PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
DOUTORADO EM FISIOPATOLOGIA E SAÚDE ANIMAL

GABRIEL DE OLIVEIRA LIMA CARAPEBA

BLOQUEIO INFILTRATIVO COM ROPIVACAÍNA ISOLADA E ASSOCIADA AO MELOXICAM EM GATAS SUBMETIDAS À OVARIOSALPINGOHISTERECTOMIA: REPERCUSSÕES INTRA E PÓS-OPERATÓRIAS

Presidente Prudente - SP
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Orientador: Profa. Dra. Renata Navarro Cassu

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Presidente Prudente, 14 de junho de 2019.

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DEDICATÓRIA

Gostaria de dedicar este trabalho a todas as pessoas que um dia sonharam em conquistar algo maior. A caminhada pelo trabalho sério e correto é uma caminhada difícil, cheia de obstáculos, que, com persistência, podem ser superados. No final da jornada, temos a capacidade de descobrir que não existe um fim e que a estrada continuará lá, à espera de quem quiser novas aventuras.

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“No princípio era o Verbo, e o Verbo estava com Deus, e o Verbo era Deus.
Ele estava no princípio com Deus. Tudo foi feito
por ele; e nada do que tem sido feito, foi feito sem ele. Nele estava a vida, e a vida
era a luz dos homens.” (João 1:1-4)

RESUMO

Objetivou-se investigar os efeitos clínicos da infiltração local de ropivacaina isolada e associada ao meloxicam em gatas submetidas à ovariosalpingohisterectomia (OSH) eletiva. Quarenta e cinco gatas foram sedadas com a associação de acepromazina (0,05 mg/kg) à meperidina (6 mg/kg), por via intramuscular (IM). A indução anestésica foi feita com propofol intravenoso (dose- efeito), seguindo-se a manutenção anestésica com isofluorano/O_2. Os animais foram distribuídos em três tratamentos (n=15), que consistiram de infiltração local de solução salina 0,9% (grupo S), infiltração local de ropivacaína 0,25% isolada (1 mg/kg, grupo R) e associada ao meloxicam (0,2 mg/kg, grupo RM). Os tratamentos foram administrados no tecido subcutâneo adjacente à linha de incisão cirúrgica, em cada um dos pedículos ovarianos e na cérvix uterina. Frequência cardíaca (FC), pressão arterial sistólica (PAS) e concentração final expirada de isofluorano (ETiso) foram registradas nos momentos: M1= basal, incisão cirúrgica da linha média, M2/M3/M4 = ligadura do pedículo ovariano direito/esquerdo/coto uterino e M5 = sutura de pele. Nas primeiras 24 horas após a extubação traqueal, o grau de analgesia foi avaliado utilizando-se a Escala Analógica Visual Interativa e Dinâmica (EAVID), a Escala Composta Multidimensional- UNESP-Botucatu (ECM) e os filamentos de Von Frey. Morfina (0,1 mg/kg, IM) foi administrada como analgesia de resgate. Empregou-se teste qui-quadrado, ANOVA com teste de Tukey e teste de Kruskall-Wallis e Friedman para dados paramétricos e não paramétricos, respectivamente ($P < 0,05$). Os valores médios registrados de ETiso foram inferiores no grupo RM em relação aos grupos S e R ($P = 0,0002$). Em relação aos grupos S e R, no grupo RM a ETiso foi reduzida em 21,9% e 15,6%, respectivamente. Valores inferiores de FC foram detectados nos momentos M2 ($P = 0,001$), M3 ($P = 0,006$) e M4 ($P = 0,002$) e de PAS no M2 ($P = 0,001$) e M3 ($P = 0,006$) no grupo RM em relação aos grupos R e S. Na 1ª hora após a extubação traqueal, escores inferiores foram observados no grupo RM pela EAVID em relação aos demais grupos ($P = 0,034$). Analgesia de resgate foi necessária em 4/15 gatas no S e em 1/15 gatas nos grupos R e RM ($P > 0,05$). Conclui-se que como parte de um protocolo de analgesia multimodal, o tratamento RM reduziu o requerimento de isofluorano e a resposta cardiovascular intra-operatória, além de diminuir os escores de dor (EAVID) na primeira hora após a OSH em gatas.

Palavras-chave: analgesia, anestésico local, anti-inflamatório não-esteroidal, infiltração.

ABSTRACT

The aim of this study was to investigate the clinical effects of the local infiltration of ropivacaine and its combination with meloxicam in cats undergoing elective ovariohysterectomy. Forty-five cats were sedated with acepromazine (0.05 mg/kg) and pethidine (6 mg/kg). Anesthesia was induced with intravenous propofol (dose effect) and maintained with isoflurane/O_2. The cats were randomly distributed into three treatments (n = 15), which consisted of the local infiltration of saline solution (group S), ropivacaine 0.25% alone (1 mg/kg, group R), and combined with meloxicam (0.2 mg/kg, group RM). The treatments were infiltrated in the midline incision, and into the ovarian pedicles and caudal uterine body. Heart rate (HR), systolic arterial blood pressure (SABP), end- tidal isoflurane concentration (FE´ISO) were recorded at T1 = baseline, skin incision, T2/T3/T4 = after the clamping of first, second ovarian pedicles and the uterine cervix, respectively, and T5 = skin suture. In the first 24 hours post-extubation, the degree of analgesia was assessed using an Interactive Visual Analog Scale (IVAS), UNESP-Botucatu Multidimensional Composite Pain Scale (MCPS) and von Frey filaments. Rescue analgesia was provided with intramuscular morphine (0.1 mg/kg). Data were analyzed using the chi-square test, Tukey test, Kruskal-Wallis test, and Friedman test ($P < 0.05$). FE´ISO was significantly lower in the RM compared to the S and R groups ($P = 0.0002$). Compared with S and R groups, mean isoflurane requirement in the RM group were decreased by a mean of 21.9% and 15.6%, respectively. Significantly lower values of HR were recorded at T2 ($P = 0.001$), T3 (0.0006), and T4 ($P = 0.002$) and of SABP at T2 ($P = 0.001$) and T3 ($P = 0.006$) in the RM compared to R and S groups. The IVAS pain scores were lower at one hour postoperatively in the RM compared to the R and S groups ($P = 0.034$). Rescue analgesia was required in 4/15 cats in the S group and in 1/15 cat in each of the R and RM groups. In conclusion, as part of a multimodal analgesic protocol, the treatment RM decreased isoflurane requirements and cardiovascular responses intraoperatively, and also decreased the postoperative pain scores (IVAS) at one hour after ovariohysterectomy in cats.

Keywords: analgesia, infiltration, local anesthetic, non-steroidal anti-inflammatory drug.

SUMÁRIO

ARTIGO CIENTÍFICO 1

Effects of local infiltration with ropivacaine and its combination with meloxicam on the isoflurane requirements and cardiovascular response during feline ovariohysterectomy

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Abstract

Background: The use of loco-regional anesthesia, as part of a multimodal analgesic protocol, has been associated with significant decreases in anesthetic requirements and cardiovascular responses induced by surgical trauma. The aim of this study was to investigate whether surgical site infiltration with ropivacaine and its combination with meloxicam could decrease the isoflurane requirements and cardiovascular responses during feline ovariohysterectomy. Forty-five cats were sedated with acepromazine and pethidine. Anesthesia was induced with propofol and maintained with isoflurane. The cats were randomly distributed into three groups (n = 15), which consisted of the local infiltration of saline solution (group S), ropivacaine 0.25% alone (1 mg/kg, group R), and combined with meloxicam (0.2 mg/kg, group RM). The solutions were infiltrated in the midline incision, and into the ovarian pedicles and caudal uterine body. Heart rate, systolic arterial blood pressure, end-tidal isoflurane concentration, end-tidal carbon dioxide concentration, respiratory rate, and esophageal temperature were recorded. Time points used for comparison were T1 = baseline, after the first skin incision, T2 and T3 = after the clamping of first and second ovarian pedicles, respectively, T4 = after the clamping of the uterine cervix, and T5 = after the last skin suture was placed.

