Original Article

Addition of magnesium sulphate to ropivacaine for spinal analgesia in dogs undergoing tibial plateau levelling osteotomy

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Abstract

The aim of this blinded, randomised, prospective clinical trial was to determine whether the addition of magnesium sulphate to spinally-administered ropivacaine would improve peri-operative analgesia without impairing motor function in dogs undergoing orthopaedic surgery. Twenty client-owned dogs undergoing tibial plateau levelling osteotomy were randomly assigned to one of two treatment groups: group C (control, receiving hyperbaric ropivacaine by the spinal route) or group M (magnesium, receiving a hyperbaric combination of magnesium sulphate and ropivacaine by the spinal route). During surgery, changes in physiological variables above baseline were used to evaluate nociception. Arterial blood was collected before and after spinal injection, at four time points, to monitor plasma magnesium concentrations. Post-operatively, pain was assessed with a modified Sammarco pain score, a Glasgow pain scale and a visual analogue scale, while motor function was evaluated with a modified Tarlov scale. Assessments were performed at recovery and 1, 2 and 3 h thereafter. Fentanyl and buprenorphine were administered as rescue analgesics in the intra- and post-operative periods, respectively.

Plasma magnesium concentrations did not increase after spinal injection compared to baseline. Group M required less intra-operative fentanyl, had lower Glasgow pain scores and experienced analgesia of longer duration than group C (527.0 ± 341.0 min vs. 176.0 ± 109.0 min). However, in group M the motor block was significantly longer, which limits the usefulness of magnesium for spinal analgesia at the investigated dose. Further research is needed to determine a clinically effective dose with shorter duration of motor block for magnesium used as an additive to spinal analgesic agents.

Introduction

Prevention and control of pain is one of the most important ethical obligations of veterinarians. As a result, various aspects of this fascinating branch of anaesthesia have been explored, and a number of novel techniques have been developed over the past decades to improve peri-operative pain management. It is likely that a multimodal approach has increased efficacy and, consequently, there has been particular interest in agents that, although not classified as analgesics, do exert antinociceptive effects (KuKanich, 2013, Madden et al, 2014, Crociolli et al, 2015; Norkus et al., 2015).

The use of magnesium has generated widespread interest as it could prevent central sensitisation by acting as a non-competitive antagonist at N-methyl-D-aspartate receptors in the dorsal horn, in a voltage-dependent fashion. Magnesium sulphate is commercially available in Europe, and the formulation developed for parenteral use is inexpensive, stable at room temperature and approved for use in dogs. Several studies in both human patients and dogs suggest that magnesium sulphate exerts antinociceptive effects (Bahrenberg et al., 2015), and consistently prolongs the duration of analgesia of various local anaesthetics and opioid combinations when administered via either the epidural or spinal route (Buvanendran et al, 2002, Oezalevli et al, 2005, Arcioni et al, 2007). Additionally, a study in dogs investigating the neurotoxicity of intrathecal magnesium sulphate found that a dose rate of 3 mg/kg did not cause neurological deficits or histopathological changes in the spinal cord (Simpson et al., 1994).

Overall, these findings supported our hypothesis that a clinical trial investigating the effects of spinally administered magnesium in client-owned dogs would be feasible and ethically acceptable. The aim of this study was to compare the intensity and duration of peri-operative analgesia and motor block in client-owned dogs undergoing elective orthopaedic surgery, after spinal administration of either ropivacaine, or a combination of ropivacaine–magnesium

sulphate. Our hypothesis was that the inclusion of magnesium sulphate would provide longer lasting, better quality analgesia than ropivacaine alone, without impairing neurological function of the pelvic limbs and/or prolonging the duration of the motor block.

Materials and methods

Animals and determination of sample size

Twenty client-owned dogs undergoing elective tibial plateau levelling osteotomy (TPLO) between May 2014 and March 2015 were enrolled in the study. On arrival, a pre-anaesthetic physical examination was performed, as well as venous blood sampling for haematology and chemistry. Exclusion criteria were an American Society of Anaesthesiologists risk category higher than 2, infectious skin diseases affecting the lumbosacral area, and bleeding disorders. The clinical trial was approved by the Committee for Animal Experimentation, Canton of Berne, Switzerland (approval no. BE11/14, 28 April 2014), and performed with informed owner consent.

