Antinociceptive effects of epidural magnesium sulphate alone and in combination with morphine in dogs

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Abstract

Objective To compare the antinociceptive effects of magnesium sulphate ($MgSO_4$) when administered epidurally alone and in combination with morphine.

Study design Experimental, randomized, 'blinded', crossover study.

Animals Six healthy adult Beagle dogs.

Methods Evaluated treatments were $MgSO_4$ (2.5 mg kg^{-1}) alone (Mg), morphine (0.1 mg kg^{-1}) alone (Mo), $MgSO_4$ in combination with morphine (Mm), and sterile water (0.115 mL kg^{-1}; Co) that were injected in the lumbosacral epidural space using an epidural catheter. Antinociception was measured using the von Frey mechanical threshold device applied to the carpal pads, both sides of the thorax and metatarsi. Measurements were obtained at time points: before treatment (baseline) and 0.5, 1, 2, 4, 6, 12, 18 and 24 hours after the epidural injection. Sedation, behaviour score and presence of motor deficits were assessed. Data were analyzed using a linear mixed model and Bonferroni adjustments, with significance set at $p < 0.05$.

Results There were significant effects of treatment and time in all regions. Overall threshold values in grammes force [median (interquartile range)] when stimulation regions were combined were significantly higher in Mg [164 (135–200)], Mo [156 (129–195)] and Mm [158 (131–192)] compared to Co [145 (120–179)]. Thresholds were significantly higher compared to Co in Mg, Mo and Mm at the thorax and metatarsi, but only in Mg and Mo at the carpal pads. No motor deficits were observed at any time point. Thresholds (combined regions) were increased from baseline at one or more time points with all treatments, including control.

Conclusion and clinical relevance Epidural $MgSO_4$ produced an antinociceptive effect characterised by an increase in the mechanical thresholds of similar magnitude to that produced by epidural morphine, compared with the control group, without causing any motor deficits. No potentiation of morphine antinociception was observed. The onset and offset times of antinociception could not be clearly established. To what extent these results can be extrapolated to clinical cases requires further investigation.

Keywords analgesia, canine, epidural, magnesium sulphate, mechanical threshold, neuraxial.

Introduction

Magnesium is the fourth most common cation in the body and the second most common intracellular ion (Dubé & Granry 2003). It has a fundamental role in many cellular functions; it possesses calcium antagonistic properties, is involved in transmembrane ion fluxes and regulates neuronal activity (Dubé &

Granry 2003). Magnesium is a natural antagonist of the *N*-methyl-D-aspartate (NMDA) receptors as the magnesium ion blocks the central canal of the ionic receptor inhibiting calcium influx and preventing neuronal depolarisation. Therefore, it has been postulated that systemic and/or neuraxial administration of $MgSO_4$ may produce analgesia and prevent development of central sensitization (Mayer et al. 1984).

Human studies have reported analgesic effects of systemic administration of $MgSO_4$ intraoperatively with a resultant reduction of intra- and post-operative opioid requirements during soft tissue surgery, such as hysterectomy or cardiac surgery (Steinlechner et al. 2006; Ryu et al. 2008), as well as during orthopaedic surgery (Levaux et al. 2003) and thoracotomy (Kogler 2009). However, other studies found no beneficial analgesic effect of systemic administration of $MgSO_4$ to human patients undergoing soft tissue surgery including caesarean section or cholecystectomy (Bhatia et al. 2004; Paech et al. 2006). In a systematic review of 14 human randomized clinical trials, it was concluded that there was no effect of systemic administration of $MgSO_4$ on post-operative pain intensity and analgesic requirements (Lysakowski et al. 2007).

