Pharmacokinetics of single-dose intragastric and intravenous pregabalin administration in clinically normal horses

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Objective—To assess pharmacokinetics of pregabalin in horses after a single intragastric or IV dose.

Animals—5 healthy adult mares.

Procedures—Horses received 1 dose of pregabalin (approx 4 mg/kg) via nasogastric tube in a crossover-design study; after a 3-week washout period, the same dose was administered IV. Food was not withheld. Plasma pregabalin concentrations in samples obtained 0 to 36 hours after administration were measured by use of ultra-performance liquid chromatography with triple quadrupole tandem mass spectrometry. Pharmacokinetic variables were estimated by means of noncompartmental analysis.

Results—Mild sedation was observed in 2 horses following intragastric and IV pregabalin administration. Signs of mild, transient colic or behavioral abnormalities were observed in all horses following IV administration. After intragastric administration, median (range) maximal plasma concentration was 5.0 µg/mL (4.4 to 6.7 µg/mL), time to maximal plasma concentration was 1.0 hour (0.5 to 2.0 hours), elimination half-life was 8.0 hours (6.2 to 9.4 hours), and area under the curve from time 0 to infinity (AUC0–∞) was 47.2 µg•h/mL (36.4 to 58.4 µg•h/mL). After IV administration, initial concentration was 22.2 µg/mL (19.8 to 27.7 µg/mL), elimination half-life was 7.74 hours (6.94 to 8.17 hours), and AUC0–∞ was 48.3 µg•h/mL (44.8 to 57.2 µg•h/mL). Bioavailability was 97.7% (80.7% to 109.8%). Median predicted values for minimal, mean, and maximal steady-state plasma concentrations after intragastric administration assuming an 8-hour dosing interval were 3.9, 5.3, and 6.3 µg/mL, respectively.

Conclusions and Clinical Relevance—At a simulated intragastric dosage of approximately 4 mg/kg every 8 hours, median pregabalin steady-state plasma concentration in healthy horses was within the therapeutic range reported for humans. Therapeutic concentrations and safety of this dosage have not been established in horses. (Am J Vet Res 2013;74:1043–1048)

Pregabalin, (S)-3-aminomethyl-5-methylhexanoic acid, has analgesic, anticonvulsant, and anxiolytic activity in humans.¹ It has also been effectively used as an adjunct anticonvulsant in dogs with idiopathic epilepsy inadequately controlled with standard treatments.²

The exact mechanisms of action for pregabalin and its predecessor, gabapentin, are unknown. Both bind the αδ subunit of presynaptic, voltage-gated calcium channels that are distributed throughout the CNS and peripheral nervous system. Binding results in inhibitory modulation of calcium influx and a reduction in the release of several excitatory neurotransmitters, including glutamate, noradrenaline, and substance P.³ The binding affinity of pregabalin for the αδ subunit is 6 times as great as that of gabapentin.¹

The pharmacokinetics of pregabalin have been studied extensively in humans. In a study of healthy human volunteers, orally administered pregabalin was absorbed rapidly, with peak plasma concentrations occurring within 0.7 to 1.3 hours after administration. In humans, bioavailability of the drug, as determined by urine clearance methods, is > 90%. The bioavailability of pregabalin is independent of dose and frequency of administration, unlike gabapentin, which relies on active dose-dependent transport from the gastrointestinal tract.³ At doses > 500 mg, gabapentin bioavailability in humans decreases due to saturation of carrier transport.⁴ In humans, administration of pregabalin with food reduces the rate of absorption and maximum plasma concentration but not total absorption, and the elimination half-life is approximately 6 hours.³ Preg-
pregabalin is not protein bound and is >90% excreted unchanged in the urine. As expected, clearance of the drug is reduced in human patients with renal impairment.

Pharmacokinetic parameters in fed healthy dogs administered a single dose of pregabalin orally were similar to those in humans, with peak plasma concentration occurring in 1.5 hours and an elimination half-life of 6.9 hours. At a dose of 4 mg/kg and a simulated dosing interval of 12 hours, plasma concentration would be within the therapeutic range of 2.8 to 8.2 µg/mL, which is clinically effective in controlling epilepsy and neuropathic pain in people. The frequency of seizures in dogs with suspected idiopathic epilepsy was significantly reduced in 9 of 9 client-owned dogs that completed a 3-month trial of pregabalin at a dosage of 3 to 4 mg/kg orally every 8 hours as an adjunct to phenobarbital, potassium bromide, or both.

