IRANIAN JOURNAL OF VETERINARY SURGERY (IJVS)
WWW.IVSA.IR

Comparison the Efficacy of Meloxicam and Ketoprofen in Alleviating Pain Following Ovariectomy in Rats

Azin Tavakoli, DVSc
Ali Akbar Shabannia, DVSc
Leila Mohammadyar*, PhD

Faculty of Veterinary Medicine, Islamic Azad University-Garmsar branch, Garmsar, Iran

Abstract

Objective: The purpose of this study was to compare the efficacy of two NSAIDs, meloxicam and ketoprofen in controlling post-operative pain following ovariectomy in rats.
Study design: Experimental study
Animals: Twenty one healthy and adult female Sprague-Dawley rats weighting approximately 200 gr.
Methods: Intramuscular injection of the combination of xylazine (10 mg/kg) and ketamine (75 mg/kg) was used to induce general anesthesia in all rats. Then the selected rat was assigned for a group prior to surgery in the following order: Group A, received no extra analgesic, group B, received subcutaneous injection of 5 mg/kg ketoprofen at the time of induction of anesthesia and group C received subcutaneous injection of 2 mg/kg meloxicam (7.5 mg/ml) at the time of induction of anesthesia. Then ovariectomy was performed through two dorso-lateral abdominal incisions. Score of pain at 2, 4 and 24 hours after the surgery using VAS, serum levels of cortisol and CPK at 2 hours after the surgery were evaluated in all rats.
Results: The score of pain was significantly different in group A, compared to group B and C (P=0). Also the concentration of cortisol was significantly increased in all groups following the surgery. This increase was significant in group A compared to group B and C ($P<0.05$). Although CPK concentration was increased significantly in all groups after the surgery, no significant correlation was found among groups.
Conclusion: It is concluded that either meloxicam or ketoprofen have similar effects in controlling pain following ovariectomy in rats.
Key words: meloxicam, ketoprofen, ovariectomy, rat

* **Corresponding author:**
Leila Mohammadyar, DVM, DVSc
Department of Basic Science, Faculty of Veterinary Medicine, Islamic Azad University-Garmsar branch, Garmsar, Iran
Email: leilamohammadyar@iau-garmsar.ac.ir

Introduction

The use of analgesics for pain management is an integral part of clinical veterinary medicine while paying attention to animals' welfare.[1]Therefore different drugs and their combinations have been used to help prevent or reduce pain. Opioids are currently known as the most powerful agents to alleviate postoperative pain in small animals. However because of variety of adverse effects like respiratory depression, bradycardia, hypotension, dysphoria, abnormal behaviors and also limitations of prescription in many rural clinics, the use of other non-opioid analgesics considered as an alternative.[2] Both meloxicam and ketoprofen are non-steroid anti-inflammatory drugs (NSAID) that possess analgesic properties and are clinically being used frequently in veterinary medicine including mice and rats.[3] They exert their analgesic effects by inhibiting cyclooxygenase enzyme (COX) which results in decreased production of prostaglandin that causes pain. Although meloxicam is a COX-2 preferential NSAID, ketoprofen is a nonselective COX inhibitor. Evidence suggests that COX- 2 is the dominant COX isoform in the spinal cord and is associated the recognition of pain by central nervous system during inflammation. In addition they are less harmful to renal and gastrointestinal system. Therefore the use of the drugs that have more affinity to COX-2 is preferred than the drugs without any selective activity to COX due to their less side effects.[4, 5] The objective of this study was to compare the efficacy of two non-opioid analgesics, ketoprofen and meloxicam in controlling pain following ovariectomy in rats.

Material and methods

Animals

Twenty one healthy and adult female Sprague-Dawley rats, weighting 200 gr (180±30 gr) were used in this experiment after the approval received from the University Research Committee in accordance with the guidelines of its Institutional Animal Experimentation Ethics Committee. The rats kept in cages in a similar condition at the ambient room temperature with a 12 h-day/night cycle. They were feed with the standard laboratory pellets and allowed free access to water. The rats used in this experiment were handled for 15 min/day for a 2-week period before the tests in order to preclude stress-induced analgesia during the study. The intramuscular injection of the combination of 10 mg/ kg xylazine (Rampon®, 2% Bayer, Germany) and 75 mg/kg ketamine (Ketalar®, 10%, Alfasan, Woerden, Holland) was used to induce general anesthesia in all rats. Then the selected rat was assigned for a group prior to surgery in the following order:
Group A: Received no extra analgesic as a control group.
Group B: Received subcutaneous injection of 5 mg/kg ketoprofen (10%, Ketophen, Razi, Iran) at the time of induction of anesthesia for analgesia.
Group C: Received subcutaneous injection of 2 mg/kg meloxicam (7.5 mg/ml, Boehringer-Ingelheim, Germany) at the time of induction of anesthesia for analgesia.

