, M, BM and C. Dogs were sedated using acepromazine at 0.1mg/kg intramuscularly. Ten minutes later, induction was achieved by administering propofol at 5mg/kg intravenously. Anaesthesia was then maintained using isoflurane. Routine ovariohysterectomy was performed on each dog and test analgesics administered at the placement of the last skin suture. Dogs in group B received butorphanol at 0.2 mg/kg, group M received meloxicam at 0.2 mg/kg, group BM received butorphanol-meloxicam combination at half the dosage of each drug (0.1 mg/kg butorphanol and 0.1 mg/kg meloxicam), and those in group C, acting as the control, received saline at 0.5ml/10kg body weight. All the test analgesics and placebo were administered subcutaneously. Wound healing was assessed at 24, 48, 72 hours and 8 days, postoperatively using clinical appearance of wounds (swelling, erythema, dehiscence, discharge) and histopathology of wound biopsies (collagen, epithelialization, neovascularization, fibroblasts, macrophages and neutrophils). In this study, parametric variables were analyzed using ANOVA and student t-test while non-parametric variables were analyzed using Kruskal Wallis rank sum test and Mann Whitney test. Statistical significant was set at $P<0.05$. Dogs treated using meloxicam had significantly lower scores for clinical appearance of the wound compared to those under butorphanol ($P=0.03$) and those in the control group ($P=0.02$) but statistically similar scores to dogs under butorphanol-meloxicam combination ($P=0.39$). Dogs in the control group had the highest scores for wound swelling, erythema and dehiscence while those under meloxicam had the lowest scores. Histologically, wound biopsies from dogs under meloxicam and the butorphanol-meloxicam combination had better scores for collagen, epithelialization, neovascularization, fibroblasts, macrophages and neutrophils compared to dogs under butorphanol and those in the control group. Better response to wound healing was elaborated by more wound collagen, better epithelialization and neovascularization, more fibroblasts and gradual diminishing levels of neutrophil and macrophage numbers in dogs treated with analgesics in the postoperative period than in those in the control. This indicates an important interplay between pain, stress response and wound healing in dogs, postoperatively. Thus, to enhance patient comfort and improve on surgical outcome treatment of pain and minimizing perioperative stress is imperative.

Key words: Wound, Ovariohysterectomy, Butorphanol, Meloxicam, Stress-response, Dogs

INTRODUCTION

Wound healing is a normal biological process that consists of four highly integrated and overlapping phases namely homeostasis, inflammation, proliferation, and tissue remodeling or resolution (Gosain and DiPietro, 2004). Studies have shown that delayed wound healing in humans and laboratory animals can be associated with post-operative pain and stress (Padgett *et al.*, 1998; Broadbent *et al.*, 2003). This is usually a biological cycle that starts by postoperative pain causing stress. Stress negatively influences the inflammatory phase of wound healing by reducing pro-inflammatory cytokines, which are supposed to function by attracting phagocytes to the wound site for clearance of infectious agents and for preparation of the site for new tissue growth (Barbul,

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1990; Broadbent *et al.,* 2003). This position is supported by a previous report in mice showing that stress increased susceptibility of wounds to bacterial infection, hence delaying wound healing (Rojas *et al.,* 2002). Stress can also affect the remodeling phase of wound healing by regulating production and activation of matrix metalloproteinase enzymes, which are involved in degradation of collagen as well as facilitation of cellular invasion and migration into the wound (Pajulo *et al.,* 1999; Broadbent *et al.,* 2003).

Assessment of wound healing in veterinary patients can be achieved through clinical appearance, histopathology and ultrasonography (Sylvestre *et al.,* 2002; Abramo *et al.,* 2004; Laiju *et al.,* 2005; Nisbet *et al.,* 2010). Ultrasound scanning of wounds enables repeated, noninvasive, quantitative assessment of structural changes deep within wounds, while histopathological assessment allows more precision but not serial examination of wounds (Abramo *et al.,* 2004). The effects of pain and stress on wound healing following surgery in dogs and the resulting quality of wound healing has not been elucidated. It was therefore considered essential to evaluate these effects by managing pain using single and multimodal analgesic drugs following ovariohysterectomy in dogs. The drugs used for management of pain in this study were butorphanol and meloxicam, either alone or in their combination.

MATERIALS AND METHODS

Study design

This was a prospective randomized controlled study in which dogs were subjected to ovariohysterectomy. The treatments involved postoperative administration of butorphanol, meloxicam, butorphanol-meloxicam combination and a placebo. Monitoring and evaluation of various parameters was done following ovariohysterectomy and administration of analgesics/placebo.