Results: End-tidal isoflurane concentration was significantly lower (P = 0.0002) in the RM (mean ± SD, 0.88 ± 0.14) compared to the S (mean ± SD, 1.13 ± 0.23) and R groups (mean ± SD, 1.05 ± 0.13). Compared with the S and R groups, the mean isoflurane requirement in the RM group was decreased by means of 21.9% and 15.6%, respectively. Heart rate was significantly lower at T2 (P = 0.001), T3 (0.0006), and T4 (P = 0.002) in the RM compared to R and S groups. Systolic blood pressure was significantly lower in the RM at T2 (P = 0.001) and T3 (P = 0.006) compared to the R and S groups.

Conclusions: Local infiltration at the surgical site with ropivacaine plus meloxicam decreased isoflurane requirements and cardiovascular responses in cats undergoing ovariohysterectomy. This technique should be considered, as an adjunctive method of analgesia, in routine anesthetic practice.

Keywords: Analgesia, Infiltration, Local anesthetic, Isoflurane, Non-steroidal anti- inflammatory drug.

Background

There is increasing evidence that multimodal analgesia is an effective method for surgical pain management, involving the use of multiple analgesics that act by different mechanisms, which can result in additive or synergistic effects [1]. Among the principle agents of multimodal analgesia, local anesthetics represent one of the most effective options for preventing the transduction and transmission of nociceptive signs and can potentially improve the overall analgesia [2,3]. Different techniques of loco-regional anesthesia have been used in combination with general anesthesia, not only to reduce the analgesic requirements [3,4] but also the consumption of anesthetic drugs [5,6], improving anesthesia safety, which represents an important advantage, particularly in cats, whose perioperative- related death rate has been reported to be five times higher than in dogs [7].

Among the different regional techniques, the infiltration of local anesthetics at the surgical site has become a popular analgesic strategy both in human and veterinary medicine, due to its simplicity, low cost, and easy application [2,3,8]. However, the analgesic and anesthetic sparing effects of the incisional blocks in small animals have been questioned. Whereas some studies reported improved intraoperative analgesia and a significant anesthetic sparing effect using perioperative infiltration of lidocaine or bupivacaine at the surgical site [5,6], other studies did not find evident benefits with this anesthetic modality for dogs or cats undergoing ovariohysterectomy [9,10].

In human surgical patients, the infiltration of local anesthetics in combination with non-steroidal anti-inflammatory drugs (NSAIDs) at the site of tissue injury has been shown to produce an intense perioperative analgesic effect [11,12]. The combination drugs of produces analgesia through a dual mechanism, including direct inhibition of noxious impulses from the site of injury and reduced local expression of mediators able to sensitize nociceptors on afferent fibers, particularly the prostaglandins [12,13]. Using a rat laparotomy model, previous studies reported pronounced analgesic benefits after infiltration of the surgical wound with the combination of different NSAIDs with levobupivacaine and epinephrine [13,14].

To date, no studies have reported the intraoperative effects following wound infiltration of ropivacaine alone or combined with NSAIDs in dogs or cats undergoing ovariohysterectomy.

The aim of this study was to investigate whether the surgical site administration of ropivacaine and its combination with meloxicam could decrease the isoflurane requirements and cardiovascular responses during feline ovariohysterectomy. The hypothesis was that the addition of meloxicam to ropivacaine could potentiate the analgesic effect, resulting in a more evident decrease in cardiovascular responses and isoflurane requirements.

Methods Study design

The study protocol was approved by the local ethics committee (protocol 3843/2017 CEUA) and informed written consent for the investigation was obtained from all owners. A prospective, randomized, blinded, positive-controlled clinical study was designed to compare the intraoperative effects of injection in the wound with saline, ropivacaine, and its combination with meloxicam.

Animals

The study involved 45 cats of different breeds, scheduled for elective OHE. For inclusion the cats were required to have normal complete blood count and serum chemistry, be aged ≥ 6 months, and to have a mild temperament. The exclusion criteria were: pregnancy, lactation, extreme aggression, body weight < 2 kg, a body condition score greater than 6 or less than 3 on a nine-point scale, and systemic diseases. The cats were admitted to the hospital at least 48 h prior to surgery to allow the observer to become familiar with each cat. Before each experiment, the cats were fasted overnight with free access to water.

Anesthesia and monitoring

All anesthetic procedures were performed by the same anesthetist who was blinded to the group allocation. The cats were sedated intramuscularly (IM) with acepromazine (0.05 mg/ kg; Acepran 0.2%, Vetnil, São Paulo, Brazil) in combination with pethidine (6 mg/kg; Dolosal, Cristália, São Paulo, Brazil). Fifteen min later, an intravenous (IV) 24-gauge catheter was aseptically placed in the cephalic vein. Anesthesia was induced with (IV) propofol (Propovan 1%, Cristália, São Paulo, Brazil) in a dose sufficient to permit endotracheal intubation. After instillation of lidocaine (2 mg) on the vocal cords, the cats were intubated with an appropriately sized cuffed-endotracheal tube and attached to a non- rebreathing system (SAT 500, Takaoka, São Paulo, Brazil). Isoflurane (Isoforine, Cristália, São Paulo, Brazil) vaporized in 100% oxygen (300 mL/kg/min) was administered for the maintenance of anesthesia. Cats were permitted to breathe spontaneously throughout the procedure. Body temperature was maintained between 37°C and 38°C using an electrical heating pad (Brasmed Veterinária, São Paulo, Brazil). Lactated Ringer’s solution was administered IV at 5 mL/kg/h until extubation.

Electrocardiography (lead II), heart rate (HR), oxygen saturation of hemoglobin (SpO_2%), and esophageal temperature were continuously measured using a multi-parametric monitor (DX 2020, Dixtal Biomédica Ind. Com. Ltda., São Paulo, Brazil); respiratory rate (RR), end-tidal carbon dioxide concentration (FE´CO_2), and end-tidal isoflurane concentration (FE´ISO) were measured by a gas analyzer (Gas analyzer module VAMOS plus, Dräger do Brazil, Barueri, Brazil). Before each experiment, the gas analyzer was calibrated with a standard gas mixture (CO_2: 5 vol %, N_2O: 70 vol %, O_2: 24 vol %, and isoflurane: 1 vol %) (White Martins Gases, São Paulo, Brazil). Systolic arterial blood pressure (SABP) was monitored indirectly by sphygmomanometry, with a Doppler ultrasound device (Doppler 841-A; Parks Medical Electronics, Oregon, USA), using an appropriately sized cuff, between 40 and 50% of the circumference of the thoracic limb, with the probe placed over the metacarpal artery on the plantar surface.

Data were recorded at specific time points throughout anesthesia, as follows: T1

=baseline, after the first skin incision, T2 and T3 = after the clamping of first and second ovarian pedicles, respectively, T4 = after the clamping of the uterine cervix, and T5 = after the last skin suture was placed.

Study groups

The cats were randomly assigned using an online software program (Research Randomizer, Computer software, http://www.randomizer.org/, Pennsylvania, USA) to receive one of the

three treatments (n =15): saline (S group), ropivacaine alone (1 mg/kg; R group), and combined with meloxicam (0.2 mg/kg; RM group). In the R group, ropivacaine (Ropi 10mg/mL, Cristália, São Paulo, Brazil) was diluted in saline solution 0.9% to obtain a final concentration of 0.25%. In the RM group, meloxicam (Maxicam 0.2%, Ourofino Saúde

Animal, São Paulo, Brazil) was diluted in a saline solution 0.9%, using an equivalent volume necessary to dilute the ropivacaine to a final concentration of 0.25%. The solution was equally divided into four parts, and an equivalent volume was administered in specific anatomical areas: subcutaneous tissue of the incision site, right and left ovarian pedicles, and caudal uterine body. The same volume of saline solution was administered into the same surgical sites. The injections were delivered to the surgical center, and the solutions were injected by a veterinary student who was not aware of the allocation status. Subcutaneous meloxicam (0.2 mg/kg) was administered to the cats of the S and R groups after the intubation.

