PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIA ANIMAL

DENIS ROBISON GOMES

ADIÇÃO DE SULFATO DE MAGNÉSIO À ROPÍVACAINA NO BLOQUEIO INTRAPERITONEAL PARA O CONTROLE DA DOR APÓS OVARIOSALPINGOHISTERECTOMIA EM CADELAS

Presidente Prudente
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Dissertação apresentada à Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciência Animal. Área de concentração: Fisiopatologia Animal.

Orientador:
Profa. Dra. Renata Navarro Cassu

Presidente Prudente
2019

636.089
G633a

Gomes, Denis Robison.
Adição de sulfato de magnésio à ropivacaína no bloqueio intraperitoneal para o controle da dor após ovariosalpingohisterectomia em cadelas / Denis Robison Gomes. – Presidente Prudente, 2019.
42f.: il.

Dissertação (Mestrado em Ciência Animal) - Universidade do Oeste Paulista – Unoeste, Presidente Prudente, SP, 2019.
Bibliografia.
Orientador: Renata Navarro Cassu

1. Analgesia. 2. Ropivacaína. 3. Sulfato de magnésio. I. Título.

Catalogação na Fonte: Michele Mologni–CRB 8-6204

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Dr. André Leguthe Rosa
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DEDICATÓRIA

Este trabalho é dedicado aos meus pais Denilson e Rosinei, os quais sempre apoiaram minhas decisões, incentivando e apoiando em todos os momentos, mesmo estando distantes, nunca mediram esforços para ajudar em meu crescimento profissional.

Aos meus avós Severino e Eiko, que desde o início da minha carreira acadêmica, estão presentes me apoiando e se emocionando com minhas conquistas.

Minha namorada Bianca pelo apoio e incentivo na decisão de iniciar esta etapa, na compreensão quando me ausentava da clínica para a conclusão deste trabalho. Ajudando a superar a dificuldades, para sempre seguir em frente.

Ao Sr. Ivam e Sra. Dalva, em que a importante decisão de iniciar este trabalho partiu do incentivo deles.

A todos os familiares e amigos que torceram para que esta etapa se concretizasse.

A vocês eu dedico este trabalho!

AGRADECIMENTOS

A Deus pelo dom da vida e pela sabedoria adquirida para utilizar em benefício dos animais.

“O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – (Brasil) CAPES – Código de Financiamento 001”

A minha orientadora Profa Dra Renata Navarro Cassu, a quem me aceitou quando um dia fui pedir a sua orientação, acreditando, confiando, ensinando e incentivando a sempre seguir em frente, sem ela nada disso seria possível, os meus mais sinceros agradecimentos.

A amiga Isabela pelo auxílio no projeto desde a primeira castração até o último ponto realizado, ao conhecimento cedido e o companheirismo nas manhãs de terça.

Aos amigos residentes da cirurgia de pequenos animais Murilo e Rafaela pela colaboração e compreensão, cedendo um precioso período na agenda tão agitada do setor.

Aos professores responsáveis pelo setor de cirurgia de pequenos animais Camila e Gabriel pela compreensão.

A amiga Leticia residente do setor de anestesiologia, a qual foi de suma importância para a realização deste projeto, responsável por anestesiar todos os animais envolvidos.

Ao amigo José Sergio Jr, o qual teve uma pequena participação, mas não menos importante.

A equipe do setor do laboratório de patologia clínica do Hospital veterinário Ana Maria, Lídia, Sidenir e a residente Marina.

A professora do setor de imagem Rejane e a residente Sheila por auxiliar com as imagens nos exames de triagem.

A todos os funcionários do Hospital Veterinário da Unoeste que de forma direta ou indireta participaram de alguma forma para que este projeto fosse realizado. Em especial aos amigos da secretária Marcelão e Pedro, pelo auxílio com o preparo das fichas e as requisições de exames dos animais selecionados.

As todas as pessoas envolvidas na ONG do município de Pirapozinho.

Muito obrigado!

"Todos os nossos sonhos podem vir a ser verdadeiros – se tivermos a coragem de segui-los."

Walt Disney

RESUMO

Adição de sulfato de magnésio à ropivacaína no bloqueio intraperitoneal para o controle da dor após ovariosalpingohisterectomia em cadelas