The neuraxial administration of $MgSO_4$ has been investigated in human clinical trials. The addition of $MgSO_4$ to epidural or intrathecal opioids and/or local anaesthetics resulted in prolonged analgesia (Buvanendran et al. 2002; Özalevli et al. 2005; Yousef & Amr 2010), a post-operative opioid sparing effect (Arcioni et al. 2007) and a decrease in post-operative pain scores (Sun et al. 2012) in patients undergoing soft tissue and orthopaedic surgeries. However, epidural administration of $MgSO_4$ alone for 48 hours did not significantly decrease the incidence of chronic post-operative pain in human patients undergoing thoracic surgery (Lee et al. 2012) and failed to prolong epidural analgesia when administered with bupivacaine in human patients undergoing lower abdominal and lower limb surgeries (Ghatak et al. 2010).

In veterinary medicine, only a few recent studies have investigated the effects of systemic $MgSO_4$ administration in relation to analgesia. Systemic administration of $MgSO_4$ reversed mechanical hyperalgesia induced by magnesium deficiency (Begon et al. 2001) and reduced allodynia in rats (Xiao & Bennett 1994). However, intraoperative intravenous (IV) administration of $MgSO_4$ failed to show a clear antinociceptive effect in dogs undergoing ovariohysterectomy (Rioja et al. 2012).

In rats, $MgSO_4$ administered intrathecally enhanced spinal anaesthesia induced by opioids (Kroin et al. 2000) and delayed the development of opioid tolerance (McCarthy et al. 1998). Furthermore, intrathecal $MgSO_4$ in rats produced sedation and sensory block (Bahar et al. 1996) and motor block (Karasawa et al. 1998). The addition of $MgSO_4$ to epidural local anaesthetics or ketamine induced a prolonged antinociceptive effect in goats (Bigham 2009), horses (Bigham & Shafiei 2008), cattle (Deghani & Bigham 2009) and sheep (DeRossi et al. 2012).

The purpose of this study was to compare the antinociceptive effects of $MgSO_4$ when administered epidurally alone and in combination with morphine in dogs. The null hypothesis was that $MgSO_4$ would not produce an antinociceptive effect when administered alone and that it would not enhance morphine antinociception when administered in combination.

Material and methods

Study design

Six healthy research Beagle dogs (three females, three males) were enrolled in this experimental, 'blinded', randomized, crossover study after obtaining approval from the University of Pretoria (South Africa) Animal Use and Care Committee (V074-11). The mean ± SD weight and age of the dogs were 15.2 ± 1.5 kg and 4.3 ± 0.9 years, respectively. Dogs were determined to be healthy prior to enrolment based on a clinical examination and blood work including a complete blood count, total serum protein and creatinine. Dogs' total serum magnesium concentrations were also determined to exclude states of hypomagnesaemia.

Dogs received four treatments in a random order with a 1-week wash-out period between treatments. Treatments consisted of an epidural injection of: $MgSO_4$ (2.5 mg kg^{-1}; Sabax Magnesium sulphate 50%; Adcock Ingram, South Africa; treatment Mg); morphine (0.1 mg kg^{-1}; Morphine Sulphate-Fresenius PF 10 mg mL^{-1}; Fresenius Kabi for Bodene, South Africa; treatment Mo); $MgSO_4$ (2.5 mg kg^{-1}) in combination with morphine (0.1 mg kg^{-1}; treatment Mm), and sterile water (0.115 mL kg^{-1}; Sabax water for injection 10 mL; Adcock Ingram Critical Care, South Africa; treatment Co). Sterile

water was added to treatment groups Mo, Mg and Mm to obtain a total volume of 0.115 mL kg^{-1}. The above-described solutions were prepared by an investigator (BTD) not involved in the antinociceptive evaluations and the investigator performing the evaluations (AB) remained unaware of the treatments throughout the study.

Treatment administration

A 20 gauge × 4.45 cm cannula (Jelco; Smiths Medical International, UK) was placed in the cephalic vein for administration of IV drugs and fluids. Dogs were anaesthetized weekly for the placement of the lumbosacral epidural catheter. Anaesthesia was induced using propofol (6–7 mg kg^{-1}; Propofol 1% Fresenius; Fresenius Kabi, South Africa) administered IV to effect to allow endotracheal intubation. Anaesthesia was maintained with isoflurane (Isofor Inhalation Anaesthetic; Saffeline Pharmaceuticals, South Africa) in oxygen at a flow rate of 1 L $minute^{-1}$ via a circle rebreathing system. Vital parameters were monitored continuously during anaesthesia using a multiparameter monitor (SurgiVet; Smiths Medical PM, WI, USA). Dogs received 4 mL kg^{-1} $hour^{-1}$ of Lactated Ringer's solution [Sabax Ringer-Lactate (Hartmann's Solution); Adcock Ingram, South Africa] during anaesthesia.

