Pharmacokinetics of maropitant citrate in New Zealand White rabbits (Oryctolagus cuniculus)

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OBJECTIVE
To determine the pharmacokinetics and adverse effects of maropitant citrate after IV and SC administration to New Zealand White rabbits (Oryctolagus cuniculus).

ANIMALS
11 sexually intact (3 males and 8 females) adult rabbits.

PROCEDURES
Each rabbit received maropitant citrate (1 mg/kg) IV or SC. Blood samples were collected at 9 (SC) or 10 (IV) time points over 48 hours. After a 2-week washout period, rabbits received maropitant by the alternate administration route. Pharmacokinetic parameters were calculated. Body weight, food and water consumption, injection site, mentation, and urine and fecal output were monitored.

RESULTS
Mean ± SD maximum concentration after SC administration was 14.4 ± 10.9 ng/mL and was detected at 1.25 ± 0.89 hours. Terminal half-life after IV and SC administration was 10.4 ± 1.6 hours and 13.1 ± 2.44 hours, respectively. Bioavailability after SC administration was 58.9 ± 13.3%. Plasma concentration at 24 hours was 2.87 ± 1.69 ng/mL after IV administration and 3.4 ± 1.2 ng/mL after SC administration. Four rabbits developed local dermal reactions at the injection site after SC injection. Increased fecal production was detected on the day of treatment and 1 day after treatment.

CONCLUSIONS AND CLINICAL RELEVANCE
Plasma concentrations of rabbits 24 hours after SC and IV administration of maropitant citrate (1 mg/kg) were similar to those of dogs at 24 hours. Reactions at the SC injection site were the most common adverse effect detected. Increased fecal output may suggest an effect on gastrointestinal motility. Additional pharmacodynamic and multidose studies are needed.

Rabbits are one of the most popular small animals kept as pets throughout the world, and they commonly require veterinary care.11 To our knowledge, there have been no studies conducted to evaluate the use of maropitant for any condition in rabbits. Thus, the purpose of the study reported here was to determine the pharmacokinetics and possible adverse effects of a single dose of maropitant citrate in New Zealand White rabbits (Oryctolagus cuniculus) after SC and IV administration. We hypothesized that maropitant citrate at a dose of 1 mg/kg administered both SC and IV would have pharmacokinetic properties similar to those of dogs and plasma concentrations similar to those of dogs at 24 hours after administration. We also hypothesized that no adverse effects would be identified after administration of a single dose of this drug.

Materials and Methods

Animals
Eleven sexually intact (3 males and 8 females) adult (age range, 8 months to 5 years) New Zealand White rabbits were used. The rabbits were housed in a six-cage facility and had free access to food (commercial dog food, Purina Pro Plan) and water. The rabbits were provided with a 12-h light:12-h dark cycle and were allowed to acclimate to the environment for 1 week before the study began.

Each rabbit received maropitant citrate (1 mg/kg) IV or SC. Blood samples were collected at 9 (SC) or 10 (IV) time points over 48 hours. After a 2-week washout period, rabbits received maropitant by the alternate administration route. Pharmacokinetic parameters were calculated. Body weight, food and water consumption, injection site, mentation, and urine and fecal output were monitored.

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M aropitant is a specific synthetic, nonpeptide, selective NK1-receptor antagonist that was developed for its ability to block substance P from binding within the chemoreceptor trigger zone.1,2 Maropitant citrate has been used in clinical practice for a variety of clinical conditions. It is effective against both central and peripheral emetogens3–5 and is approved for use in the treatment and prevention of emesis in dogs and cats (1 mg/kg, IV or SC, q 24 h; 2 mg/kg, PO, q 24 h).2,6

The NK1-receptor antagonists may also affect visceral pain. Tachykinins such as substance P have been detected in the gastrointestinal, respiratory, urogenital, and integument systems and are involved in inflammation, nociception, smooth muscle contractility, and epithelial activity.7 The NK1-receptor antagonists can decrease the response to colorectal stimulation in rabbits,8 decrease the sensitivity of the colon to chemical irritants in guinea pigs,9 and increase colonic peristalsis in rabbits.10

ABBREVIATIONS
Cmax  Maximum concentration
NK1  Neurokinin 1
White rabbits were used in 2 experiments. The mean ± SD body weight of the rabbits was 3.63 ± 0.46 kg. Six rabbits were used for a preliminary experiment, and 9 rabbits (4 of which were used in the preliminary study) were used for the primary experiment. All rabbits had been used previously for teaching purposes and had previously been administered sedatives. There was a 2-week washout period before the start of the preliminary experiment. The rabbits were considered healthy on the basis of results of a full physical examination and measurement of PCV and total protein concentration performed 1 week before the start of the preliminary experiment. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of California-Davis.