Objetivou-se comparar a eficácia analgésica e os efeitos adversos decorrentes do bloqueio intraperitoneal (IP) com ropivacaína isolada e associada ao sulfato de magnésio ($MgSO_4$) em cadelas encaminhadas para ovariosalpingohisterectomia (OSH). Quarenta e cinco cadelas foram sedadas com a associação de acepromazina (0,05 mg/kg) à meperidina (5 mg/kg), por via intramuscular (IM). A indução anestésica foi feita por via intravenosa (IV) com propofol (dose-efeito), seguindo-se a manutenção anestésica com isofluorano/O_2. Em delineamento encoberto e randomizado, os animais foram distribuídos em três tratamentos (n=15): S: solução salina 0,9% (1,2 mL/kg); R: ropivacaína 0,25% (3 mg/kg); R-Mg: associação de ropivacaína 0,25% (3 mg/kg) e sulfato de magnésio (MgSO4) (20 mg/kg). Nos grupos R e R-Mg, os fármacos foram diluídos em solução salina 0,9%. Após a abertura cirúrgica da cavidade abdominal, as soluções foram instiladas no espaço peritoneal (pedículos ovarianos e cérvix uterina). Durante a cirurgia, suplementação analgésica foi realizada com fentanil (2,5 µg/kg, IV) com base nos parâmetros cardiovasculares (incremento de 20% na frequência cardíaca e/ou pressão arterial, em relação à mensuração prévia). A concentração plasmática de MgSO4 foi mensurada antes (basal) e após (15, 60, 120 e 240 minutos) o tratamento IP. 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 de Dor de Glasgow -forma abreviada (ECG) e o limiar nociceptivo mecânico (LNM). Morfina (0,5 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 ($P < 0,05$). Durante a cirurgia, a incidência de suplementação analgésica e de hipotensão foram superiores no grupo R-Mg em relação ao grupo S ($P = 0,034$ e $P = 0,018$, respectivamente). Os escores de dor (ECG), o LNM e a concentração plasmática de MgSO4 não diferiram entre os grupos ($P > 0,05$). Escores inferiores foram detectados pela EAVID entre 0,5 ($P = 0,004$) e 1 hora ($P = 0,003$) após a extubação traqueal no grupo R-Mg em relação ao demais grupos. A incidência de suplementação analgésica pós-operatória não diferiu entre os grupos ($P > 0,05$). Conclui-se que o tratamento IP com MgSO4 associado à ropivacaína reduziu o requerimento analgésico intra-operatório e os escores de dor (EAVID) na primeira hora após a cirurgia, porém foi associado à maior incidência de hipotensão.

Palavras-chave: Analgesia. Anestésico Local. Cães. Intraperitoneal. Ropivacaína. Sulfato de Magnésio.

ABSTRACT

Addition of magnesium sulfate to intraperitoneal ropivacaine for perioperative analgesia in canine ovariohysterectomy

The aim of this study was to investigate the analgesic efficacy and adverse effects of the intraperitoneal ropivacaine and its combination with magnesium sulfate ($MgSO_4$) in dogs undergoing ovariohysterectomy. Forty-five dogs were sedated with acepromazine (0.05 mg/kg) and pethidine (5 mg/kg). Anesthesia was induced with intravenous (IV) propofol (dose effect) and maintained with isoflurane/O_2. In a masked and randomized design, the dogs were randomly distributed into three treatments (n = 15): S = saline solution 0.9% (1.2 mL/kg); R: 0.25% ropivacaine (3 mg/kg); R-Mg: 0.25% ropivacaine (3 mg/kg) combined with $MgSO_4$ (20 mg/kg). The solutions were instilled into the peritoneal space (ovarian pedicles, and uterine cervix). Intraoperatively, fentanyl (2,5 µg/kg, IV) was administered based on cardiovascular parameters. The magnesium plasma concentration was measured before (baseline) and 15, 60, 120 and 240 minutes after IP treatment. Analgesia was assessed for 24 hours post-extubation using an Interactive Visual Analog Scale (IVAS), the short form of the Glasgow Composite Pain Scale (CMPS-SF), and mechanical nociceptive thresholds (MNT). Morphine was administered as rescue analgesia. Data were analyzed using the chi-square test, Tukey test, Kruskal-Wallis test, and Friedman test ($P < 0.05$). Intraoperatively, the incidence of analgesic supplementation and hypotension were higher in the R-Mg group compared to S group ($P = 0.034$ e $P = 0.018$, respectively). The CMPS-SF pain score, MNT and the magnesium plasmatic concentration did not differ between groups. The IVAS pain scores were lower in the R-Mg group at 0.5 ($P = 0.004$) and 1 ($P = 0.003$) hour post-extubation when compared with the other groups. The incidence of postoperative analgesic supplementation did not differ between groups ($P > 0.05$). In conclusion, IP treatment with ropivacaine in combination with $MgSO_4$ decreased intraoperative requirements and the postoperative pain scores (IVAS) in the first hour after ovariohysterectomy in dogs, however was associated with a higher incidence of hypotension.

Keywords: Analgesia. Local Anesthetic. Dogs. Intraperitoneal. Ropivacaine. Magnesium Sulfate.

SUMÁRIO

Addition of magnesium sulfate to intraperitoneal ropivacaine for perioperative analgesia in canine ovariohysterectomy

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Abstract

Given the antinociceptive effects of magnesium, its use as an adjunctive analgesic has been proposed for perioperative pain management. This study investigated the analgesic efficacy and adverse effects of intraperitoneal ropivacaine alone and in combination with magnesium sulfate (Mg) in canine ovariohysterectomy. Forty-five dogs were sedated with acepromazine plus pethidine. Anesthesia was induced with propofol and maintained with isoflurane. The dogs were randomly distributed into three treatments (n = 15): intraperitoneal instillation of saline solution 0.9% (group S), ropivacaine 0.25% (3 mg/kg, group R) alone in combination with Mg (20 mg/kg, group R-Mg). Intraoperatively, intravenous fentanyl was given as rescue analgesia. Postoperative pain was assessed using an Interactive Visual Analog Scale (IVAS), the short form of the Glasgow Composite Pain Scale (CMPS-SF), and mechanical nociceptive thresholds (MNT). Morphine was administered as rescue analgesia. The R-Mg group required significantly less intraoperative fentanyl, and exhibited a higher incidence of intraoperative hypotension compared to the S group. Significantly lower IVAS pain scores were recorded in the first hour post-extubation in the R-Mg compared to the other groups. Postoperative rescue

analgesia did not differ significantly between groups. The R-Mg treatment decreased the intraoperative analgesic requirements and early postoperative pain, however was associated with a higher incidence of hypotension.