Study design

The study was designed as an investigator-blinded, block-randomised, prospective clinical trial. Dogs were randomly allocated to one of two treatment groups using a block randomisation method, based on shuffle and drawing of treatment assignments inside an opaque, sealed envelope. One operator not involved in the study was in charge of the allocations list, which was disclosed only at the end of the trial.

A sample size calculation determined that 10 dogs were needed in each treatment group, to achieve a power of 0.9 with an α of 0.05, to detect a minimum difference of 60 min in the mean

duration of analgesia (defined as the time elapsed from the spinal injection to the first administration of rescue analgesics, either intra-operative fentanyl or post-operative buprenorphine), between groups.

Anaesthetic protocol and procedures

After IM premedication with acepromazine (0.03 mg/kg, Prequillan, Aprovet), an appropriately sized IV catheter was placed in a cephalic vein. General anaesthesia was induced with IV propofol (Propofol, Fresenius Kabi) titrated to effect to enable orotracheal intubation, and maintained with isoflurane (IsoFlo, Abbott) vaporised in an oxygen–air mixture and delivered via a circle system. All dogs received IV lactated Ringer's solution (Ringer-Lactate, Fresenius Kabi) at a rate of 10 mL/kg/h during anaesthesia. The dorsal pedal artery of the non-surgical pelvic limb was catheterised to allow blood sampling and continuous measurement of the systolic (SAP), mean (MAP) and diastolic (DAP) arterial blood pressures. A multiparametric monitor was used to assess cardiovascular (SAP, MAP, DAP, heart rate [HR]) and respiratory (end-tidal carbon dioxide, $P_{E'}CO_2$; peak inspiratory pressure, PIP; respiratory rate, RR; tidal volume, TV; inspired fraction of oxygen, FIO_2; end-tidal isoflurane tension, $P_{E'Iso}$) variables, as well as oesophageal temperature (T, °C). Data were manually recorded every 5 min until the end of anaesthesia. The dogs were allowed to breathe spontaneously unless $P_{E'}CO_2$ was >45 mmHg, in which case pressure-controlled ventilation with PIP set at 10 cm H_2O was used to maintain $P_{E'}CO_2$ within the normal range. A constant $P_{E'Iso}$ of 1.3%, equivalent to the minimum alveolar concentration (MAC) for the species (Steffey and Mama, 2007), was targeted during anaesthesia.

Hypotension, defined as MAP lower than 60 mmHg, was treated with a crystalloid bolus (10 mL/kg lactated Ringer's delivered IV over 10 min). Non-responsive hypotension was

126 treated initially with a colloid bolus (2 mL/kg tetrastarch delivered IV over 10 min), and then
127 with dopamine infusion, starting at a rate of 5 µg/kg/min. The dose was increased by
128 2.5 µg/kg/min every 10 min until MAP was above 60 mmHg.
129
130 Bradycardia, defined as HR lower than 45 beats per min (bpm), was treated with IV
131 glycopyrronium, 10 µg/kg, administered as a bolus. Any clinical signs compatible
132 with hypermagnesaemia, including cardiac bradyarrhythmias and persistent hypotension,
133 were recorded.
134
135 After tracheal extubation, carprofen (4 mg/kg) was administered IV to all dogs. Dogs were
136 discharged from the hospital 24 h after surgery.
137
138 *Spinal injection*
139 Once the plane of anaesthesia was judged as adequate on the basis of clinical assessments (jaw
140 relaxation, absence of active blinking, slight or absent palpebral reflex, immobility and
141 physiological variables within normal ranges for the species), spinal injection was performed
142 by one of two anaesthetists (C.A. or D.C.), who were blinded to the treatment. The dogs were
143 positioned in lateral recumbency with the limb to be operated on in a dependent position, with
144 both pelvic limbs pulled symmetrically cranially to maximise the length of the dorsal lumbar
145 intervertebral spaces. The iliac wings and the dorsal spinous processes of L5, L6 and L7 were
146 used as anatomical landmarks. After surgical preparation of the area, a 75 mm × 19 G spinal
147 needle was inserted towards the epidural space, with the bevel facing cranially, through the
148 interspinous ligament between L6 and L5. The stylet was then withdrawn and the needle slowly
149 advanced until cerebrospinal fluid was observed at the hub of the needle. A ‘dry tap’ after the
150 third attempt of needle insertion was considered an exclusion criterion.

