

Effects of ropivacaine combined with morphine at 0.15 and 0.2 mg kg⁻¹ in bitches undergoing epidural anesthesia¹

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ABSTRACT

PURPOSE: To investigate cardiorespiratory effects and serum concentration of ropivacaine combined with morphine at different doses.

METHODS: Sixteen healthy adult female dogs weighting 9.8±4.1 kg were included in the study. Twenty minutes after being premedicated with acepromazine and midazolam, the animals were randomly assigned to receive an epidural injection according to each group: RM0.15 = ropivacaine + morphine (0.15 mg kg⁻¹) and RM0.2 = ropivacaine + morphine (0.2 mg kg⁻¹). Variables recorded consisted of: heart rate and cardiac rhythm, respiratory rate, oxyhemoglobin saturation, inspired oxygen fraction, end-tidal carbon dioxide tension, systolic, mean and diastolic arterial pressures, serum cortisol, plasma ropivacaine and morphine.

RESULTS: SAP, MAP and DAP were significantly increased at TPR in RM0.15 but returned to normal values at the end of the procedure. Arterial pH was decreased in T30 and TESu in both groups and also returned to acceptable ranges at TR. Both PaO₂ and PaCO₂ were increased along the duration period of the epidural blockade (T30 and TESu) and returned to acceptable values at TR. Serum cortisol was lower at TB, T30 and TR when compared to TESu.

CONCLUSION: The procedures were performed safely and minimal changes in cardiovascular and respiratory variables.

Key words: Analgesics, Opioid. Chromatography, High Pressure Liquid. Hydrocortisone. Dogs.

Introduction

Ropivacaine is a local anesthetic that provides prolonged duration and has intermediate vasoconstrictive properties, so it does not require adrenalin in its formulation¹. It is 3 to 4 times more potent than lidocaine and lasts longer. When low concentrations and doses are used, sensitive analgesia can be achieved without prolonging motor blockade, due to ropivacaine's lesser effect over motor fibers².

Studies conducted by³ have demonstrated that 0.75% Ropivacaine is most indicated for anesthesia for being less toxic to the heart when compared to bupivacaine. However, although the onset of motor blockade is similar to that obtained with 0.75% bupivacaine, sensitive blockade happens much later.

Some authors^{4,5} have reported safe associations between ropivacaine and opioids for epidural anesthesia, with no changes on respiratory pattern or acid-base balance, but only a few transient adverse effects.

Morphine is widely used in veterinary medicine for its efficacy in treating post-operative pain after moderate and large surgeries². When given epidurally with 0.9% sodium chloride, morphine has been shown to provide intense sedation and lower serum cortisol levels in dogs⁶. If local anesthetics are combined to it, the duration and efficacy of analgesia are greater⁷.

Therefore, the purpose of this study was to investigate the cardiorespiratory and analgesic effects, incidence of adverse reactions and serum concentrations of cortisol of female dogs undergoing constant rate infusion (CRI) of propofol and epidural anesthesia with ropivacaine combined with morphine at 0.15 and 0.2 mg kg⁻¹ for elective ovariohysterectomy.

We have hypothesized that ropivacaine combined with morphine in higher doses (0.15 and 0.2 mg kg⁻¹) would enable the surgery to be performed under continuous infusion rate infusion of propofol at 0.4 mg kg⁻¹ minute⁻¹ without causing significant cardiovascular changes, adverse effects or increased cortisol levels.

Methods

The study has been approved by the local Animal Usage Ethics Committee under the protocol number 004391.

Sixteen healthy (based on physical examination, complete blood count, serum dosage of urea, creatinine, alkaline phosphatase and alanine transaminase, electrocardiography and *leishmaniasis* test) adult female dogs of various breeds and mean weight of 9.8±4.1 kg were selected from the local community

and included in the study for elective ovariohysterectomy (OH). Animals were fasted for 12 hours and water was withheld for 4 hours before the procedures.

Before premedication, baseline variables (TB) were obtained: heart rate (HR), respiratory rate (f_R), electrocardiogram, systolic (SAP), mean (MAP) and diastolic (DAP) non-invasive arterial pressures and serum cortisol levels, dosed from venous blood samples. Premedication consisted of intramuscular acepromazine (0.05 mg kg⁻¹, Acepran 0.2%, Univet, São Paulo, Brazil) and midazolam (0.2 mg kg⁻¹, Dormire, Cristalia, Itapira-SP, Brazil).