Epidural catheters were inserted by a single investigator (ER) in the anaesthetized dogs placed in sternal recumbency. The lumbosacral area was clipped and aseptically prepared using chlorhexidine and 90% alcohol. An 18 gauge × 4.45 cm Tuohy needle was first inserted in the lumbosacral epidural space with the bevel pointing cranially. Correct epidural needle placement was verified by lack of resistance to injection of a small volume of sterile water using a 3 mL syringe. A 20 gauge catheter (Epidural Catheterization Set with Flex-Tip Plus Catheter for Pedriatric Lumbar Placement; Arrow International Special Order Products, South Africa) was then introduced through the needle and was advanced 2–4 cm into the epidural space. Drugs were administered immediately after placement of the epidural catheter, whilst the dog was still anaesthetized and in sternal recumbency, and the injection time was recorded. The epidural catheter was removed after treatment administration and the dogs were allowed to recover from anaesthesia. Dogs were recovered in sternal recumbency and under continuous observation.

Data collection

Antinociceptive threshold testing was performed using a von Frey device [Electronic von Frey, Model 23931 (modified); IITC Life Science, CA, USA]. The device consisted of a load cell, a handle, a recording device and a rigid tip. The plastic tip (4.5 cm in length, 0.5 cm diameter) was modified to increase the rigidity by filling it with an epoxy putty (Repair Metal power Epoxy; Pattex, Germany). The load cell was capable of measuring an applied force of 1–1000 gramme force (gf). The instrument was calibrated each day of the study prior to data collection following manual instructions. The investigator performing the measurements increased the applied force in a slow constant manner and the maximum applied force was recorded by the instrument.

The repeatability of the von Frey measurements was assessed prior to commencement of the experimental evaluation of treatments (phase 1 of the study). Three investigators (AB, ER and BTD) performed two sets of measurements on two separate days, with three measurements on each region on the six study dogs. Tested areas included (for the pectoral limb) the carpal pad (Cp), lateral epicondyle of the humerus, thorax (Th) at the intercostal spaces 6 or 7, lateral surface of the thigh and (for the pelvic limb) metatarsus (Mt), on both sides. These areas were tested bilaterally for consistency. Threshold testing was performed on clipped skin in a temperature-controlled room, with minimal restraint of the dogs.

During the experimental evaluation (phase 2 of the study), threshold testing was performed by a single investigator (AB), prior to anaesthesia and prior to administration of the treatment (baseline). Threshold testing was then repeated at 30 minutes, and 1, 2, 4, 6, 12, 18 and 24 hours after the epidural injection. Three measurements were obtained bilaterally at three regions (Cp, Th and Mt) at each time point. Briefly, the tip of the von Frey device was applied at each region perpendicular to the body surface and pressure was applied in a consistently increasing manner until a nociceptive response was obtained. A nociceptive response was considered withdrawal of the limb (Cp and Mt), a skin twitch or turning of the head (Th).

A withdrawal reflex obtained in response to the first touch with the tip before applying any pressure

was not recorded as a nociceptive response. The maximum force at which a response was noted (the von Frey threshold) was recorded by a second observer also unaware of treatment group. The measured von Frey thresholds were expressed in grammes force (gf) and the three measurements averaged for statistical analysis. A maximum cut off of 600 gf was set. The investigator was notified to stop if this force was reached and it was recorded as the von Frey threshold. Tested regions were inspected visually each week for signs of tissue damage caused by the applied force.