151 *Treatment groups*

152 Group C (control) received ropivacaine (Naropin 1%, AstraZeneca), at a dose of 1 mg/kg
153 (0.1 mL/kg). Group M (magnesium) received a mixture of magnesium sulphate (2 g/10 mL,
154 Magnesio Solfato, Galenica Senese), at a dose of 2 mg/kg (equivalent to a volume of
155 0.01 mL/kg), and ropivacaine at a dose of 1 mg/kg. All treatments were administered spinally.
156
157 For both treatments, the solution for injection was made hypertonic immediately before the
158 injection by adding 50% glucose (Glucose 50% BBraun, 0.002 mL/kg) to the solution. The
159 specific gravity of the solutions, measured with a refractometer, was 1.032 and 1.035 at 25 °C
160 for groups C and M, respectively. The solution was injected over 1 min. Doses and volumes
161 were based on previous reports in both human and veterinary medicine (Oezalevli et al, 2005,
162 Arcioni et al, 2007, Bilir et al, 2007, Sarotti et al, 2011).
163
164 *Assessment of nociception*

165 Intra-operatively, any increase in HR, MAP and/or RR of 20% above baseline values
166 (defined as the values recorded before skin incision, after $P_{E'ISO}$ values of 1.3% had been
167 recorded consecutively for 15 min) was considered indicative of nociception. When such
168 increases were seen for at least two of these parameters, fentanyl (3 µg/kg, IV) was
169 administered as rescue analgesia.
170
171 Post-operatively, pain was assessed with a modified multifactorial pain score (Sammarco et
172 al, 1996, Adami et al, 2012) and the Glasgow pain scale (Holton et al., 2001). Additionally, a
173 10 cm visual analogue scale (VAS) with end points labelled ‘worst pain imaginable’ (0) and
174 ‘no pain’ (10) was used. Cut-off values to administer rescue buprenorphine (Temgesic,
175 10 µg/kg IV) were one or more pain scores exceeding 40% of the maximum value possible

(>4 for the VAS, >6 for the Sammarco pain score, and >10 for the Glasgow pain scale). Neurological function of the pelvic limbs and the degree of motor block were assessed with a modified Tarlov scale (Table 1) (Buvanendran et al., 2002).

Blood sampling

The assessments were performed as soon as the dogs were conscious enough to respond to stimulation (vocal call and incitement to sit or stand up) and then at 60, 120, 180, 240 and 300 min thereafter. The evaluations were performed by one of two observers (C.A. and D.C.), who were blinded to the treatment. During preliminary tests, comparable pain and motor block scores were determined by the two observers when independently evaluating the same dogs.

Statistical analysis

Normality of data was tested with the Kolmogorov–Smirnov test and the Shapiro–Wilk test. Repeated measures ANOVA, followed by Tukey–Kramer's multiple comparison test, was used for the plasma magnesium concentrations, for physiological variables and for post-operative pain and Tarlov scores, with treatment (group) and time of data collection as factors. Physiological variables used for statistical analysis were recorded at three predefined time points: (1) before the beginning of surgical stimulation (used as baseline); (2) immediately after skin incision; and (3) immediately after the beginning of tibial osteotomy.

The duration of anaesthesia and analgesia, as well as the number of intra-operative fentanyl and post-operative buprenorphine boluses received by each group, was tested using either one way ANOVA followed by a Bonferroni multiple comparison test, or Kruskal–Wallis ANOVA on ranks followed by Dunn's test. The proportion of dogs within each group showing hypotension and/or bradyarrhythmias was analysed with Fisher's exact test.

Commercially available software (NCSS-2007, SigmaStat; SigmaPlot 12, Systat Software) was used. P values < 0.05 were considered statistically significant.

Results

Eleven female dogs (seven of which were neutered) and nine males (six of which were neutered) were enrolled in the study. The dogs weighed 35.5 ± 22.0 kg, with a mean age of 12.5 ± 5.8 years. Data for age, bodyweight, intra-operative fentanyl requirement, duration of anaesthesia and analgesia and plasma magnesium concentrations were normally distributed. The number of cases per treatment group was equally distributed between the two observers.

Duration of anaesthesia was 267.3 ± 36.0 min (mean ± standard deviation) in group M and 282 ± 36.0 min in group C; this difference was not statistically significant (P = 0.37). Group M experienced significantly longer analgesia (527.0 ± 341.0 min; Fig. 1) and required fewer intra-operative fentanyl boluses (median 0, range 0–1; Fig. 2) than group C (176.0 ± 109.0 min, P = 0.015; median 1.5, range 0–4 boluses, P = 0.0018; respectively).

