Ciência Rural, Santa Maria, v.47: 01, e20160027, 2017 http://dx.doi.org/10.1590/0103-8478cr20160027
ISSNe 1678-4596
CLINIC AND SURGERY

Minimum alveolar concentration of isoflurane in dogs administered a single intramuscular injection of racemic or S (+)-ketamine after premedication with acepromazine-morphine

Thais Feres Bressan[1] Eduardo Raposo Monteiro[1,2] Karina Coelho[1] Betânia Souza Monteiro[1] Daniela Campagnol[1]

[1]Escola de Medicina Veterinária, Universidade Vila Velha (UVV), Vila Velha, ES, Brasil.
[2]Faculdade de Medicina Veterinária, Universidade Federal do Rio Grande do Sul (UFRGS), 91540-000, Porto Alegre, RS, Brasil. E-mail: eduardo.monteiro@ufrgs.br. Corresponding author.

ABSTRACT: *The present study evaluated the minimum alveolar concentration of isoflurane (ISO_{MAC}) in twenty three dogs premedicated with acepromazine (0.02mgkg^{-1}) and morphine (0.5mgkg^{-1}) and administered racemic (RK) or S(+)-ketamine (SK). Dogs randomly received a single dose (3mgkg^{-1}, IM) of either RK or SK 15minutes after anesthetic induction with propofol. The ISO_{MAC} was determined by the up-and-down method. Approximately 20 minutes after administration of RK or SK, a surgical noxious stimulus was applied and the response evaluated. The ISO_{MAC} was 0.50±0.01% in the RK group (n=10) and 0.31±0.04% in the SK group (n=13). The ISO_{MAC} was 38% lower in the SK group compared to the RK group. Results of the present study revealed that in dogs premedicated with acepromazine and morphine, IM administration of 3mgkg^{-1} ketamine approximately 20 minutes before the noxious stimulus produced clinically important reduction in the ISO_{MAC} and the MAC-sparing effect was significantly greater with SK compared to RK.*
Key words: *dissociative anesthesia, MAC, NMDA antagonist, phencyclidine derivative, preemptive analgesia.*

Concentração alveolar mínima do isoflurano em cães tratados com dose única intramuscular de cetamina racêmica ou S (+) após premedicação com acepromazina-morfina

RESUMO: *No presente estudo, foi avaliada a concentração alveolar mínima do isoflurano (CAM_{ISO}) em vinte e três cães premedicados com acepromazina (0,02mgkg^{-1}) e morfina (0,5mgkg^{-1}) e tratados com cetamina racêmica (CR) ou S(+) (CS). Os cães receberam aleatoriamente dose única (3mgkg^{-1}, IM) de CR ou CS, decorridos 15 minutos da indução anestésica com propofol. A CAM_{ISO} foi determinada pelo método up-and-down. Aproximadamente 20 minutos após a administração da CR ou CS, um estímulo nociceptivo (cirúrgico) foi aplicado e a resposta avaliada. A CAM_{ISO} foi 0,50±0,01% no CR (n=10) e 0,31±0,04% no CS (n=13). A CAM_{ISO} foi 38% menor no CS comparado ao CR. Os resultados deste estudo revelaram que, em cães premedicados com acepromazina e morfina, a administração IM de 3mgkg^{-1} de cetamina, aproximadamente 20 minutos antes do estímulo nociceptivo, causou redução clinicamente importante na CAM_{ISO} e o efeito redutor na CAM_{ISO} é significativamente maior com CS do que com CR.*
Palavras-chave: *anestesia dissociativa, CAM, antagonista NMDA, derivados da fenciclidina, analgesia pré-emptiva.*

INTRODUCTION

Ketamine is a phencyclidine derivative classified as a dissociative anesthetic. This class of drugs binds to a variety of receptors within the central nervous system and induces a cataleptic state. Ketamine has been used for anesthetic induction or for chemical restraint in several species (BERRY, 2015). Commercially, ketamine is available as a racemic mixture and, more recently, as a formulation containing only the S(+)-isomer. The S-isomer was reported to be 1.5 times more potent than the racemic mixture in dogs (CASONI et al., 2015).