Twenty minutes after premedication, all variables were again recorded (T20). The cephalic veins were then catheterized for fluid administration (right cephalic vein – 0.9% NaCl at 10 mL kg⁻¹ h⁻¹) and for propofol (Propovan, Cristalia, Itapira-SP, Brazil) continuous rate infusion (CRI) (left cephalic vein) at 0.4 mg kg⁻¹ minute⁻¹ after a loading dose of 4 mg kg⁻¹ (induction of anesthesia). The CRI was maintained by a syringe pump (680Samtronic, São Paulo, Brazil).

After induction and intubation, animals were kept under 100% oxygen flow and were allowed to breathe spontaneously. The injection site ($L_7 - S_1$) was prepared for aseptic epidural anesthesia. Animals were put in prone sphinx position and a Tuohy needle (22G, B-Braun Sharing Expertise, São Gonçalo-RJ, Brazil) was inserted through the intervertebral space until it reached below the interacuate ligament. Location of the needle was confirmed by the “hanging-drop” sign (0.9% NaCl).

Animals were then randomly assigned to one of two groups: RM0.15 (n=8) = ropivacaine (Ropi 0.75%, Cristalia, Itapira-SP, Brazil) + morphine (Dimorf, Cristalia, Itapira-SP, Brazil) (0.15 mg kg⁻¹) and RM0.2 (n=8) = ropivacaine + morphine (0.2 mg kg⁻¹). Ropivacaine was added up to a final volume of 0.3 mL kg⁻¹.

Epidural anesthesia was performed slowly along one minute and the position was maintained for 30 minutes⁸ so that drugs would be symmetrically distributed⁹. The position was switched to dorsal recumbency for skin sensitivity test, which would define whether the surgical procedure could be started. During surgery, body temperature was maintained above 36.5°C by a warm blanket (Warm Air Blower Unit TC3000, Gaymar, Orchard Park, New York, USA).

The same surgeon performed all procedures with mean duration of 30 minutes. Incisions were of 10 cm below the navel and pedicle clamping was always started at the left side. Every ligature and suture was made using nylon thread of various diameters, according to each animal.

If general anesthesia became superficial (10% increase in HR, f_r and arterial pressure and/or reactions to noxious stimuli), propofol infusion would be increased in 0.1 mg kg⁻¹ minute⁻¹.

Variables were collected at various time points: 30 minutes after epidural anesthesia (T30), at surgical incision (TS), left and right ovarian pedicle clamping (TPL and TPR respectively), cervix clamping (TC) and start and end of skin suture (TSu and TESu).

For each time point, parameters were recorded from the multiparameter monitor (Monitor multiparameter Mod Cardiacap 5, Datex Ohmeda, Madison, Wisconsin, USA) and HR was assessed through electrocardiography (TEB C10, São Paulo, Brazil) as well as cardiac rhythm, duration and amplitude of P-wave, QRS complex, ST segment and QT interval. Monitored parameters were respiratory rate (f_r), oxyhemoglobin saturation (SpO₂), inspired oxygen fraction (FiO₂), end-tidal carbon dioxide tension (P_E'CO₂) as well as systolic (SAP), mean (MAP) and diastolic (DAP) arterial pressures (through catheterization of the right metatarsal artery). Rectal temperature was assessed using a digital thermometer (BD, São Paulo, Brazil). Blood gases (Omni-C, Ribeirão Preto-SP, Brazil), lactate (Roche, São Paulo, Brazil) and glucose (Roche, São Paulo, Brazil) were also evaluated.

After surgery, variables were collected every 15 minutes until full recovery (TR) from epidural blockade (standing position without signs of ataxia). At these times, SAP was no longer obtained by the invasive method, but rather through Doppler pressure recordings (DV10 Microem, Ribeirão Preto-SP, Brazil). Also, HR was assessed using a stethoscope (3M™ Littmann Classic II, Sumaré-SP, Brazil) and f_r was directly obtained by observing thoracic movement.

Serum cortisol (Siemens, São Paulo, Brazil) was assessed from a venous blood sample drawn from the jugular vein at TB, T30, TESu and TR through radioimmunoassay. Plasma ropivacaine and morphine were also dosed from venous blood samples drawn into heparin syringes (Macromed, Diadema-SP, Brazil) at T30, T1h (one hour after epidural), TESu and TR. Dosages were performed by high-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (Class-LC 10, Shimadzu, Kyoto, Japan).

Onset time of local anesthesia was evaluated through anal sphincter reflex (direct stimulation of the perianal skin with a needle), muscle tone and interdigital reflex (using a Cocker hemostat coated with rubber in order to avoid skin lacerations and closed to the second ratchet on both limbs).