Intra-operative physiological variables remained within normal ranges for the species and no differences were detected between treatments or time points (Fig. 3). However, one dog in group M and one dog in group C showed moderate sinus bradycardia (40 beats per min) and arterial hypotension (MAP, 50 and 55 mmHg, respectively) shortly after the spinal injection, which responded to glycopyrronium and colloid administration, respectively. None of the dogs required rescue buprenorphine before the final pain assessment.

Post-operatively, there were no significant differences between groups or time points in VAS ($P = 0.36$ and $P = 0.57$) and Sammarco ($P = 0.17$ and $P = 0.16$) pain scales (Fig. 4). However, group M had significantly lower scores for the Glasgow pain scale ($P = 0.012$) and the Tarlov scale ($P = 0.049$) compared to group C (Fig. 4). The Glasgow ($P = 0.08$) and the Tarlov ($P < 0.001$) scores significantly increased over time in both groups. Two dogs in group M showed a persistent motor block, accompanied by loss of deep pain sensation, which lasted 24 and 18 h; neurological function of the pelvic limbs normalised progressively and no long-term complications were observed, although these two dogs required longer hospitalisation and were discharged 72 h after surgery.

Total plasma magnesium concentrations remained within physiological ranges for the species (Fig. 5) and no significant differences were observed between subjects ($P = 0.015$) or between time points ($P = 0.61$).

Discussion

The main finding of this study is that spinal administration of magnesium potentiates the analgesia provided by ropivacaine in dogs undergoing elective orthopaedic surgery. However, magnesium also prolongs the duration of motor block, which makes it a less attractive adjunctive analgesic for peri-operative pain in client-owned dogs, especially in those undergoing TPLO, as they are frequently large breed dogs. Persistent motor block is likely to cause discomfort and to increase the costs of hospitalisation. This is in contrast with the findings of most reports focusing on the neuroaxial use of magnesium in both humans and canine patients, which indicated a lack of effect of magnesium on motor function when administered by either epidural or spinal routes (Buvanendran et al, 2002, Yousef, Amr, 2010, Shahi et al, 2014, Bahrenberg et al, 2015). Nonetheless, magnesium was found to prolong and

enhance brachial plexus motor block when used with lidocaine in human patients (Haghighi et al., 2015).

The mechanisms by which magnesium causes motor block are unknown. Magnesium sulphate is an inorganic salt which readily dissolves in water and becomes almost completely dissociated across a wide pH range, from the low pH of the stomach to the neutral pH of extracellular and cerebrospinal fluids1. An in vitro study on isolated mammalian dorsal root ganglion neurons showed that bivalent and trivalent metal cations transiently block voltage-activated calcium channel currents (Busselberg et al., 1994). The ionised magnesium released by its salt could have acted so, blocking the calcium currents by altering the resting potential of the neuronal membrane within the spinal cord.

Another possible explanation is that, because the magnesium sulphate solution used was hyperosmolar, it might have altered the osmotic homeostasis of cerebrospinal fluid and spinal cord, leading to axonal shrinking and transient neurological dysfunction. In vitro studies have shown that osmotically perturbed neurons are capable of regulating their membrane capacitance, structural organisation and topology, and that these changes are reversible (Wan et al, 1995, Mills, Morris, 1998). Furthermore, dynamic changes in neuronal volume and surface area caused by osmotic manipulation of isolated ganglia resulted in blockade of transmembrane sodium channels (Mills and Morris, 1998), which is also a well-recognised mechanism of action by which local anaesthetics interrupt sensory and motor transmission. For most solutions, however, osmolarity and specific gravity usually change in parallel, and therefore this explanation is less likely because the solution for injection in both groups had very similar specific gravity.

Spinal administration of local anaesthetics has been shown to provide adequate analgesia to dogs undergoing orthopaedic procedures (Sarotti et al., 2011), and is a commonly used technique in clinical practice. For this reason, ropivacaine was selected for use in the positive control group in this trial.

Spinal administration of magnesium did not increase total plasma magnesium concentrations in the dogs enrolled in this trial. However, one limitation of our methods is that plasma magnesium concentrations do not correlate with tissue concentrations, with the exception of interstitial fluid and bone, nor does it reflect total body magnesium (Elin, 2010). Moreover, only total magnesium, rather then the ionised, biologically active form of the ion, could be measured. Another limitation is that blood was collected over a relatively short period of time; more frequent sampling over a longer period, though not feasible in client-owned animals, would have provided a more complete picture of magnesium uptake and distribution. However, because clinical signs compatible with hypermagnesaemia were not observed, it is reasonable to assume that ionised magnesium stayed within acceptable ranges for the species.

