Effect of Refrigeration of the Antiemetic Cerenia (Maropitant) on Pain on Injection*

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CLINICAL RELEVANCE

Injection pain has been associated with veterinary use of the antiemetic maropitant (Cerenia, Pfizer Animal Health). Cerenia is formulated using sulphobutylether-β-cyclodextrin to bind maropitant and mitigate injection pain. The objective of this study was to determine whether the temperature of Cerenia alters binding between maropitant and sulphobutylether-β-cyclodextrin and affects injection pain. Binding decreased as temperature increased, and Cerenia-elicited injection pain increased at warmer drug temperatures. These data suggest that the amount of free unbound maropitant increases with temperature and that injection pain increases with temperature in a similar fashion. Clinically, these studies suggest that injection of refrigerated Cerenia may significantly reduce or eliminate pain associated with SC injection of Cerenia.

INTRODUCTION

A large body of literature indicates that substance P is involved in the mediation of emesis and that neurokinin-1 (NK-1) receptor antagonists block this effect of substance P.¹,² Maropitant (Cerenia, Pfizer Animal Health) is a highly potent and selective NK-1 receptor antagonist and is the first drug of this class developed specifically to prevent and treat emesis in dogs. Maropitant has been shown to effectively control emesis produced by challenge with centrally acting agents (apomorphine) and peripherally acting emetogens (syrup of ipecac).³ Additionally, maropitant effectively controls cisplatin-induced emesis in tumor-bearing dogs.⁴ Maropitant has been shown to be effective in preventing motion sickness in client-owned dogs.
with a previous history of vomiting during transportation. Maropitant administered 1, 2, or 10 hours before transportation significantly reduced the incidence of motion sickness–induced emesis by 79%, 92%, and 82%, respectively, compared with placebo treatment.5,6

Cerenia Injectable Solution has been developed as a sterile, multi-dose, ready-to-use formulation intended for SC administration. The solution contains the active drug maropitant (10 mg/ml), sulphobutylether-β-cyclodextrin (SBEC D; 63 mg/ml), and the preservative meta-cresol (3.3 mg/ml). SBEC D above, there was very little evidence that the drug product produced pain on injection.4 However, in Pfizer’s postmarketing surveillance program, an increase in the frequency of injection pain reports was noticed. In the majority of the registration studies, refrigerated drug product was used because the long-term stability of Cerenia was still under investigation. Commercial Cerenia Injectable Solution is recommended to be stored at room temperature because the long-term stability of the drug product has been established. These observations suggest the possibility that the temperature of the Cerenia solution may affect the injection pain when given SC to dogs. The following experiments were performed to test this hypothesis.

### MATERIALS AND METHODS

#### In Vitro Studies

Phase-solubility measurements were carried out by the method of Higuchi and Connors15 by adding excess amount of maropitant to 1 ml of deionized water containing increasing amounts of SBEC D (0 to 0.09 M). The resulting mixture was equilibrated on a shaking thermostatic heating block for more than 24 hours at various temperatures (4°C, 14°C, 22°C, and 37°C). Suspensions were filtered through 0.45 μm cellulose acetate membrane filters to remove undissolved solids. The phase-solubility experiments were conducted in triplicate at each temperature. An aliquot from each vial was diluted and analyzed for maropitant using the isocratic high-pressure liquid chromatography (HPLC) method, which utilizes 25 mmol ammonium acetate in 75% methanol–25% water as mobile

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This experiment was conducted to determine whether the temperature of Cerenia affects injection pain.
phase on C8 column (Eclipse XDB, Agilent Technologies, Santa Clara, CA) with a flow rate of 1.0 ml/minute and a detection wavelength of 220 nm. The apparent binding constants (Ka) of the complexes were calculated from the phase-solubility diagrams according to the following equation, in which So (i.e., the intrinsic solubility of maropitant in the absence of SBECD at a particular temperature) and the slopes were derived from the maropitant molar concentration versus SBECD molar concentration graphs:

$$Ka = \frac{\text{Slope}}{So \times (1 - \text{Slope})}$$

The phase-solubility measurement is a widely accepted method for evaluating the effect of cyclodextrin complexation on drug solubility and is used to measure the kinetics of the complex association and dissociation processes.\textsuperscript{12,14,16}

In Vivo Studies

All experiments involving animals were carried out in compliance with national legislation and subject to local ethical review. Two studies evaluated injection pain produced by a single 1 mg/kg SC injection of commercial Cerenia. In the first study, Cerenia was evaluated in 51 bred-for-research beagles (Liberty Research Inc., Waverly, NY). Dogs received Cerenia at either 14°C, 22°C, or 37°C. These temperatures simulate scenarios in which Cerenia might be removed from a refrigerator and allowed to warm slightly in a syringe (14°C), Cerenia at room temperature (22°C), and warmed Cerenia (37°C). Temperature was controlled by placing vials of Cerenia in temperature-controlled water baths an hour before the start of the experiment. Injections were given between the scapulae. Dogs were randomized to treatment group and pen location. Dogs were monitored continuously for 1 hour after injection and intermittently for an additional 23 hours after Cerenia administration. Injection pain was evaluated using a four-compartment scoring system (see box, above).