Each rabbit was housed separately and allowed to acclimate to its surroundings before the preliminary or primary experiments. Rabbits were fed commercially available rabbit pellets and 1 large cube of timothy hay daily. Water was provided ad libitum via a water bottle. Absorbent pads were placed in the bottom of each cage to collect urine and feces; pads were changed daily.

At least 1 week before the start of the preliminary experiment, blood was collected from the rabbits (total volume, 3 to 6 mL/rabbit) to provide a blank sample for assay validation. Blood collected represented < 1% of the body weight of each rabbit.

Preliminary experiment

Six rabbits were used in a preliminary experiment. In this preliminary experiment, rabbits were randomly assigned to receive maropitant citrate SC at a dose of 1 mg/kg (n = 2 rabbits), 2 mg/kg (2), and 4 mg/kg (2). Blood samples were collected from peripheral venipuncture sites immediately before (0 minutes) and 2, 5, 10, 15, and 30 minutes and 1, 2, 4, 8, 12, 24, and 48 hours after drug administration.

Primary experiment

Procedures (dose and times of sample collection) for the primary experiment were determined on the basis of results from the preliminary experiment and results for a study of dogs. Nine rabbits were assigned by use of a random number generator to initially receive maropitant SC (1 mg/kg) by SC (n = 4) or IV (5) administration.

The day before the start of the primary experiment, the sites for SC injection (interscapular) and peripheral venipuncture (right and left saphenous veins) were shaved on each rabbit. The morning of the experiment, an Elizabethan collar was placed on each rabbit. Topical anesthetic cream was applied to the skin overlying the right and left lateral saphenous veins and covered with a transparent film dressing for 1 hour before catheter placement. A catheter was placed in the right lateral saphenous vein for the rabbits receiving the IV treatment. Maropitant at refrigerator temperature (2° to 8°C) was administered SC at the shaved interscapular location, and a permanent marker was used to circle the injection site after administration. Blood samples (0.4 mL/sample) were collected by venipuncture (lateral or medial saphenous veins, jugular veins, or cephalic veins) before (time 0) and 15 and 30 minutes and 1, 2, 4, 8, 12, 24, and 48 hours after SC administration. For IV administration, maropitant citrate was administered over a 30-second period via the catheter in the right lateral saphenous vein; the catheter was then flushed with saline (0.9% NaCl) solution (0.5 mL/rabbit). Blood samples were collected before (time 0 minutes) and 5, 10, 15, and 30 minutes and 1, 2, 4, 8, 12, 24, and 48 hours after IV administration. Catheters were removed after collection of the blood sample at 15 minutes. Blood samples were collected from the same veins as for SC administration, except the right lateral saphenous vein in which maropitant was administered was not used until at least 4 hours after IV administration. After a 2-week washout period, rabbits received maropitant by the opposite route of administration (SC = 5 and IV = 4) in an identical manner to that of the initial administration.

Blood samples were stored in heparinized tubes on ice until centrifugation (10 minutes at 3,800 X g); all samples were centrifuged within 4 hours after blood collection. Plasma was separated immediately and stored in cryotubes at -80°C until analysis.

Maropitant plasma concentrations

Maropitant plasma concentrations were measured with liquid chromatography-tandem mass spectrometry by use of a method described elsewhere. Analyses were performed with a triple quadrupole mass spectrometer equipped with an electrospray ionization source coupled to a liquid chromatography system. Extracted samples (injection volume, 30 μL) were separated on a C18 column (2.1 X 100 mm; inner diameter, 3 μm) maintained at 30°C. Gradient mobile phase consisted of 2 components (component A, water and 0.2% formic acid; component B, 0.1% formic acid in acetonitrile). Flow rate was 0.35 mL/min. For the tandem mass spectrometry analysis, protonated molecular ions were isolated and fragmented by use of helium gas collisions; collision energy was 45 eV for maropitant and 30 eV for d4-buprenorphine. Resulting mass spectra were acquired by use of selective reaction monitoring mode (m/z for maropitant, 469.3→119.1; m/z for d4-buprenorphine, 472.3→101.1).

Partial validation was performed with rabbit plasma obtained before drug administration as a matrix. Precision and accuracy of the assay were determined by use of quality control samples (3 concentrations within the standard curve) as outlined in the FDA recommendations for bioanalytic method validation.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed by use of pharmacokinetic software. Noncompartmental
pharmacokinetic parameters were determined for each rabbit and reported as mean ± SD.