The duration of motor blockade was assessed by comparing the response to interdigital clamping from when it was first lost to when it returned. Furthermore, the posture was observed from when muscle tone was lost to when animals were able to stand and walk with no signs of ataxia.

Finally, possible signs of intoxication by local anesthetics or complications arisen from general anesthesia were carefully observed until animals could be discharged.

Statistical analysis

For statistical analysis data were tested through ANOVA for repeated measures and residual analysis in order to estimate variances homogeneity. All analyses were performed by the Statistical Analysis System (SAS) software and significance was considered when p<0.05. For normally distributed variables, multiple comparisons were performed by Tukey's test. For all other variables, differences between groups were assessed through Kruskal-Wallis' test and between time measures through Friedman's test, followed by Dunn's multiple comparisons.

Results

There was no significant difference between groups in HR, cardiac rhythm, QRS complex and QT interval. Other cardiovascular variables showed a few significant differences of no clinical relevance and values were within reference ranges for dogs.

Arterial pressures (SAP, MAP and DAP) were significantly increased at TPR and at subsequent measures in RM0.15, but returned to normal values at the end of the procedure. No difference was found between groups (Table 1).

TABLE 1 - Mean ± standard deviation of systolic, mean and diastolic arterial pressures (mmHg) of female dogs undergoing epidural anesthesia.

Variable	Group	Times						
		T30	TS	TPR	TPL	TC	TSu	TESu
Systolic arterial pressure (mmHg)	RM0.15	106±11bc	103±17c	115±21abc	132±19a	120±19ab	108±19bc	109±14bc
	RM0.20	109±25	105±35	111±20	134±33	120±33	113±40	114±45
Mean arterial pressure (mmHg)	RM0.15	77±17cd	77±22cd	95±21abc	108±15a	96±18abc	87±20bcd	83±15cd
	RM0.20	79±18	74±19	87±18	101±17	91±19	84±23	84±26
Diastolic arterial pressure (mmHg)	RM0.15	60±21cd	62±22bcd	79±23abc	95±16a	83±18ab	74±21abc	68±17bcd
	RM0.20	60±17	59±16	78±19	84±16	76±14	66±17	69±20

Means followed by the same letter in lines do not differ significantly according to Tukey's test (P > 0.05).

Rectal temperature and blood glucose did not differ between groups, whereas lactate was significantly lower in TESu compared to TB in RM0.15 (2.7±0.9; 1.4±0.3 and 2.4±0.6mmol L⁻¹, respectively in TB, TESu and TR).

Respiratory variables (f_R, SpO₂, FiO₂ and PE'CO₂) did not vary over time inside each group.

Regarding blood gas evaluations, arterial pH gradually decreased in T30 and TESu in both groups, which accompanied the duration of the epidural blockade, and returned to baseline values

at TR. Arterial oxygen (PaO₂) and carbon dioxide (PaCO₂) were increased during epidural (T30 to TESu), returning to acceptable ranges at TR (Table 2). Serum cortisol was lower at TB, T30 and TR when compared to TESu in both groups (Table 3).

Plasma morphine and ropivacaine were similarly altered over time and along the epidural blockade. Differences were found when comparing T30 to T1h and TESu as well as when these three variables were compared to TR. Morphine levels differed significantly between groups (Table 3).

TABLE 2 - Mean ± standard deviation of pH, PaO₂ and PaCO₂ (mmolL⁻¹) of female dogs undergoing epidural anesthesia.

Variable	Group	Times			
		TB	T30	TESu	TR
pH	RM0.15	7.373±0.035a	7.275±0.076b	7.256±0.056b	7.357±0.032a
	RM0.20	7.369±0.029a	7.277±0.047b	7.264±0.056b	7.363±0.026a
PaO ₂ (mmHg)	RM0.15	105.1±26b	298.2±131.1a	322.3±178.2a	96.6±18.5b
	RM0.20	96.3±21.8b	344.3±112.1a	222.7±170.6	91.4±23.1b
PaCO ₂ (mmHg)	RM0.15	34.4±5.2b	50.1±12.3a	51.9±10.5a	36.5±5.0b
	RM0.20	36.5±4.0b	50.2±7.8a	48.2±9.3a	37.1±4.4b

Means followed by the same letter in lines do not differ significantly according to Tukey's test (p>0.05).

TABLE 3 - Mean ± standard deviation of serum cortisol and plasma levels of morphine and ropivacaine (ngmL⁻¹) of female dogs undergoing epidural anesthesia.