Keywords

analgesia, canine, intraperitoneal, local anesthetic, NMDA antagonist receptors

1. INTRODUCTION

The provision of adequate perioperative analgesia has been well recognized, not only for ethical reasons, but also to improve the pain management outcome of veterinary patients. In recent decades, the combination of regional anesthetic techniques with systemic analgesics has been considered an effective method for postoperative pain management in small animal practice (Campagnol, Teixeira-Neto, Monteiro, Restitutti, & Minto, 2012; Kalchofner Guerrero, Campagna, Bruhl-Day, Hegamin-Younger, & Guerrero, 2016).

Among the different regional techniques, the intraperitoneal (IP) instillation of local anesthetics has become a popular analgesic strategy in both human and veterinary medicine, due to its simplicity, low cost, and easy application. Clinical evidence indicates that IP analgesia decreases postoperative pain and supplemental analgesic requirements after ovariohysterectomy in both dogs and cats (Benito et al., 2016; Campagnol et al., 2012).

Despite the beneficial analgesic effects of IP administration of local anesthetics for routine ovariohysterectomy, this technique has been associated with a limited duration of action (Campagnol et al., 2012). The use of many types of adjuvant drugs has been proposed to improve the duration of analgesia of different techniques of local anesthesia. Magnesium is a non-competitive N-methyl-D-aspartate (NMDA) antagonist and has been shown to decrease intra and postoperative requirements in human surgical patients (Abd-Elsalam et al., 2017; Yadava, Rajput, Katiyar, & Jain, 2016).

In veterinary medicine, reports of the effectiveness of additive administration of Mg to local anesthetics are limited. In dogs undergoing orthopedic surgery, the addition of spinal Mg to ropivacaine increased the intensity and duration of analgesia (Adami, Casoni, Noussitou, Rytz, & Spadavecchia, 2016). In contrast, no analgesic benefits were reported following the epidural combination of ropivacaine with Mg in dogs undergoing hip arthroplasty (Lardone et al., 2017). To date, the additive IP administration of Mg has not been reported in small animal practice. In humans, the combination of IP ropivacaine with Mg decreased the systemic analgesic requirements and duration of postoperative analgesia after laparoscopy (Yadava et al., 2016).

The aim of this study was to investigate the analgesic effects and adverse events of IP instillation of ropivacaine alone and in combination with Mg in dogs undergoing elective ovariohysterectomy. The hypotheses were that the addition of Mg to ropivacaine would decrease the intra-and postoperative analgesic requirements and postoperative pain scores, as well as delay the time to rescue analgesia.

2. MATERIALS AND METHODS

2.1 Study design

A prospective, randomized, blinded, placebo-controlled clinical study was designed to compare the perioperative analgesic effects of IP administration of saline, ropivacaine, and its combination with Mg. 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.

2.2 Animals

The study involved 45 dogs of different breeds, scheduled for elective OHE. For inclusion, dogs were required to present normal complete blood count and serum chemistry, be aged ≥ 6 months, and have a good temperament. The exclusion criteria were: pregnancy, lactation, extreme aggression, body condition score greater than 6 or less than 3 on a nine-point scale, and systemic diseases. The animals were admitted to the hospital at least 24 hours prior to surgery for acclimatization.

2.3 Anesthetic protocol and randomization

All anesthetic procedures were performed by the same anesthetist who was blinded to the group allocation. After fasting for 8 hr, dogs were sedated intramuscularly (IM) with acepromazine (0.05 mg/ kg; Acepran 0.2%, Vetnil, Louveira, SP, Brazil) in combination with pethidine (5 mg/kg; Dolosal, Cristália, Itapira, SP, Brazil). Thirty min later, an intravenous (IV) 22-gauge catheter was aseptically placed in the cephalic vein. Anesthesia was induced with IV propofol (Propovan 1%, Cristália, Itapira, SP, Brazil) in a sufficient dose to permit endotracheal intubation. Isoflurane 1.0 -1.5% (Isoforine, Cristália, Itapira, SP, Brazil) vaporized in 100% oxygen was administered for the maintenance of anesthesia. 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, SP, 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 Brasil, Barueri, SP, 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, SP, Brazil). Dogs were permitted to breathe spontaneously throughout the procedure, unless $FE'CO_2$ exceeded 45 mmHg, when mechanical ventilation was used to maintain eucapnia. Systolic arterial blood pressure (SABP) was monitored indirectly by sphygmomanometry, with a Doppler ultrasound device (Doppler 841-A; Parks Medical Electronics, OR, 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. Body temperature was maintained between 37°C and 38°C using an electrical heating pad (Brasmed Veterinária, São Paulo, SP, Brazil). Lactated Ringer's solution was administered IV at 5 mL/kg/hr until extubation.