Although cardiovascular variables remained within physiologically acceptable limits, spinal injection in both groups resulted in a transient decrease in heart rate and arterial blood pressure. Additionally, one dog in each group experienced persistent hypotension and bradycardia, which required treatment with colloids and anticholinergics. Administration of ropivacaine by the spinal route might result in decreased sympathetic outflow to the cardiovascular system (Levin et al., 1998). However, because the incidence of cardiovascular side effects did not differ between treatments, it is unlikely that they were caused by magnesium.

Conclusions

The addition of magnesium sulphate to spinal ropivacaine increased the intensity and the duration of peri-operative analgesia in dogs undergoing orthopaedic surgery, but the potential for prolonged motor block could limit its utility in clinical practice. Further research might help identify a dose with similar analgesic effects but with less potential for prolonged motor block.

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Conflict of interest

None of the authors have financial or personal relationships with individuals or organisations that could inappropriately influence or bias the content of the paper.

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387

Figure legends:

Fig. 1. Duration of analgesia (min) in dogs receiving a spinal injection of either ropivacaine alone (group C, $n = 10$) or a combination of ropivacaine and magnesium (group M, $n = 10$). The asterisk indicates a statistically significant difference between treatments ($P < 0.05$). The lines indicate median values. The upper and lower boxes indicate the 75% and 25% of the values which fall below the upper and lower quartiles, respectively. The upper and lower whiskers indicate the maximum and minimum values.

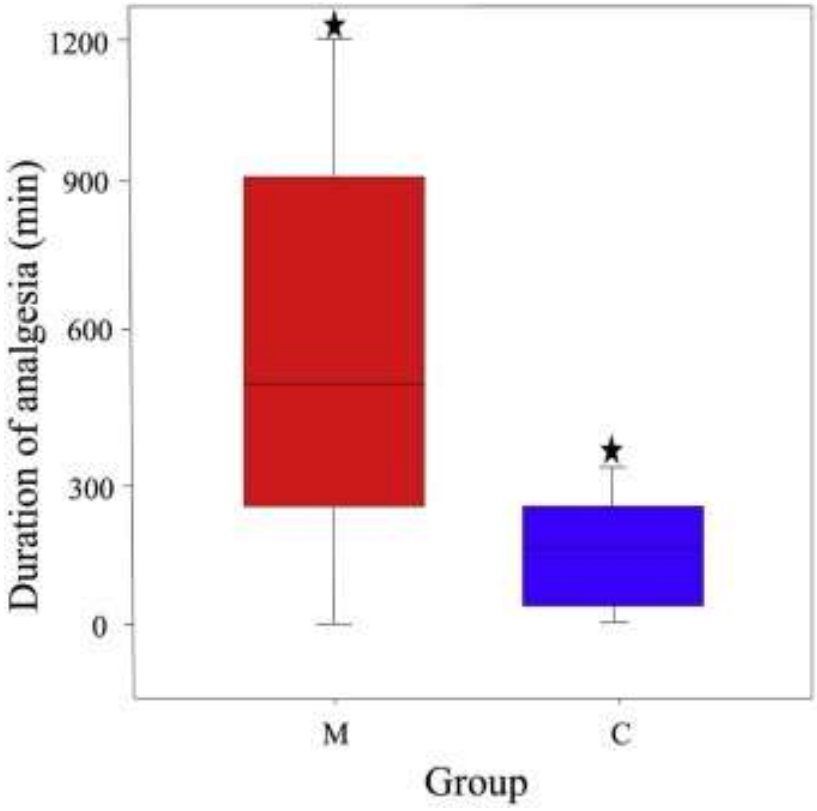


Fig. 2. Number of intra-operative fentanyl boluses (3 µg/kg each bolus) administered to 20 dogs receiving a spinal injection of either ropivacaine alone (group C, $n = 10$) or a combination of ropivacaine and magnesium (group M, $n = 10$). The lines indicate median values. The upper and lower boxes indicate the 75% and 25% of the values which fall below the upper and lower quartiles, respectively. The upper and lower whiskers indicate the maximum and minimum values. The dots indicate the outliers. The asterisks indicate a statistically significant difference between treatments ($P < 0.05$).