The study animals

Forty-eight entire female dogs were used in the study. The dogs were acquired from clients who presented them to the University of Nairobi, Small Animal Clinic for ovariohysterectomy and were willing to have the dogs included in the study. Once acquired, the dogs were subjected to routine clinical examination to screen them for presence of any diseases. Only dogs free of diseases were selected for the study. They were dewormed (Vermic total®, Microsules laboratories, Uruguay), treated for ectoparasites (Frontline Plus®, Merial, Duluth-Georgia USA) and allowed 14 days to acclimatize to the new environment. During this period, dogs were subjected to weekly clinical examination and regular handling to make them get acquainted with handling and manipulation. The dogs that never accepted easy handling after the acclimatization period were excluded from the study, but were spayed and released to the owners. All the dogs excluded from the study were replaced by recruiting others that were easy to handle.

The dogs were housed individually in kennels at the Department of Clinical Studies and fed on commercial dog feed once per day but water was provided *ad libitum.* The 48 dogs were randomly assigned to 4 treatment groups of 12 dogs each. The groups were randomly generated via computer random number table and designated as B, M, BM and C. The 4 treatment groups are outlined in the treatment sub-section below.

Experimental drugs and dosages

The following analgesics were used in this study at the specified dosages:

a. Butorphanol hydrochloride (Turbusegic®- SA, Zoeitis, New Jersey- USA) (0.2 mg/kg BW) was administered subcutaneously as the test opioid analgesic drug.
b. Meloxicam hydrochloride (Mobic®, Boehringer Ingelheim Pharmaceuticals, Ridgefield, Connecticut, USA) (0.2 mg/kg BW) was administered subcutaneously as the test NSAID analgesic drug.
c. Butorphanol hydrochloride and Meloxicam hydrochloride (0.1 mg/kg and 0.1 mg/kg, respectively) were administered subcutaneously as the test opioid-NSAID drug combination.

In addition, the following drugs were used to facilitate ovariohysterectomy:

a. Acepromazine hydrochloride (Labistress® Labiana Life Sciences SA, Barcelona-Spain) 2 % (0.1 mg/kg BW) administered intramuscularly for sedation.
b. Propofol 1% (Propofol-® Lipuro10mg/ml B-Braun, Melsungen-Germany) (5 mg/kg BW) administered intravenously for induction of anaesthesia.
c. Isoflurane (Forane® Isofluranum, Abbott Laboratires Ltd, Queenborough, Kent England) inhalant anaesthetic for maintenance of anaesthesia during surgery.

Treatment 1: Dogs in group B received butorphanol hydrochloride at 0.2 mg/kg BW, injected subcutaneously.
Treatment 2: Dogs in group M received meloxicam hydrochloride at 0.2 mg/kg BW, injected subcutaneously.
Treatment 3: Dogs in group BM received butorphanol-meloxicam drug combination at half the dosage of each individual drug (i.e butorphanol hydrochloride at 0.1 mg/kg BW and meloxicam hydrochloride at 0.1 mg/kg BW), injected subcutaneously.
Treatment 4: Dogs in group C served as a control and received a placebo in form of sterile saline at a dose rate of 0.5 ml/10kg BW, injected subcutaneously.

Experimental procedure

Food and water were withheld from the dogs 12 hours prior to the surgery as a routine pre-anaesthetic preparation. The dogs were weighed each time immediately preceding the experiments.

All dogs were sedated with acepromazine hydrochloride at 0.1mg/kg BW by intramuscular injection into the lateral thigh muscles. The ventral abdominal region was shaved, scrubbed and 70% ethyl alcohol applied on the site in preparation for aseptic surgery. Propofol at 5mg/kg BW was administered intravenously as a bolus for induction of anaesthesia. After induction, dogs were then intubated for maintenance of anaesthesia with isoflurane vaporized in oxygen, using a rebreathing anaesthesia circuit.

After anaesthesia and preparation, each dog was positioned on a surgical table in dorsal recumbency. The operative site was draped and routine ovariohysterectomy performed. Warm Lactated Ringers solution was administered intravenously (10ml/kg/hour) to each dog throughout the period of anaesthesia until the endotracheal tube was removed. Immediately after placing the last skin suture, the test analgesic drugs were administered as described in treatment sub-section above. All the test analgesic drugs were administered subcutaneously on the dorsal part of the neck. The drug combination was injected as a mixture in the same syringe.