In the second study, 46 dogs received either saline or Cerenia at either 4°C or 25°C. The allocation of animals to treatments, pens, and rooms was done according to a randomized block design, with treatments having a two-way treatment structure in temperature (4°C or 25°C) and test article (saline or Cerenia). Blocking was based on pretreatment body condition scores. On day –1, all dogs were injected between the scapulae with room temperature (25°C) saline (0.1 ml/kg); pain on injection was measured using a visual analog scale (VAS; described below) by a trained observer who was masked to treatment. The following day (day 0), animals were injected with either saline (n = 6 at each temperature) or Cerenia (n = 17 at each temperature). Injection pain was measured using both the four-compartment system and the VAS system.

To distinguish between an animal’s reaction to the needle insertion and potential pain produced by the injected solution, in both studies the dose administrator inserted the needle beneath the animal’s skin, waited briefly, and then announced when he or she started to push the syringe plunger. The observer, who was blinded to treatment group, began assessing pain on injection when the administrator made the announcement. In the second study,
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a VAS was also recorded by a second observer (also blinded to treatment group) to assess pain on a 10-cm line scale, with 0 indicating no reaction and 10 indicating prolonged yelping or aggression. Pain was recorded by the observer placing a single vertical line transecting the 10-cm VAS scale. The more severe the clinical sign, the farther to the right the mark was made, with no pain or reaction marked at the far left on the scale and “worst possible pain” (defined as prolonged howling, aggression, and/or being unapproachable and trying to hide) marked at the far right. In the second experiment, the VAS score from day –1 (saline) was subtracted from the score on the day of injection (Cerenia or saline) to help control for dogs that reacted to the needle poke.

Data Analysis

A biometrics representative from Pfizer Biometrics group was responsible for all summary and statistical analysis of data. The pain compartment scale response was summarized descriptively with frequency tables by treatment but was not analyzed statistically. VAS for pain was analyzed using a mixed linear model. The model contained the fixed effects temperature, test article, and the interaction between temperature and test article. The random effects included room, block within room, and error. In the second in vivo study, pretreatment VAS was used as a covariate for the analysis for VAS for pain. Least squares were used as estimates of the treatment means. Standard errors were calculated, and 90% CIs were constructed. A priori contrasts were used to assess treatment differences. Treatment differences were assessed at the 5% level of significance (P < .05).

RESULTS

Phase-Solubility Data

Phase-solubility curves were constructed from the solubility data generated in triplicate at multiple temperatures. The binding constant of the complex Ka at each temperature was calculated from the slope and the intercepts of the phase-solubility curves as explained in Materials and Methods, and the results are tabulated in Table 1. Table 1 illustrates that there is nearly a 10-fold difference in the binding complex at 4˚C versus 37˚C.

Cerenia Injection Pain in Dogs

Cerenia was evaluated in dogs in two separate studies. In the first study, 51 dogs received Cerenia (1 mg/kg SC; n = 17 per group) at either 14˚C, 22˚C, or 37˚C. After receiving the injection, dogs were observed and scored for signs of pain by an observer masked to treatment group. In the cooled (14˚C) Cerenia treatment group, one animal reacted (compartment score, 2) and the other 16 animals did not show a painful reaction to the injection of Cerenia. When Cerenia was injected at room temperature (22˚C), 11 dogs injected had no reaction, 2 animals reacted with a compartment score of 1, and 4 animals reacted with a score of 2. In the warmed Cerenia (37˚C) group, 14 animals scored 0 on the compartment pain scale, 2 animals scored a 1, and 1 animal scored a 3. Overall, 1 of 17 dogs receiving 14˚C Cerenia

<table>
<thead>
<tr>
<th>Temperature (˚C)</th>
<th>Ka (M⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>1,227.46</td>
</tr>
<tr>
<td>22</td>
<td>2,826.20</td>
</tr>
<tr>
<td>14</td>
<td>8,102.91</td>
</tr>
<tr>
<td>4</td>
<td>11,524.69</td>
</tr>
</tbody>
</table>

Ka = apparent binding constants.

TABLE 1. Binding Constants for Maropitant–Sulphobutylether-β-Cyclodextrin Complexes at Various Temperatures
reacted with some degree of pain to injection while 9 of 34 dogs receiving room temperature or warm Cerenia reacted with pain to the injection of the drug (Table 2).