Surgical Technique

All surgeries were performed by the same surgeon. Following induction of general anesthesia, the animal was positioned to dorsal recombency and the abdominal area was prepared for aseptic surgery. The locations of the ovaries in rats were first detected on a cadaver. A one cm dorso-lateral skin incision of the flank was made sharply by scissors. The incision was

approximately near the inguinal area right over the ovary. Then the underneath abdominal muscles dissected bluntly. The ovary was found at the cranio-lateral border of the incision. If the ovary could not be located, a uterus horn was followed cranially to reach the ovary. The connection of the oviduct and uterine horn was found and a ligature of 3-0 polyglyconate was used to ligate the ovarian vessels. Then the connection was cut, ovary was removed and the pedicle checked for hemorrhage. The muscles bring back together without sutures, but either a cruciate suture or two simple interrupted sutures were used to close the skin incision with 4-0 polyamide. The similar procedure was repeated for the other ovary. (Fig 1& 2).

Figure 1. Ovary and oviduct with the associated fat tissues is being ligated.

Figure 2. The skin incision is closed using two simple interrupted sutures.

Measured parameters

The surgical time, intra and post-operative complication was recorded in all rats. Serum level of cortisol and creatine phosphokinase (CPK) was assessed prior to surgery and 2 hours after the surgery. Video recording of each rat was done before, 2 hours, 4 hours and 24 hours after the surgery. A trained observer scored pain in all rats at the recorded times by reviewing recorded movies using Visual Analogue Scale (VAS).

Statistical analysis

Single point measurements, at any particular times, were analyzed using repeated measure ANOVA. Kruskal-Wallis test was implemented as a post hoc test using SAS. Changes in parameters over time were analyzed using GLM procedure in SAS, including repeated measures in the model. A spearman's rank correlation coefficient was used to identify any relation between concentration of cortisol and cpk. Data were presented as Mean±SEM. A value of $P < 0.05$ was considered significant.

Results

Mean±SEM duration of surgery was 4.2 ± 0.56 minutes in all rats. Two rats died due to severe hypothermia after the surgery and excluded from the study. The score of pain was significantly higher in group A, compared to group B and C at any particular time (P=0). However it does not differ significantly between group B and C (P=0.77) (Table 1). The

highest pain scores animal suffered was in 2 hours after surgery which were 5, 4 and 3 in group A, B and C respectively. Also the highest pain scores observed 4 hours after surgery (5, 3 and 3 in group A, B and C respectively). The animals observed to be free of pain 24 hours following ovariectomies.

Table 1. Comparing Mean scores of pain among groups of the study at different times following ovariectomy in rats.

Groups	2 hours after the surgery	4 hours after the surgery	24 hours after the surgery
Group A (control)	4^{Aa}	4^{Aa}	No pain
Group B	3^{Ba}	2^{Ba}	No pain
Group C	2^{Ba}	1^{Bb}	No pain

^{ABC} Values within columns with different superscripts differ (P<0.05).
^{abc} Values within rows with different superscripts differ (P<0.05).

Results regarding serum levels of cortisol indicated that the concentration of cortisol increased after the surgery in all groups. This increase was significantly higher in group A compared to group B and C (P<0.05). However no significant difference was recorded between group B and C (*P*=0.07). There was no correlation in concentration of CPK among groups, although CPK was increased significantly within groups following surgery. No significant correlation detected between the concentration of cortisol and CPK prior and after surgery (*P*>0.05). (Table 2).

Table 2. Comparing serum levels of cortisol (µg/dl) and CPK (IU/l) (Mean±SEM) among groups of the study before and 2 hours after the surgery.

Groups	Cortisol (µg/dl)		CPK (IU/l)	
	Before the surgery	2 hours after the surgery	Before the surgery	2 hours after the surgery
Group A (control)	2.6 ± 0.76^{Aa}	5.7 ± 1.14^{Bb}	636.8 ± 279^{Dd}	3845.1 ± 310^{Ee}
Group B	2.5 ± 0.33^{Aa}	4.48 ± 0.73^{Cc}	1993.1 ± 180^{Dd}	5920.4 ± 1940^{Ee}
Group C	1.8 ± 0.16^{Ab}	4.2 ± 0.79^{Cc}	751 ± 245^{Dd}	4140.4 ± 270^{Ee}

^{ABC} Values within columns with different superscripts differ (P<0.05).
^{abc} Values within rows with different superscripts differ (P<0.05).