Evaluation of parameters

Following each treatment, surgical wound on every dog was subjected to assessment clinical appearance and histopathology as described below.

Clinical appearance of the surgical wound

Clinical appearance of the surgical wound was scored at 24 hours, 48 hours, 72 hours and day 8 postoperatively. The surgical wound was scored by the investigator based on swelling, erythema, dehiscence, and discharge (exudation) as outlined in Table 1. This scoring system is adapted from Sylvester *et al.*, (2002).

Histopathological evaluation of the surgical wound

Histopathological evaluation was done by taking a biopsy of the surgical wound at 24 hours, 48 hours, 72 hours and day 8 postoperatively. Three dogs were systematically chosen from the 12 dogs in a group at each sampling time (24 hours, 48 hours, 72 hours and day 8). The 3 dogs were anaesthetized as described in the experimental procedure sub-section and a full thickness biopsy (extending from the skin to the peritoneum) of the surgical wound and part of the surrounding tissues collected. The dimensions of the collected biopsy were 1 cm wide and 6 cm long. The wound created after collection of the biopsy was sutured routinely in three layers. Following which meloxicam at 0.2mg/kg subcutaneously was administered once daily for 3 days in all dogs.

The biopsy samples were placed in appropriately labeled eppendorf tubes and fixed in 10% buffered formalin. The samples were then processed routinely, cut and mounted on microscope slides as described by Nisbet *et al.* (2010). The tissue sections were examined under a light microscope and photo-micrographs taken using a digital camera coupled to a microscope.

The histopathology parameters that were assessed are: the population of neutrophils, macrophages and fibroblasts; the extent of neovascularization; collagen lay-down; and epithelialization. Subjective measures/score used in the current study for collagen, epithelialization and fibroblast population as well as the counts for neovascularization in wound healing are as reported by Nisbet *et al.* (2010) and these are given in Table 2

Data management and analysis

Data were entered into Microsoft Office Excel, verified and validated as correct entries based on the data collection sheets. Data were then imported into StatPlus pro 5.9.8 statistical software for computation of means and P values. Statistical significance was set at $P<0.05$.

Non-parametric data were expressed as median and parametric data as means±SD for analysis and comparison within and between the four groups. The median values were compared using the Kruskal-Wallis rank sum test. Where statistical differences were observed, Mann Whitney rank sum test was used as a post-hoc test. Means±SD values were compared using ANOVA for repeated measures. Where significant difference was indicated by ANOVA, a Bonferroni corrected student T-test was applied to determine statistical differences between treatments.

RESULTS

Clinical wound appearance

Wound Swelling

Wound swelling was observed in all dogs in the four treatment groups after ovariohysterectomy. The swelling increased gradually beginning 24 hours postoperatively, with maximal swelling at 48 hours for dogs in butorphanol, butorphanol-meloxicam combination and control groups, but at 72 hours for those in meloxicam group. Measurements of the wound swellings (means±sd) in the four treatment groups are shown in table 3.

Wound swelling was significantly more in dogs treated with butorphanol at 48 hours (3.1±3.2 cm) and 72 hours (3.0±0.0 cm) when compared to the value at 24 hours (0.2±0.4 cm). Wound swelling was still present on day 8 but this was not significant when compared to what was observed at 24 hours. Similar observations were made in dogs treated with the butorphanol-meloxicam combination, in which wound swelling increased significantly from a value of 0.2±0.3 cm recorded 24 hours postoperatively to 3.0±0.0 cm at 48 hours and 2.4±1.6 cm at 72 hours, postoperatively. There was still some swelling on day 8 postoperatively (0.3±0.6 cm), but the swelling was not significantly more than what was observed at 24 hours.

Dogs in the control group had significantly more wound swelling at 48 hours (3.3±1.0 cm), 72 hours (3.3±0.8 cm) and on day 8 (2.4±0.5 cm) postoperatively, compared to a value of 0.7±1.2 cm recorded at 24 hours postoperatively. In dogs treated with meloxicam, wound swelling increased relatively from a baseline value of 0.1±0.2 cm, reaching a peak of 2.0±2.7 cm at 72 hours postoperatively, but reducing to zero (no swelling at all) by day 8, postoperatively.

There were no significant ($P=0.32$) differences in mean wound swelling between the treatment groups. However, among the dogs treated with analgesics, the least wound swelling was in the meloxicam-treated group (0.7±0.9 cm) and the most was in the control group (2.4±1.2 cm). (Figure 1).