In addition to the anesthetic effect, ketamine was also reported to possess analgesic and antihyperalgesic properties at lower than anesthetic doses via a non-competitive antagonism at N-methyl-D-aspartate (NMDA) receptors (SCHMID et al., 1999). In dogs undergoing ovariohysterectomy, preoperative ketamine (2.5mgkg^{-1}, IM) resulted in lower postoperative pain scores and reduced the need for postoperative rescue analgesia in comparison to a control group in which ketamine was not administered (SLINGSBY & WATERMAN-PEARSON, 2000). In the same study, the same dose of ketamine administered at extubation failed to significantly decrease the need for postoperative rescue analgesia, which might suggest a preemptive analgesic effect of the drug in dogs.

Another benefit of including ketamine as part of a balanced anesthetic technique is its effect

Received 01.12.16 Approved 08.11.16 Returned by the author 10.26.16
CR-2016-0027.R1

on the minimum alveolar concentration (MAC). Administration of ketamine as an intravenous target-controlled infusion reduced the isoflurane MAC (ISO_{MAC}) in dogs in a plasma concentration-dependent manner (SOLANO et al., 2006). Reduction on the ISO_{MAC} was associated to the improvement in ventilation and hemodynamics compared with isoflurane alone (BOSCAN et al., 2005).

In previous studies, which evaluated the effect of ketamine on MAC, the drug was administered intravenously as a target-controlled infusion (SOLANO et al., 2006). However, a recent study revealed that preemptive administration of a single intramuscular dose of ketamine improved postoperative pain control in dogs undergoing ovariohysterectomy (SLINGSBY & WATERMAN-PEARSON, 2000). However, the effect of intramuscular administration of ketamine on the MAC has not been evaluated in dogs. In addition, ketamine is rarely used as the only analgesic agent for dogs undergoing surgery. Therefore, the effect of ketamine alone on the MAC may not reflect routine clinical practice where it is administered as part of a balanced anesthetic technique. The present study evaluated the ISO_{MAC} in dogs premedicated with an acepromazine-morphine combination followed by a single intramuscular dose of racemic or S(+)-ketamine administered after anesthetic induction with propofol.

MATERIALS AND METHODS

Twenty three female dogs scheduled for elective ovariohysterectomy were included in the study. Any dog having clinical signs of systemic disease, abnormal laboratory data (CBC and serum chemistry), or aged <6 months or >8 years was excluded from the study.

Food was withheld for 12 hours prior to each experiment. Dogs were administered $0.02mgkg^{-1}$ of acepromazine and $0.5mgkg^{-1}$ of morphine as premedication. Drugs were mixed in a single syringe and administered IM in a pelvic limb. A 20 or 22G catheter was placed into a cephalic vein. Fifteen minutes after premedication, anesthetic induction was performed with propofol administered IV until endotracheal intubation could be accomplished. Anesthesia was maintained with isoflurane (Isoforine, Cristália) in oxygen (1.5 to $2.0Lmin^{-1}$) administered through a circle breathing system. An infrared gas analyzer (ILCA Sensor Module, Dräger) was used to monitor the end-tidal carbon dioxide ($ETCO_2$) and isoflurane (ETISO) concentrations. Samples of airway gases were obtained from between the endotracheal tube and the breathing system. Before each experiment, the calibration of the analyzer was checked by use of a calibration gas mixture containing a single concentration of isoflurane. Dogs were positioned in dorsal recumbency on a thermal pad to maintain esophageal temperature between 37°C and 38°C. All dogs received lactated Ringer's solution IV at $10mLkg^{-1}\ h^{-1}$ throughout anesthesia and had their lungs mechanically ventilated to maintain the $ETCO_2$ between 30 and 35mmHg.