Discussion

Rats are principal laboratory animals that are used widely to study osteoporosis as an experimental model for humankind. Ovariectomy is considered to be the procedure that gives reliable model of osteoporosis. [6] Also it produces mild to moderate postoperative pain and is used as a standard surgery to assess pain beside ovariohysterectomy in animals.[2] The technique used in this study to perform ovariectomy was the one explained by Lasota et al. in 2004 to be superior to other techniques.[6] The score of pain and serum level of cortisol was significantly different in group A compared to group B and C. This result insisted on the use

of analgesics, which are not only effective but also necessary to alleviate pain following the surgery in laboratory animals like rat.

Ketamine and xylazine that used in this experiment to induce analgesia, reported to have no analgesic properties after 15 to 30 minutes following one administration.[7] So because the combination was given once to each animal, it is expected to have similar effect on the results. The analgesics were administered at the time of induction of anesthesia in this experiment. It may take 30 to 60 minutes for NSAID to exert their analgesic effects.[8] By considering 15 to 30 minutes analgesic duration of the combination of ketamine and xylazine, the use of either meloxicam or ketoprofen as analgesic at the time of inducing anesthesia seems to be appropriate. Cortisol concentration was used to interpret post-operative pain in this study. Serum cortisol concentration is recognized as one of the most objective criteria for pain assessment in animals and found to have direct relation with post-operative pain[9]. Significant increase of cortisol following the surgery indicated that the animals in all groups suffered from pain disregarding the use of analgesia. But this increase was higher in group A. However no significant difference observed between the use of meloxicam and ketoprofen. The increase in CPK has been shown to occur in response to anesthesia as well as with intramuscular injections.[10, 11] CPK also increased by skeletal muscle injury and recumbency. Therefore, it was considered as unreliable pain indicator in dogs.[10, 12] These findings could suggest that CPK may not be a good pain indicator in rats as well. No correlation was found between groups and within groups after ovariectomy in this regard. Therefore the result indicated that CPK could not be a reliable indicator of pain in rats. This finding needs specific investigation on serum levels of CPK in rats following the surgery.

Mathews et al compared the analgesic efficacy and safety of meloxicam with ketoprofen and butarphanol in dogs undergoing abdominal surgery.[13] They reported that analgesic effects of meloxicam is comparable to ketoprofen and superior to butarphanol. Also Baba et al. studied the efficacy of ketoprofen and meloxicam to manage post-operative pain following castration in dogs and reported that both drugs have equal effects in controlling pain.[14] Similar result was obtained in this experiment in a rat model. Therefore being a COX-2 preferential did not influence the analgesic efficacy of meloxicam.

It is concluded that the administration of meloxicam or ketoprofen to control post-operative pain following ovariectomy in rats has similar effect without any clinical priority. Also to obtain a right time analgesia, administration of these two analgesics at the time of inducing anesthesia is recommended.

References

1. Kurt K, Sladky A. Evaluation of epidural morphine for postoperative analgesia in ferrets. *Am Assoc Lab Anim Sci* 2000; 6:33-38.
2. Tavakoli A, Kazime Mehrjerdi H, et al., Analgesic Effects of Metoclopramide Following Conventional Ovariohysterectomy in Bitches. *IJVS* 2009; 4(1 &2): 77-84.
3. Montoya L, Ambros L, Kreil V, et al. A paharmacokinetic comparison of meloxicam and ketoprofen following oral administration to healthy dogs. *Vet Res Commu* 2004; 28: 415-428.
4. Canduz B, Aktug H, Maviog˘lu O, et al. Epidural lornoxicam administration – innocent. *J Clinic Neurosci*. 2007; 14: 968–974.
5. Karnik PS, Johnston S, Ward D, et al. The effects of epidural deracoxib on the ground reaction forces in an acute stifle synovitis model. *Vet Surg*. 2006; 35(1):34-42.