Before the beginning of the surgery, the cats received an incisional subcutaneous infiltration with one of the three treatments. After the abdomen was surgically opened and the uterus and ovaries exposed, the local anesthesia or saline solution were infiltrated on the ovarian pedicles (left and right) and uterine body caudal to the bifurcation. Ten minutes later, the excisions of the pedicles and uterus were initiated.

Ovariohysterectomy was performed using a standard technique through median laparotomy access in supine cats. All surgical procedures were performed by the same surgeon using a 3-cm ventral midline approach and 3-clamp technique [3].

Intraoperatively, vaporizer settings were adjusted according to the conventional signs of anesthesia (rotation of the eyeball, loss of palpebral reflex, and loss of jaw tone) and autonomic responses to surgical stimulation. If a cat showed an increase in jaw tone or a palpebral reflex, propofol (1 mg/kg) was administered intravenously, and FE´ISO was increased by 0.1-0.2%. If SABP or HR increased or decreased by more than 20% from the previously recorded values, FE´ISO was adjusted accordingly in increments of 0.1- 0.2%. If an FE´ISO above 1.6% was required, additional analgesia was provided with fentanyl (1µg/kg, IV).

The anesthesia time (time elapsed from the administration of propofol to discontinuation of isoflurane), surgery time (time elapsed from the first incision until placement of the last suture), time to extubation (time elapsed from termination of isoflurane until extubation), and recovery time (time elapsed from the time of discontinuation of isoflurane to voluntary movement into a sternal position) were recorded for each cat. Extubation was performed when the cat recovered the swallowing reflex.

Adverse events

The occurrence of complications related to the infiltrative injection, such as hemorrhage during surgery, accidental intravascular injection, and hematoma formation were recorded.

Outcome measures

The primary outcome measures were the cardiovascular responses and isoflurane requirements. Secondary outcome measures included the intraoperative requirement for the rescue analgesia and adverse events.

Statistical analysis

A sample size of at least 14 cats per group was estimated to achieve an 80% statistical power to detect a mean difference in the SABP of 20% between the treated groups (R and RM) and the placebo group (S), and a standard deviation (SD) of 19 mmHg, at an overall alpha level of 0.05. Data were estimated from a pilot study.

A Shapiro-Wilk test was performed to assess the normality of the variables. Data are expressed as mean ± standard deviation.

Body weight, age, time to extubation, and surgical, anesthetic, and recovery times were compared between groups using one-way ANOVA followed by a Tukey´s test. For physiological parameters and FE´ISO, two way-ANOVA and the Tukey test were performed to compare groups and time points. Differences between baseline and other time point values in each group were assessed by repeated measure ANOVA and the Tukey post-test.

All analyses were performed using GraphPad Prism7.0 (GraphPad Software Inc., San Diego, USA). Differences were considered significant when P < 0.05.

Results

From the 50 cats initially enrolled in the study, five were excluded due to aggressiveness (n=3) and pregnancy (n =2).

There were no significant differences between groups in age, body weight, condition score, dose of propofol, duration of anesthesia and surgery, and time to extubation or recovery (P > 0.05) (Table 1).

HR was significantly lower at T2 (P = 0.001), T3 (0.0006), and T4 (P = 0.002) in the RM compared to R and S groups. Similarly, SABP was significantly lower in the RM at T2 (P = 0.001) and T3 (P = 0.006) compared to the R and S groups. Compared to T1 (baseline), HR increased at T2, T3 and T4 (P < 0.0001) in the R and S groups, and SABP increased at T2 and T3 (P < 0.0001) in the R group and at T2, T3 and T4 (P < 0.0001) in the S group. No significant differences were recorded in the RM group over time. No significant differences were observed between groups with regards to the RR, FE´CO_2, and esophageal temperature (Table 2).

Mean FE´ISO was significantly lower (P = 0.0002) in the RM group (mean ± SD,

0.88 ± 0.14) compared to the R (mean ± SD, 1.05 ± 0.13) and S groups (mean ± SD, 1.13 ± 0.23) (Fig.1). When each specific time point was compared between groups, mean FE´ISO was significantly lower in the RM group compared to the R and S groups at T2, T3 (P < 0.0001), and T4 (P = 0.0001) (Table 2). Compared with the S and R groups, mean isoflurane requirement in the RM group was decreased by means of 21.9% and 15.6%, respectively. Compared to T1, FE´ISO was significantly higher at T2 and T3 in the S group (P < 0.0001), and at T2 in the R group (P = 0.0016). In the RM group no significant differences were observed in the FE´ISO over time (P = 0.62) (Table 2). Intraoperative supplementation with propofol or fentanyl was not necessary.

The occurrence of adverse effects was minimal. Hematoma was observed in the ovarian pedicles after the injection in 3/15, 2/15, and 2/15 cats in the S, R, and RM groups, respectively.

Discussion

The results of this study demonstrated that the administration of ropivacaine combined with meloxicam at the surgical site significantly reduced the isoflurane requirements and cardiovascular responses during ovariohysterectomy in cats. However, no intraoperative analgesic benefits were found when ropivacaine was administered alone. These findings support our hypothesis that the addition of meloxicam to ropivacaine could improve the intraoperative analgesia.

This study was designed to investigate the use of local anesthesia, as part of a multimodal analgesic protocol, in cats under general anesthesia. For ethical reasons, due to the inclusion of a saline treated group, and in order to approximate this experimental design with daily clinical situations, all cats received an opioid (pethidine) prior to surgery. In addition, the cats in the R and S groups also received subcutaneous meloxicam.

Ovariohysterectomy involves the surgical manipulation of ovaries, which are richly innerved by sensory and autonomic fibers, being extremely responsive to noxious stimulation [10]. Traction of the ovarian ligament and clamping of its pedicle triggers an autonomic response, signaled by the increase in physiologic parameters, such as heart rate and arterial pressure, which have often been used as indicators of nociception in anesthetized patients [10,15,16]. Furthermore, as in other types of surgery, ovariohysterectomy may induce pro- inflammatory cytokine production, which leads to sensitization of peripheral nociceptors, resulting in inflammatory pain [12,13]. Thus, it seems that the addition of meloxicam to ropivacaine in the current study provided an effective reduction in peripheral nociceptive transmission, since this treatment was associated with significant decreases in isoflurane requirements and cardiovascular responses to the surgical stimulation. The low isoflurane requirement may be attributed to the antinociceptive effects of combined administration of ropivacaine and meloxicam. While ropivacaine could block the afferent sensory signal transmission [8], meloxicam could reduce the release of peripheral inflammatory mediators, preventing sensitization of the afferent fibers and decreasing the nociception process [17]. On the other hand, when ropivacaine was administered alone, no additional analgesic intraoperative effects were detected. It is important to emphasize that in the R group, meloxicam was administered subcutaneously preoperatively. Thus, it appears that the infiltration of meloxicam directly at the site of tissue injury really resulted in superior intraoperative analgesia than its systemic administration. With local administration, higher drug concentration is reached at the point of the origin of the inflammatory process, which could improve the analgesic effect [13,14,17]. In humans, the maximum plasma concentration of meloxicam was achieved at 1.8 h, with a plasma concentration plateau maintained up to 24 h after local administration [17]. Although the plasmatic concentration of meloxicam was not measured in this study, it is likely that a high concentration of meloxicam was maintained at the surgical site through the OHE, as the mean surgical time was around 20 min.