Tail tone and ataxia of the pelvic limbs were assessed to evaluate motor effects immediately prior to the von Frey threshold measurements at the same time points. The degree of tail tone was scored using a numeric descriptive scale, with 0 having a normal tail tone, 1 having a mild decrease in tail tone, 2 having a moderate decrease in tail tone, and 3 having no tail tone. Ataxia of the pelvic limbs was scored with the dog walking three metres in a straight line using a numeric descriptive scale, with 0 being no ataxia, 1 mild ataxia, 2 moderate ataxia, and 3 severe ataxia.

Additionally, behaviour was assessed using a numeric descriptive scale, with 0 being frightened, 1 calm and cooperative, 2 anxious and unsettled, and 3 excited and non-cooperative. The level of sedation was scored using a numeric descriptive scale, with 0 being not sedated, 1 mildly sedated, 2 moderately sedated, and 3 severely sedated. Room temperature and humidity were recorded at each observation time during data collection.

Statistical analysis

Data were assessed for normality through the plotting of histograms, calculation of descriptive statistics and the Anderson-Darling test for normality. Outcome variables violating the normality assumption were transformed using natural logarithms or ranks prior to statistical analysis. Repeatability was assessed by calculating the coefficient of variation (standard deviation divided by the mean) of the three repeated measurements and by performing a variance components analysis. A linear mixed model was used to analyze the effect of treatment and time on the von Frey thresholds. Dog was included as a random effect in the model and behaviour, side, region and week were included as fixed effects. Week when treatments were administered was evaluated as a potential confounder or effect modifier in the evaluation of treatment effects. Multiple pairwise comparisons were adjusted using Bonferroni correction. A non-parametric Freidman test was used to compare the distance of the epidural catheter within the canal among treatments. Data were analyzed using commercially available software (SPSS version 17.0; SPSS Inc, IL, USA) and results interpreted at the 5% level of significance.

Results

Data are expressed as mean ± SD unless otherwise specified. Clinical examination and haematology prior to the study revealed no abnormalities in any dog. Serum magnesium concentration was 0.8 ± 0.1 mmol L^{-1}, which was within the normal range of the laboratory.

Anaesthesia was induced using 6.6 ± 1.3 mg kg^{-1} of propofol and total anaesthesia time was 13.0 ± 4.3 minutes. Induction, maintenance and recovery from anaesthesia were uneventful in all dogs. There was no evidence of tissue damage, injury or lameness due to the applied pressure of the von Frey mechanical threshold testing at any time. The total volume administered into the epidural space was 3.17 ± 0.68 mL including injected drugs and the additional sterile water used to test catheter placement. There was no difference in the distance that the epidural catheter was advanced in the canal among treatments ($p = 0.717$).

During phase 1 of the study, data collected from the Cp, Th and Mt had the highest repeatability (data not shown) and therefore, were selected for the evaluation of treatment effects. The mean coefficients of variation (range) of the von Frey thresholds for the three regions were 20.8% (3.2–40.3%), 27% (13.6–49.8%) and 18.9% (3.7–42.4%) at the Cp, Th and Mt sides, respectively. The majority (74%) of variability in the von Frey mechanical thresholds was unexplained, but 18.4% was attributed to the operator, 3.4% to the dog, 3.3% to the region and 0.7% to the day.

Mechanical von Frey threshold values in gf are presented as median (interquartile range). Baseline thresholds at each region were not significantly different throughout the study and did not significantly vary by week. During phase 2 of the study, overall threshold values (pooled for all treatments and time) varied significantly by region ($p < 0.001$), with values for Th being the highest 172 (140–214),

followed by Mt 162 (136–192) and Cp 138 (118–165).

There was a significant effect of treatment in all regions. Overall von Frey threshold values (when regions were combined) were significantly higher in treatments Mg, Mo and Mm than in treatment Co ($p < 0.001$), and significantly higher in treatment Mg than in Mm ($p = 0.022$; Table 1). At the Cp, von Frey threshold values were significantly higher in treatments Mg and Mo than in treatment Co, but there was no difference between treatment Mm and Co. At the Th and Mt, threshold values were significantly higher in treatments Mg, Mo and Mm than in treatment Co (Table 1). There were no significant differences in threshold values comparing Mg, Mo and Mm in independent analyses for each region.