Gabapentin, although well tolerated in horses, has somewhat poor absorption following oral administration. In horses that had food withheld for 0.5 hours before and 1 hour after administration of a single 20 mg/kg dose of orally administered gabapentin, bioavailability was 16.2%. In a clinical report describing a pregnant draft horse mare with leptomeningeal retraction to conventional analgesia following colic surgery, gabapentin administered orally at 2.5 mg/kg every 8 to 12 hours for 6 days appeared to reduce signs of discomfort.

To our knowledge, the pharmacokinetics of pregabalin have not been investigated in horses. Given its favorable pharmacokinetic properties in humans, compared with gabapentin, and some evidence that gabapentin may be beneficial for treatment of neuropathic pain in horses, pregabalin has the potential to be both well absorbed and efficacious in horses. The objectives of the study reported here were to determine the pharmacokinetics of pregabalin in clinically normal horses after a single dose given via nasogastric tube (to mimic oral administration) or IV and to predict steady-state plasma concentrations for various dosing regimens following intragastric administration.

Materials and Methods

Animals—Five healthy adult (median age, 14 years [range, 12 to 19 years]) university-owned warmblood (n = 4) and Thoroughbred (n = 1) mares weighing 518 kg (range, 509 to 600 kg) were used in the first part of the study and additionally at 5 months before and 1, 2, 4, 8, 12, 24, and 36 hours after drug administration. Horses were monitored continuously from time 0 to 2 hours, then at every blood collection time. Attitude, appetite, and manure and urine production were subjectively evaluated and recorded. Rectal temperature, heart rate, and respiratory rate were measured every 24 hours. Blood samples were obtained via jugular venipuncture and collected in evacuated tubes containing sodium heparin. Samples were centrifuged at 1,000 × g for 10 minutes; plasma was removed and maintained at −20°C until shipped overnight on dry ice to the testing laboratory.

Following a 3-week washout period, the same horses received the compounded pregabalin solution IV in the second part of the study. An area over the left jugular vein of each horse was clipped of hair, aseptically prepared, and infiltrated with local anesthetic, and the vein was catheterized. Blood samples were obtained from the right jugular vein at the same time points used in the first part of the study and additionally at 5 minutes following the completion of the IV infusion, and plasma was separated and handled as previously approved by the Cornell University Institutional Animal Care and Use Committee.

Preparation of pregabalin for IV administration—The pregabalin solution (200 mg/mL aqueous solution with propylene glycol [8% wt/wt]) was provided as a single source product by a compounding pharmacy and subjected to quality control measures, including purity and sterility testing. Quantitative analysis of the IV pregabalin formulation was performed at a laboratory with HPLC-MS-MS. In summary, isotopically labeled D6-pregabalin was added as the internal standard to an aliquot of the compounded pregabalin product. Samples were diluted, mixed, and injected into the HPLC system for separation. Analysis was performed with a C18 column (3-µm particle size, 2.1 × 10 mm) and triple quadrupole MS-MS detector. Quantitation was achieved by monitoring the following transitions after HPLC separation with positive-ion electrospray MS-MS: 160.2 to 142.2 and 160.2 to 97.2 for pregabalin and 166.2 to 148.2 and 166.2 to 103.2 for D6-pregabalin. Each analytic run was independently calibrated at concentrations of 0.2, 0.5, 1.0, and 1.5 µg/mL. Two levels of control were run in each analytic batch. The HPLC-MS-MS method used had a lower limit of quantification of 0.20 µg/mL and quality control accuracy of >98%. The compounded product was shipped on ice and was stored at approximately 4°C until used (2 days after receipt).

Drug administration and sample collection—in the first part of the crossover-design study, each horse was administered pregabalin capsules that were completely dissolved in 100 mL of warm water and administered as a slurry via a nasogastric tube into the stomach. For each horse, approximately 1,000 mL of warm water to rinse any remaining medication from the tube into the stomach. Because the pregabalin capsules could not be divided, the exact dose varied slightly among horses; the median dose was 4.1 mg/kg (range, 4.0 to 4.3 mg/kg). Blood samples (18 mL) were collected immediately prior to drug administration (time 0) and at 15 and 30 minutes and 1, 2, 4, 8, 12, 24, and 36 hours after drug administration. Horses were monitored continuously from time 0 to 2 hours, then at every blood collection time. Attitude, appetite, and manure and urine production were subjectively evaluated and recorded. Rectal temperature, heart rate, and respiratory rate were measured every 24 hours. Blood samples were obtained via jugular venipuncture and collected in evacuated tubes containing sodium heparin. Samples were centrifuged at 1,000 × g for 10 minutes; plasma was removed and maintained at −20°C until shipped overnight on dry ice to the testing laboratory.

