

Comparison of pain response after subcutaneous injection of two maropitant formulations to beagle dogs

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ABSTRACT

The antiemetic maropitant, with metacresol as preservative (Cerenia, Zoetis), has been associated with pain after subcutaneous injection in dogs and cats. Recently, a generic formulation containing benzyl alcohol was authorised (Prevomax, Le Vet). Benzyl alcohol is reported to have local anaesthetic properties and reduce injection pain. This study compared local pain after subcutaneous injection of the two maropitant formulations, administered at approximately 4°C and 25°C, to dogs. Thirty-two healthy beagle dogs were enrolled into a blinded, randomised, cross-over study. Dogs received subcutaneous injections of maropitant injection containing metacresol as preservative and maropitant injection containing benzyl alcohol as preservative, both at approximately 4°C and 25°C, with at least three days in between treatments. Injection pain was evaluated by two blinded observers using a visual analogue scale immediately after injection and a simple descriptive scale at two minutes after injection. In healthy beagle dogs, subcutaneous injection of maropitant with benzyl alcohol is significantly less painful than injection of maropitant with metacresol.

INTRODUCTION

Maropitant is a centrally acting neurokinin-1 receptor antagonist that acts by inhibiting the binding of substance P, the key neurotransmitter involved in vomiting. It is the first drug in its class to be approved to treat and prevent vomiting in dogs and cats. It has been shown to significantly decrease vomiting from both centrally acting and peripherally acting emetogens.¹ The proprietary injectable formulation of maropitant (Cerenia, Zoetis) contains maropitant (10 mg/ml), sulphobutylether- β -cyclodextrin and metacresol as preservative. Transient pain and vocalisation during subcutaneous injection occur frequently, especially in cats where moderate to severe responses (retreating, vocalisation, hissing, scratching) are observed very commonly.² It was found that these may be reduced by injection of the product at refrigerated temperature.^{3,4}

Recently, a generic maropitant injectable formulation was authorised containing benzyl alcohol as preservative (Prevomax, Le Vet). It has been reported that benzyl alcohol has local

anaesthetic properties and can thus reduce injection pain.^{5,6} Indeed, clinical studies in human beings indicate that growth hormone formulations containing benzyl alcohol are less painful on injection than formulations containing metacresol as preservative.⁷ It was therefore hypothesised that the generic maropitant formulation containing benzyl alcohol as preservative would be less painful on injection than the proprietary formulation containing metacresol as preservative.

The aim of this study was to compare local pain after subcutaneous injection of the two formulations of 10 mg/ml maropitant solution for injection, administered both at refrigerated temperature of 4°C and standardised room temperature of 25°C to dogs.

MATERIALS AND METHODS

Animals

Thirty-two purpose-bred beagle dogs (15 male and 17 female, between eight months and three years of age and weighing between 6.2 and 12.0 kg) were enrolled in the study. All animals came from a stock colony and were housed per pair (same sex) in a climate-controlled facility, and were deemed healthy based on a veterinary inspection before the start of the study. Dogs received their daily ration of pelleted diet in the morning, and water was provided ad libitum via an automatic watering system. After the study, the animals were returned to the stock colony in good health.

Experimental design

The study was a blinded, randomised, cross-over study according to a four-period, four-sequence, four-treatment Latin square design, and was conducted in accordance with the principles of good laboratory practice (GLP). Randomisation was performed on Microsoft Excel using a random number generator. Male and female dogs were randomised separately. Each dog received four subcutaneous injections of 1 mg/kg maropitant, with at least three days



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between treatment periods. Two different formulations of maropitant 10mg/ml solution for injection were tested: metacresol-preserved formulation (formulation MM; Cerenia, Zoetis) and benzyl alcohol-preserved formulation (formulation MBA; Prevomax, Le Vet). Each formulation was tested at refrigerated temperature (4°C) and at room temperature (25°C). Approximately 1.5–2 hours before administration, one vial of each test item was placed on an ice bath (approximately 4°C) and another vial of each test item was put on a preheated water bath (of approximately 25°C).