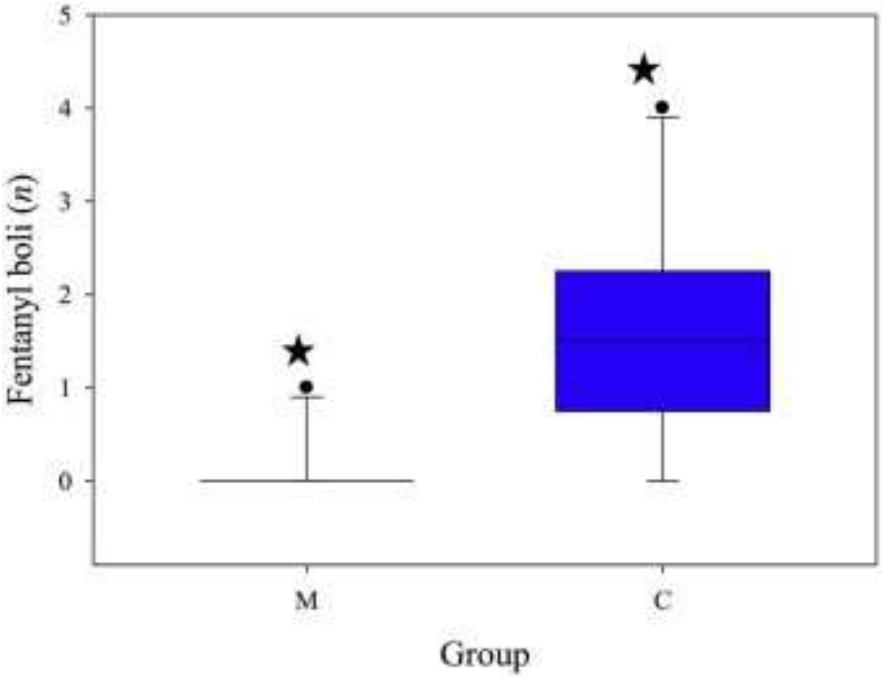


Fig. 3. Values for heart rate (HR, beats per min [bpm]) and mean arterial pressure (MAP, expressed in mmHg) for 20 dogs receiving a spinal injection of either ropivacaine alone (group C, n = 10) or a combination of ropivacaine and magnesium (group M, n = 10). Data were recorded at three different time points: (1) before the beginning of surgical stimulation (used as baseline); (2) immediately after skin incision; and (3) immediately after the beginning of tibial osteotomy. The lines indicate median values. The upper and lower boxes indicate the 75% and 25% of the values which fall below the upper and lower quartiles, respectively. The upper and lower whiskers indicate the maximum and minimum values.