This first study implies that injection of cooled Cerenia reduces injection pain. However, this study described the level of pain using a descriptive compartmental scoring method that was unsuitable for statistical analysis. Therefore a second study was performed using both descriptive (compartmental scoring) and statistical (VAS scoring) analysis of injection pain. In the second experiment, injection pain produced by Cerenia and saline at 4°C (the typical temperature of a drug stored in a refrigerator) and 25°C (room temperature) was compared in 46 dogs. Figure 1 summarizes the compartmental pain scores elicited by cold and room temperature saline and Cerenia. Two of six dogs receiving either the cold or room temperature saline exhibited a mild to moderate pain response to injection. Room temperature Cerenia produced a pain response when injected in 9 of 17 dogs. In 4 dogs this response was rated as moderate to severe by the blinded observer. In contrast, only 1 of 17 dogs had a mild response to the injection of cold Cerenia. Figure 2 summarizes the VAS pain scores produced by cold and room temperature saline and Cerenia. VAS scores illustrated in Figure 2 are the difference between the VAS score (in millimeters) recorded on the day of the experiment and the previous day’s score in reaction to injection of room temperature saline. These data indicate that room temperature Cerenia elicited a significantly \( P < .05 \) greater VAS pain score than did cold Cerenia or room temperature or cold saline. Furthermore there was no statistical difference in the VAS pain score elicited by room temperature or cold saline and cold Cerenia. Figure 3 illustrates the VAS pain scores (in millimeters) using least squares means to estimate treatment means. In this analysis, pretreatment VAS was used as a covariate for the analysis for VAS for pain. These data indicate that injections of room temperature Cerenia produced significantly \( P < .05 \) more injection pain than did injections of cold Cerenia.

### DISCUSSION
Cerenia is a selective, potent NK-1 receptor antagonist with broad-spectrum antiemetic activity in dogs.\(^3\)\(^-\)\(^6\) The present study was designed to determine whether injection pain noted during postregistration surveillance of Cerenia may be related to the temperature of the injected Cerenia. The data indicate that at

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**TABLE 2. Categorical Pain Scores—Frequency Distribution by Treatment**

<table>
<thead>
<tr>
<th>PAIN SCORE</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT GROUP</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Cerenia at 14°C ( n = 17 )</td>
<td>16</td>
<td>94.1</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Cerenia at 22°C ( n = 17 )</td>
<td>11</td>
<td>64.7</td>
<td>2</td>
<td>11.8</td>
</tr>
<tr>
<td>Cerenia at 37°C ( n = 17 )</td>
<td>14</td>
<td>82.4</td>
<td>2</td>
<td>11.8</td>
</tr>
<tr>
<td>Total ( n = 51 )</td>
<td>41</td>
<td>80.4</td>
<td>4</td>
<td>7.8</td>
</tr>
</tbody>
</table>

*The pain scale ranged from 0–3: 0 = no reaction to injection; 1 = twitching of the skin, licking of the fur, or digging at the injection site; 2 = short-term vocalization, jumping, or wincing; 3 = prolonged yelping or aggression.
warmer temperatures, maropitant has an increased likelihood to dissociate from the SBECED, thereby producing more unbound Cerenia and increasing the potential to cause injection pain.

In this study we found that the solubility of maropitant increased linearly as a function of SBECED concentration, showing formation of an aqueous soluble complex with a 1:1 stoichiometry. This type of drug–cyclodextrin complex is typical of binding in which a single drug molecule is in the cavity of a cyclodextrin, with a binding constant $K_a$ for the equilibrium between the bound and unbound species. This type of complex does not precipitate at the site of injection. The bound and unbound forms of drug will be in equilibrium, and the free drug will be available for the local tissues and physiologic fluids for further distribution. However, the fraction of unbound drug on dilution and the dissociation time depend on the strength of the binding or binding constant, which for most drug–cyclodextrin complexes ranges between 100 and 20,000 M$^{-1}$. The higher the binding strengths are, the lower will be the fraction available as unbound form on dilution at the site of injection. This is important as it is thought that unbound maropitant has local irritant properties that are responsible for injection pain.
The magnitude of binding constant Ka for the maropitant–SBECD complex varied with temperature and demonstrated an inverse relationship with temperature, with values varying between 1,227.46 M$^{-1}$ at 37°C and 11,524.69 M$^{-1}$ at 4°C (Table 1). The thermodynamic calculations (data not shown) for the molecular complexation of maropitant–SBECD suggest it is an exothermic process and enthalpy driven (i.e., temperature will have a significant effect on the stability of the complex). In most cases, increasing the temperature decreases the magnitude of the apparent binding constant of the complexation, and the effect was reported to be a result of possible reduction of drug–cyclodextrin interaction forces, such as van der Waals and hydrophobic forces with rise of temperature. On the other hand, temperature can have the opposite effect; that is, increasing temperature can increase the magnitude of the binding constant. However, temperature changes may have negligible effect when the drug–cyclodextrin interaction is predominantly entropy driven (i.e., resulting from the liberation of water molecules hydrated around the charges of guest and host molecules through inclusion complexation). The phase-solubility experiments indicate that the binding strength of the maropitant–SBECD complex is very high at cold temperatures and hence less unbound or free drug will be available to the local tissues immediately after injection, thus reducing the potential for injection-site reactions such as pain. Based on the relationship between percent of a drug bound to cyclodextrin as a function of the strength of the binding constant (Ka) and dilution, the phase-solubility data generated in this study suggest that there may be about 25% free Cerenia at 37°C and minimal to no free Cerenia at 4°C.