The same dose volume was administered throughout the four treatment periods, based on the bodyweight measured during acclimatisation. The products were administered via subcutaneous injection using a 25-G needle between the scapulae (at approximately 2.5 cm from the spine). The order of injection was as follows: cranial right; caudal right, caudal left; cranial left. One needle stayed in the temperature-conditioned vials for withdrawing the product and a new needle was used for each injection. The needle was inserted beneath the skin, and after approximately two seconds the product was injected. The administrator announced when the products were dosed and left the cage immediately after dosing. The other dog housed in the same cage was temporarily kept outside the cage during dosing and observation.

Injection pain was assessed independently by two veterinarians, blinded to treatment allocation. Immediately after the administrator announced injection of the product, pain was scored using a visual analogue scale (VAS), by placing a vertical line transecting a 10-cm long line scale, with 0 being no pain and 10 being the worst possible (injection) pain. The dog was then observed for two minutes, after which a simple descriptive scale (SDS) score was assigned, with scores ranging from 0 (no pain) to 3 (severe reaction) (Table 1).

Specific clinical signs observed during or shortly after injection were also recorded. Twenty-four hours after the injection, the injection site was checked for swelling and pain on palpation.

The total number of animals used in this study was based on Narishetty and others⁴ and calculated using specific software (FARTSSIE V.2.2, Free Analysis Research Tool for Sample Size Iterative Estimation, David Dubins 2007–2017). The following factors were taken into account: power 80 per cent, alpha 5 per cent, expected

intrasubject sd of 4, and estimated minimal difference in mean VAS scores between treatments of between 2.75 and 3. The results indicated that approximately 30–36 dogs were required to generate meaningful results.

Statistical analysis was performed using SAS V.4.3 and Microsoft Excel 2016. Analysis of variance (ANOVA) (PROC GLM) was used to analyse VAS scores, with the following terms specified as fixed effects in the model: sequence, subject within sequence, period and treatment. A log transformation of the VAS scores (mean of the two observers) was used in order to fit the ANOVA model. Least square means were used as an estimate of the treatment means. The SDS scores were analysed using a logistic regression model (PROC GENMOD), with the same terms specified in the model as fixed effects. If treatment effect, as defined in the above statistical models, was found statistically significant, pairwise comparisons were performed. All statistical tests on the VAS and SDS were conducted at the 5 per cent significance level. All pairwise comparisons were conducted using two-sided tests and were reported at the 5 per cent levels. The individual dog served as the experimental unit.

Cohen's kappa was calculated for interobserver reliability of SDS scores, and Lin's concordance correlation coefficient (ρ_c) was calculated to assess agreement between observers on VAS scores. The kappa statistic for interobserver agreement was considered as fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80) and almost perfect (0.81–1.00). Agreement as calculated with Lin's concordance correlation coefficient was considered as fair (>0.70–0.80), moderate (>0.80–0.90), substantial (>0.90–0.95) and excellent (>0.95–1.00).

RESULTS

Thirty-two dogs were enrolled in the study. All dogs completed the study.

The mean VAS scores per group are depicted in Fig 1. The mean logVAS for MBA 25°C (1.7±1.0) and MBA 4°C (1.4±1.0) were both significantly lower ($P<0.0001$) than that of MM 25°C (3.1±1.3) and MM 4°C (2.8±1.3). There were no statistically significant differences in VAS score between MBA 4°C compared with MBA 25°C, and MM 4°C compared with MM 25°C. A significant period effect was observed, with VAS scores in periods 2 and 3 being higher compared with periods 1 and 4 (mean logVAS of 2.9±1.2 and 2.5±1.4 in periods 2 and 3, v 2.0±1.3 and 1.7±1.1 in periods 1 and 4, respectively). Overall agreement between the two observers on the VAS scores was substantial with $\rho_c=0.90$.