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described. Each horse received the same dose of pregabalin IV as had been administered via the intragastric route; the drug was administered through the catheter as an infusion over 5 minutes (timed with a stopwatch). Following IV administration of the pregabalin, the catheters were flushed with 30 mL of heparinized saline (0.9% NaCl) solution and then removed. Physiologic variables were monitored as described for the first part of the study.

Sample processing—Quantitative analysis for pregabalin plasma concentration was performed at a laboratory by use of UPLC-MS-MS. Briefly, 100 µL of isotopically labeled pregabalin-13C3 was added as the internal standard to 0.20-mL aliquots of equine plasma samples. Samples were extracted by means of protein precipitation with trichloroacetic acid; samples were mixed and centrifuged to yield supernatants that were transferred to vials for instrumental analysis. Analysis was performed on a UPLC system with a 2.0 X 50 mm, 2.5-µm analytic column and triple quadrupole MS-MS detector. Quantitation was achieved by monitoring 2 transition ions (160.0 to 97.0 and 160.0 to 83.0 for pregabalin, and 163.0 to 99.0 and 163.0 to 84.0 for the internal standard pregabalin-13C3 for equine plasma samples) following UPLC separation with positive-ion electrospray MS-MS. Each analytic run was independently calibrated at concentrations of 0.10, 0.25, 1.0, 2.5, 7.5, and 15 µg/mL. Two concentrations of control were included in each analytic batch. This UPLC-MS-MS method had a lower limit of quantification of 0.10 µg/mL and between-run coefficient of variation of 6.32% and 8.64% at 0.23 and 9.25 µg/mL, respectively.

A matrix-matching experiment was performed on equine plasma to ensure adequate pregabalin recovery. Samples from 5 horses were tested in triplicate: 1 blank and 2 at different concentrations of spiked standard (1.0 and 10 µg/mL). All acceptable criteria were met, so it was concluded that the equine plasma matrix did not affect recovery of pregabalin. The blank samples were < 25% of the response of the low calibrator, and the spiked samples were all within 20% above or below the known spiked standard values at both concentrations.

Pharmacokinetic analysis—Plasma pregabalin concentration versus time data for individual horses were plotted on linear and semilogarithmic graphs for analysis. Data were subjected to noncompartmental analysis with commercially available software. The program uses curve stripping, which resolves the concentration-time curves into a series of exponential terms corresponding to absorption, distribution, and elimination phases of the drug over time in circulation. These exponential terms are used to calculate single- and multiple-dose parameters following established pharmacokinetic calculations. The curve-stripping approach in this program assumes the disposition phases of the drug follow apparent first-order processes, as evidenced by linearity of the terminal portion of the semilogarithmic plots. The AUC data were computed on the basis of the trapezoidal rule.

The single-dose pharmacokinetic data were used to simulate predicted steady-state plasma pregabalin concentrations. The multiple-dose pharmacokinetics program operates as an extension of the single-dose pharmacokinetic data. It draws on the pharmacokinetic results calculated for single-dose information (eg, elimination half-life) and combines this with user input of a multiple dosing interval to compute and graph estimates of multiple-dose pharmacokinetics parameters. For any selected time, it assumes that the dosing interval is regular and equal, that repeated doses are administered during the postdistributive or elimination phase, and that the drug behavior is characterized by linear pharmacokinetics.

The following parameters were determined for each horse after intragastric administration of pregabalin: maximal plasma concentration, time to reach maximal plasma concentration, absorption half-life, elimination half-life, AUC, and MRT. The following parameters were determined for each horse after IV administration of the drug: initial concentration at 5 minutes, distribution half-life, elimination half-life, AUC, MRT, volume of distribution, and clearance. The volume of distribution determined was the apparent volume of distribution based on the trapezoidal AUC∞ after intragastric administration divided by the AUC∞ after IV administration; data were reported as median (range). Predicted maximum, minimum, and mean steady-state plasma concentrations at proposed dosing intervals of 8 and 12 hours were calculated.

Figure 1—Median (range) plasma pregabalin concentrations versus time in 5 healthy adult horses following administration of a single median dose of 4.1 mg/kg (range, 4.0 to 4.3 mg/kg) via intragastric (dotted line, circles) and IV (solid line, squares) routes. Pregabalin was administered via nasogastric tube (intragastric route) as a slurry prepared from capsules in warm water to mimic oral administration. After horses had a 3-week washout period, a pregabalin product for injection obtained from a compounding pharmacy was administered to the same horses as a slow infusion through a jugular catheter over 5 minutes. Circles and squares represent median values; vertical lines represent range.
Statistical analysis—Pharmacokinetic data were not subjected to tests of normality. Descriptive statistics (median and range values) were determined with commercially available software.a

Results

Animals—Two of 5 horses developed mild sedation characterized by lowered head position starting 1 hour after intragastric administration of pregabalin and continuing for up to 12 hours. The horses were easily roused and continued to eat and drink normally. All measured physiologic variables were within the respective reference ranges.