**Evaluation of adverse effects after maropitant administration**

During both experiments, rabbits were monitored for adverse drug reactions for 48 hours before the start of the experiments, during blood collection, and for at least 48 hours after collection of the last blood sample. Water intake of the rabbits was not monitored during the preadministration period for the initial administrations in the primary experiment, but it was monitored daily for the remainder of the administrations and time periods. Daily food consumption (measured by weight of the diet), water consumption (measured in milliliters), body weight, and mentation were evaluated every 24 hours. Fecal output was evaluated by counting the number of fecal pellets on each absorbent pad and subjectively assessing changes in the size of the fecal pellets. Urine output was evaluated on a scale of 1 to 4, with 1 indicating urine covering one-fourth of the absorbent pad and 4 indicating urine covering the entire pad. The SC injection site of each rabbit was evaluated at each blood sample collection time and then every 24 hours for 4 days by visual examination and palpation to detect erythema or swelling or to elicit signs of pain. Rabbits were monitored after drug administration to detect any immediate changes in mentation, irritation at the injection site, or differences in respiratory rate, compared with results obtained before drug administration.

**Statistical analysis**

Statistical analysis was performed with commercial statistical software. A Shapiro-Wilk test was used to test for normality of the model residuals.

A mixed-effects ANOVA was used to evaluate the effect of time and administration route on urination, defecation, water intake, food intake, and body weight. Results for the day of administration and days after administration were compared with results for the first day of measurement (2 days before maropitant administration). Data were assessed for normality by use of a normality probability plot. Significance was defined as values of $P \leq 0.05$.

**Results**

The mean ± SD plasma concentrations of maropitant after IV and SC administration at a dose of 1 mg/kg were determined (Figure 1). Pharmacokinetic parameters were calculated by use of noncompartmental analysis; concentration data for 1 rabbit after IV administration appeared to be erroneous, and these data were excluded from the final analysis. Noncompartmental pharmacokinetic parameters were summarized (Table 1). The mean ± SD bioavailability after SC administration was 58.9 ± 13.3%.

All rabbits remained healthy for the duration of the study. Four of 9 rabbits developed local reactions at the SC injection site. Three rabbits licked or scratched at the injection site approximately 2 to 3 minutes after SC injection; 2 of these 3 rabbits developed local reactions at the SC injection site. One of the 2 rabbits developed localized erythema cranial to the SC injection site that lasted < 12 hours, and the other rabbit developed a localized dermal reaction characterized by erythema and thickening at the SC injection site that progressed to horizontal abrasions; the thickened and raised skin persisted for 3 days, and the horizontal abrasions evolved into scabs that lasted for a total of 12 days after SC injection. Palpation of the area did not elicit signs of pain in that rabbit. The remaining 2 rabbits developed small bruises and erythema at the SC injection site; however, the bruising and erythema completely resolved by 5 days after SC injection.

Five of 9 rabbits became hyperexcited (increased activity, movement, or resistance to cap-
Table 1—Mean ± SD values for pharmacokinetic parameters of maropitant in plasma, determined by use of noncompartmental analysis after SC and IV administration of a single dose of maropitant citrate (1 mg/ kg) to 9 New Zealand White rabbits (Oryctolagus cuniculus).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IV</th>
<th>SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>ND</td>
<td>14.4 ± 10.9</td>
</tr>
<tr>
<td>C&lt;sub&gt;0&lt;/sub&gt; (ng/mL)</td>
<td>101.8 ± 88.5</td>
<td>ND</td>
</tr>
<tr>
<td>C&lt;sub&gt;ss&lt;/sub&gt; (ng/mL)</td>
<td>2.9 ± 1.7</td>
<td>3.4 ± 1.2</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>1.25 ± 0.89</td>
<td>ND</td>
</tr>
<tr>
<td>t&lt;sub&gt;ss&lt;/sub&gt; (h)</td>
<td>10.4 ± 1.6</td>
<td>13.1 ± 2.4*</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0&lt;/sub&gt; (h*ng/mL)</td>
<td>348.9 ± 66.7</td>
<td>208.5 ± 52.9†</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;ex&lt;/sub&gt; (%)</td>
<td>3.2 ± 1.8</td>
<td>7.2 ± 3.0†</td>
</tr>
<tr>
<td>CI (mL/h/kg)</td>
<td>2,960 ± 571</td>
<td>NA</td>
</tr>
<tr>
<td>V&lt;sub&gt;d&lt;/sub&gt; (L/kg)</td>
<td>3.5 ± 1.3</td>
<td>NA</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>NA</td>
<td>58.9 ± 13.3</td>
</tr>
</tbody>
</table>

*Value differs significantly (P = 0.001) from the value for IV administration. †Value differs significantly (P < 0.001) from the value for IV administration.