Time had a significant effect on the von Frey threshold values. Overall von Frey threshold values (when all treatments and regions were combined) were significantly increased at 30 minutes, 2, 4, 6 and 12 hours after injection of the treatments compared with baseline values ($p < 0.001$, $p = 0.002$, $p < 0.001$, $p = 0.01$, $p < 0.001$, respectively; Table 2).

The threshold values obtained within the separate treatments over time (pooled for regions) are summarised in Table 3. Threshold values were significantly higher than baseline at 30 minutes in treatments Co, Mg and Mo ($p = 0.006$, $p = 0.026$, $p < 0.001$, respectively). Additionally, thresholds were significantly increased over baseline in treatment Co at 2, 4 and 12 hours ($p = 0.002$, $p = 0.007$, $p = 0.005$); in Mo at 4 hours ($p = 0.05$); and in Mm at 2 hours ($p = 0.022$).

Threshold values obtained on the left side were significantly higher than on the right side at all three regions ($p < 0.001$).

No ataxia or decrease in tail tone was detected in any dog throughout the study.

Sedation score 2 (moderate) was given to 13%, 4% and 0% of the dogs; sedation score 1 (mild) to 58%, 29%, and 8% of the dogs; and sedation score 0 to 29%, 66% and 92% of the dogs at 30 minutes, 1 and 2 hours after the epidural injection, respectively.

Seventy-eight percent of the dogs had a behaviour score of 1, 15% behaviour score 2, 5% behaviour score 3 and 3% behaviour score 0. Dogs with behaviour score 0 had significantly higher overall threshold values [166 (137–189)] compared to dogs with a behaviour score 2 [164 (133–197); $p = 0.045$]. At the Mt, dogs with a behaviour score 3 had significantly higher threshold values [185 (156–215)] than dogs with a behaviour score 2 [172 (145–197); $p = 0.029$].

Discussion

When compared over the whole time course of the experiment (24 hours) epidural $MgSO_4$ produced an antinociceptive effect in dogs characterised by an increase in mechanical thresholds compared with the control treatment that was of similar magnitude as epidural morphine. Potentiation of antinociception was not observed when $MgSO_4$ and morphine were administered in combination; on the contrary, antinociception produced by $MgSO_4$ alone seemed to be of greater magnitude than when $MgSO_4$ and morphine were combined. When individual time points were examined, a statistically significant

Table 1 Overall mechanical threshold values in gramme force [median (interquartile range)] obtained from six Beagle dogs with the von Frey device. Individual epidural treatments consisted of Mg (2.5 mg kg^{-1} of $MgSO_4$), Mo (0.1 mg kg^{-1} of morphine), Mm (0.1 mg kg^{-1} of morphine and 2.5 mg kg^{-1} of $MgSO_4$) and Co (0.115 mL kg^{-1} of sterile water). Results are shown for the carpal pads (Cp), both sides of thorax (Th) and metatarsi (Mt), and for all regions combined

	Treatments				
Region	**Co**	**Mg**	**Mo**	**Mm**	***p* Values**
Combined regions	145 (120–179)	164 (135–200)*, §	156 (129–195)*	158 (131–192)*	<0.001*, 0.022§
Cp	130 (111–155)	144 (124–174)*	137 (118–168)†	140 (119–166)	<0.001*, 0.019†
Th	160 (125–199)	186 (152–224)*	171 (142–216)†	174 (139–213) ‡	<0.001*, 0.014†, 0.012‡
Mt	153 (128–182)	170 (136–199)*	162 (136–197)†	166 (141–192)‡	<0.001*, <0.001†, 0.003‡

*,†,‡Value significantly different from Co. §Value significantly different from Mm.

