Levetiracetam pharmacokinetics in healthy dogs following oral administration of single and multiple doses

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Objective—To measure pharmacokinetics of levetiracetam (LEV) after single-dose oral administration in healthy dogs and determine whether pharmacokinetics changed after repeated oral dosing.

Animals—6 healthy adult dogs.

Procedures—Pharmacokinetics were calculated following administration of a single dose (mean, 21.7 mg/kg, PO; day 1) and after administration of the last dose following administration for 6 days (20.8 to 22.7 mg/kg, PO, q 8 h; days 2 to 7). Plasma LEV concentrations were determined by use of high-pressure liquid chromatography. Pharmacokinetic data were analyzed by use of a 1-compartment model with first-order absorption.

Results—Peak concentration occurred 0.6 hours after administration of the first dose, with an absorption half-life of 0.06 hours. Minimal accumulation occurred over the 7 days, with only a slight increase in total area under the concentration-versus-time curve from 268.52 ± 56.33 h•µg/mL (mean ± SD) to 289.31 ± 51.68 h•µg/mL after 7 days. Terminal half-life was 2.87 ± 0.21 hours after the first dose and 3.59 ± 0.82 hours after the last dose on day 7. Trough plasma concentrations were variable, depending on the time of day they were measured (morning trough concentration, 18.42 ± 5.16 µg/mL; midday trough concentration, 12.57 ± 4.34 µg/mL), suggesting a diurnal variation in drug excretion.

Conclusions and Clinical Relevance—Results indicated that the pharmacokinetics of LEV did not change appreciably after administration of multiple doses over 7 days. Administration of LEV at a dosage of 20 mg/kg, PO, every 8 hours to healthy dogs yielded plasma drug concentrations consistently within the therapeutic range established for LEV in humans. (*Am J Vet Res* 2010;71:337–341)

Seizures are the most common neurologic disorder encountered in small animal practice and have been reported to occur in 0.5% to 5.7% of dogs.[1,2] Primary, or idiopathic, epilepsy (ie, recurrent seizures for which an underlying cause is not found) is diagnosed in approximately 80% of dogs with seizures.[1,2] The mainstay of treatment for dogs with idiopathic epilepsy is AEDs, of which phenobarbital and potassium bromide are most commonly prescribed. However, between 20% and 30% of dogs with epilepsy are refractory to conventional medical therapy[3] and, as such, continue to have recurrent seizure activity despite appropriate treatment with AEDs at established therapeutic serum concentrations. For this subset of patients, evaluation of new AEDs is vital to seizure management and improved quality of life.

Abbreviations

AED	Antiepileptic drug
AUC	Area under the concentration-versus-time curve
HPLC	High-performance liquid chromatography
LEV	Levetiracetam

Levetiracetam is a structurally novel AED that was approved as an adjuvant treatment for partial-onset seizures in humans in 1999. The mechanism of action of LEV has been postulated to involve inhibition of excitatory neurotransmitter release by binding to the synaptic vesicle protein SV2A.[4] Following its approval for treatment of partial-onset seizures, LEV was demonstrated to be effective for treatment of a wide variety of seizure types in human patients, including simple partial, complex partial, and secondary generalized seizures.[5] Furthermore, LEV is preferred in critical or geriatric patients because of minimal hepatic metabolism and primary renal excretion.[4,6,7] Levetiracetam also has minimal effect on the disposition of other AEDs in humans, making it an ideal adjuvant medication for patients with refractory epilepsy.[6,7] A therapeutic range for LEV administration in people has not

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been definitively established, but doses that achieve serum concentrations of 5 to 45 μg/mL have been proposed to be effective.[8]

On the basis of promising results in humans, LEV is being used with increasing frequency for the management of dogs with epilepsy.[9] The pharmacokinetics of LEV in healthy dogs after administration of a single dose PO, IM, or IV have been reported.[8,10,11] However, treatment of dogs with refractory epilepsy would require repeated dosing, and there is no published information on the pharmacokinetics of LEV after repeated oral administration. The objective of the study reported here was to measure the pharmacokinetics of LEV in healthy dogs after administration of a single oral dose and to determine whether the pharmacokinetics changed after repeated oral dosing.

Materials and Methods

Animals—Six adult dogs (5 Beagles and 1 hound mix) obtained from the North Carolina State University Laboratory Animal Resources unit were used in the study. The dogs ranged in weight from 11.0 to 23.6 kg (mean, 14.7 kg) and were housed in runs throughout the study, except that they were moved to cages on days when blood samples were collected. In each dog, a central venous catheter (jugular or long saphenous vein) was inserted 24 hours prior to initiation of the study by use of a standard aseptic technique. Dogs were sedated with medetomidine (5 to 10 μg/kg, IV) for catheter insertion; sedation was reversed with atipamezole after completion of catheter placement. The study protocol was approved by the North Carolina State University Institutional Animal Care and Use Committee.