A blood pressure cuff was placed proximal to the carpus for measurement of indirect systolic (SAP), mean (MAP) and diastolic (DAP) blood pressures by an oscillometric device. The cuff width was 40-50% of limb circumference and all measurements were taken at the level of the sternal manubrium. The mean of three consecutive measurements of SAP, MAP and DAP was used for analysis. Heart rate (HR) was monitored by an ECG. A vital signs monitor (Life windowTM 6000Vet, Digicare Animal Health) was used for measurement of the above mentioned variables. On all occasions, values of ETISO, HR, SAP, MAP, DAP, $ETCO_2$ and esophageal temperature were registered before the noxious stimulus.

Following instrumentation (approximately 15min after anesthetic induction), dogs were randomly administered one of two treatments as follows: in the RK group, $3mgkg^{-1}$ of 5% racemic ketamine (Clortamina, BioChimico) was administered whereas in the SK group, $3mgkg^{-1}$ of 5% S(+)-ketamine (Ketamin-s; Cristália) was administered. On all occasions, the experimental treatment was administered IM in a pelvic limb.

The ISO_{MAC} was determined by use of the up-and-down method (DIXON, 1965) with modifications (AGUADO et al., 2011; MONTEIRO et al., 2016). After administration of the experimental treatment (RK or SK), the concentration of isoflurane was adjusted to the ETISO to be tested and was kept constant for 15min. Thereafter, noxious stimulus was applied: surgical drapes were fixed to the dog's skin by four Backhaus towel clamps and a midline skin incision and subcutaneous tissue dissection were performed (AGUADO et al., 2011; MONTEIRO et al., 2016). The same surgeon performed the noxious stimulus on all occasions. The response to the noxious stimulus was classified as positive if movement of the head, trunk, or limbs was observed at any time during the noxious stimulus or within 1min after subcutaneous tissue dissection was completed. Response to the noxious stimulus was evaluated a single time in each dog. If a positive response was observed, the ETISO for the subsequent dog in that

group was increased by 0.1%. Otherwise, the ETISO for the next dog in the same group was decreased by 0.1%. The ETISO for the first dog in each group was set at 0.7%.

After evaluating the response to the noxious stimulus, all dogs received 0.2mgkg^{-1} of meloxicam IV and the concentration of isoflurane was increased to maintain surgical depth of anesthesia to perform the ovariohysterectomy. Dogs presenting a positive response were administered 0.5 to 1.0mgkg^{-1} of propofol IV to interrupt purposeful movement.

The estimated ISO_{MAC} for the population was calculated as the mean of the ETISO values from four crossovers. A crossover consisted of two dogs and was observed when a positive response was followed by a negative response in the subsequent dog from the same group or vice versa (a negative response was followed by a positive response in the subsequent dog). One dog could not appear in more than one crossover. This means that the estimated ISO_{MAC} was always calculated as the mathematical average of ETISO values from eight dogs.The ISO_{MAC} was also calculated by quantal analysis using the equation: $P(X) = 1 / [1 + (ED_{50} / x)^n]$; where P(X) is the probability of non movement, "x" is the tested ETISO, "n" is hill slope and ED_{50} is the estimated ISO_{MAC} value (SONNER, 2002).

Statistical analysis was carried out using a statistical software package. Normality of data was evaluated by the Shapiro-Wilk test. A Mann Whitney test was used to compare differences in age between the RK and SK groups. For the other variables, differences between groups were compared by unpaired t tests. A P value of <0.05 was considered significant.

RESULTS

Twenty three dogs completed the study. There were no significant differences between the groups for weight and age. The mean±SD weight was 14.3±8.0kg and 16.7±7.3kg, and the median (range) age was 12 (6-60) months and 24 (8-72) months in the RK and SK groups, respectively.

Times elapsed from premedication to anesthetic induction were 17±3 and 16±1 minutes (P=0.52); the times elapsed from anesthetic induction to the noxious stimulus were 35±7 and 37±5 minutes (P=0.48); and times elapsed from administration of the experimental treatment to the noxious stimulus were 18±4 and 21±4 minutes (P=0.09) in the RK and SK groups, respectively.

Propofol dose necessary for anesthetic induction was lower (P=0.0050) in the SK group (4.0±0.7mgkg^{-1}) than in the RK group (4.9±0.8mgkg^{-1}). The ISO_{MAC} calculated by mathematical average was 34% lower in the SK group (0.33%) compared to the RK group (0.50%). Individual response to the noxious stimulus for each dog is shown in figure 1. The ISO_{MAC} values calculated by quantal analysis were similar to those obtained by mathematical average (Figure 2).