Table 2 Mechanical threshold values (pooled for all treatments) in gramme force [median (interquartile range)] over time obtained from six Beagle dogs with the von Frey device. For details of individual epidural treatments see Table 1. Results are shown for the carpal pads (Cp), both sides of thorax (Th) and metatarsi (Mt), and for all regions combined, at different time points after epidural injection of the treatments

	Time (hours)								
Region	**Baseline**	**0.5**	**1**	**2**	**4**	**6**	**12**	**18**	**24**
Combined regions	146 (124–178)	166 (138–208)*	155 (128–190)	159 (130–198)*	161 (132–199)*	157 (129–195)*	162 (131–198)*	14 (122–173)	153 (123–190)
Cp	134 (118–155)	155 (126–199)*	132 (113–159)	137 (117–164)	142 (120–176)	138 (118–160)	143 (121–174)	130 (114–162)	133 (114–162)
Th	152 (122–195)	175 (148–220)*	174 (139–217)*	180 (141–220)*	178 (149–230)*	188 (148–228)*	177 (141–216)*	162 (128–189)	167 (135–207)
Mt	158 (135–185)	168 (141–208)*	160 (134–188)	178 (140–204)*	166 (132–200)	163 (131–193)	165 (137–193)	150 (132–168)	165 (133–197)

*Value significantly different from baseline within a region ($p < 0.05$).

Table 3 Mechanical threshold values (pooled for regions) in gramme force [median (interquartile range)] over time obtained from six Beagle dogs with the von Frey device. Individual epidural treatments consisted of Mg (2.5 mg kg^{-1} of $MgSO_4$), Mo (0.1 mg kg^{-1} of morphine), Mm (0.1 mg kg^{-1} of morphine and 2.5 mg kg^{-1} of $MgSO_4$) and Co (0.115 mL kg^{-1} of sterile water). Results are shown for the four separate treatments at different time points after epidural injection

	Time (hours)								
Treatment	**Baseline**	**0.5**	**1**	**2**	**4**	**6**	**12**	**18**	**24**
Co	126 (110–159)	153 (123–184)*	139 (118–177)	148 (122–197)*	151 (126–180)*	148 (125–178)	153 (125–185)*	141 (117–163)	150 (116–177)
Mg	158 (134–192)	179 (149–227)*	158 (127–191)	159 (137–191)	171 (133–211)	171 (135–211)	170 (142–205)	155 (135–187)	153 (131–199)
Mo	152 (127–181)	175 (147–227)*	158 (136–185)	153 (128–196)	165 (134–215)*	161 (133–211)	154 (131–193)	141 (117–167)	160 (125–192)
Mm	148 (132–175)	161 (135–197)	160 (127–204)	174 (143–208)*	164 (134–190)	156 (133–189)	165 (138–203)	148 (123–177)	149 (119–188)

*Value significantly different from baseline within a treatment ($p \leq 0.05$).

increase in von Frey thresholds from baseline was seen with the control administration of water at more time points than the actual treatments. However, the baseline values for this control treatment were lower than those prior to other treatments, and the von Frey values for the control overtime also tended to be lower than those for the other treatments, which may in part explain the difference observed between control and the other treatments when all time points were pooled.

Magnesium deficiency may induce hyperalgesia, which can subsequently be reversed by $MgSO_4$ administration (Begon et al. 2001). In the current study, serum magnesium levels were measured in the dogs before inclusion in the study, and were within normal limits in all dogs; therefore reversal of hypomagnesaemia could not be an explanation for any of our results.

The doses of neuraxial $MgSO_4$ described in the literature are variable. In humans, a dose of approximately 0.5–0.7 mg kg^{-1} of $MgSO_4$ has been most commonly used intrathecally with no apparent adverse effects (Buvanendran et al. 2002; Özalevli et al. 2005; Yousef & Amr 2010). Epidural doses of 0.18 mg kg^{-1} have been administered in horses (Bigham & Shafiei 2008), 0.21 mg kg^{-1} in cattle (Deghani & Bigham 2009) and 2 mg kg^{-1} in goats (Bigham 2009) in combination with local anaesthetics. A dose of 3 mg kg^{-1} of $MgSO_4$ administered intrathecally to dogs did not cause any adverse effects and it seemed to possess neuroprotective effects (Simpson et al. 1994). Based on the great variability of reported doses, the lack of a clinically recommended dose and the apparent safety of epidural $MgSO_4$, a relatively high dose of 2.5 mg kg^{-1} was used in the present study.