Wound Erythema

Wound erythema was a clinical feature observed in all dogs in the four treatment groups. Generally, wound erythema was observable from 24 hours postoperatively, and its extent increased with increasing time such that at 48 hours and 72 hours, the extent was relatively more than what was observed at 24 hours postoperatively (Table 3). The most extensive wound erythema was observed in the butorphanol-treated group, where it increased from a baseline value of 0.10±0.2 cm at 24 hours to a value of

0.64±0.6 cm at 72 hours, postoperatively. The least extent of wound erythema was observed in the meloxicam-treated group where its size increased from a baseline value of 0.08±0.2 cm at 24 hours to a value of 0.17±0.4 cm at 48 hours, postoperatively. Dogs in the butorphanol-meloxicam group and those in the control group had a moderate extent of wound erythema. In dogs treated with meloxicam, erythema peaked at 48 hours, while in dogs treated with butorphanol, butorphanol-meloxicam and those in the control group, wound erythema was at its peak at 72 hours (Table 3). In dogs treated with meloxicam, wound erythema had cleared completely by day 8, postoperatively.

When the extent (means±sd) of wound erythema in dogs in the four treatment groups was compared, it was established that dogs treated with meloxicam had significantly less extensive wound erythema (0.08±0.1 cm) as compared to that observed in dogs in the control group (0.59±0.3 cm). (Figure 2)

Wound Dehiscence

More wound dehiscence (as measured by the percentage suture removal) was observed in dogs in the control group (24.8±16.9%), followed by dogs in the butorphanol group (14.3±13.2%), then meloxicam group (6.4±7.9%) and butorphanol-meloxicam drug combination group (2.3±1.9%) [Table 3, Figure 3 and Figure 4]. However, there was no significant difference in wound dehiscence between the treatment groups (P=0.07).

Histopathological findings of the wounds

Collagen score

There was a significant difference in the median collagen score from 24 hours through to 8 days postoperatively in all the treatment groups as shown in table 4. The amount of collagen in wounds of butorphanol-treated dogs and those in the control group, was significantly (P<0.05) higher at 72-hour and day 8 monitoring time-points (Score 2) as compared to baseline score (Score 0). For wounds in meloxicam treated dogs, the amount of collagen increased significantly (P<0.05) from a baseline score of 1 to a median score of 2 at 48-hour period and to median score of 3 at 72-hour as well as day 8 of monitoring. Butorphanol-meloxicam drug combination-treated dogs also had the amount of collagen in their wounds increasing significantly ($p < 0.05$) to a median score of 3 at 72-hour and day 8 of monitoring, from a median score of 1 at 24 hours postoperatively.

Epithelialization score

There were no significant differences in the levels of wound epithelialization in dogs across the four treatment groups. However, median epithelialization scores increased towards day 8 of monitoring postoperatively and the epithelialization was relatively more complete in wounds of dogs treated with butorphanol, meloxicam and butorphanol-meloxicam drug combination as compared to those in the control group (Table 4).

Neovascularization

The number of blood vessels in wounds of dogs treated with butorphanol increased significantly from baseline values (median score of 0) through to day 8 (score of 3), postoperatively. In dogs treated with the butorphanol-meloxicam drug combination, the wound neovascularization increased significantly (P<0.05) from baseline values through to day 8 postoperatively (median score of 1 to median score of 2 at 72 hours and median sore of 3 at day 8). Comparisons of neovascularization scores between the treatment groups did not reveal any significant differences.

Fibroblasts

The number of fibroblasts in wounds of dogs in the control group increased significantly (P<0.05) in the control group from the baseline values through to day 8 postoperatively (score 1 and to score 2 at 72 hours and at day 8 of postoperatively). The fibroblast scores at various monitoring time-points in wounds of dogs treated with butorphanol, meloxicam and butorphanol-meloxicam drug combination were not significantly different from their respective baseline values (Table 4).

Table 1: Parameters used as criteria for scoring the appearance of surgical wounds in dogs

Parameters	Descriptions
Swelling	Wound edges thicker than the surrounding tissues. Measurement from cranial, mid and caudal section of the wound to be taken and averaged to get the final wound swelling score.
Erythema	Redding of the skin around the wound. Measure the distance from the wound margins. Measurement from cranial, mid and caudal section of the wound to be taken and averaged to get the final erythema score.
Dehiscence	Percentage of sutures removed by the dog. Record taken of the total number of sutures used to close the skin incision. At each examination period, record the number of sutures removed. Calculate the percentage of sutures removed in each dog.
Discharge	Any serous, serosanguinous and purulent discharge observed from the surgical wound at each examination period recorded.