Mean, median and range of the SDS scores are summarised in Table 2. Statistically lower SDS scores were observed after treatment with MBA 4°C and MBA 25°C than after treatment with MM 4°C and MM 25°C. After treatment with MM 25°C, dogs had 36.8 and 15.8 times higher odds of having higher pain scores than after treatment with MBA 4°C and 25°C, respectively ($P<0.0001$). After treatment with MM 4°C, dogs had 27.2 and 11.7

TABLE 1: Simple descriptive scale scores

Score	Description
0	No reaction to injection
1	Mild reaction (eg, twitching of the skin, looking at injection site, one-time licking or scratching of the injection site)
2	Moderate reaction (eg, repeated licking or scratching, short-term vocalisation, jumping or shuddering)
3	Severe reaction (eg, prolonged yelping, hiding or circling with tucked tail or aggression)

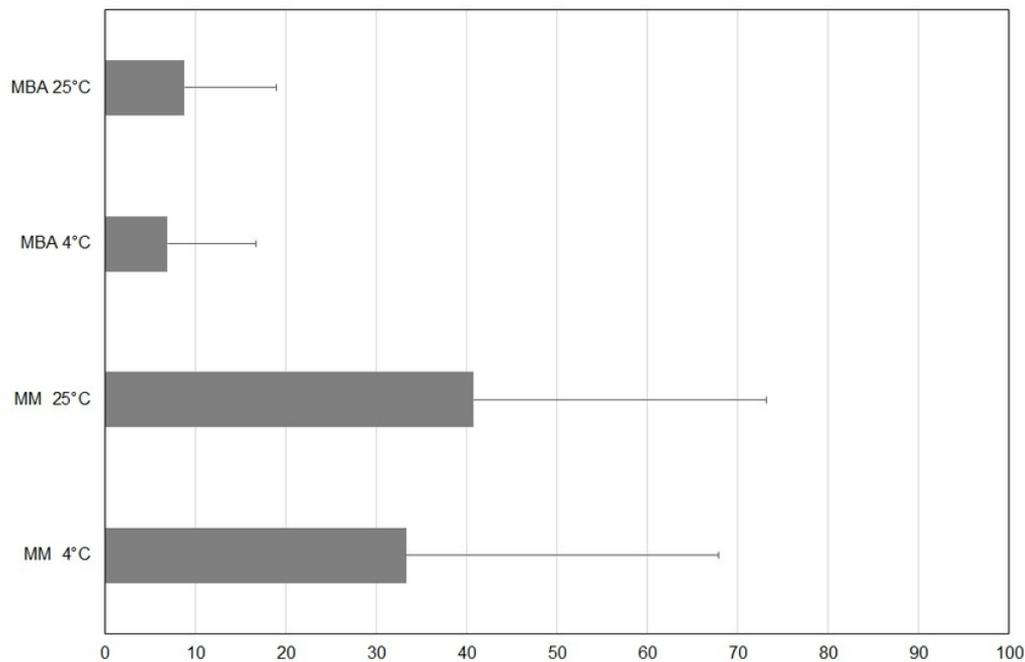


FIG 1: Mean and sd of visual analogue scale scores (in mm) comparing injection pain after subcutaneous injection of two formulations of maropitant solution for injection (at 4°C and 25°C) to 32 beagle dogs. MBA, benzyl alcohol-preserved maropitant solution for injection (Prevomax, Le Vet); MM, metacresol-preserved maropitant solution for injection (Cerenia, Zoetis).

times higher odds of having higher pain scores than after treatment with MBA 4°C and 25°C, respectively ($P < 0.0001$). After treatment with MBA 25°C, dogs had slightly higher pain scores than after treatment with MBA 4°C (odds ratio: 2.3, $P = 0.03$), whereas no statistically significant difference in SDS pain scores was observed after treatment with MM 25°C versus MM 4°C.

For the SDS scores, agreement between observers was moderate ($\kappa = 0.59$). On 11 occasions, observer A assigned a higher score than observer B; on 26 occasions observer A assigned a lower score than observer B. When observers assigned a different score, this difference was not more than 1.