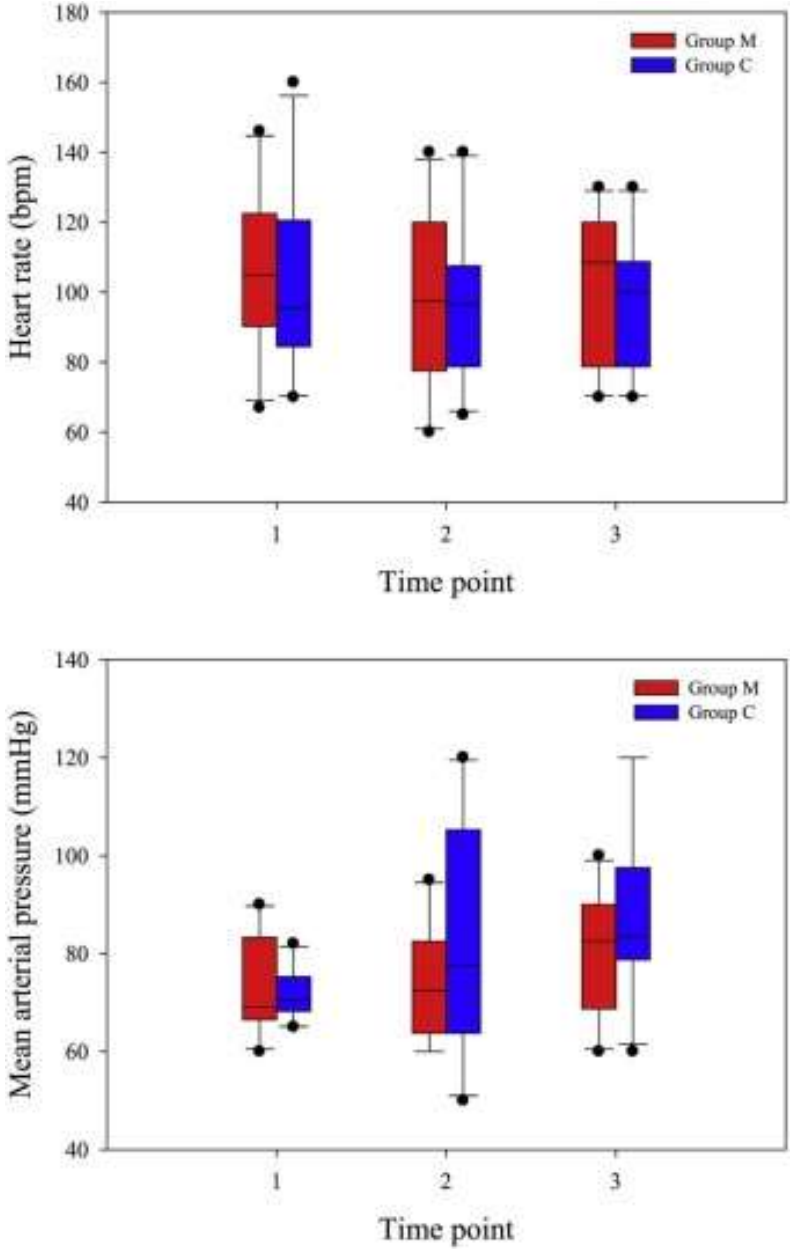


Fig. 4. Values for Sammarco pain score, Glasgow pain scale, Visual Analogue Scale (VAS) and Tarlov scale for 20 dogs receiving a spinal injection of either ropivacaine alone (group C, n = 10) or a combination of ropivacaine and magnesium (group M, n = 10). Data were recorded at four different time points: at recovery, as soon as the dogs were conscious enough to be examined (time point 1), and then 1, 2 and 3 h after that (time points 2, 3 and 4, respectively). The lines indicate median values. The upper and lower boxes indicate the 75% and 25% of the values which fall below the upper and lower quartiles, respectively. The upper and lower whiskers indicate the maximum and minimum values. The asterisks and the daggers indicate statistically significant differences between treatments and between time points, respectively ($P < 0.05$).