Table 2: Scoring system for histopathological tissues evaluation of various parameters

Parameter	Score			
	0	1	2	3
Collagen	None	Scanty	Moderate	Abundant
Epithelialization	None	Partial	Complete but immature/thin	Complete and mature with keratinization
Neovascularization	None	Up to 5 vessels/ HPF	6-10 vessels/ HPF	>10 vessels/ HPF
Fibroblast	None/minimal	Few	Moderate fibroblast	Predominant
Macrophages	None	Up to 20 macrophages/ HPF	20-40 macrophages/ HPF	>41 macrophages/ HPF
Neutrophils	None	Few	Moderate number	Predominant

Key: HPF-High Power Field.

Table 3: Clinical wound appearance in dogs treated with butorphanol, meloxicam, butorphanol-meloxicam drug combination and in the control group after ovariohysterectomy.

Clinical Features	Observation time-points	Treatment Groups			
		Butorphanol	Meloxicam	Butorphanol-Meloxicam	Control
Wound Swelling (in centimeters)	24 Hours	0.2±0.4	0.1±0.2	0.2±0.3	0.7±1.2
	48 Hours	3.1±3.2*	0.6±0.6	3.0±0.0*	3.3±1.0*
	72 Hours	3.0±0.0*	2.0±2.7	2.4±1.6*	3.3±0.8*
	08 Days	0.8±0.7	0.0±0.0	0.3±0.6	2.4±0.5*
Wound Erythema (in centimeters)	24 Hours	0.10±0.2	0.08±0.2	0.04±0.1	0.39±0.9
	48 Hours	0.41±0.4	0.17±0.4	0.34±0.5	0.75±0.7
	72 Hours	0.64±0.6	0.08±0.1	0.53±0.7	0.90±0.9
	08 Days	0.33±0.4	0.00±0.0	0.26±0.3	0.33±0.6
Wound Dehiscence (% of sutures removed)	24 Hours	2.8±8.3	0.4±1.4	2.0±6.3	3.3±10.5
	48 Hours	11.1±18.2	8.3±17.7	4.6±8.5	23.8±37.1
	72 Hours	10.0±22.4	16.7±40.8	2.8±6.8	27.8±39.0
	08 Days	33.3±57.7	0.0±0.0	0.0±0.0	44.4±50.9

KEY: *Indicate value is significantly higher compared to the respective 24-hour value

Table 4: Median scores for histopathological parameters evaluated in dogs treated with butorphanol, meloxicam, and butorphanol-meloxicam drug combination and in the control group after ovariohysterectomy.

Histopathological Parameters	Assessment Time-point	Treatment groups			
		Butorphanol	Meloxicam	But-Mel	Control
Collagen	24 Hours	0	1	1	0
	48 Hours	1	2*	1	0
	72 Hours	2*	3*	3*	2*
	08 Days	2*	3*	3*	2*
Epithelialization	24 Hours	2	2	2	1
	48 Hours	2	2	2.5	1
	72 Hours	2	2	3	2
	08 Days	2.5	3	3	3
Neovascularization	24 Hours	0	2	1	0
	48 Hours	1	2	2	1
	72 Hours	1.5	2.5	2*	1
	08 Days	3*	3	3*	1.5
Fibroblasts	24 Hours	1	1	0.5	1
	48 Hours	2	2	1.3	1
	72 Hours	2	3	2	2*
	08 Days	2	3	3	2*
Macrophages	24 Hours	2	2	1	0.5
	48 Hours	3	3	3	2
	72 Hours	1	2	2	2
	08 Days	1	1	1	2
Neutrophils	24 Hours	2	1	2	1
	48 Hours	1	1	1	3
	72 Hours	0*	0	0*	1
	08 Days	0*	0	0*	1

KEY: *indicates that the value is significantly different at $p < 0.05$ compared to the 24-hour baseline value.

Fibroblasts

The number of fibroblasts in wounds of dogs in the control group increased significantly ($P<0.05$) in the control group from the baseline values through to day 8 postoperatively (score 1 and to score 2 at 72 hours and at day 8 of postoperatively). The fibroblast scores at various monitoring time-points in wounds of dogs treated with butorphanol, meloxicam and butorphanol-meloxicam drug combination were not significantly different from their respective baseline values (Table 4).

Macrophages

The number of macrophages in wounds of dogs generally increased from their baseline values to reach the peak at 48 hours postoperatively then declined towards day 8 of monitoring, in all treatment groups, except for those in the control group. In the control group, the median macrophage score increased from a baseline value of 0.5 to a score of 2 at 48 hours and remained at that level through day 8 of monitoring (Table 4). When compared, all these changes in macrophage in all the treatment groups were not significant.