Variable	Group	Times			
		T30	T1h	TESu	TR
Serum cortisol (µgdL ⁻¹)	RM0.15	6.6±4.3 ^a	3.3±1.8a	15.6±6.8b	5.5±4.6a
	RM0.20	7.0±5.3 ^a	4.8±2.9a	15.7±3.1b	8.3±3.8a
Plasma ropivacaine (µgdL ⁻¹)	RM0.15	30.9±9.7 ^a	16.2±3.8b	12.9±4.0b	0.8±0.5c
	RM0.20	41.3±8.6 ^a	22.8±4.4b	17.4±5.0b	0.8±0.6c
Plasma morphine (µgdL ⁻¹)	RM0.15	477.3±165.0a	376.5±95.2b	293.4±91.6b	79.0±45.6c
	RM0.20	536.5±138.2a	380.6±109.8b	338.3±106.2b	72.5±15.4c

Means followed by the same letter in lines do not differ significantly according to Tukey's test (p>0.05).

No difference was observed regarding onset time of blockade and time of recovery from anesthesia between groups. Propofol CRI started at 0.4 mg kg⁻¹ minute⁻¹ in both groups and the adjustments on infusion rate varied between groups. Most subjects in RM0.15 (50%) were kept under 0.4 mg kg⁻¹ minute⁻¹ whereas in RM0.2 the rate was increased to 0.5 mg kg⁻¹ minute⁻¹ in 50% of the animals (Table 4). Adverse effects arising from the epidural technique, such as muscle tremors, opisthotonos, Schiff-Sherrington-like posture, diarrhea and vomiting were observed (Table 4).

TABLE 4 - Percentage of propofol infusion rates and of adverse effects in female dogs undergoing epidural anesthesia.

Variable	Group	
	RM0.15	RM0.2
Infusion rate		
0.4mg kg ⁻¹ min ⁻¹	50%	25%
0.5mg kg ⁻¹ min ⁻¹	25%	50%
0.6mg kg ⁻¹ min ⁻¹		12.50%
0.8mg kg ⁻¹ min ⁻¹	25%	12.50%
Adverse effects		
Diarrhea		12.50%
Limb spasticity	12.50%	25%
Opisthotonos	12.50%	25%
Muscle tremors	12.50%	12.50%

Discussion

Cardiovascular variables were the most expected to change with the increase of morphine doses. However, most of the variables studied did not differ in a statistically or clinically significant manner. Likewise, cardiovascular stability with the combination of morphine (0.1 mg kg⁻¹) to 0.9% sodium chloride has been demonstrated in female dogs⁶. Other studies reported by Albuquerque *et al.*⁵, however, have reported significant increases in the HR of female dogs receiving epidural ropivacaine and morphine (0.1 mg kg⁻¹) using the same methodology for elective OH.

In regard of arterial pressure there has been an increase, even if not significant, at the times of pedicle or cervix clamping (TPR, TPL and TC), which was also observed by other authors when using ropivacaine alone¹⁰. At those time points the incidence of increased rates of propofol was greater and could be a reason for decreasing arterial pressure and thus eliminating possible differences. These findings can be related to the property of the drugs used epidurally or intravenously in potentiating one another.

Hypotension has not been observed in all studies concerning epidural anesthesia. Values of SAP above 80 mmHg have been found after epidural administration of ropivacaine combined with fentanyl or tramadol in female dogs⁴. Other studies have reported a decrease in SAP and DAP after one hour of epidural anesthesia with ropivacaine in conscious dogs, and have ascribed this effect to sympathetic blockade caused by the technique¹¹. Similarly, reduced SAP has been observed after epidural administration of ropivacaine in dogs¹².

In this study, lactate levels varied along measures, starting with a decrease after epidural anesthesia and returning to baseline values at recovery. However, in none of the measures were these values above or below reference ranges for the canine species (lower and upper limits of 1.4±0.3 and 2.8±1.2 mmol L⁻¹, respectively). In humans, decreased lactate levels have been observed after intravenous administration of morphine, and the authors indicate reduced muscle tone, increased clearance by the liver and improved glycolysis as possible causes¹³.