The dogs 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 (3 mg/kg; R group), and ropivacaine combined with Mg (20 mg/kg; R-Mg group).

Treatments were administered after the abdomen had been surgically opened and before ligation of the ovarian pedicles or uterus. Solutions were instilled into the peritoneal space: in S, 0.9% saline (1.2 mL/kg), in R, ropivacaine 0.25% (3 mg/kg), and in R-Mg, ropivacaine at the same dose with Mg (20 mg/kg). Ropivacaine 1% was diluted in saline to a concentration of 0.25%, achieving a final volume of 1.2 mL/kg. In the R-Mg, magnesium sulfate was added to ropivacaine after the dilution. One-third of the total volume was administered on to each ovarian pedicle and uterine cervix, using a syringe attached to a 22-gauge catheter. The ovariohysterectomy commenced 5 min later, which was performed using a standard technique through median laparotomy access in supine dogs. All surgical procedures were performed by the same surgeon using a 3-cm ventral midline approach and 3-clamp technique (Howe, 2006).

During anesthesia, 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 SABP or HR increased by more than 20% of previously recorded values, additional analgesia was provided with fentanyl (2.5 µg/kg, IV). Data were recorded at specific time points throughout anesthesia, as follows: T0 =baseline, T1= 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. The number of dogs requiring rescue analgesia and the number of fentanyl rescue doses were recorded.

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 dog. Extubation was performed when the dog recovered the swallowing reflex.

2.4 Postoperative monitoring

The same single observer, unaware of the treatment groups, was responsible for the pain and sedation assessments, which were performed 24 hr prior to surgery (baseline), and 0.5, 1, 2, 4, 6, 8, 12, 18, and 24 hr after extubation. The observer was a veterinary post-graduate student, with experience in the assessment of pain in dogs 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 the short form of the Glasgow composite pain scores (CMPS-SF), from 0 = no pain to 24 = maximum pain) (Reid, Nolan, & Hughes, 2007). The CMPS-SF pain scoring included 30 descriptor options with six behavioral categories. For scoring, each dog was initially evaluated for one minute in the kennel. Following this, the dog was stimulated to move around, for observation of mobility, reactions, and behavior. Finally, the incision and surrounding area of the abdomen was gently palpated using 2-3 digits, and the reaction of the dog was assessed and recorded.

The pain scores were also evaluated through mechanical nociceptive thresholds (MNT) using an electronic von Frey device (electronic von Frey anesthesiometer, IITC Life Science, Los Angeles, CA, USA). 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 dogs 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 dog exhibited signs of pain, such as a withdrawal movement, contraction of the abdominal wall, attempts to bite/scratch, and vocalization. The MNT was assessed after the IVAS and CMPS-SF measurements at the same time points.

Morphine was administered (0.5 mg/kg IM; Cristália, Itapira, SP, Brazil) as rescue analgesia if the CMPS-SF scores were ≥ 6/24, as previously suggested (Reid et al., 2007). Thirty min after the first supplemental analgesia, if the CMPS-SF score remained ≥ 6, meloxicam (0.2 mg/kg IM; Movatec, Boehringer-Ingelheim, São Paulo, SP, Brazil) was administered as a single dose. The number of dogs requiring rescue analgesia and the number of rescue doses were recorded.

Numerical rating scores were used for the assessment of the degree of sedation, including four items: spontaneous posture, eye position, response to noise (handclap), and general appearance/attitude (scale range = 0 -11 points) (Wagner, Hecker, & Pang, 2017).

2.5 Blood sampling

Venous blood samples (2 mL) were collected in non-heparinized tubes prior to surgery and at 15, 60, 120, and 240 min after IP injections for measurement of plasma concentration of magnesium (mmol/L), using a commercial colorimetric assay (Roche Farmacêutica Química Ltda, São Paulo, SP, Brazil).

2.6 Adverse events

The occurrence of adverse events during the study period such as seizures, nausea, vomiting, and cardiovascular effects (bradycardia, arrhythmias, or hypotension) were recorded. Bradycardia and hypotension were defined as a HR < 60 beats/minute, SABP < 90 mmHg, respectively for longer than 5 min consecutively. Bradycardia was treated with IV atropine (0.022 mg/kg), as required. Hypotension was treated with IV crystalloid bolus (10 mL/kg lactated Ringer´s, for 10 min, repeated if necessary). In cases of non-responsive hypotension, IV dopamine (5-10 µg/kg/min), was administered.

2.7 Statistical analysis

A sample size of at least 15 dogs per group was estimated to achieve 80% statistical power to detect a prevalence of treatment failure of 70% in the Control group and 20% in the treated groups (R and R-Mg). From Zanuzo et al. (2015), the prevalence of rescue analgesia in dogs receiving pethidine before ovariohysterectomy ranged from 40-70%.