Neutrophils

There was significant decrease ($P<0.05$) in the number of neutrophils (median neutrophil score) in wounds of dogs treated with butorphanol and butorphanol-meloxicam. In these two groups, median neutrophil score decreased from a baseline score of 2 to score 0 at 72-hour and day 8, postoperatively. Unlike in the other groups where an initial decrease was observed, the number of neutrophils (neutrophil score) in the control group increased from a median score of 1 recorded at 24 hours to 3 at 48 hours after surgery. Thereafter, the neutrophil count started to decrease and reached score 1 at 72 hours and remaining so up to day 8, postoperatively (Table 4).

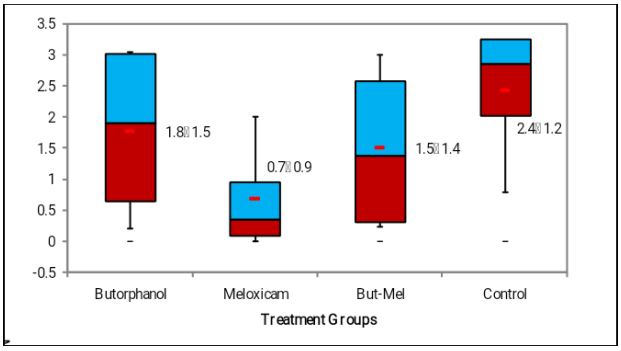

Fig. 1: Mean wound swelling in dogs treated with butorphanol, meloxicam, butorphanol-meloxicam drug combination and in the control group after ovariohysterectomy. Key: But-Mel = Butorphanol-Meloxicam combination.

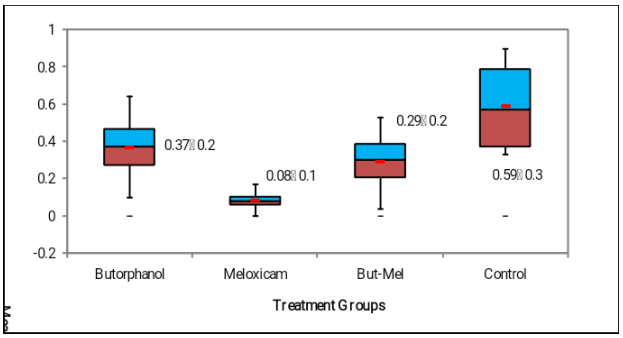

Fig. 2: Wound erythema in dogs treated with butorphanol, meloxicam, butorphanol-meloxicam drug combination and in the control group after ovariohysterectomy. Key: But-Mel = Butorphanol-Meloxicam combination.

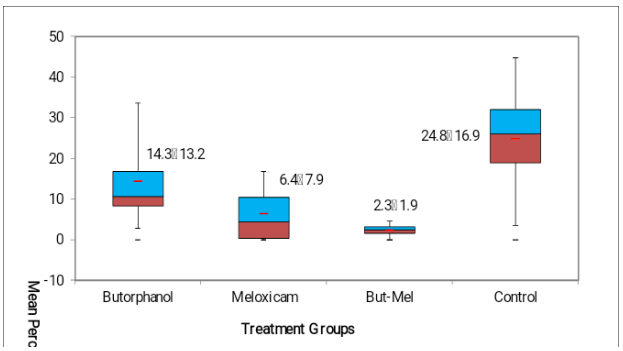

Fig. 3: Mean percentage suture removal (by dogs themselves) in dogs treated with butorphanol, meloxicam, butorphanol-meloxicam drug combination and in the control group after ovariohysterectomy. Key: But-Mel = Butorphanol-Meloxicam combination.

Changes in the amount of collagen, degree of epithelialization, neovascularization, numbers of fibroblast, macrophages and neutrophils in the wounds of dogs were not significantly different when compared between the four treatment groups at all sampling time-points, postoperatively.

DISCUSSION

The results of this study indicate no significant difference in the clinical effects of the individual drugs, butorphanol and meloxicam and their combination, on wound healing in dogs following ovariohysterectomy. The butorphanol-meloxicam combination only showed a slight advantage over butorphanol on its own. The finding that meloxicam-treated dogs had significantly less extensive wound erythema, less swelling and dehiscence than those that were treated with butorphanol as well as those in the control group can be explained by meloxicam's preferential blockade of cyclooxygenase 2 (COX-2) enzyme, which results in antipyretic, analgesic and anti-inflammatory effects (Lee *et al*., 1991; Mathew, 1996).