Conflicting data have been reported regarding the efficacy of local anesthesia for decreasing the autonomic responses and anesthetic requirements during OHE in small animals [5,10,18]. In dogs undergoing OHE, a mesovarian injection of lidocaine did not decrease the isoflurane requirements or the intraoperative autonomic responses [10]. On the other hand, the infiltration of lidocaine into the surgical site, involving the subcutaneous, mesovarian, and ovarian pedicles, significantly decreased the need for injectable anesthetics during OHE in cats [5]. Similarly, infiltration with lidocaine or bupivacaine at the incision site reduced cardiovascular responses during OHE in cats [18].

In the current study, aiming to blockade the main anatomical areas involved in the OHE, the local analgesia was administered at four points, involving ovarian pedicles, uterine cervix, and subcutaneous tissues of skin incision, as reported by Fudge et al [3]. Failure to show any additional analgesic intraoperative effects with the infiltration of ropivacaine into the surgical site may be attributed to the dose, concentration, and volume administered. To the author´s knowledge, there are no reports on the use of surgical site infiltration with ropivacaine, as an adjunctive method of analgesia, in dogs or cats undergoing OHE. Thus, the dose administered was based on the veterinary literature in order not to exceed the maximum recommended dose for cats, especially because there is limited information regarding toxic dosing of this local anesthetic in cats [19]. Additionally, the ideal concentration-volume ratio necessary to block the nerve endings involved in the surgical manipulation of the ovaries and uterus has not been established. Both concentration and volume play an important role in the onset, duration, and efficacy of sensory block [8,20]. The dilution of ropivacaine to 0.25% in the current study may have interfered in the local anesthetic onset of action and in the intensity of analgesia. In humans, a previous study reported prolonged onset of sensory blockade in patients receiving an interscalene block with ropivacaine 0.25% (20 min) when compared to 0.5% (10 min) and 0.75 % (5 min) [20]. In the present study, the excisions of the pedicles and uterus were initiated 10 min after the local infiltration, which may have been insufficient to achieve a complete sensory blockade to decrease the peripheral nociceptive receptor sensitivity to the mechanical stimulation induced by the traction of the suspensory ligament of the ovarian, and the clamping of the ovarian pedicles and uterine cervix.

The most common complications related to the local infiltration of drugs at the incision site include minor wound hematoma, edema, drainage, fluid accumulation, and infection [21]. In the current study, hematoma in the ovarian pedicles was the only wound- related complication, and occurred in few cats (15%), suggesting that the injection technique used was safe. In addition, systemic toxicity may occur if an excessive amount of local anesthetic is absorbed, resulting in cardiovascular and neurological side-effects [22]. The classical signs of local anesthetic toxicity are related to activation of the central nervous system, including shivering, muscle twitching, seizure, and tremor [19]. Moreover, cardiovascular dose-dependent effects can also be identified, such as direct myocardial depression, arrhythmias, prolonged conduction, and total cardiovascular collapse [22]. To date, no studies have reported the systemic toxicity related to overdosing of ropivacaine in cats. In the current study, due to concerns regarding systemic toxicity, the total dose administered of ropivacaine was 1 mg/kg, which is inferior to the maximum dose recommended for cats (1.5 mg/kg) [19], and was apparently safe, since no neurological or cardiovascular signs compatible with local anesthetic toxicity were observed intraoperatively or during the recovery time.

This study has some limitations. The dilution of expired inhalant concentration by the high fresh gas flow rate required in a non-rebreathing system may have interfered in the measured end-tidal partial pressures, including both FE´ISO and FE´CO_2. To avoid this dilution effect, a sampling catheter could be introduced through the lumen and advanced to the distal end of the endotracheal tube [23]. In the present study, the end-tidal concentrations of anesthetic gases were measured using an adapter inserted in the proximal end of the tracheal tube, which could justify the lower isoflurane concentrations recorded through the surgical procedure compared to those reported in a previous study in cats undergoing OHE, where a long sampling catheter was used to measure isoflurane concentration [23]. An additional limitation was the absence of ropivacaine and meloxicam plasma concentration measurements, which could improve understanding of the pharmacokinetic profile of these drugs and their systemic absorption following infiltrative wound administration. Further studies are needed to quantify the analgesic potential of these protocols on the intraoperative inhalant anesthetic requirements and cardiovascular responses.

Conclusions

In conclusion, local infiltration at the surgical site with ropivacaine combined with meloxicam decreased both isoflurane requirements and cardiovascular responses to the surgical stimulus in cats undergoing elective OHE. This technique should be considered, as an adjunctive method of analgesia, in routine anesthetic practice.

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

GOLC: study design, drug preparation, and drafting of manuscript. IPGAN: perioperative care, behaviour scoring, rescue analgesia, drafting of manuscript. ABFS: recruitment and enrolling study animals and local solution infiltration. TSB: data acquisition, data management. JSCJ: anaesthesiologist, postoperative care. GMN: surgical procedure. RNC: study design, data analysis, helped with statistical analysis, writing of manuscript. All authors approved the final manuscript.

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Table 1. Demographic data, dose of propofol and procedural times (mean ± standard deviation) of cats undergoing ovariohysterectomy treated with local infiltration with saline solution (S, *n* = 15), ropivacaine (R, *n* = 15) and ropivacaine/meloxicam (RM, *n* = 15).

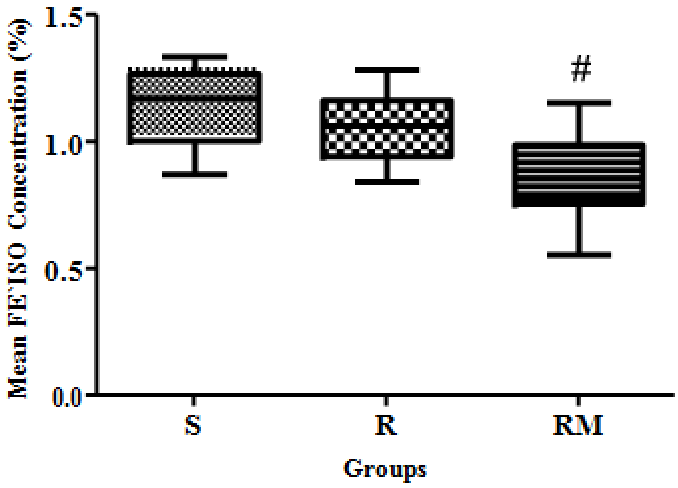
Variables	**Group**			
	S	R	RM	P value
Body weight (kg)	2.5 ± 0.4	2.6 ± 0.5	2.4 ± 0.4	0.63
Age (months)	15.6 ± 8	17.4 ± 7.7	15 ± 8	0.68
Propofol (mg/kg)	7.8 ± 2.3	7.4 ± 2.4	7.7 ± 2.2	0.92
Anesthesia time (min)	38.6±8.5	46.9±10	42.4±9.9	0.14
Surgery time (min)	21 ± 4.0	23.2 ± 4.3	19.8 ± 4.1	0.19
Extubation time (min)	7.8 ± 5	7.8 ± 3.2	7.3 ± 3.9	0.23
Recovery time (min)	39 ± 15.2	38±13.7	33 ± 15.1	0.35

Table 2. Mean ± standard deviation of heart rate (HR), systolic arterial blood pressure (SABP), end-tidal isoflurane concentration (FE´ISO), end-tidal carbon dioxide concentration (FE´CO_2), respiratory rate (RR) and esophageal temperature (T) of cats undergoing ovariohysterectomy treated with local infiltration with saline solution (S, *n* = 15), ropivacaine (R, *n* = 15) and ropivacaine/meloxicam (RM, *n* = 15).