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 appropriate.

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.

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.

The number of dogs requiring rescue analgesia intra-and postoperatively was compared between groups using the Fisher´s exact test. A Kruskal-Wallis test was used to compare the number of morphine and fentanyl doses administered to the groups.

All analyses were performed using GraphPad Prism 7.0.[p] Differences were considered significant when $P < 0.05$.

3. RESULTS

3.1 Population data and procedural times

Fifty-five dogs were initially enrolled in the study, however only 45 of these met the inclusion criteria. Ten dogs were excluded (three dogs were diagnosed with ehrlichiosis, and three with pyometra; two dogs exhibited aggressive behavior, and two were pregnant). Age, weight, and surgery, anesthesia, and extubation times were not different between groups (P > 0.05) (Table 1).

3.2 Intraoperative fentanyl requirements

The prevalence of intraoperative rescue analgesia was significantly lower in the R-Mg compared to the Control (P = 0.034), but not compared to the R group (P = 0.127) (Table 2). Fentanyl supplementation was needed in three dogs in the R-Mg (20%), nine dogs (60%) in the S, and eight dogs (53.3%) in the R group.

3.3 Postoperative assessments

The IVAS pain scores were lower in the R-Mg at 0.5 (P = 0.004) and at 1 hour (P = 0.003) post-extubation compared to the R and S groups. Compared to the corresponding baseline values, the IVAS scores were significantly increased from 0.5 to 1 hr in all groups (P < 0.001). The CMPS-SF scores were not significantly different between groups (P > 0.05). Compared to the baseline values, significant increases were recorded in the CMPS-SF scores from 0.5 to 1 hr (P = 0.004) in the R-Mg group and from 0.5 to 2 hr (P = 0.006) in the R and S groups (P > 0.05) (Table 3).

The MNT measurements were not significantly different between groups at any time point (P > 0.05) (Table 3).

Sedation scores did not differ between groups during the 24-hr period. When compared with baseline values, increased scores were recorded from 0.5 to 1 hr post-extubation (P < 0.001) (Table 3).

3.4 Postoperative rescue analgesia

The number of dogs (P = 0.67) that required rescue analgesia and the number of rescue analgesic doses (P = 0.83) administered did not differ statistically between groups. In the S and R-Mg groups, each of the dogs required rescue analgesia on two occasions (one dose of morphine and one dose of meloxicam). In the R group, four dogs received rescue analgesia on two occasions (one dose of morphine and one dose of meloxicam), and one dog received rescue analgesia on one occasion (one dose of morphine) (Table 4).

3.5 Magnesium plasma concentration

The magnesium plasma concentrations were comparable between groups and remained within normal values (Figure 1).

3.6 Adverse events

During anesthesia, the incidence of hypotension was higher in the R-Mg (p = 0.018) compared to the S group, but not compared with the R group (P = 0.147). The occurrence of hypotension was recorded in three dogs (20%) in the R and in seven dogs (46.6%) in the R-Mg. Hypotension was not detected in the S group. No other adverse events were observed during the study period.

4. DISCUSSION

The results of this study demonstrated that addition of IP magnesium to ropivacaine reduced the intraoperative analgesic requirements and also the postoperative pain (lower IVAS scores) during the first hour following OHE in dogs. However, the combination of the drugs did not prolong the postoperative analgesia, which is partially in accordance with the authors´ hypotheses.

Clinical studies have shown a reduction in the intraoperative opioid requirements when magnesium was administered by intravenous and intrathecal routes in both dogs and humans (Adami et al., 2016; Kara, Sahin, Ulusan & Aydogdu, 2002; Koinig et al., 1998). In the current study, the addition of magnesium to ropivacaine significantly reduced the intraoperative fentanyl requirements compared to the other groups. More than 50% of dogs in the S and R groups required analgesic supplementation during the clamping of the ovarian pedicles, suggesting that the analgesia provided by both protocols was insufficient to decrease the peripheral nociceptive receptor sensitivity to the mechanical stimulation induced by surgical trauma. In contrast, when Mg was added to ropivacaine, intraoperative analgesic supplementation was required in only 20% of dogs, which is supported by a previous study, where the addition of spinal Mg to ropivacaine decreased the intraoperative analgesic requirements compared to the sole use of ropivacaine in dogs undergoing orthopedic surgery (Adami et al., 2016). Given the antinociceptive effects of magnesium, including the inhibition of calcium influx and the antagonism of the NMDA receptors (Cavalcante et al., 2013; Nowak, Bregestovski, Ascher, Herbet & Prochiantz, 1984), it is likely that the analgesia was potentiated by the combination of the drugs, preventing central sensitization elicited by the surgical noxious stimulation (Woolf & Thompson, 1991). In animal studies, the analgesia induced by morphine was improved by the calcium channel blockade, which resulted in a decrease in total opioid consumption (Mccarthy et al., 1998; Omote, Sonoda, Kawamata, Iwasaki & Namiki., 1993).