Oedema and soft tissue swelling that characterize inflammation, continuously stimulate nerve endings as well as nociceptors and cause increasingly more pain and stress. Inflammation-related pain can also be caused by production of neuropeptides that include substance P, neurokinin A, bradykinin and prostaglandins (Woo, 2012). The pain is likely to cause wound mutilation and pulling out of sutures by the dog, resulting in wound dehiscence, contamination and possible infection. Hence, the reason for the more extensive wound erythema and increased dehiscence in the wounds of dogs in the control group compared to those in groups treated with analgesics. This finding further shows the advantage of pain management in enhancing postoperative wound healing.

The finding that the combination of butorphanol and meloxicam did not demonstrate any significant additive benefit over the individual drugs, can probably be attributed to the small number of dogs per group which was low for detection of minor inter-group differences as previously observed (Tsai *et al*., 2013). It could also be due to failure of butorphanol and meloxicam to exert their maximal effects on pain (Tsai *et al*., 2013), which may probably be attributable to use of half of their individual dosages when the two analgesics were combined. The fact that meloxicam has more anti-inflammatory effects than butorphanol explains the lower scores of clinical wound parameters in butorphanol-meloxicam combination-treated dogs than in those treated with butorphanol.

The persistent slightly high neutrophil and macrophage counts in the control group indicated that inflammation phase remained fairly active in the wound tissues throughout to day 8 postoperatively, compared to that in dogs treated with the analgesic drugs, in which these inflammatory cells diminished towards the 72-hour and day 8 of evaluation. Inflammation is essential for wound healing with neutrophils and macrophages functioning at the local wound-level to destroy bacteria and debride the wound in preparation for neovascularization and regeneration (Walburn *et al*., 2009). These cells also release substances such as interleukin-1 (IL-1α, IL-1β), interleukin-6, interleukin-8, tumor necrosis factor and matrix metalloproteinases that are vital for tissue healing (Loo *et al*., 2007). However, studies have shown that excessive and prolonged inflammation causes significant delay in wound healing (Kiecolt-Glaser *et al*., 1995; Padgett *et al*., 1998; Mercado *et al*., 2002). Moreover, studies suggest that the main factor influencing inflammation-related delay in wound healing is neutrophilia (Sroussi *et al*., 2009). This is due to consumption of large amounts of oxygen during neutrophil activation, which when coupled with low blood supply contributes to wound hypoxia (Gajendrareddy *et al*., 2005; Sroussi *et al*., 2009) and these consequently delay wound healing.

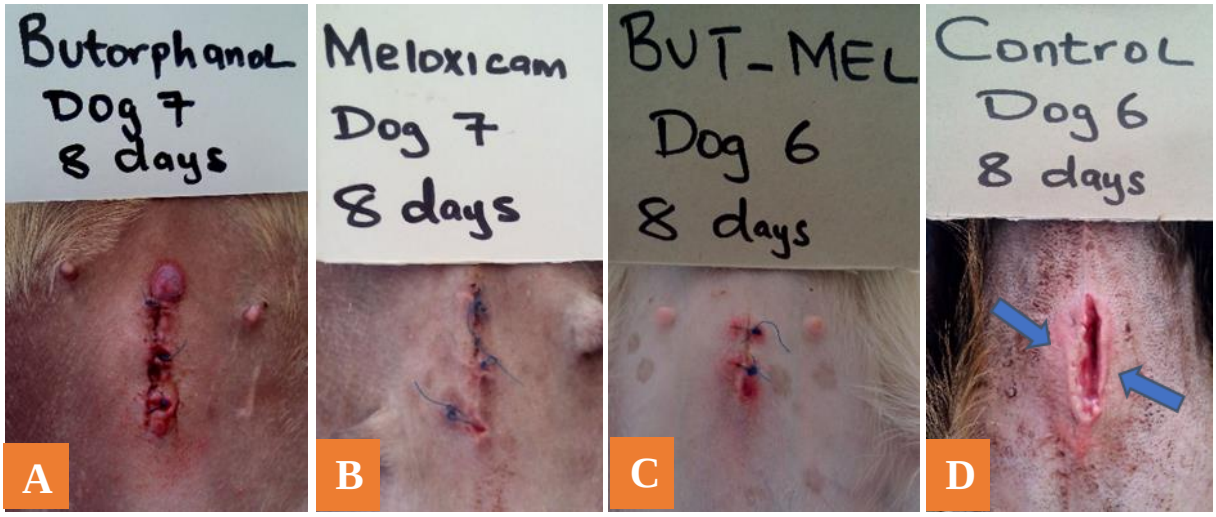

Fig. 4: Pictorial representation of the surgical wounds in the four treatment groups 8-days postoperatively. **A** demonstrates wound swelling in a dog treated with butorphanol, **B** demonstrates a wound in one of the dog under meloxicam that had healed without dehiscence, erythema or swelling, **C** demonstrates slight wound erythema in a dog treated with butorphanol-meloxicam combination and **D** demonstrate complete wound dehiscence from a dog in the control group. Also notice the wound swelling (Blue arrows).