The clinical signs observed during and within two minutes after injection are summarised in Table 3. Although not statistically analysed, it is clear that more clinical signs were observed after injection of MM than

MBA ($n = 99$ v $n = 33$). Scratching at the injection site and vocalisation were observed most often; scratching was observed in 30 dogs after injection with MM ($n = 13$ after MM 4°C and $n = 17$ after MM 25°C) and in only 8 dogs after injection with MBA ($n = 4$ after both MBA 4°C and 25°C). Vocalisation was observed in 38 dogs after injection with

TABLE 3: Frequency of clinical signs observed during and within two minutes after subcutaneous injection of two formulations of maropitant solution to 32 beagle dogs at 4°C and 25°C

Clinical signs	MM		MBA	
	4°C	25°C	4°C	25°C
Hunched or cramped posture	4	2	3	4
Shaking head	6	5	3	3
Scratching injection site	13	17	4	4
Stereotype behaviour	1	4	0	1
Slight aggressive behaviour	3	1	0	1
Slight tremors/muscle twitching	1	2	1	0
Quick breathing	0	1	0	0
Slight restless behaviour	0	1	0	0
Lateral recumbency	0	0	0	1
Howling	7	12	0	1
Squeaking	7	12	2	5
Total	42	57	13	20

MBA, benzyl alcohol-preserved maropitant solution for injection (Prevomax, Le Vet); MM, metacresol-preserved maropitant solution for injection (Cerenia, Zoetis).

TABLE 2: Simple descriptive scale scores (mean, median and range) for 32 beagle dogs receiving subcutaneous injections of two formulations of maropitant solution for injection at 4°C and 25°C

	Mean	Median (range)
MM 4°C	1.4	1 (0–3)
MM 25°C	1.5	2 (0–3)
MBA 4°C	0.5	0 (0–2)
MBA 25°C	0.7	1 (0–3)

MBA, benzyl alcohol-preserved maropitant solution for injection (Prevomax, Le Vet); MM, metacresol-preserved maropitant solution for injection (Cerenia, Zoetis).

MM (n=14 after MM 4°C and n=24 after MM 25°C) and in only 8 dogs after injection with MBA (n=2 after MBA 4°C and n=6 after MBA 25°C).

Twenty-four hours after injection, swelling of the injection site was observed in four dogs in the MM 4°C group (slight), six dogs in the MM 25°C group (2 slight, 4 moderate), six dogs in the MBA 4°C group (5 slight, 1 moderate) and in eight dogs in the MBA 25°C group (5 slight, 1 moderate, 2 severe). Swelling of the injection site was only observed during periods 2 and 3 (ie, injection caudal right and caudal left between the scapulae). The swollen injection sites were not scored as painful, and as the injection sites were only swollen for a short period of time (24 hours), these findings were considered to be not adverse. Other incidentally observed minor clinical observations (eg, slight salivation, vomiting, abnormal faeces) occurred only sporadically and solely on non-dosing days and were thus considered not treatment-related.

DISCUSSION

Maropitant, a centrally acting neurokinin-1 receptor antagonist, is the first drug in its class to be approved to treat and prevent vomiting in dogs and cats. Currently, two different formulations of maropitant solution for injection are available, the proprietary injectable formulation of maropitant (Cerenia, Zoetis), containing metacresol as preservative, and the generic formulation (Prevomax, Le Vet), containing benzyl alcohol as preservative. Post-marketing surveillance of the proprietary formulation revealed that pain and vocalisation on subcutaneous injection frequently occur. Maropitant solution for injection is formulated with cyclodextrin to improve solubility of the active ingredient. The cyclodextrin forms a molecular cavity that entraps maropitant and limits the amount of free drug. This binding relationship is said to be temperature-dependent. The cyclodextrin complex of maropitant is preserved at cold temperatures and is more stable and intact when the formulation is refrigerated. It is thought that the unbound maropitant, which increases with temperature, is responsible for the local irritation and injection pain.⁴

The proprietary injectable formulation of maropitant contains metacresol as preservative. According to the European public assessment report, metacresol was selected among others because of its acceptable injection site tolerance.⁸

The generic maropitant solution for injection, on the other hand, contains benzyl alcohol as preservative. Benzyl alcohol has been reported to possess local anaesthetic properties. For instance, it has been shown that benzyl alcohol attenuates the pain of intradermal lidocaine injections by 27 per cent.⁹ Another study found that there was a significant difference in pain perception, as assessed by VAS and verbal descriptive pain scales, between formulations of epoetin alfa, with the multidose formulation, containing benzyl alcohol as preservative, causing less pain than the single-dose formulation.¹⁰ Similarly, preinjection

with physiological saline solution containing benzyl alcohol decreases the incidence of pain associated with intravenous administration of propofol and is comparable to that of mixing lidocaine with propofol.⁶ It was therefore hypothesised that the generic maropitant formulation containing benzyl alcohol as preservative would be less painful on injection than the proprietary formulation containing metacresol as preservative.