There was no significant difference between the groups for SAP, MAP, DAP, $ETCO_2$ and esophageal temperature (Table 1). Bradycardia (HR<70beatsmin^{-1}) was observed in 3/10 dogs (30%) and 3/13 dogs (23%) and hypotension (MAP<60mmHg) was observed in 7/10 dogs (70%) and 10/13 dogs (77%) in the RK and SK groups, respectively. In all cases, hypotension was resolved after the surgery started.

DISCUSSION

In a previous study employing the same experimental design as in the present study, the ISO_{MAC} was 1.20±0.11% in dogs (MONTEIRO et al., 2016). In the same study, it was reported that administration of acepromazine (0.02mgkg^{-1}) and morphine (0.5mgkg^{-1}) before anesthetic induction decreased the ISO_{MAC} by 33% (from 1.20±0.11% to 0.80±0.13%) (MONTEIRO et al., 2016). In the present study, the ISO_{MAC} values determined in dogs given acepromazine and morphine combined with RK or SK were consistently lower than the 0.80% value reported in dogs administered acepromazine-morphine only (MONTEIRO et al., 2016). In goats, the effect of ketamine and lidocaine on the ISO_{MAC} were additive (DOHERTY et al., 2007) and in dogs, this combination produced nearly additive effects on the MAC of sevoflurane (WILSON et al., 2008). Effects of acepromazine, morphine and ketamine on the ISO_{MAC} may be additive in dogs, but this study was not designed to determine the nature of the interaction.

The propofol dose for anesthetic induction was lower in the SK compared to the RK group. This was an unexpected finding because the experimental treatments were administered after anesthetic induction. In addition, all dogs in this study received the same drugs for premedication and the same person was responsible for administration of propofol and for evaluating the clinical endpoints during induction of anesthesia. Despite the significant difference in the propofol dose, this finding can not account for the differences in ISO_{MAC} values observed between the RK and SK groups. Because propofol can reduce MAC, a higher propofol dose in the RK group should result in a lower, rather than higher, ISO_{MAC} value.