The better scores for fibroblasts, epithelialization, neovascularization and collagen in the wounds of meloxicam-treated and butorphanol-meloxicam combination-treated dogs than in butorphanol-treated and control group dogs, suggest that the former analgesia protocols have more effective pain management outcomes than the latter. This also suggests that when pain is well managed, stress is minimized and subsequently wound healing would be faster and possibly of more superior quality. The effects of analgesic pain management on histopathologic responses of operative wounds have not been reported previously in dogs. The mechanisms through which pain and associated stress may negatively affect wound healing have been described (Woo, 2008 and Woo, 2012). This includes response to painful stimuli by C sensory nerve fibers to release neuropeptides like substance P, which activate leukocytes and other immunoactive cells, such as glial cells to release pro-inflammatory cytokines. These proinflammatory cytokines have been shown to play a role in augmenting pain signals and stress response.

Consequent to stress, there is overproduction of glucocorticoids, specifically cortisol and catecholamines through stimulation of ACTH on the anterior pituitary gland and adrenal medulla (Blackburn-munro, 2004; Bomholt *et al*., 2004). These hormonal changes negatively affect wound healing as a result of changes in immune system as well as the resulting tissue hypoxia (Kiecolt-Glaser *et al*., 1995).

Slow healing of dermal biopsy wounds was observed in human patients with higher cortisol levels Ebrecht *et al*., 2004). Furthermore, the relationship between stress and skin barrier recovery from damage caused by tape stripping was found to be significant in a study carried out in human subjects, indicating that high stress level, slowed the skin barrier recovery rate (Garg *et al*., 2001).

Glaser *et al.* (1999) examined psychological stress and the levels of pro-inflammatory cytokines in experimentally induced skin blisters on the forearm of 36 women. Women who reported more stress on the Perceived Stress Scale produced significantly lower levels of interleukin-1 and interleukin-8. Kiecolt-Glaser *et al.* (1995) demonstrated that the rate of complete biopsy punch wound closure increased by 24% or 9 days longer in caregivers stressed from providing care for their relatives with Alzheimer disease compared to those in control group. Further, blood leukocytes from stressed caregivers exhibited a diminished ability to express interleukin-1 gene in response to lipopolysaccharide stimulation *in vitro*. Broadbent *et al.* (2003) investigated the relationship between psychological stress and wound repair in 36 patients following inguinal hernia operation. They reported that perceived stress before the operation was a significant predictor of low interleukin-1 levels in wound fluids accounting for 17% of the variance. In contrast, worry about the operation significantly predicted lower levels of matrix metalloproteinase 9 in the wound fluid as well as increased pain over the first 20-hours postoperatively. Interleukins play an important role of protecting the host against infection and preparing injured tissue for repair by enhancing phagocytic cell recruitment and activation (Glaser and Keicolt-Glaser, 2005).

This study has in previous chapters also demonstrated higher cortisol, glucose and neutrophil-lymphocytes ratio levels in dogs under control group as compared to those in butorphanol, meloxicam, and butorphanol-meloxicam combination groups. This further reinforces the important interaction and the negative impact of stress response on wound healing, considering that dogs in the control groups had poor wound healing parameters. Thus to enhance patient comfort and improve on surgical outcome treatment of pain and minimizing perioperative stress is imperative.

Conclusions

The following conclusions can be made from the current study: There was no significant difference in wound healing response between butorphanol-meloxicam drug combination-treated dogs and those treated with either meloxicam alone or butorphanol alone. Despite this, the butorphanol-meloxicam drug combination gave better wound healing outcome than butorphanol alone.

Better response to wound healing was elaborated by more wound collagen, better epithelialization and neovascularization, more fibroblasts and gradual diminishing levels of neutrophil and macrophage numbers in dogs treated with analgesics in the postoperative period than in those in the control. This indicates an imperative interplay between pain, stress response and wound healing in dogs, postoperatively.

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