The antinociceptive effect of $MgSO_4$ extended up to the thoracic limbs in the present study. The dispersion of a drug in the epidural space is dependent on the injected volume, the pressure within the epidural space (Torske & Dyson 2000) and the lipid solubility of the drug, as these factors facilitate the absorption across the dura membrane and into the cerebrospinal fluid (Valverde 2008). In the present study, the total administered epidural volume approximated a volume of 0.2 mL kg^{-1}, which has been described to migrate up to the thoracolumbar area (Torske & Dyson 2000; Valverde 2008). The observed antinociceptive effect on the thoracic limbs has been previously described with lumbosacral epidural morphine (Valverde et al. 1989), which was explained by the absorption of the drug into the cerebrospinal fluid promoting its cranial migration (Valverde et al. 1992).

No potentiation of antinociception was observed when $MgSO_4$ was co-administered with morphine in the present study, which contrasts with observations from a previous study in rats, where intrathecal $MgSO_4$ co-administered with morphine enhanced analgesia in a dose dependent manner (Kroin et al. 2000). Likewise, additional administration of intrathecal $MgSO_4$ enhanced fentanyl analgesia detected with a decrease in pain score and opioid requirements in humans undergoing orthopaedic surgery (Bilir et al. 2007). One possible explanation for this discrepancy between studies is that the nociception elicited by the von Frey device is of different quality and intensity than clinical pain elicited by surgeries. The mechanical pressure elicited by the von Frey device activates mechanoreceptors and subsequently Aδ and C-fibres (Le Bars et al. 2001), but elicites no inflammation and central sensitization. It is suspected that $MgSO_4$ analgesia is mainly mediated by its antagonistic action on the NMDA receptors (Melzack & Wall 1965), which only become activated during states of central sensitization. Therefore, the potentiating effect of $MgSO_4$ when administered in combination with opioids observed in patients undergoing surgeries might be due to the difference in type of fibres and/or receptor activation. This hypothesis is supported by a study in rats where antinociceptive effects of intrathecal $MgSO_4$ were not observed in acute models of pain, but were demonstrated during the second (tonic) phase of the formalin test when inflammation and activation of NMDA receptors were involved (Takano et al. 2000). Alternatively, the chosen dose of $MgSO_4$ and the power of the present study, diluted by the use of several testing sites and multiple time points, may have been insufficient to observe a potentiation of the antinociceptive effect of morphine caused by $MgSO_4$.

In the present study, a significant increase in thresholds was observed from 0.5 to 12 hours (excluding 1 hour) when all regions and all treatment groups were pooled together. However, a significant increase in thresholds compared with baseline could only be observed at 30 minutes in the control, $MgSO_4$ and morphine treatments (when regions were pooled), but not consistently at later time points in the actual treatment groups. In the literature, the onset of action of epidural morphine in dogs is reported to be between 20–60 minutes (Valverde 2008), and the analgesia is reported to