In contrast from other studies that reported superior intensity and duration of analgesia when adding magnesium to local anesthetics (Adami et al., 2016; S. M. Vučković et al., 2015), in the current study the combination of these drugs did not provide significant postoperative analgesic benefits, which is consistent with the results reported by Lardone et al. (2017), who investigated the epidural addition of magnesium to ropivacaine in dogs undergoing hip arthroplasty. Based on the CMPS-SF scores, MNT measurements, and postoperative analgesic requirements, both IP protocols resulted in similar analgesic effects and did not improve analgesia compared to IP saline. Through the IVAS, lower pain scores were recorded in the R-Mg group only during the first hour post-extubation. Specific experimental conditions, including an experienced surgeon, minimal tissue trauma, and the provision of preoperative analgesia could explain in part the lack of significant effects on CMPS-SF pain scores and MNT testing. Lascelles et al. (1997) reported that the preoperative administration of a single dose of pethidine prevented allodynia and decreased hyperalgesia in dogs after OHE. Additionally, to minimize selection bias, in the current study the dogs 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 analgesic supplementation. Moreover, given the antihyperalgesic effects of morphine and meloxicam (Kukanich, Lascelles, & Papich, 2005; Zanuzzo et al., 2015), the postoperative analgesic supplementation may have interfered in the response to the von Frey filaments in this study. In addition, the interference of sedation on animal pain responses has been well established. However, it is unlikely that our results were influenced by the degree of sedation, since the highest sedation scores were detected only until the first hour post-extubation in all treatment groups.

In our study, the percentage of dogs that required postoperative recue analgesia was near to the percentage reported by Lambertine et al. (2108), where the analgesic effects of IP ropivacaine were considered similar to those provided by an equivalent dose of bupivacaine in dogs undergoing OHE. However, in that study, the majority of dogs treated with IP ropivacaine received rescue analgesia at 8 hr after extubation, while in our study the need for rescue analgesia was more evident at 2 hr after extubation. Explanations for these discrepancies may include the systemic analgesics combined with the IP block, and the different concentrations of administered ropivacaine. While Lambertine et al. (2018) administered buprenorphine and carprofen to all dogs prior to surgery, in the current study only meperidine was given as a preventive analgesic. The decision to use pethidine was based on its short duration of action, approximately 1-2 hr in dogs (Yamashita et al., 2015), aiming to provide intraoperative analgesia with little influence on post-operative pain. Additionally, in our study ropivacaine was administered at a concentration of 0.25%, while a concentration of 0.5% was reported by Lambetine et al. (2018). As concentration and volume play an important role in the onset, duration, and efficacy of sensory block(Su, Zhang, Zhang, Li, & Shi, 2015; Zhai, Wang, Rong, Li, & Wang, 2016), it is possible that the dilution of ropivacaine to 0.25% in the current study may have interfered in our results. In humans, a previous study reported prolonged onset of sensory blockade in patients receiving an interscalene block with ropivacaine at a concentration of 0.25% when compared with concentrations of 0.5% and 0.75% (Zhai, Wang, Rong, Li, & Wang, 2016). Similarly, in rats, administering a low volume with high concentration of local anesthetic (1.5% mepivacaine) decreased the onset and increased the intensity of sensory block when compared to a more diluted solution (1% mepivacaine) (Muniz et al., 2008).

Measurement of serum or plasma magnesium has been recommended to assess both therapeutic concentrations and adverse events. Although the magnesium plasma concentration in this study remained within the normal limits, suggested to range from 0.66-1.23 mmol/L in dogs (Nakayama, Nakayama, Miyamoto, & Hamlin, 1999), a significantly higher incidence of hypotension was recorded in the R-Mg group. Previous studies also reported the occurrence of hypotension when magnesium was administered alone and in combination with loco-regional anesthetic blocks in ewes (Sipes et al., 1992; Vincent et al., 1991). Due to the calcium channels blockade, a decrease in both systemic vascular resistance and arterial blood pressure may be expected following Mg administration (Shechter, 2010). The hypotension in all dogs was transient and reverted during surgery using only a crystalloid bolus. Other adverse events related to Mg administration, such as cardiac arrhythmias, nausea, and vomiting, were not observed during the study period, which is in agreement with previous reports in dogs (Adami et al., 2016; Lardone et al., 2017).

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 CMPS-SF 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 Control group and 20% in the treatment groups (R and R-Mg). However, the differences in the frequencies of rescue analgesia were smaller than this, limiting the statistical power of our study. Moreover, the optimal dose of magnesium for perioperative analgesia in dogs has not been established. The dose used in the current study was based on previous pain animal model studies, which reported antinociceptive effects using systemic magnesium at doses ranging from 0.5 to 30 mg/kg (Srebro, Vučković, Vujović, & Prostran, 2014; Vuckovic et al., 2015). 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 dogs have not been reported. Moreover, the addition of Mg to ropivacaine could interfere in the onset of action and duration of the effect.

