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

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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 15 minutes 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.

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...
on the minimum alveolar concentration (MAC). Administration of ketamine as an intravenous target-controlled infusion reduced the isoflurane MAC (ISO\textsubscript{MAC}) in dogs in a plasma concentration-dependent manner (SOLANO et al., 2006). Reduction on the ISO\textsubscript{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 and the MaC has not been evaluated in dogs. In addition, a balanced anesthetic technique. The present study evaluated the ISO\textsubscript{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\textsuperscript{-1} of acepromazine and 0.5mgkg\textsuperscript{-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, anesthesia induction was performed with propofol administered IV until endotracheal intubation could be accomplished. Anesthesia was maintained with isoflurane (Isorofine, Cristália) in oxygen (1.5 to 2.0Lmin\textsuperscript{-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\textsubscript{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\textsuperscript{-1} h\textsuperscript{-1} throughout anesthesia and had their lungs mechanically ventilated to maintain the ETCO\textsubscript{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\textsubscript{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\textsuperscript{-1} of 5% racemic ketamine (Clortamina, BioChimico) was administered whereas in the SK group, 3mgkg\textsuperscript{-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\textsubscript{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
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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 was 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 (0.02mg/kg) and morphine (0.5mg/kg) at the respective end-tidal isoflurane concentration tested (ETISO). RK group, dogs were administered 3mg/kg racemic ketamine after anesthetic induction with propofol (n=10); SK group, dogs were administered 3mg/kg 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$_{50}$ with the respective standard deviation. 95\% confidence intervals (CI) for the ED$_{50}$ are also reported.
The recommended dose of ketamine for anesthetic induction in dogs ranges from 5 to 22mgkg⁻¹ 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 ISoMaC 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 (APPÉL 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.

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