6. Lasota A, Danowska-Klonowska D. Experimental osteoporosis- different methods of ovariectomy in female white rats. In Proceedings *Annales Academiae Medicae Bialostocensis* 2004; 49: 129-131.
7. Gross ME. Drugs acting on central nervous system. In: Veterinary Pharmacology and Therapeutics. Adams HR, 8th eds. Blackwell Publishing Professionals, Lowa, 2001:318.
8. Mathews KA. Pain assessment and general approach to management. *Vet Clin North Am* 2000; 30: 729-755.
9. Short C.E. Pain in animals. In : Wall P D and Melzack R (eds) Textbook of Pain , 4th eds, Churchill Livingstone : Edinburgh , UK . 1999: 1932-1944.
10. Austin B, Lanz O, Hamilton SM, et al., Laparoscopic ovariohysterectomy in nine dogs. *J Am Anim Hos Assoc* 2003; 39:391-396.
11. Aktas BM, Vinclair P, Autefage A, et al: In vivo quantification of muscle damage in dogs after general anaesthesia with halothane and propofol. *JSAP* 1997; 38:565-569.
12. Hancock RB, Lanz OI, Warldon DR, et al. Comparison of postoperative pain following ovariohysterectomy via harmonic scalpel assisted laparoscopy versus traditional celiotomy in dogs. *Vet Surg* 2005; 34: 273-282.
13. Mathews KA, Pettifer G, Foster R, et al. Safety and efficacy of preoperative administration of meloxicam, compared with that of ketoprofen and butorphanol in dogs undergoing abdominal surgery. *Am J Vet Res* 2001 62(6):882-8.
14. Baba MA, Fazili MR, Molvi BA, et al. Comparison of the analgesic effects of meloxicam with those of ketoprofen in male stray dogs undergoing pinhole castration. *Asi J Anim Sci* 2012; 6(4): 164-173.

چکیده

مقایسه تاثیر کتوبروفن و ملوکسیکام در کاهش درد پس از عمل جراحی برداشت تخمدان در رت

آذین توکلی ، علی اکبر شعبان نیا، لیلا محمدیار*

[1]گروه علوم درمانگاهی، دانشکده دامپزشکی دانشگاه آزاد اسلامی، واحد گرمسار، گرمسار، ایران.

هدف- هدف از این مطالعه مقایسه میزان تاثیر دو داروی ضد التهاب غیر استرویدی در کنترل درد پس از عمل جراحی برداشت تخمدانها در رت می باشد.

مطالعه- مطالعه تجربی

حیوانات- ۲۱ سر رت سالم ماده با وزن تقریبی ۲۰۰ گرم.

مواد و روش کار- از تزریق عضلانی ترکیب دارویی زایلازین (۱۰ میلی گرم بر کیلوگرم) و کتامین (۷۵ میلی گرم بر کیلوگرم) جهت القای بیهوشی در رتها استفاده شد. سپس رت بیهوش شده به صورت تصادفی در یکی از گروهها به ترتیب زیر قرار گرفت: گروه A، هیچ ضد درد اضافه ای دریافت ننمود. در گروه B و C به ترتیب کتوبروفن (۵ میلی گرم بر کیلوگرم) و ملوکسیکام (۲ میلی گرم بر کیلوگرم) به عنوان ضد درد در زمان القای بیهوشی به صورت زیر جلدی تزریق شد. سپس جراحی اوریکتومی از محل برش شکمی- جانبی انجام شد. امتیاز درد پس از عمل در زمانهای ۲، ۴ و ۲۴ ساعت پس از جراحی به روش VAS ، میزان کورتیزول و کراتین فسفوکیناز سرم نیز در قبل و ۲ ساعت پس از جراحی اندازه گیری شد.

نتایج- امتیاز درد پس از عمل به صورت معنی داری در گروه A، بیشتر از گروه B و C بود.(P=0). همچنین میزان کورتیزول سرم پس از عمل جراحی به صورت معنی داری در گروه A بیشتر از گروه B و C افزایش یافته بود. هر چند این تغییر بین دو گروه B و C فاقد اختلاف معنی داری بود (P>0.05). اگر چه میزان کراتین فسفوکیناز پس از جراحی به صورت معنی داری بیشتر از قبل از جراحی بود، اما میزان آن بین گروهها فاقد اختلاف معنی داری بود. (P>0.05)

نتیجه گیری- دو داروی کتوبروفن و ملوکسیکام تاثیر مشابهی در کاهش درد پس از عمل در جراحی اوریکتومی در رتها دارند.

کلمات کلیدی- ملوکسیکام، کتوبروفن، اوریکتومی، رت