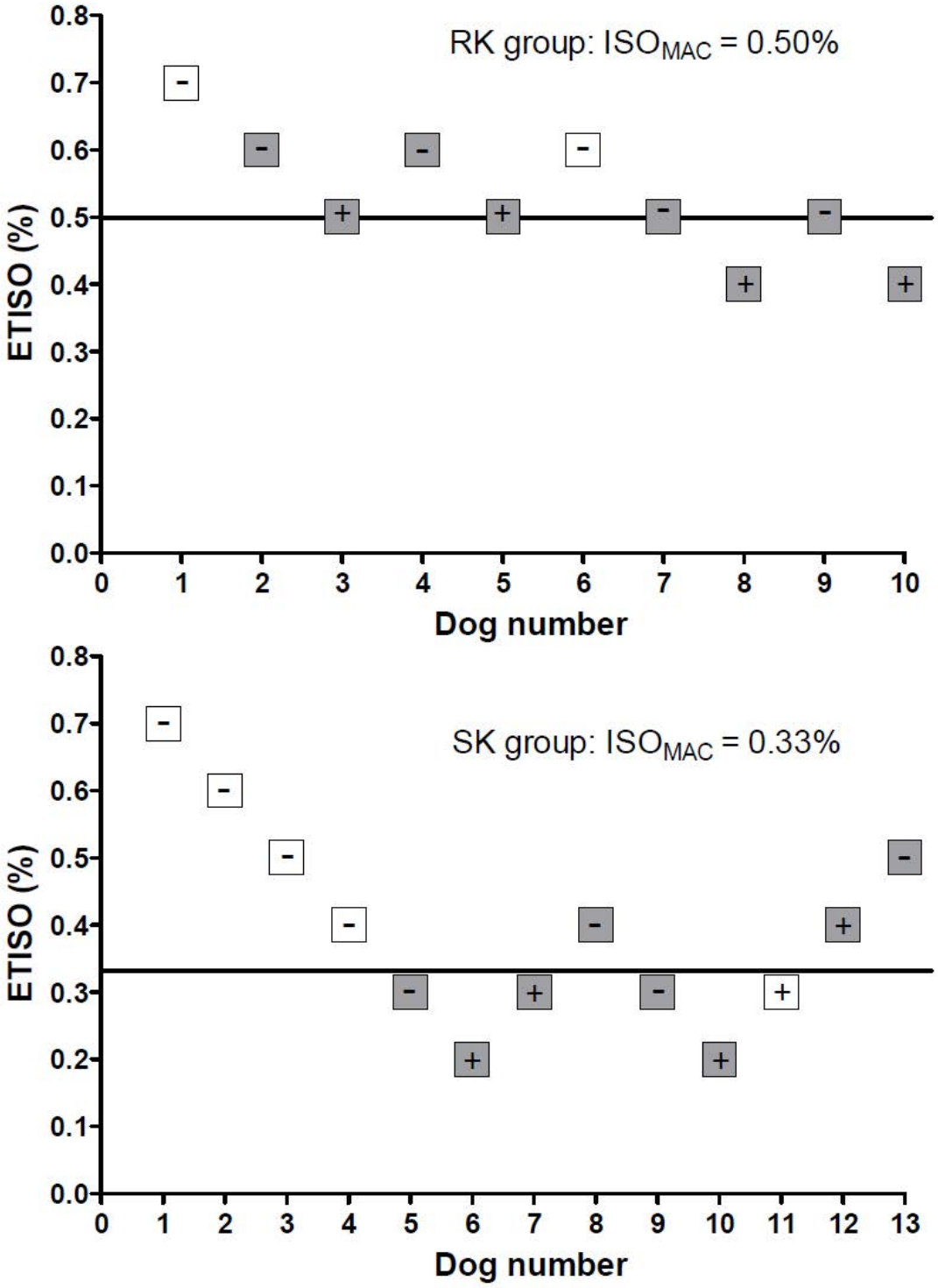

Figure 1 - Response (positive or negative) for each of the 23 anesthetized dogs premedicated with acepromazine (0.02mgkg^{-1}) and morphine (0.5mgkg^{-1}) at the respective end-tidal isoflurane concentration tested (ETISO). RK group, dogs were administered 3mgkg^{-1} racemic ketamine after anesthetic induction with propofol (n=10); SK group, dogs were administered 3mgkg^{-1} S(+)-ketamine after anesthetic induction with propofol (n=13). Noxious stimulus was delivered approximately 20 minutes after the experimental treatment (RK or SK), at stable ETISO concentration. (+) indicated a positive response (purposeful movement); (-) indicated a negative response (no purposeful movement); shaded squares indicate the crossovers. Horizontal lines indicate the calculated mean ISO_{MAC} value.