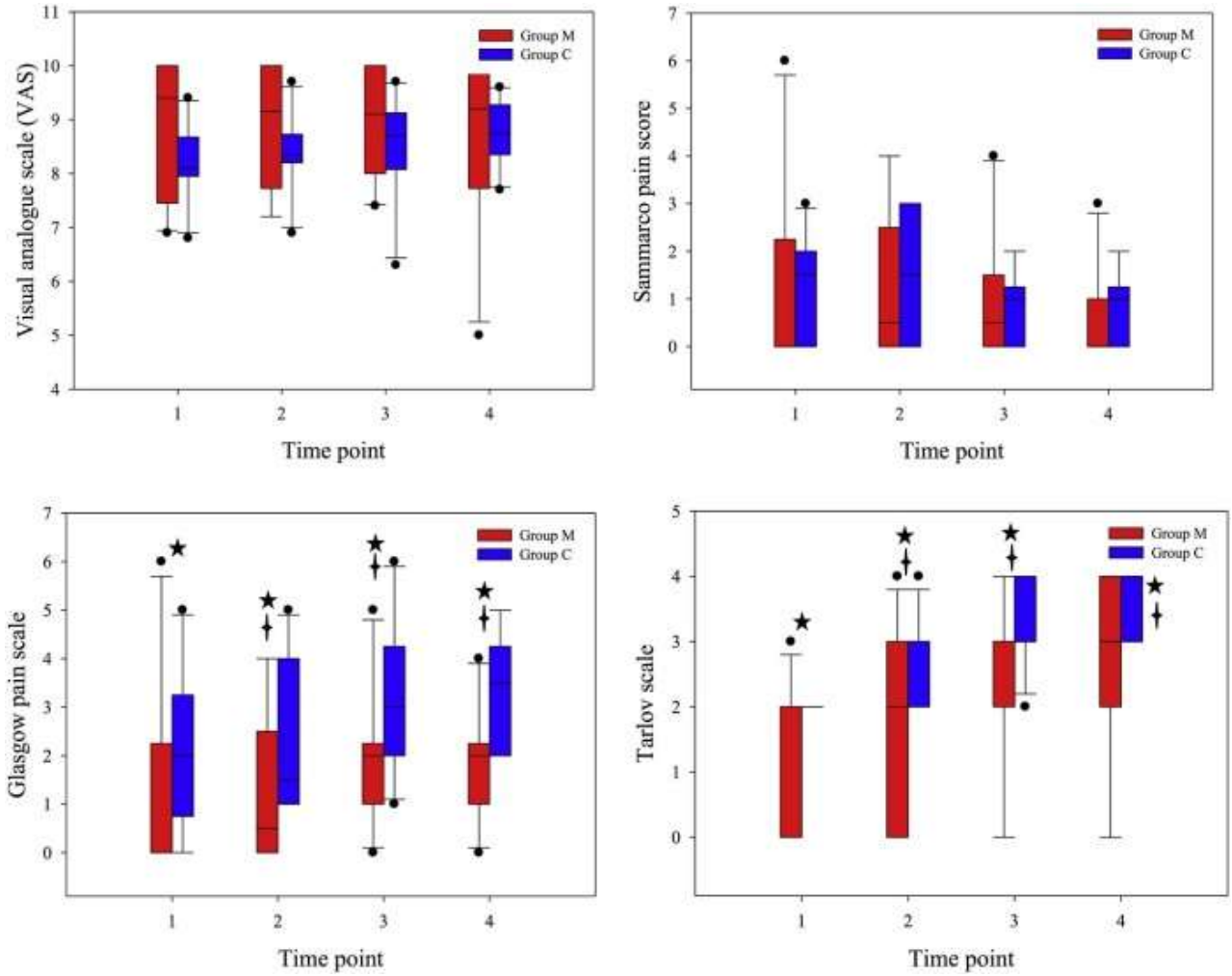
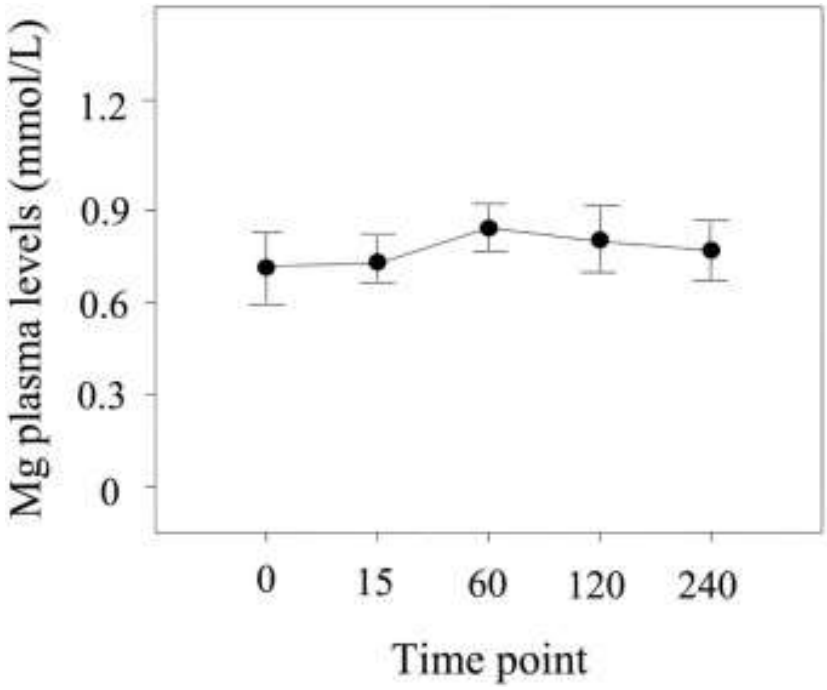


Fig. 5. Mean values (±standard deviations) of total plasma magnesium concentrations (mmol/L) for 20 dogs receiving a spinal injection of either ropivacaine alone (group C, n = 10) or a combination of ropivacaine and magnesium (group M, n = 10). Blood was sampled at the following time points: before spinal injection (0, baseline), and then at 15, 60, 120 and 240 min thereafter.



434 Table 1. Tarlov's scale (modified from Buvanendran et al., 2002) used for neurological

435 assessment.

Grade	Description
Grade 0	Flaccid paraplegia, no movements of the pelvic limbs, possible loss of bowel/urinary bladder control
Grade 1	Spastic paraplegia with moderate or vigorous purposeless movements of the pelvic limbs. No sitting, unable to walk
Grade 2	Good movements of the pelvic limbs but unable to stand
Grade 3	Able to stand but unable to walk normally, hips and pelvic limbs obviously unstable, moderate to severe ataxia
Grade 4	Able to stand and walk normally, some muscle weakness of the pelvic limbs may be seen

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