Figure 2—Mean ± SE number of fecal pellets over time after IV (white circles and dashed line) or SC (black circles and solid line) administration of maropitant citrate (1 mg/ kg) to 9 New Zealand White rabbits. Day 0 was the day of maropitant administration. *,†Value differs significantly (*P < 0.001 and †P = 0.049) from the value before maropitant administration.

dose (1 mg/kg, SC or IV) in New Zealand White rabbits was evaluated. Although maropitant has been used empirically in rabbits in clinical practice, to our knowledge, there have been no reports of the pharmacokinetics of this drug in rabbits.

In the study reported here, mean plasma concentrations of maropitant citrate were similar to those associated with antiemesis in dogs<sup>13,14</sup>. The mean ± SD C<sub>max</sub> in rabbits of the present study (14.4 ± 10.9 ng/mL) was much lower than the C<sub>max</sub> identified in studies of dogs<sup>2</sup> (92.0 ± 33.8 ng/mL), cats<sup>6</sup> (269 ng/mL), and macaques<sup>15</sup> (113 ng/mL) in which the drug was administered at the same dose. In dogs, the drug has dose-dependent pharmacokinetics at higher doses.<sup>5</sup> In cats, maropitant appears to have linear pharmacokinetics, at least at lower doses.<sup>6</sup> Because only a single dose was used in the present study, additional studies to evaluate higher doses and repeated administrations of maropitant are required to determine whether there are differences in pharmacokinetics with differing dosages in rabbits.

Mean time to C<sub>max</sub> after SC administration in the present study was similar to that for both dogs<sup>2</sup> and cats<sup>6</sup> after SC administration. Bioavailability after SC administration in the study reported here was only approximately 60%, which is much lower than that found in dogs<sup>2</sup> (90.7%) or cats<sup>6</sup> (117%). In canine and feline patients, the high bioavailability after SC administration explains the reason that equivalent doses can be used for both SC and IV administration. The cause for the low bioavailability in rabbits is not known.

The higher rate of maropitant clearance in rabbits, compared with clearance in other species,<sup>2,6</sup> may have contributed to the overall lower plasma concentrations (and therefore lower area under the curve extrapolated to infinity) of maropitant in the present study. Maropitant is eliminated by hepatic metabolism and renal clearance; however, renal clearance is minimal in other species. In dogs, the

Discussion

In the study reported here, the pharmacokinetics of maropitant citrate after administration of a single
The drug is metabolized by 2 cytochrome P450 isoforms that lead to oxidation of the molecule.\(^2\) There are important species and even breed differences in hepatic metabolism. For example, different strains of rabbits can express various activities of enzymes involved in hepatic drug metabolism.\(^10\) Because maropitant is primarily cleared by hepatic metabolism, species differences in hepatic blood flow may affect the rate of clearance. Hepatic blood flow in New Zealand White rabbits is approximately 2.662 mL/kg/h.\(^17\) This is extremely similar to the value for clearance of maropitant (2.960 mL/kg/h) in the present study, which would support the contention that maropitant is primarily cleared via hepatic metabolism in rabbits.

The most common adverse effect reported for maropitant citrate is injection site reactions after SC administration.\(^18\) In the present study, 4 of 9 rabbits developed local reactions at the injection site after SC administration; only 1 developed a severe reaction. Additionally, 3 rabbits licked or scratched at the injection site after SC injection (2 of these 3 rabbits developed local reactions at the injection site). Evaluation of biopsy specimens of the skin of cats after SC administration of maropitant revealed hemorrhage and inflammation at the injection site.\(^6\) Refrigeration of the drug may reduce irritation at the injection site.\(^19\) For the present study, maropitant was refrigerated prior to administration, which may have contributed to the low number of injection site reactions. Commercially available maropitant is composed of sulfobutylether-\(\beta\)-cyclodextrin and metacresol, which binds maropitant. Binding decreases as temperature increases, and pain induced by injection is likely caused by the amount of unbound maropitant.\(^19\) Local reactions of the rabbits in the study reported here were mild to moderate and self-limiting, but monitoring of the injection site after administration of maropitant is recommended because of the more extensive dermal reaction that developed in 1 rabbit. It is unknown whether allergic sensitization to the drug could occur, which would predispose animals to more severe reactions after future administrations. The rabbit that developed the most extensive dermal lesion subsequently received the drug by IV administration with no complications; however, repeated SC administrations were beyond the scope of this study. Because of the number of local injection site reactions detected in this study, caution should be used for repeated SC injection of this drug in rabbits.