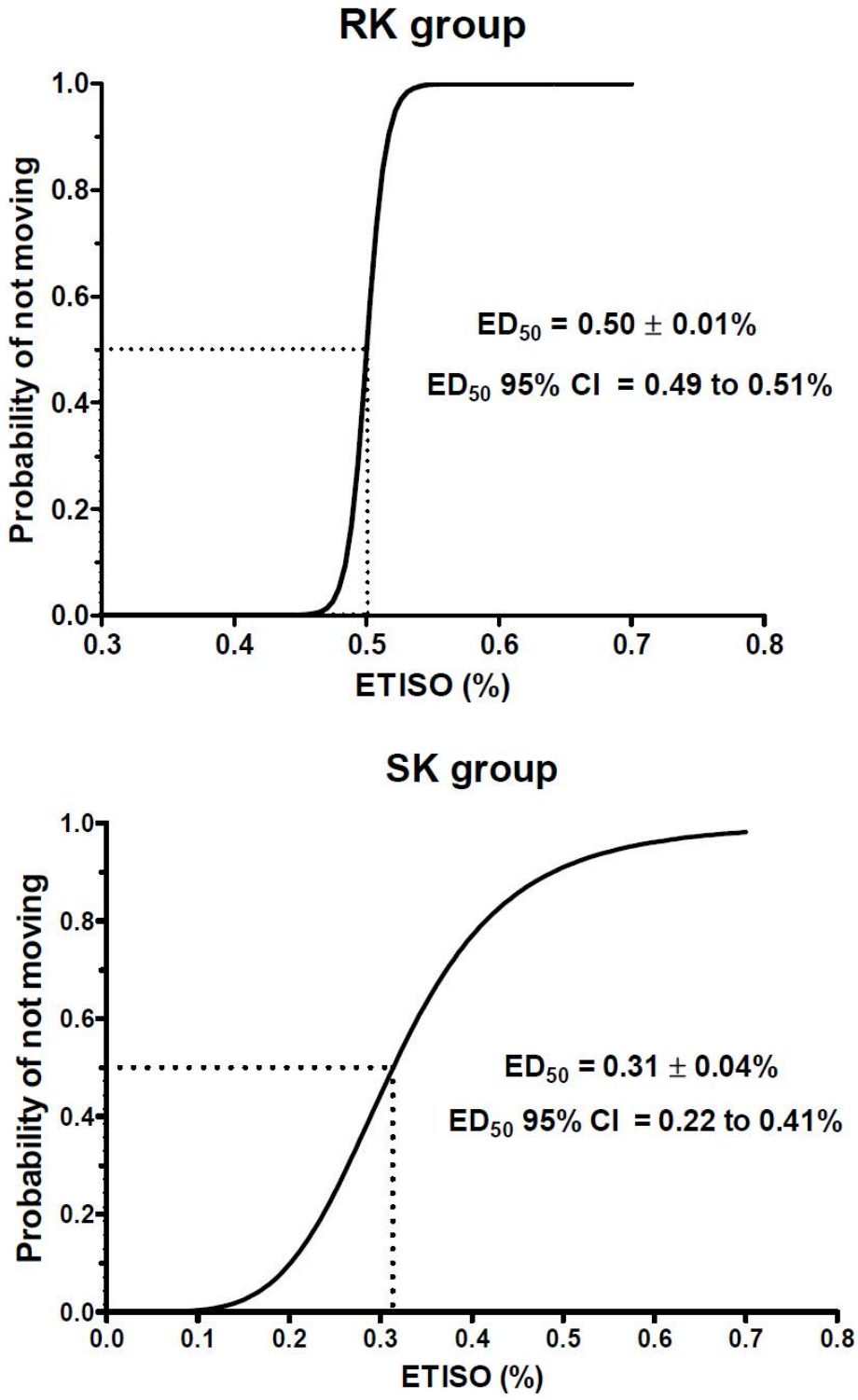

Figure 2 - Sigmoid curves obtained from quantal analysis for dogs in the racemic ketamine group (RK, *n*=10) and the S(+)-ketamine group (SK, *n*=13). The ED_{50} for either group corresponds to the ISO_{MAC} with the respective standard deviation. 95% confidence intervals (CI) for the ED_{50} are also reported.

Table 1 - The mean±standard deviation (SD) for heart rate (HR), systolic (SAP), mean (MAP) and diastolic (DAP) arterial blood pressures, end-tidal concentration of carbon dioxide ($ETCO_2$) and esophageal temperature (T) in 23 dogs administered 0.02mgkg^{-1} acepromazine and 0.5mgkg^{-1} morphine as premedication, and propofol given to effect for anesthetic induction. The experimental treatment (3mgkg^{-1} racemic ketamine [RK group] or 3mgkg^{-1}S(+)-ketamine [SK]) was injected intramuscularly after anesthetic induction and during isoflurane anesthesia. All variables were collected approximately 20 minutes after the experimental treatment, at stable ETISO concentration, and before the noxious stimulus was delivered.