The phase-solubility data suggest that injection of cold Cerenia should decrease injection pain. In the present study, we conducted two experiments comparing pain on injection produced by Cerenia at varying temperatures. In the first experiment, Cerenia was injected at 14°C, 22°C, or 37°C. These temperatures simulate scenarios in which Cerenia might be removed from a refrigerator and allowed to warm slightly in a syringe (14°C), Cerenia at room temperature (22°C), and warmed Cerenia (37°C). In this experiment the cooled Cerenia produced a mild pain response (i.e., skin twitch) in 1 of 17 (6%) dogs (Table 2). Interestingly, information in the Cerenia label notes pain on injection in 5% of the animals tested; those data were collected using refrigerated Cerenia that was pulled into a syringe and...
allowed to warm slightly. In contrast, room temperature or warmed Cerenia elicited pain in 9 of 34 (26%) dogs tested (Table 2). The incidence of injection pain seen with Cerenia in the veterinary office is currently unknown but is believed to be 20% to 30%. The Cerenia label indicates that the drug should be stored at room temperature; thus, the higher rates of injection pain seen with room temperature and warm Cerenia in the current study are consistent with veterinarian observations using commercial Cerenia.

These data suggest that injection pain produced by Cerenia is temperature dependent. The optimal way for veterinarians to control the temperature of Cerenia intended for SC injection would be to remove a vial from the refrigerator and inject the solution immediately. Therefore, we compared injection pain of Cerenia at 4°C, a temperature approximating that of refrigerated drug, and at 25°C. One dog treated with 4°C Cerenia responded with a mild skin twitch (incidence rate, 6%). Observers blinded to treatment noted a much higher incidence of mild reactions to cold and room temperature saline (Figure 1). Room temperature Cerenia produced mild to severe pain responses in 50% of the dogs (Figure 1).

The VAS pain scores support these findings. Figure 2 illustrates the differences in VAS pain scores observed on the treatment day and those observed on day –1 when room temperature saline was injected SC to all animals. These data indicate that room temperature Cerenia produced a significantly ($P < .05$) greater pain response than either cold Cerenia or cold or room temperature saline. In addition, there was no statistical difference in the pain response to cold Cerenia and either saline group in the experiment (Figures 2 and 3). These data suggest that injection of cold Cerenia will significantly ($P < .05$) reduce the injection pain observed with injection of room temperature Cerenia.

The Cerenia label indicates that the drug can be stored at room temperature. Long-term stability studies have previously demonstrated that drug stored at room temperature remains stable and does not degrade. The present study suggests that decreasing the temperature of Cerenia will increase the binding of maropitant with SBECMD and decrease the incidence and severity of injection pain produced by SC administration of Cerenia. Although data indicate that Cerenia can be stored at room temperature, the findings of the study presented here suggest that as a veterinarian begins to use a vial of Cerenia, the vial should be stored in the refrigerator and that the drug should be injected immediately after removal from the refrigerator to minimize injection pain.

**CONCLUSION**

Maropitant has previously been shown to be a selective, potent NK-1 receptor antagonist with broad-spectrum antiemetic activity in dogs. It is safe and highly effective in preventing or treating apomorphine-, ipecac-, and chemotherapy-induced (e.g., cisplatin) emesis.
and in treating vomiting in canine patients in the field. It also markedly reduces the incidence of motion sickness–induced emesis in dogs.\textsuperscript{13–15,19} The present study was designed to determine whether injection pain noted in postregistration surveillance of Cerenia may be related to the temperature of the injected Cerenia. The data indicate that room temperature maropitant has an increased likelihood to disassociate from the SBEC, thereby producing more unbound Cerenia and increasing the potential to cause injection pain. The data suggest that Cerenia should be removed from the refrigerator and injected immediately to minimize injection-site reactions.

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