Variable	Group	T1	T2	T3	T4	T5
HR (beats/min)	S	128±20	163±29*	165±27*	157±23*	149±23
	R	127±14	157±27*	159±31*	156±29*	147±30
	RM	113±17	124±30#	123±22#	124±26#	120±24
SABP (mmHg)	S	94±24	127±27*	123±24*	113±24*	106±26
	R	91±13	123±26*	126±20*	110±26	97±22
	RM	91±14	95±18#	97±20#	97±23	92±21
FE´ISO (%)	S	1.10±0.15	1.23±0.27*	1.23±023*	1.13±0.14	1.0±0.23
	R	1.02±0.10	1.18±0.15*	1.16±0.18	1.10±0.20	0.96±0.15
	RM	1.0±0.20	0.99±0.21#	0.99±0.11#	0.95±0.13#	0.90±0.17
FE´CO_2 (mmHg)	S	39±6	37±7	38±7	37±7	37±7
	R	38±5	37±9	35±6	35±9	38±8
	RM	40±4	38±8	39±6	36±7	37±6
RR (breats/min)	S	15±6	18±8	16±8	15±7	16±6
	R	12±5	16±8	16±6	15±7	14±7
	RM	14±8	15±10	16±7	14±8	13±7
T (°C)	S	37.4±0.6	37.3±0.6	36.5±0.5	36.4±0.4	36.3±0.5
	R	37.4±0.4	37.4±0.6	37.0±0.5	36.7±0.5	36.5±0.4
	RM	37.4±0.7	37.1±0.5	36.5±0.5	36.3±0.4	36.3±0.4

*Significantly different from baseline values (Tukey´s Test, $P < 0.05$). # Significantly different from S and R groups (Tukey´s Test, $P < 0.05$).

Fig. 1 Mean ± standard deviation (SD) of end-tidal isoflurane concentration (FE`ISO) during ovariohysterectomy (T1-T5) in cats treated with local infiltration with saline solution (S, *n* = 15), ropivacaine (R, *n* = 15) and ropivacaine/meloxicam (RM, *n* = 15).



Significantly different from S and R groups (Tukey´s Test, P = 0.0002).

ARTIGO CIENTÍFICO 2

Effectiveness of the infiltration of the surgical site with ropivacaine alone and in combination with meloxicam for the provision of postoperative analgesia in cats undergoing ovariohysterectomy

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Abstract

Background: Evidence has shown that perioperative administration of local anesthesia, as an adjunctive analgesic method plays an important role in the postoperative pain management. This prospective, randomized, blinded, controlled clinical trial aimed to compare the analgesic efficacy of infiltration of the surgical site with ropivacaine and its combination with meloxicam in cats undergoing ovariohysterectomy. Forty-five cats were sedated with acepromazine (0.05 mg/kg) in combination with pethidine (6 mg/kg). Anesthesia was induced with intravenous propofol (dose effect) and maintained with isoflurane/O_2. Intraoperatively, the cats were randomly distributed into three treatment groups (n = 15), which consisted of the local infiltration of saline solution (group S), ropivacaine alone (1 mg/kg, group R), and combined with meloxicam (0.2 mg/kg, group RM). The solutions were infiltrated in the midline incision, and into the ovarian pedicles and caudal uterine body. Postoperative analgesia was assessed for the first 24 hours post- extubation using an Interactive Visual Analog Scale (IVAS), UNESP-Botucatu Multidimensional Composite Pain Scale (MCPS) and mechanical nociceptive thresholds (MNT). Sedation was assessed at the same time points. Rescue analgesia was provided with intramuscular morphine (0.1 mg/kg) if the MCPS was ≥ 6. Data were analyzed using the chi- square test, Tukey test, Kruskal-Wallis test, and Friedman test ($P < 0.05$).

Results: Significantly lower IVAS pain scores were recorded at one hour after extubation in the RM compared to the R and S groups ($P = 0.034$). The MCPS pain scores, the MNT, and the sedation scores did not differ between groups at any time point ($P > 0.05$). Rescue analgesia was required in 4 cats in the S group (total of 7 analgesic interventions), and 1 cat in each of the R and RM groups (total of 2 analgesic interventions).

Conclusions: As part of multimodal pain therapy, both methods of local infiltration analgesia resulted in a trend towards decreased need for supplemental systemic analgesics and the combination of ropivacaine with meloxicam reduced IVAS pain scores at the first hour postoperatively.

Keywords: Analgesia, Feline, Infiltration, Local anesthetic, Non-steroidal anti- inflammatory drug.

Background

The beneficial effects of regional anesthesia as part of a multimodal analgesia protocol are well known in both human [1,2] and veterinary medicine [3,4,5]. Different local anesthetic techniques have been administered in the perioperative period, resulting in decreased postoperative pain scores and analgesic and anesthetic-sparing effects [4-7].

Infiltration of the surgical site with local anesthetics is a simple, safe, and low-cost technique, which may decrease postoperative pain due to the inhibition of the noxious impulse transmission at the site of the wound [8]. In dogs, the perioperative infiltration of the incision site combined with intraperitoneal block reduced the postoperative pain scores and analgesic requirements after ovariohysterectomy [3]. In cats, however, reports of the effectives of infiltration of the surgical wound with local anesthetics are limited [7,9,10]. Recently, one study reported that intraoperative infiltration of bupivacaine in specific anatomical areas was an effective method for early postoperative pain management in cats undergoing ovariohysterectomy [7]. Until now, no studies have reported the effects of ropivacaine as part of a multimodal protocol of analgesia in cats. Ropivacaine has a relatively fast onset (10-15 minutes) and a prolonged duration of action (240-300 minutes), with less risk of cardiac and systemic toxicity than bupivacaine [11,12] which represents an advantage, especially for cats, which are considered more susceptible than dogs to the toxicity induced by local anesthetics.

Additionally, increasing evidence has shown that the local administration of non- steroidal anti-inflammatory drugs (NSAIDs) plays an important role in peripheral nociceptive transmission, due to a reduction in the local production of mediators that sensitize nociceptors on afferent fibers [13,14]. In animal models, have been demonstrated that infiltration of local anesthetics combined with NSAIDs into the surgical site provides clinically effective analgesia after laparotomy [15,16].

To date, there are no clinical studies focused on the administration of local anesthetics combined with NSAIDs into the surgical site for postoperative pain relief in dogs or cats undergoing soft tissue surgical procedures.

The aim of this study was to investigate the analgesic effects of the administration of ropivacaine and its combination with meloxicam at specific surgical sites in cats undergoing ovariohysterectomy. The hypothesis was that the local infiltration of ropivacaine and its combination with meloxicam would decrease postoperative pain scores and analgesic requirements when compared with saline infiltration. Moreover, we hypothesized that the addition of meloxicam could potentiate the analgesic effect of ropivacaine in terms of intensity and duration of analgesia.

Methods Animals

After obtaining informed consent, 45 crossbreed client-owned cats admitted for elective ovariohysterectomy were enrolled. The study was approved by the Institutional Animal Care Committee (protocol 3843/2017 CEUA). Only cats with normal complete blood count and serum chemistry, aged ≥ 6 months, and with an American Society of Anesthesiologists physical status I (ASA I) were included in the study. The exclusion criteria were: pregnancy, lactation, extreme aggression, body weight < 2 kg, body condition score greater than 6 or less than 3 on a nine-point scale, and systemic diseases. The cats arrived at the hospital at least 48 hours prior to surgery to allow the observer to become familiar with each cat. Preoperatively, all cats were evaluated by abdominal ultrasonography for confirmation of the absence of pregnancy. Before each experiment, the cats were fasted overnight with free access to water.