	RK	SK	*P* value
HR (beatsmin^{-1})	80 + 11	74 ± 12	0.36
SAP (mmHg)	82 + 9	88 ± 10	0.22
MAP (mmHg)	55 + 10	55 ± 8	0.93
DAP (mmHg)	39 + 11	37 ± 6	0.75
$ETCO_2$ (mmHg)	32 + 3	32 ± 2	0.92
T(ºC)	37.7 + 0.4	37.6 ± 0.5	0.54

The recommended dose of ketamine for anesthetic induction in dogs ranges from 5 to 22mgkg by either IV or IM routes (BERRY, 2015). In the present study, ketamine was included as part of a balanced anesthetic technique and not as the anesthetic induction agent. The ketamine dose of 3mgkg^{-1} was chosen with the aim of producing analgesia and not anesthesia. A similar dose (2.5mgkg^{-1}, IM) administered preoperatively improved postoperative pain control after ovariohysterectomy in dogs (SLINGSBY & WATERMAN-PEARSON, 2000).

The SK is more potent than RK, and potency ratio (SK/RK) in dogs was estimated to range between 1.29/1 and 1.5/1 (DUQUE et al., 2008; CASONI et al., 2015). In the present study, the ISO_{MAC} in SK was significantly lower than in RK based on the 95% confidence intervals for the calculated ISO_{MAC} (SK: 0.22 to 0.41%; RK: 0.49 to 0.51%). Our results are in agreement with previous studies that SK is more potent than RK in dogs such that administration of the same dose of either drug resulted in a significantly lower ISO_{MAC} in the SK compared to the RK group. However, potency ratio cannot be calculated from our data. For such calculation, doses of SK and RK resulting in a similar effect (e.g. doses producing a similar MAC reduction) would be necessary which was not the design of this study.

Ketamine increased HR, MAP and cardiac index in a plasma concentration-dependent manner in dogs (BOSCAN et al., 2005). In man, this cardiovascular stimulating effect of ketamine was mainly attributable to sympathetic stimulation via a central mechanism resulting in increased circulating levels of norepinephrine and epinephrine (APPEL et al., 1979). Despite the expected cardiovascular stimulating effects of ketamine, bradycardia was observed in 23 to 30% of dogs and hypotension was observed in 70 to 77% of dogs in the present study. This high prevalence of hypotension may be explained by a combination of factors. First, the dose of ketamine (3mgkg^{-1}) administered IM may have been insufficient to achieve plasma ketamine concentrations that result in cardiovascular stimulation. Second, concurrent acepromazine and isoflurane administration may have blunted any increase in MAP by ketamine as both acepromazine and isoflurane decreased systemic vascular resistance and blood pressure (PAGEL et al., 1991; MONTEIRO et al., 2007).

CONCLUSION

In dogs premedicated with acepromazine and morphine, IM administration of 3mgkg^{-1} ketamine approximately 20 minutes before the surgical noxious stimulus produced clinically important reduction in the ISO_{MAC} and the MAC-sparing effect was significantly greater with SK compared to RK.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study was approved by the Institutional Animal Care Committee (protocol 210/2011) and all owners gave their informed consent.

ACKNOWLEDGEMENTS

Financial support was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant number 552488/2011-9). We thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing a scholarship to T.F. Bressan and K. Coelho.

REFERENCES

AGUADO, D. et al. Reduction of the minimum alveolar concentration of isoflurane in dogs using a constant rate of infusion of lidocaine-ketamine in combination with either morphine or fentanyl. **Veterinary Journal**, v.189, p.63-66, 2011. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20594878>. Accessed: Nov. 30, 2015. doi: 10.1016/j.tvjl.2010.05.029.

APPEL, E. et al. Sympathoneuronal and sympathoadrenal activation during ketamine anesthesia. **European Journal of Clinical**

Pharmacology, v.16, p.91-95, 1979. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/499313>. Accessed: Nov. 30, 2015.

BERRY, S.H. Injectable anesthetics. In: GRIMM, K.A. et al. **Veterinary anesthesia and analgesia**. Ames:Wiley-Blackwell, 2015. Cap.15,p.277-296.