In contrast to previous studies made by Baraka *et al.*¹⁴, Valadão *et al.*⁷, Silva *et al.*¹⁵, Pereira and Marques⁶ which demonstrate significant changes in respiratory variables after morphine administration, respiratory stability has been observed in both groups of this study. There has been no change in f_R , SpO₂, FiO₂ and P_E'CO₂ over time and no difference between groups. Similarly, PaO₂ and PaCO₂ did not vary significantly in dogs after epidural injection of morphine alone or combined to low doses

of bupivacaine¹⁶. Minimal respiratory changes have been reported when 5 mg of morphine were given epidurally to dogs with 0.9% sodium chloride¹⁷. Therefore, even though many reports state that great volumes of local anesthetics combined with hydrophilic opioids, such as morphine, may cause cranial distribution and late onset respiratory depression¹⁷, there has been no evidence of such findings in this study.

Decreased arterial pH and increased PaCO₂ and bicarbonate (HCO₃) may indicate respiratory acidosis caused, probably, by the epidural anesthesia. These variables, however, were within reference ranges at the end of recovery (TR).

The high PaO₂ values that were found in this study were ascribed to 100% oxygen delivered by the rebreathing circuit (T30 to TESu). In addition, these values could be a reason for hypoventilation which would then cause increased PaCO₂. In contrast to these results, some authors¹⁴ have compared epidural tramadol and morphine and have reported significant respiratory changes caused by morphine, such as decreased f_R and PaO₂, without any consequences to PaCO₂. In humans that received epidural injection of morphine, respiratory changes on f_R and PaCO₂ are frequent¹⁸.

Propofol administration, on the other hand, may be a cause of respiratory depression and can result in hypoventilation, which would be associated with high PaCO₂. The lower dose morphine group (RM0.15) required a high infusion rate of propofol (0.8 mg kg⁻¹ minute⁻¹ in 25% of the subjects) which resulted in high P_E'CO₂ values, however changes in PaCO₂ were not observed.

Serum cortisol is known to increase significantly after noxious stimuli and together with post-operative pain¹⁹, which corroborates the findings of this study. These results, however, differ from a study in dogs that has reported reduced neuroendocrine response to stress after epidural administration of morphine combined with bupivacaine²⁰. It is possible that those authors could not reach the same results for not using a potential noxious stimulus such as the one in this study.

Plasma levels of morphine and ropivacaine behaved equivalently between one another, decreasing over time and detectable only during epidural blockade. Similar findings have been reported in rats that were given subarachnoid morphine at 0.1, 0.3, 0.5 and 1 µg¹⁵. These authors have found serum morphine concentrations of 7.38, 111.26, 151.18 and 561.37 pg 100 µL⁻¹, respectively, 60 minutes after administration. In this study, the levels after 60 minutes were of 16.2 and 22.8 ng mL⁻¹, respectively at RM0.15 and RM0.2.

In a study conducted in dogs that were given 5 mg of morphine epidurally the authors have concluded that peaks of

concentration in the brain and the spinal cord happen fast (less than 5 minutes after injection) and decrease exponentially (mean duration of 210 minutes)¹⁷. Such results are similar to those in this study, where morphine levels at RM0.15, for instance, peaked at T30 (30.9±9.7ngmL⁻¹) and were close to zero at 378 minutes, when epidural blockade ended (0.8±0.5 ngmL⁻¹).

Onset time of epidural anesthesia was not different between groups regarding motor blockade (1.5±0.5 and 1.5±0.8 minutes, respectively) and sensitive blockade (2.1±0.5 minutes in both groups). These findings are different from other studies in dogs, which have reported epidural ropivacaine onset of 7 to 20 minutes that lasted for 115 to 140 minutes¹¹. The lower numbers in this study could be ascribed to the opioid added to ropivacaine, which has also been observed by Albuquerque *et al.*⁵ after epidural administration of ropivacaine combined with 0.1 mg kg⁻¹ morphine (3.6±0.8 minutes).

Long periods of duration have been observed in this study (414±114 and 377±87 minutes for RM0.15 and RM0.2, respectively). Ropivacaine combined with 0.1 mg kg⁻¹ morphine through the epidural route has been shown to last longer (447.3±41.0 minutes) when compared to ropivacaine alone (375.8±54.8 minutes) and results of the association are similar to this study⁵. The hydrophilic property of morphine can be a reason for longer duration of effect¹⁵.

The minimal incidence of adverse effects in this study corroborates the findings of others that have reported few complications and low incidence of late onset adverse reactions after epidural anesthesia^{21,22}.

Conclusions

Increased doses of morphine through the epidural route have allowed elective ovariohysterectomy to be performed safely, with minimal changes on cardiac and respiratory variables, as well as on blood gases and serum cortisol in female dogs undergoing continuous rate infusion of propofol.

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