Study design

Prospective, randomized, blinded, positive-controlled clinical study,

Anesthesia and surgery

All anesthetic procedures were performed by the same anesthetist who was blinded to the group allocation. The cats were sedated intramuscularly (IM) with acepromazine[a] (0.05 mg/ kg) in combination with pethidine[b] (6 mg/kg). Fifteen minutes later, an intravenous (IV) 24- gauge catheter was aseptically placed in the cephalic vein. Anesthesia was induced with (IV) propofol[c] in a sufficient dose to permit the endotracheal intubation. The cats were attached in a non-rebreathing system[d] and isoflurane[d] in 100% oxygen (400 mL/kg/min) was administered for the maintenance of anesthesia. Cats were permitted to breathe spontaneously throughout the procedure. Body temperature was maintained between 37°C and 38°C using a heating pad[f]. Lactated Ringer's solution[g] was administered IV at 5 mL/kg/hour until extubation.

Intraoperatively, heart rate (HR), electrocardiogram (lead II), respiratory rate (RR), oxyhemoglobin saturation and esophageal temperature were continuously monitored using a multi-parametric monitor[h]; capnography ($ETCO_2$) end tidal isoflurane concentration (ETiso) were measured by a gas analyzer[i] and non-invasive systolic blood pressure was measured with the ultrasonic Doppler method.[j]

Ovariohysterectomy was performed using a standard technique through median laparotomy access in supine cats. All surgical procedures were performed by the same surgeon using a 3-cm ventral midline approach and 3-clamp technique (Fossum 2018).

The anesthesia time (time elapsed from the administration of propofol to discontinuation of isoflurane), surgery time (time elapsed from the first incision until placement of the last suture), time to extubation (time elapsed from termination of isoflurane until extubation), and recovery time (time elapsed from the time of discontinuation of isoflurane to voluntary movement into a sternal position) were recorded for each cat. Extubation was performed when the cat recovered the swallowing reflex.

Study groups

The cats were randomly assigned using an online software program[k] to receive one of the three treatments (n =15): saline (S group), ropivacaine alone (1 mg/kg; R group) and combined with meloxicam (0.2 mg/kg; RM group). In the R group, ropivacaine[l] was diluted in saline solution 0.9% to obtain a final concentration of 0.25%. In the RM group, meloxicam[m] was diluted in a saline solution 0.9%, using an equivalent volume necessary to dilute the ropivacaine to a final concentration of 0.25%. The solution was equally divided in four parts, and an equivalent volume was administered in specific anatomical areas: subcutaneous tissue of the incision site, right and left ovarian pedicles, and caudal uterine body. The same volume of saline solution was administered into the same surgical sites.

Before the beginning of the surgery, the cats received an incisional subcutaneous infiltration with one of the three treatments. After the abdomen was surgically opened and the uterus and ovaries were exposed the local anesthesia or saline solution were infiltrated on the ovarian pedicles (left and right) and uterine body caudal to the bifurcation. Ten minutes later, the excisions of the pedicles and uterus were initiated. Subcutaneous meloxicam (0.2 mg/kg) was administered to the cats of the S and RM groups after the intubation.

Post-operative monitoring

The same single observer, unaware of the treatment groups, was responsible for the pain and sedation assessments, which were performed 24 hours prior to surgery (baseline), and 0.5, 1, 2, 4, 6, 8, 12, 18, and 24 hours after extubation. The observer was a veterinary post-graduate student, with experience in the assessment of pain in cats using behavioral indices. Pain was assessed by two different pain scoring systems, including the Interactive Visual Analogue Scale (IVAS, from 0 mm = no pain to 100 mm = maximum pain) and UNESP-Botucatu Multidimensional Composite Pain Scale (MCPS, from 0 = no pain to 24 = maximum pain). The MCPS pain scoring involved only two domains (pain expression, scale range = 0-12 points; psychomotor change, scale range = 0-12 points) [17]. For scoring, each cat was initially evaluated for 1 minute in its cage. Following this, the cat was stimulated to move around, for observation of reactions and behavior. Finally, the incision and surrounding area of the abdomen was palpated using 2-3 digits, and the reaction of the cat was assessed and recorded.

The pain scores were also assessed with mechanical nociceptive thresholds (MNT) using an electronic von Frey device.[n] For the MNT testing, the peak force exerted by the tip of the electronic von Frey device was recorded in grams (maximum 700 g). The tip was applied with the cats in lateral recumbency, approximately 1 cm from the surgical wound, at three points: cranial, caudal, and lateral. The final MNT was the median of the three recorded values. The device was removed immediately if the cat exhibited signs of pain, such as withdrawal movement, contraction of the abdominal wall, attempts to bite/scratch, and vocalization. The MNT was assessed after the DIVAS and MCPS measurements at the same time points.

Morphine[o] was administered (0.1 mg/kg IM) as rescue analgesia if the MCPS scores

were ≥ 6 (0-24 points), as reported by previous studies [5, 18]. The number of cats requiring rescue analgesia and the number of morphine doses were recorded.

A numerical rating score was used for the assessment of the degree of sedation, where: 0 = Completely awake, able to stand and walk; 1 = Stands, but staggers when attempting to walk; 2 = With encouragement is unable to stand but laying in sternal recumbency with head elevated; 3 = Able to lift head with encouragement, but resting head down, sternal recumbency; 4 = Responsive to light stroking, lateral recumbency; 5 = Unresponsive to light stroking, lateral recumbency [19].

Adverse events

The occurrence of adverse events during the study period such as seizures, nausea, and cardiovascular effects (bradycardia, arrhythmias, hypertension, or hypotension) were recorded. Bradycardia, hypertension, and hypotension were defined as a HR < 90 beats per minute, NIBP < 90 mmHg, and NIBP > 140 mmHg, respectively for longer than 5 minutes consecutively.

Outcome measures

The primary outcome measures were the pain scores assessed by the IVAS and MCPS pain scales and the requirement for the rescue analgesia. Secondary outcome measures included the sedation scores, von Frey thresholds, and adverse effects.

Statistical analysis

A sample size of at least 15 cats per group was estimated to achieve 80% statistical power to detect a prevalence of a treatment failure of 70% in the S group and 20% in the treated groups (R and RM). The sample calculation was based on pilot data.

A Shapiro-Wilk test was performed to assess the normality of the variables. Data are expressed as mean ± standard deviation (parametric variables) or median (range) (non- parametric variables) as appropriated.

Bodyweight, age, time to extubation, and surgical, anesthetic, and recovery times were compared between groups using one-way ANOVA followed by a Tukey´s test.

The incidence of adverse events in the three groups was compared using the Fisher exact probability test. A Kruskal-Wallis test was used to compare MNT, pain and sedation scores between groups. A Friedman test was used to compare differences in MNT, pain and sedation scores over time within each group. Corresponding areas under the curves (AUCs) of MNT, IVAS and MCPS were calculated from baseline until 24 hours using the trapezoidal method and compared between groups using a Kruskal-Wallis test.

The number of cats that required rescue analgesia was compared between groups using the Fisher´s exact test. A Kruskal-Wallis test was used to compare the number of morphine doses administered post-operatively in the groups.

All analyses were performed using GraphPad Prism 7.0.[p] Differences were

considered significant when $P < 0.05$.

Results

From the 50 cats initially enrolled in the study, five were excluded due to aggressiveness (n=3) and pregnancy (n =2).

There were no significant differences between groups in age, body weight, condition score, duration of anesthesia and surgery, and time to extubation or recovery ($P > 0.05$) (Table 1).

In the first hour post-extubation, the IVAS pain score was lower in the RM compared to R and S groups ($P = 0.034$). The IVAS scores were significantly increased compared to the corresponding baseline values from 1 to 24 hours in all the groups (Table 2) ($P < 0.001$). The MCPS scores were not significantly different between groups ($P > 0.05$). Compared to the baseline values, significant increases were recorded in the MCPS scores at 2 hours ($P = 0.004$) in the S group and between 6 to 8 hours ($P = 0.006$) in the R group, whereas no significant differences from baseline were recorded in the RM group. In the analysis of AUC, comparable results were detected between groups based on both IVAS and MCPS scores ($P > 0.05$).