To investigate this, local pain (as assessed by VAS and SDS) was compared after subcutaneous injection of the two commercially available formulations of 10 mg/ml maropitant solution for injection (MM and MBA), administered at 4°C and 25°C to dogs, using a cross-over design.

Injection of the generic maropitant formulation (MBA) caused significantly less pain than the formulation with metacresol (MM), as assessed by both VAS and SDS ($P<0.0001$). Notably, injection of MBA at 25°C was still significantly less painful than injection of MM at 4°C. Although injection of MBA at 4°C was somewhat less painful than injection of MBA at 25°C (based on the lower SDS score, $P=0.033$), it would not seem necessary to inject this formulation at refrigerated temperature (VAS not significantly different).

Previously, Narishetty and others⁴ evaluated pain on injection with the proprietary formulation (MM) at 4°C and 25°C in 46 beagle dogs and found that VAS score for pain was significantly higher in dogs administered room temperature (25°C) versus refrigerated (4°C) maropitant solution for injection (MM). In the current study, however, no significant difference in injection pain was found between MM at 4°C and MM at 25°C, although pain scores tended to be slightly lower for MM at 4°C. In this respect, it must be noted that these authors employed a parallel design, whereas the current study was conducted according to a cross-over design. Furthermore, pretreatment VAS (injection with saline at 25°C on day -1) was used as a covariate for the analysis of VAS, making comparison between the two studies difficult. Finally, post-hoc analysis demonstrated that, given an intrasubject sd of 0.976, a difference in logVAS (least squares mean) of 0.31 between MM 4°C and MM 25°C, and a difference of 0.27 between MBA 4°C and MBA 25°C, the current study was underpowered to demonstrate a significant difference.

A significant period effect was observed for VAS scores, with higher VAS scores observed in periods 2 and 3 versus periods 1 and 4. This is most likely due to the location of injection, with more loose skin available more cranial between the scapulae. This is corroborated by the fact that local swelling at the injection site was only observed during periods 2 and 3 (injection more caudal between the scapulae). Nevertheless, it cannot be fully excluded that a possible systematic difference in scoring contributed to the period effect.

This study had some limitations. For one, the location of injection was not randomised, most likely causing the observed period effect, as discussed above. Also, at the time of injection, different people restrained the dogs in



the different treatment periods, which in theory could contribute to a possible systematic difference in VAS scoring. Indeed, if a dog was restrained more stringently in one period compared with another period, subtle differences in pain expression (eg, twitching of the skin or slight flinching) might have gone unnoticed. Finally, although beagle dogs are considered to be a representative breed for the general dog population, the results of the current study may not be fully predictive for an individual client-owned dog in a clinical setting.

CONCLUSION

In healthy beagle dogs, subcutaneous injection of the generic maropitant solution for injection (with benzyl alcohol as preservative), both at room temperature and at refrigerated temperature, is significantly less painful than maropitant solution for injection with metacresol as preservative. It does not seem necessary to inject the generic maropitant solution for injection at refrigerated temperature.

After logarithmic transformation, the mean VAS scores for MBA 25°C and MBA 4°C were both significantly lower than that of MM 25°C and MM 4°C ($P < 0.0001$). The SDS scores for MBA 4°C and MBA 25°C were also significantly lower than the SDS scores for MM 4°C and MM 25°C ($P < 0.0001$).

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Contributors ND was involved in the study design, data acquisition, analysis and interpretation, and writing of the manuscript. CAR was involved in the study design, data acquisition, and review and approval of the manuscript. HPV was involved in the study design, and review and approval of the manuscript. NL was involved in the study design, data acquisition, analysis and interpretation, and review and approval of the manuscript.

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Competing interests ND, CAR and HPV are employees of Regivet. Regivet has developed the generic maropitant formulation.

Ethics approval The study was approved by the ethical committee as required by the Dutch Act on Animal Experimentation (February 1997).

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Data sharing statement There are no additional unpublished data.

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