Immediately after IV administration of pregabalin, all 5 horses developed mild, transient signs of colic or behavioral abnormalities, including stretching, pawing, yawning, and flehmen response. These signs resolved without treatment ≤ 5 minutes after the first observation. Two horses had subjectively soft manure and signs of mild sedation characterized by lowered head position for up to 8 hours after IV pregabalin administration. All horses continued to eat and drink normally and had measured physiologic variables within the respective reference ranges throughout the monitoring period after administration via this route.

Pharmacokinetics—The mean ± SD pregabalin concentration in the IV preparation was 193 ± 0.97 µg/mL. Pregabalin plasma concentrations in horses following intragastric and IV administration were displayed graphically (Figure 1). The plasma concentration was within the therapeutic range reported for control of epilepsy and neuropathic pain in humans (2.8 to 8.2 µg/mL) by 0.5 hours after intragastric administration in 4 of 5 horses (concentration ranged from 3.1 to 6.1 µg/mL in these 4 horses and was 1.4 µg/mL in the remaining horse); by 1 hour after administration, this targeted range of concentrations was achieved in all of the horses (Table 1). Although the therapeutic concentration of pregabalin in horses is unknown, after a single median dose of 4.1 mg/kg, plasma concentrations remained within the targeted range for ≥ 2 hours in all horses and for 4 hours in 3 of 5 horses (with concentrations of 2.7 µg/mL detected in the remaining 2 horses) after delivery via this route. Plasma concentrations of the drug remained above the lower limit of quantification in all horses for the 36-hour sampling period after IV administration. Following intragastric administration, only 1 horse had a plasma concentration less than the lower limit of quantification at 36 hours. Pharmacokinetic parameters for each route of drug administration were summarized (Table 2). Elimination half-lives of the drug following intragastric and IV administration were similar, and the median bioavailability after intragastric administration was 97.7% (range, 80.7% to 109.8%). Evaluation of predicted steady-state plasma concentrations suggested that, at a median dose of 4.1 mg/kg via the intragastric route, an 8-hour dosing interval would be sufficient to maintain drug concentrations between 2.6 and 7.3 µg/mL (Table 3).

Discussion

The most common adverse effects reported by healthy human volunteers administered pregabalin include somnolence and dizziness.3 In a clinical trial of pregabalin (3 to 4 mg/kg, PO, q 8 h for 3 months) as an adjunct treatment to phenobarbital, potassium bromide, or both in dogs with suspected idiopathic epilepsy...
The elimination half-life of pregabalin following intragastric administration in horses of our study (median, 8.0 hours [range, 6.2 to 9.4 hours]) was similar to that found in healthy dogs (median, 6.9 hours) and in humans (mean, 3.48 to 6.64 hours), where elimination half-life was independent of dose. Because pregabalin is largely excreted unchanged in the urine, in human subjects with renal impairment, AUC and elimination half-life are expectedly higher than in healthy individuals, suggesting that a dose reduction may be necessary in these patients. In humans receiving a dosage of 50 to 200 mg of pregabalin orally every 8 hours, pregabalin was more effective than a placebo in relieving pain and improving sleep and health-related quality of life in patients with diabetic peripheral neuropathy and postherpetic neuralgia. Given the linear pharmacokinetics of pregabalin and the daily dose to maximal plasma concentration relationship in humans reported by Ben-Menachem, the safety of 8-hour dosing and therapeutic concentrations of pregabalin have not been established in horses. In humans, pregabalin toxicosis has been reported at serum concentrations of 29 to 66.5 µg/mL in patients with normal renal function, with signs of toxicosis including severe neurologic depression and coma.

Limitations of the present study included the small sample size and lack of randomization of the treatment protocol. The standardized treatment protocol (in which all horses received pregabalin via the intragastric route and then had a 3-week washout period before the drug was administered IV) was performed because the pregabalin preparation for injection was compounded as a single source, and stability of the product was unknown at the time. In the event that the product was unstable, IV administration to all of the horses on the same day potentially reduced a potential source of variability. Our results indicated that pregabalin concentrations approximating the reported therapeutic range in humans could be achieved in horses for up to 4 hours following a single intragastric dose of the drug. The bioavailability of pregabalin in this study appeared to be substantially greater than that reported for gabapen-
tin, even though the horses in this study were continually fed. The adverse effects observed in this study were minimal. However, further studies are needed to determine the safety and efficacy of pregabalin in horses.

References