Additional adverse effects in the rabbits of the present study were hyperexcitability and tachypnea after SC and IV administration of the drug. A safety study\(^20\) of cats administered maropitant citrate (1 to 5 mg/kg, SC, for 15 days) resulted in moderate tachypnea 1 week after administration. When maropitant citrate was given SC at a dose of 5 mg/kg, 1 cat developed tremors, but they were evident only while the cat was asleep; the effect was not apparent when the cat was awake.\(^6\) Respiratory rates were not monitored at scheduled intervals after injection for each rabbit because of personnel limitations; thus, only marked differences from before administration were recorded.

The number of fecal pellets was higher on the day of administration and 1 day after administration, compared with the number of fecal pellets before administration. However, this was not associated with an increase in food intake. The NK1 receptors are present on interstitial cells of smooth muscle involved in gastrointestinal motility.\(^21\) Selective NK1-receptor antagonists can enhance the velocity of colonic peristalsis in rabbits.\(^19\) It is possible that the increased number of fecal pellets was secondary to increased colonic motility in the rabbits. Further studies are needed to evaluate the effect of maropitant on gastrointestinal motility in rabbits.

Water intake was higher on the day of maropitant administration and remained high for the duration of the study period. The authors are not aware of any reports of increased water consumption associated with the administration of maropitant. Some rabbits used the water bottle as a source of environmental enrichment and hit the bottle repeatedly, which caused some water leakage. Unfortunately, the amount of water leakage could not be quantified; therefore, caution should be used regarding the effects of maropitant on water intake.

Limitations of the study reported here included a small sample size. A small number of rabbits was used to complete the pharmacokinetic analysis, but a larger sample size with a more even distribution of rabbits of both sexes would be advantageous to help decrease effects attributable to an individual rabbit or sex. However, SDs relative to the mean values for the pharmacokinetic parameters in the present study were relatively low, which could indicate that the inclusion of additional rabbits may not have had a great effect on the pharmacokinetic variables.

In the present study, SC and IV administration of maropitant at a dose of 1 mg/kg resulted in measurable plasma concentrations for 24 hours in New Zealand White rabbits that were similar to plasma drug concentrations identified in dogs administered maropitant to prevent emesis.\(^12\) However, bioavailability, plasma concentrations, and volumes of distribution in the rabbits were lower and clearance was higher than those in dogs\(^2\) and cats.\(^6\) The association between plasma concentration and pharmacodynamics in rabbits is unknown. The dose of 1 mg/kg was chosen on the basis of results of the preliminary experiment and a targeted 24-hour plasma concentration reported for dogs.\(^13\) That plasma concentration was targeted because at the time the present study was conducted, only plasma concentrations associated with antiemesis in dogs were available for comparison. Maropitant has nonlinear kinetics in other species\(^2\); thus, studies to evaluate pharmacokinetics in rabbits after higher doses or repeated administrations are needed. Rabbits should be monitored for major adverse effects (eg, localized skin reactions) after SC
administration of maropitant. Pharmacodynamic information is needed to assess clinical use of this drug in rabbits.

Acknowledgments
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The authors declare that there were no conflicts of interest.
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Footnotes
a. Laboratory rabbit diet HF No. 5236, LabDiet, St Louis, Mo.
b. Kaytee timothy hay blend cubes, Kaytee, Chilton, Wis.
c. Cerenia, 10 mg/mL, Zoetis, Kalamazoo, Mich.
e. Lidocaine 1.5% and prilocaine 2.5% cream USP, Tolmar Pharmaceuticals Inc, Fort Collins, Colo.
f. Tegaderm film, 3M Health Care, Saint Paul, Minn.
g. Monoject IV catheter, Coviden, Mansfield, Mass.
h. TSQ Vantage, Thermo Scientific, San Jose, Calif.
i. 1100 LC system, Agilent Technologies, Palo Alto, Calif.
j. ACE C18, Mac-Mod Analytical, Chadds Ford, Pa.
k. WinNolin, version 8.0, Pharsight, Princeton, NJ.
l. Stata, version 15.1/IC, StatCorp LP, College Station, Tex.
m. Pelligrand L, Associate Professor, Veterinary Anaesthesia and Clinical Pharmacology, Royal Veterinary College, University of London, North Mymms, England: Personal communication, 2019.

References