The MNT measurements were not significantly different between groups at any time point ($P > 0.05$) (Table 2).

Sedation scores did not differ between groups during the 24-h period. When compared with baseline values, increased scores were recorded from 0.5 to 1 hour post-extubation ($P < 0.001$).

There were no differences between groups in the number of cats receiving rescue analgesia ($P = 0.32$) or in the number of total rescue analgesic interventions during the observational period ($P = 0.15$). A total of 6 cats required rescue analgesia (4 cats in the S group and 1 cat in each of the R and RM groups). In both the R and RM groups, each cat received rescue analgesia on one occasion (one dose of morphine each), whereas in the S group three cats received rescue analgesia on two occasions, and one cat received rescue analgesia on one occasion (total of 7 doses of morphine) (Table 3).

No signs of local anesthetic toxicity were observed during the study period.

Discussion

The results of this study demonstrated that cats receiving both protocols of local infiltration analgesia presented a trend towards lower requirement for postoperative rescue analgesia, and that the addition of meloxicam to ropivacaine significantly decreased the IVAS pain scores in the first hour following OHE in cats. These findings supported in parts the hypothesis of this study.

The apparent lack of benefits in administering ropivacaine at the surgical site, as an adjunctive analgesic method, might be attributed partially to the insensitivity of the measurement scales, or bias. As the recognition and quantification of pain in animals represents an important challenge, in the current study pain was assessed using three methods, including objective (LNM) and subjective measures (IVAS and MCPS). Both the IVAS and MCPS have been widely used for postoperative pain assessment in cats [5, 20,21]. The MCPS is a validated, sensitive, and reliable method for pain assessment in cats [17]. Although the IVAS is not a validated scale for pain assessment in cats, several studies have reported reasonable correlations between the IVAS and pain intensity [3,20]. In the current study, the MCPS was not sufficiently sensitive to detect differences between groups. Other studies have also not found enough sensitivity to detect a treatment effect using MCPS for assessing pain in cats after ovariohysterectomy [7, 20, 21]. Through the IVAS, significant differences between groups were recorded only at 1 h post-extubation, with lower pain scores in the RM group. To minimize selection bias, in the current study the cats were not excluded from the statistical analysis if they required rescue analgesia. This approach may have decreased the differences between groups, due to artificially lower pain scores related to postoperative morphine supplementation. However, both methods were able to detect significant changes between preoperative and postoperative scores. The duration of action of ropivacaine is approximately 6 hours [11], which could explain the elevation in the MCPS observed between 6 and 8 hours in the R group. On the other hand, at 2 hours the Control group demonstrated the highest MCPS scores, while in the RM group the MCPS scores did not exceed the baseline values, suggesting that the local effect of meloxicam was superior to the systemic effect. By inhibiting COX-2 and reducing the level of inflammatory mediator synthesis, it is likely that the local administration of meloxicam potentiated the analgesia provided by ropivacaine, decreasing the peripheral pain receptor sensitivity to mechanical stimulation and reducing the inflammatory component related to the early postoperative pain elicited by surgical trauma [22]. Clinical reports in humans have also found that the addition of an NSAID to a local anesthetic prolonged the postoperative analgesia when compared with a control group, following different surgical procedures [23,24].

Regarding the objective method of pain assessment, in the current study MNT testing was unable to detect any antinociceptive effect in either protocol of local infiltration analgesia, which is in agreement with previous data published in cats and dogs treated with different local anesthetic techniques [5,9]. Particular experimental conditions, including an experienced surgeon, minimal tissue trauma, and the provision of preoperative analgesia could explain in part the lack of significant differences in the MCPS scores and MNT measurements. In the current study, pethidine was chosen as a preventive analgesic due to its short duration of effect, aiming to provide intraoperative analgesia with minimal interference in the postoperative pain assessment. As it is well established that OHE is a painful procedure, for ethical reasons meloxicam was administered to the cats in all groups. Pharmacokinetic studies have reported that meloxicam has an elimination half-life of approximately 24 hours in cats following subcutaneous administration [25] and it has been shown to be an effective analgesic for pain relief after OHE in cats [26,27]. Additionally, the antihyperalgesic effects of meloxicam have been demonstrated in experimental and clinical settings [25,28]. Moreover, Lascelles et al. [29] reported that the preoperative administration of a single dose of pethidine prevented allodynia and decreased hyperalgesia in dogs after OHE. Thus, perioperative administration of both meloxicam and pethidine to all the cats may have interfered in the response to the von Frey filaments in this study.

Although the frequency of recue analgesia did not differ between treatments, the local administration of ropivacaine alone and combined with meloxicam appeared to provide effective analgesia in the majority of the cats, since only one cat of each group required analgesic supplementation during the 24 hour-period after OHE. The infiltration of a local anesthetic at the surgical site reduces the transmission of pain from the injured tissues, due to the blockage of the afferent nociceptive fibers, preventing peripheral sensitization which may justify the reduced need for postoperative rescue analgesia in the R and RM groups. However, whether or not the infiltration of a local anesthetic at the surgical wound contributed to the control of postoperative pain is controversial. Previous clinical reports did not found relevant analgesic effects following the infiltration of bupivacaine or lidocaine at the incision site for the control of pain after OHE in dogs [4, 30] or cats [31]. In the current study, besides the midline incision site, the ropivacaine was infiltrated at other anatomical areas, including the ovarian pedicles and the uterine cervix, aiming to block the sensitization of the main structures involved in the tissue trauma induced by OHE. In a similar study, the infiltration of small volumes of bupivacaine 0.5% (2mg/kg), in the same anatomical sites, provided adequate early postoperative analgesia in cats undergoing ovariohysterectomy [7]. In view of these results it appears that the local infiltration of both ropivacaine and bupivacaine at anatomical structures closely related to the sensitization elicited by the surgical stimulus may result in superior analgesia when compared with a single application of a local anesthetic at the incision site. Moreover, the time for the first rescue analgesia in the RM group was a little longer than in the R group, suggesting that the analgesic effect may have been prolonged by the addition of the meloxicam to the ropivacaine. The pharmacokinetic parameters of meloxicam in human patients showed that the mean plasma drug concentration was significantly lower during the first three hours after local compared to intravenous administration [21]. Although the plasmatic concentration of meloxicam was not measured in this study, it is possible that the prolonged time for the first rescue analgesia in the RM (4 h) compared to the R (1 h) may be attributed to the high concentration of meloxicam at the surgical site. To date, no studies have analyzed the pharmacokinetic profile after local administration of meloxicam in cats. In humans, the maximum plasma concentration of meloxicam was achieved at 1.8 h with a terminal elimination half-life of 53.1 h after local administration, whereas a terminal elimination half-life of 19.2 h was detected after intravenous administration [21]. These data suggest that after local administration, meloxicam remained stored in the damaged surgical tissue, being slowly released into the systemic circulation.

The most common complications related to the local infiltration of drugs at the incision site include minor wound hematoma, edema, drainage, fluid accumulation, and infection [32]. Additionally, adverse events related to the toxicity of the local anesthetic, such as cardiac arrhythmias, shivering, muscle twitching, seizure, and tremor have been reported in experimental conditions [33]. In this study, complications regarding local infiltration at the surgical site or toxicity related to the local anesthetic were not recorded, suggesting that the local infiltration technique, as well as the dose and concentration of ropivacaine administered in this study were safe for cats.