BOSCAN, P. et al. Cardiovascular and respiratory effects of ketamine infusions in isoflurane-anesthetized dogs before and during noxious stimulation. **American Journal of Veterinary Research**, v.66, p.2122-2129, 2005. Available from: <http://avmajournals.avma.org/doi/abs/10.2460/ajvr.2005.66.2122?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed>. Accessed: Nov. 30, 2015. doi: 10.2460/ajvr.2005.66.2122.

CASONI, D. et al. S-ketamine versus racemic ketamine in dogs: their relative potency as induction agents. **Veterinary Anaesthesia and Analgesia**, v.42, p.250-259, 2015. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25041216>. Accessed: Nov. 30, 2015. doi: 10.1111/vaa.12200.

DIXON, W. The up-and-down method for small samples. **Journal of the American Statistical Association**, v.60, p.967–978, 1965. Available from: <http://www.jstor.org/stable/2283398.>. Accessed: Nov. 30, 2015.

DOHERTY, T. et al. Effect of intravenous lidocaine and ketamine on the minimum alveolar concentration of isoflurane in goats. **Veterinary Anaesthesia and Analgesia**, v.34, p.125-131, 2007. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17316393>. Accessed: Nov. 30, 2015. doi:10.1111/j.1467-2995.2006.00301.

DUQUE, J.C. et al. Relative potency of ketamine and S(+)-ketamine in dogs. **Journal of Veterinary Pharmacology and Therapeutics**, v.31, p.344-348, 2008. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18638295>. Accessed: Nov. 30, 2015. doi: 10.1111/j.1365-2885.2008.00965.

MONTEIRO, E.R. et al. Effects of acepromazine on the cardiovascular actions of dopamine in anesthetized dogs. **Veterinary Anaesthesia and Analgesia**, v.34, p.312-321, 2007. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17686118>. Accessed: Nov. 30, 2015. doi: 10.1111/j.1467-2995.2006.00328.

MONTEIRO, E.R. et al. Effects of acepromazine-morphine and acepromazine-methadone premedication on the minimum alveolar concentration of isoflurane in dogs. **Veterinary Anaesthesia and Analgesia**, v.43, p.27-34, 2016. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25880906>. Accessed: Jun. 15, 2016. doi:10.1111/vaa.12265.

PAGEL, P.S. et al. Comparison of the systemic and coronary hemodynamic actions of desflurane, isoflurane, halothane, and enflurane in the chronically instrumented dog. **Anesthesiology**, v.74, p.539-551, 1991. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/2001033>. Accessed: Nov. 30, 2015.

SCHMID, R.L. et al. Use and efficacy of low-dose ketamine in the management of acute postoperative pain: a review of current techniques and outcomes. **Pain**, v.82,p.111-125, 1999. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/8250308>. Accessed: Nov.30, 2015.

SLINGSBY, L.S.; WATERMAN-PEARSON, A.E. The post-operative analgesic effects of ketamine after canine ovariohysterectomy--a comparison between pre- or post-operative administration. **Research in Veterinary Science**, v.69, p.147-152, 2000. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11020366>. Accessed: Nov. 30, 2015. doi:10.1053/rvsc.2000.0406.

SOLANO, A.M. et al. Effect of intravenous administration of ketamine on the minimum alveolar concentration of isoflurane in anesthetized dogs. **American Journal of Veterinary Research**, v.67, p.21-25, 2006. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16426207>. Accessed: Nov. 30, 2015. doi:10.2460/ajvr.67.1.21.

SONNER, J.M. Issues in the design and interpretation of minimum alveolar anesthetic concentration (MAC) studies. **Anesthesia and Analgesia**, v.95, p.609-614, 2002. Available from: <http://http://www.ncbi.nlm.nih.gov/pubmed/12198046>. Accessed: Jun. 15, 2016. doi: 10.1213/00000539-200209000-00021.

WILSON, J. et al. Effects of intravenous lidocaine, ketamine, and the combination on the minimum alveolar concentration of sevoflurane in dogs. **Veterinary Anaesthesia and Analgesia**, v.35, p.289-296, 2008. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/6989292>. Accessed: Nov. 30, 2015. doi: 10.1111/j.1467-2995.2007.00389.