As part of a multimodal pain therapy, the addition of magnesium to ropivacaine decreased the intraoperative analgesic requirements and early postoperative pain, however, this protocol was associated with a higher incidence of hypotension. Further studies are required to determine the ideal dosage for IP administration of magnesium together with the pharmacokinetic profile of IP ropivacaine in dogs.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. Rejane Batista Brinholi for her technical support. The authors also thank the owners of the dogs for their contributions and the clinicians in the Department of Veterinary Surgery and Anesthesiology, University of Western São Paulo, for their support.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION

DRG: study design, surgical procedure, and drafting of manuscript. IPGAN: perioperative care, pain assessment, and rescue analgesia. LD: recruitment and enrolling study animals, data acquisition, and local solutions infiltration. LMLC: anesthesiologist and postoperative care. 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 dogs undergoing ovariohysterectomy treated with IP instillation of saline solution 0.9% (S, *n* = 15), ropivacaine 0.25% (R, *n* = 15) and its combination with magnesium sulfate (R-Mg, *n* = 15)

Variables	**Group**			
	S	R	R-Mg	P-value
Body weight (kg)	10.5 ± 5.8	10.2 ± 5	13.4 ± 5.4	0.23
Age (months)	36 ± 29	29 ± 16	27 ± 16	0.54
Surgery time (min)	27.8 ± 3.9	27.1 ± 3	27.9 ± 6.2	0.51
Anesthesia time (min)	45.6 ± 8.7	44.8 ± 6.9	44.5 ± 7.2	0.92
Extubation time (min)	6.8 ± 3.3	7.1 ± 3.6	6.6 ± 3.6	0.94
Recovery time (min)	28.7 ± 19	36.3 ± 23	32.6 ± 21	0.78

Table 2. Number of fentanyl doses administered during ovariohysterectomy in dogs treated with IP instillation of saline solution 0.9% (S, *n* = 15), ropivacaine 0.25% (R, *n* = 15) and its combination with magnesium sulfate (R-Mg, *n* = 15)

Group	T0	T1	T2	T3	T4	T5	Total rescue doses (n°)
S	0	2	6	1	0	0	9
R	0	1	5	2	0	0	8
R-Mg	0	0	3	0	0	0	3†

†Significantly different from S group ($P < 0.05$)

Table 3. 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 dogs undergoing ovariohysterectomy treated with IP instillation of saline solution 0.9% (S, *n* = 15), ropivacaine 0.25% (R, *n* = 15) and its combination with magnesium sulfate (R-Mg, *n* = 15)

Test	Group	Time (hours)									
		BL	0.5	1	2	4	6	8	12	18	24
IVAS	S	0 (0-0)	10 (0-60)*	5 (0-70)*	0 (0-50)	0 (0-30)	0 (0-30)	0 (0-15)	0 (0-5)	0 (0-5)	0 (0-5)
	R	0 (0-0)	20 (0-35)*	15 (0-40)*	0 (0-40)	0 (0-40)	0 (0-30)	0 (0-30)	0 (0-15)	0 (0-15)	0 (0-0)
	R-Mg	0 (0-0)	0 (0-20)†*	0 (0-20)†*	0 (0-15)	0 (0-15)	0 (0-10)	0 (0-10)	0 (0-5)	0 (0-5)	0 (0-0)
CMPS-SF	S	0 (0-2)	4 (1-9)*	3 (1-11)*	2 (0-10)*	2 (0-5)	2 (0-5)	1 (0-5)	1 (0-5)	0 (0-4)	0 (0-4)
	R	0 (0-3)	5 (1-6)*	3 (1-6)*	3 (0-13)*	2 (0-9)	1 (0-8)	1 (0-5)	1 (0-5)	0 (0-5)	0 (0-4)
	R-Mg	0 (0-4)	4 (0-8)*	3 (0-10)*	1 (0-6)	2 (0-8)	2 (0-4)	2 (0-6)	1 (0-2)	0 (0-3)	0 (0-3)
MNT (g)	S	502	210	250	274	244	206	250	284	278	273
		(186-583)	(148-474)	(124-532)	(80-582)	(114-531)	(96-471)	(98-587)	(152-525)	(129-616)	(118-600)
	R	389	205	327	306	262	270	244	254	259	260
		(116-602)	(105-583)	(99-528)	(104-537)	(122-540)	(110-480)	(86-508)	(102-594)	(109-526)	(83-645)
	R-Mg	455	292	296	273	268	252	285(	306	250	251
		(177-650)	(79-567)	(150-499)	(181-477)	(176-548)	(106-487)	149-538)	(82-528)	(97-605)	(70-565)
Sedation	S	1 (0-1)	3 (0-10)*	2 (1-6)*	2 (1-5)	1 (1-5)	1 (1-5)	1 (1-4)	1 (1-4)	1 (1-4)	1 (1-3)
	R	1 (0-1)	5 (1-9)*	3 (1-5)*	2 (1-4)	1 (1-4)	1 (1-3)	1 (1-1)	1 (1-1)	1 (1-1)	1 (1-0)
	R-Mg	1 (0-1)	4 (1-8)*	3 (1-7)*	2 (1-6)	1 (1-4)	1 (1-4)	1 (1-3)	1 (1-3)	1 (1-3)	1 (0-0)

Note: IVAS = Interactive Visual Analogue Scale; CMPS-SF = short form of the Glasgow Composite Pain Scale; MNT = Mechanical Nociceptive Thresholds;
*Significantly different from baseline values (P < 0.05). †Significantly different from S and R groups (P < 0.05)