This study has some limitations. One potential reason for failure to demonstrate any significant differences between groups in the frequency of rescue analgesia and in the MCPS pain scores could be attributed to the small sample size. The sample size was estimated considering a frequency of rescue analgesia of 70% in the S group and 20% in the treatment groups (R and RM). However, the differences in the frequencies of rescue analgesia were smaller than this, limiting the statistical power of our study. Moreover, as the dose and volume play an important role in the action of local anesthetics it is possible that these factors may have interfered in our results. To date, there are no studies concerning the local infiltration of ropivacaine for postoperative pain relief in cats. Thus, the dose of ropivacaine administered was based on the veterinary literature in order not to exceed the maximum recommended dose for cats, especially as there is limited information regarding toxic dosing of this local anesthetic in cats. However, it is possible that although apparently safe, the dose of ropivacaine administered in this study was insufficient for the control of pain following OHE. Furthermore, the dilution of ropivacaine from 1% to 0.25% could interfere in the duration of action, decreasing the postoperative analgesic efficacy. Additionally, due to the lack of studies using local administration of meloxicam in small animals undergoing abdominal surgeries, the decision was made to use the same dose administered systemically. In addition, pharmacokinetic parameters of ropivacaine were not determined in this study. Until now, to the author´s knowledge, pharmacokinetic studies following local administration of ropivacaine to cats have not been reported. Moreover, the addition of meloxicam to ropivacaine could interfere in the onset of action and duration of effect. Further studies are needed to determine the efficacy and safety of local administration of ropivacaine, using different doses and concentrations and its combination with meloxicam in cats.

Conclusions

As part of multimodal pain therapy, both methods of local infiltration analgesia resulted in a trend towards decreased need for supplemental systemic analgesics and the combination of ropivacaine with meloxicam reduced IVAS pain scores at the first hour postoperatively.

Endnotes

[a]Acepran 0.2%, Vetnil, São Paulo, Brazil. [b]Dolosal, Cristália, São Paulo, Brazil. [c]Propovan 1%, Cristália, São Paulo, Brazil. [d]SAT 500, Takaoka, São Paulo, Brazil [e]Isoforine, Cristália, São Paulo, Brazil.

[f] Colchão Térmico, Brasmed, São Paulo, Brazil.

[g]Ringer com Lactato, JP Pharmaceutical Ind., Ribeirão Preto, Brazil.

[h]DX 2010; Dixtal, São Paulo, Brazil.

[i] Gas analyzer module VAMOS plus, Dräger do Brazil, Barueri, Brazil

[j] Doppler 841-A; Parks Medical Electronics, OR, USA.

[k] Research Randomizer, Computer software, http://www.randomizer.org/, Pennsylvania,

USA

[l]Ropi 10mg/mL, Cristália, São Paulo, Brazil.

[m]Maxicam 0.2%, Ourofino Saúde Animal, São Paulo, Brazil.

[n] Electronic von Frey anesthesiometer, IITC Life Science, CA, USA

[o] Dolosal, Cristália, São Paulo, Brazil.

[p]GraphPad Software Inc., CA, USA

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Authors' contributions

GOLC: study design and drafting of manuscript. IPGAN: perioperative care, behaviour scoring, rescue analgesia, drafting of manuscript. ABFS: recruitment and enrolling study animals. TSB: data acquisition, data management. JSCJ: anaesthesiologist, postoperative care. GMN: surgical procedure. RNC: study design, data analysis, helped with statistical analysis, writing of manuscript. All authors approved the final manuscript.

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Table 1. Demographic data and procedural times (mean ± standard deviation) of cats undergoing ovariohysterectomy treated with local infiltration with saline solution (S, *n* = 15), ropivacaine (R, *n* = 15) and ropivacaine/meloxicam (RM, *n* = 15).

Variables	**Group**			
	S	R	RM	P value
Body weight (kg)	2.5 ± 0.4	2.6 ± 0.5	2.4 ± 0.4	0.63
Age (months)	15.6 ± 8	17.4 ± 7.7	15 ± 8	0.68
Anesthesia time (minutes)	38.6±8.5	46.9±10	42.4±9.9	0.14
Surgery time (minutes)	21 ± 4.0	23.2 ± 4.3	19.8 ± 4.1	0.19
Extubation time (minutes)	7.8 ± 5	7.8 ± 3.2	7.3 ± 3.9	0.23
Recovery time (minutes)	39 ± 15.2	38±13.7	33 ± 15.1	0.35

Table 2. Pain and sedation scores [median (range)] measured prior to ovariohysterectomy (BL) and at 0.5, 1, 2, 4, 6, 8, 18 and 24 hours after extubation in cats treated with local infiltration with saline solution (S, *n* = 15), ropivacaine (R, *n* = 15) and ropivacaine/meloxicam (RM, *n* = 15).

Test	**Group**	**Time (hours)**									
		BL	0.5	1	2	4	6	8	12	18	24
IVAS	S	0 (0-0)	5 (0-10)*	5 (0-35)*	5 (5-35)*	10 (0-25)*	5 (0-20)*	5 (0-20)*	5 (0-15)*	5 (0-10)*	5 (0-10)*
	R	0 (0-0)	5 (0-15)*	5 (0-20)*	5 (0-20)*	5 (0-15)*	5 (5-15)*	5 (0-20)*	5 (0-10)*	5 (0-10)*	5 (0-10)*
	RM	0 (0-0)	5 (0-10)*	5 (0-10)*†	5 (0-20)*	5 (0-35)*	5 (5-20)*	5 (0-15)*	5 (0-20)*	5 (0-10)*	5 (0-15)*
MCPS	S	0 (0-5)	0 (0-2)	0 (0-15)	1 (0-15)*	1 (0-12)	1 (0-6)	1 (0-6)	1 (0-4)	1 (0-2)	1 (0-2)
	R	0 (0-1)	0 (0-2)	1 (0-7)	1 (0-4)	1 (0-5)*	1 (0-5)*	1 (0-4)	1 (0-2)	1 (0-2)	0 (0-2)
	RM	0 (0-5)	0 (0-4)	0 (0-1)	0 (0-5)	0 (0-6)	1 (0-5)	1 (0-3)	1 (0-2)	0 (0-2)	0 (0-2)
MNT (g)	S	201.3 (91-306)	213.4 (95- 483)	219.7 (100- 304)	189.6 (99- 337)	167.4 (70-370)	166.2 (61- 278)	147.8 (113- 358)	172.2 (113-290)	170.0 (120- 223)	180.0 (133-244)
	R	221.3 (91-306)	213.4 (99- 384)	219.7 (100- 304)	189.6 (105-337)	167.4 (70 -370)	166.2 (61- 278)	147.8 (113- 358)	172.2 (113-290)	170.0 (120- 223)	180.0 (133-244)
	RM	243.1 (98-308)	251.6 (83- 329)	251.4 (132-311)	200.4 (117-312)	191.0 (93-299)	158.1 (61-231)	174.2 (106 -227)	155.1 (102-282)	152.3 (90-253)	187.1 (108-291)
Sedation	S	0 (0-0)	1 (0-3)*	0 (0-1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
	R	0 (0-0)	1 (0-3)*	0 (0-1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
	RM	0 (0-0)	1 (0-3)*	0 (0-1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)

*Significantly different from baseline values ($P < 0.05$). † Significantly different from S and R groups ($P = 0.034$).

IVAS = Interactive Visual Analogue Scale; MCPS = UNESP-Botucatu Multidimensional Composite Pain Scale; MNT = Mechanical Nociceptive Thresholds

Table 3. Number of rescue doses administered over time following ovariohysterectomy in cats treated with local infiltration with saline solution (S, *n* = 15), ropivacaine (R, *n* = 15) and ropivacaine/meloxicam (RM, *n* = 15).

Group	Postoperative time (hours)									Total number of rescue doses	Total number of rescued cats
	0.5	1	2	4	6	8	12	18	24		
S	0	2	4	1	0	0	0	0	0	7	4/15
R	0	1	0	0	0	0	0	0	0	1	1/15
RM	0	0	0	1	0	0	0	0	0	1	1/15

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Healthwise Knowledgebase. US Pharmacopeia, Rockville. 1998. http://www.healthwise.org. Accessed 21 Sept 1998.

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- **Results:** the main findings

- **Conclusions:** a brief summary and potential implications

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