last 10–24 hours (Torske & Dyson 2000), 12–24 hours (Valverde 2008) and 16 hours (Troncy et al. 2002). Little is known about the onset and duration of antinociception of epidurally administered $MgSO_4$. In rats, onset of sensory block is reported to be 8.4 ± 1.5 minutes (Bahar et al. 1996) with a duration of 52 ± 5.1 minutes after intrathecal injection of 0.05 mg kg^{-1} $MgSO_4$. In the present study, the increased mechanical thresholds observed at 30 minutes post-injection might have been due to mild to moderate sedation observed in 70% of the dogs, and not due to the effects of the epidural drugs. A sedated dog is less likely to respond to a nociceptive stimulus and the reaction time might also be prolonged (Beecher 1957). However, a previous study reported that the von Frey mechanical thresholds could distinguish between sedation and antinociception in dogs as the reported sedation in the dogs outlasted the antinociceptive effect detected by the von Frey device after IV morphine administration (KuKanich et al. 2005). Other possible explanations for the increase in thresholds 30 minutes post-injection is the rapid initial systemic absorption of the injected drugs, as has been shown following epidural administration of morphine in dogs (Valverde et al. 1992), or a mechanical effect due to the pressure exerted by the fluid in the spinal canal. The fact that in the actual treatment groups thresholds were not increased consistently at later times points may reflect a lack of power when treatments were separated *versus* when they were pooled. Another possible explanation is that the onset and offset times of antinociception might be different (shorter in more caudal regions compared to more cranial regions), and therefore, it is possible that these opposing onset and offset times counteracted each other when regions were pooled and prevented more significant differences to be observed in the actual treatment groups over time.

As discussed above, in the control group the threshold values increased at some time points compared with baseline. This could have been due to a number of factors such as the effect of injecting water, epidural pressure changes related to the volume injected, unexplained circadian variations in the thresholds, or a learning process by the dogs. The latter could reflect that the dogs became accustomed to the stimulus over time within the same day. Even though external distractions were controlled and avoided as much as possible, there are some external and intrinsic (dog) factors impossible to control that could have influenced the results over time (for example when the dogs were hungry or fed or needed to urinate or defecate). Nonetheless, learning did not seem to occur, as the results did not vary by week and the fact that the treatments were randomized from week to week (and week was included as a factor in the statistical models) should have also avoided any such possible influence on the results.

No motor deficits were observed with the $MgSO_4$ dose utilized. This contrasts with a study in rats where intrathecal $MgSO_4$ induced motor deficits in a dose dependent manner (Karasawa et al. 1998). Another study in rats also reported that the motor deficits induced by intrathecal $MgSO_4$ outlasted the sensory block (Bahar et al. 1996). High magnesium concentrations in the spinal cord can reduce presynaptic release of acetylcholine, thereby altering neuromuscular transmission and increasing the threshold for axonal excitation (Fawcett et al. 1999). Higher drug concentrations are reached in the spinal cord following intrathecal *versus* epidural administration, which could be the reason for the lack of motor effects observed in the present study.

The von Frey device has been validated for antinociceptive threshold testing in dogs and other species (Redua et al. 2002; Vivancos et al. 2004; KuKanich et al. 2005). Baseline thresholds were similar throughout the present study and did not vary by week, indicating no learning effect or aversion to its use when used on different days. No tissue damage occurred in the tested areas during the study, which is a mandatory feature for validation of any mechanical threshold device (Beecher 1957). Threshold values obtained in the present study were influenced by the behaviour of the dogs, as has been previously suggested (Beecher 1957; Bove 2006). In an attempt to minimize its influence on the results, behaviour was quantified and included in the statistical model.

The thresholds measured on the left side were significantly higher compared to the right side. Positioning of the patient after epidural injection is known to influence the contact of the agent with the target tissue and therefore influence the migration of drugs during the first 5 minutes post-injection (Valverde 2008). Dogs were kept in sternal recumbency during the time of epidural injection and recovery from anaesthesia, and therefore, positioning is unlikely to have caused unequal migration of drugs. Another possible explanation is the lateralization of the epidural catheter during its introduction into the epidural canal. The same person performed all the catheter placements and used the

same technique. This investigator is right handed and this could have influenced the lateralization of the catheter towards the left side. The cranial spread of the solution can also be influenced by the distance that the epidural catheter is advanced into the epidural canal. In the present study this distance was slightly different among dogs; however, no difference in distance was detected among treatments and therefore should not have influenced the obtained results.

In conclusion, $MgSO_4$ administered into the lumbosacral epidural space of dogs produced an antinociceptive effect without causing any motor deficits but did not enhance morphine analgesia. The onset and offset times of antinociception could not be clearly established. To what extent these results can be extrapolated to clinical cases requires further investigation.

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