Table 4. Number of rescue doses administered over time following ovariohysterectomy in dogs treated with IP instillation of saline solution 0.9% (S, *n* = 15), ropivacaine 0.25% (R, *n* = 15) and its combination with magnesium sulfate (R-Mg, *n* = 15)

	Post-operative time (hr)										
Group	0.5	1	2	4	6	8	12	18	24	Rescue doses (n°)	Rescued dogs (n°)
S	2	2	4	0	0	0	0	0	0	8	4/15
R	1	0	5	1	2	0	0	0	0	9	5/15
R-Mg	2	2	1	0	0	1	0	0	0	6	3/15

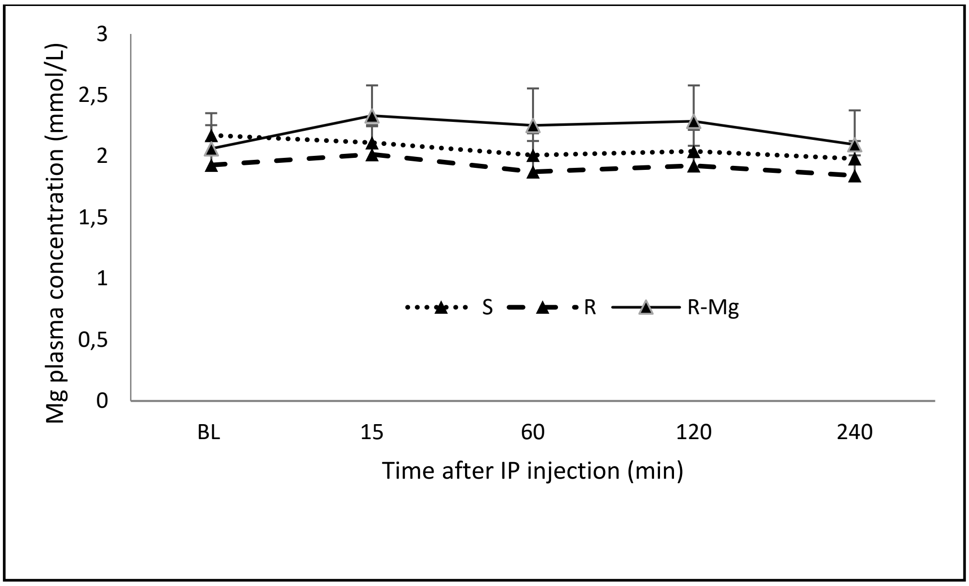

Figure 1 Mean values (±standard deviation) of plasma magnesium concentration of dogs undergoing ovariohysterectomy treated with IP instillation of saline solution 0.9% (S, *n* = 15), ropivacaine 0.25% (R, *n* = 15) and its combination with magnesium sulfate (R-Mg, *n* = 15)

ANEXO- NORMAS PARA PUBLICAÇÃO - JOURNAL OF VETERINARY PHARMACOLOGY AND THERAPEUTICS

GUIDE FOR AUTHORS

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Thank you for your interest in the *Journal of Veterinary Pharmacology and Therapeutics*. Note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium.

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Short Communications: these should differ from full papers on the basis of scope or completeness, rather than quality of data. They may report significant new data arising from problems with narrow, well-defined limits, or important findings that warrant rapid publication before broader studies are complete. The text should neither exceed 1,500 words nor be divided up into conventional sections. A standard abstract not to exceed 200 words is required and should include a list of five keywords. To aid rapid publication the Editors may undertake responsibility for correcting proofs. When submitting short communications, authors should make it clear that their work is to be treated as such.

Letters to the Editor and Commentaries: Letters to the Editor should be in response to issues arising from a specific recently published article. Commentaries should be a short, free-standing piece expressing an opinion. No abstract is required, and text should be formatted in one continuous section. These articles are limited to 1,000 words.

Reviews: the journal welcomes reviews providing an updated state of the art on topics associated with Veterinary Pharmacology, Veterinary Therapy and Veterinary Toxicology. It is however recommended that authors who are planning or preparing larger review-type articles should contact the Review Editor for detailed information.

Mark Papich
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Pharmacokinetic Reports: the aim of Pharmacokinetic Reports is to efficiently report on the pharmacokinetics of a drug from a defined study using a single dose by a single route of administration in a single species. The goal is to reduce lengthy introductions and discussions. More complex comparative studies (multiple doses, route comparisons, multiple species, and disease effects) should be submitted as full papers. The layout should be as follows:

• **Abstract** - The abstract should not exceed 200 words.

• **Introduction** - Introduce the drug class and provide a very short summary of the clinical applications.

• **Material and Methods** – Overview of experimental design, analytical methods, pharmacokinetic modeling approach and software used and statistical criteria for optimal model selection.

• **Results** - Provide both a table of pharmacokinetic parameters (without repeating parameters in the text) and plots of model predicted versus observed data or other graphical approaches to demonstrate model fit.

• **Discussion** - Limit to 4-5 paragraphs, compare results to previous publications and briefly discuss implications of the analysis.

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