Experimental protocol and sample collection—Food was withheld from dogs for 12 hours, and dogs were administered a single oral dose of LEV[a] (mean dose, 21.7 mg/kg; range, 20.8 to 22.7 mg/kg; day 1). Blood samples were collected immediately before; 20, 40, and 60 minutes after; and 1.5, 2, 3, 4, 6, 8, 10, 12, 16, and 24 hours after administration of the dose of LEV. After LEV was administered, 12 mL of water was administered orally to ensure that the tablet passed to the stomach. Blood samples were collected from the central venous catheters into heparinized tubes. The tubes were immediately placed on ice and subsequently centrifuged for 5 minutes at 1,163 × g. Plasma was harvested and stored at –70°C until analyzed for LEV concentration. One dog (dog 6) vomited 7 minutes and again 20 minutes after administration of LEV. Subsequent analysis indicated that plasma drug concentrations in this dog were much lower than concentrations for the other 5 dogs. Therefore, data obtained from this dog after administration of the first dose of LEV were not used for analysis.

After the initial single-dose experiment, dogs were administered LEV every 8 hours (20.8 to 22.7 mg/kg, PO) for an additional 6 days (days 2 to 7). Doses were administered at 7:30 AM, 3:30 PM, and 11:30 PM in a small meatball of canned dog food. Dogs were fed at 7:30 AM (ie, at the same time as drug administration) and 5:00 PM each day. Beginning on the evening of day 6, food was withheld for 12 hours, and a single dose of LEV was administered on day 7. Blood samples were collected and handled as described for the initial single-dose experiment.

Measurement of plasma LEV concentration—Plasma samples were analyzed for LEV concentration by use of an HPLC method developed by one of the authors (MGP). An LEV reference standard obtained from the drug manufacturer was dissolved in methanol to produce a 1 mg/mL stock solution that was stored in a tightly sealed, light-resistant vial refrigerated at approximately 4°C. As needed, the stock solution was further diluted in distilled water and added to control plasma from dogs that had not been given LEV, to produce calibration standards with LEV concentrations from 0.05 to 80 μg/mL.

The mobile phase for HPLC analysis consisted of phosphate buffer (93%) and acetonitrile (7%). The pH of the buffer solution was adjusted to 5.5. Fresh mobile phase was prepared and degassed each day. The HPLC system consisted of a quaternary solvent delivery system[b] (flow rate, 1 mL/min), an autosampler,[c] and a UV detector[d] at a wavelength of 205 nm. The chromatograms were integrated with a computer program.[e] The analytic column was a reverse-phase, 4.6-mm × 15-cm C8 column[f] maintained at a temperature of 40°C.

All study, calibration, and control plasma samples were prepared in an identical manner for HPLC analysis. Solid-phase extraction cartridges[g] were conditioned with 1 mL of methanol followed by 1 mL of distilled water. Each plasma sample (500 μL) was added to a conditioned cartridge, followed by a wash step of 1 mL of a distilled water:methanol (95:5) solution. Levetiracetam was eluted with 1 mL of 100% methanol and collected in clean glass tubes. The tubes were evaporated at 40°C for 15 minutes to achieve a dry residue. Each tube was then reconstituted with 200 μL of mobile phase, vortexed briefly, and transferred to an HPLC injection vial. Fifty microliters of each sample was then injected into the HPLC system. Retention time for the peak of interest was 6.2 to 6.5 minutes. Each day, a new set of calibration and control samples was prepared. All calibration curves were linear with an r^2 value of 0.99 or higher. Limit of quantification for LEV in canine plasma was 0.05 μg/mL, which was determined from the lowest point on a linear calibration curve that produced acceptable precision and accuracy. Quality-control samples were analyzed at the same time as incurred and calibrated samples.

If LEV concentration in an incurred sample was higher than the highest value on the calibration curve (> 80 μg/mL), the sample was diluted and analyzed again. Spiked samples were diluted in a similar manner to ensure that the dilution produced a valid response. Control plasma samples were analyzed to determine the level of background noise and to ensure that there were no interfering peaks eluting at the same time as LEV.

Pharmacokinetic analysis—Plasma LEV concentrations obtained after administration of the first dose were plotted on linear and semilogarithmic graphs for visual analysis. Analysis of curves and pharmacokinetic modeling were conducted with a commercial software program.[h] Data for each animal were analyzed with a

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compartmental model. A weighting factor of 1/(predicted Y)^2, where Y is the plasma concentration, was used for pharmacokinetic analysis. One- and 2-compartment models were tested for best fit on the basis of a smaller value for the Akaike information criterion and visual examination of observed versus predicted concentrations.[12] Examination of the plasma concentration-versus-time curves and goodness-of-fit analyses indicated that the best pharmacokinetic model was a 1-compartment model with first-order input (absorption) and first-order elimination. A lag-time effect was added to account for formulation dissolution and gastric emptying times. The general formula for the best-fit 1-compartment model was as follows:

$$C_T = D \bullet F \bullet k_{01} / V(k_{01} - k_{10}) \bullet [\exp(-k_{10} \bullet T) - \exp(-k_{01} \bullet T)$$

where C_T is the plasma concentration at time T, k_{01} is the absorption rate constant following oral administration, k_{10} is the elimination rate constant, V is the apparent volume of distribution, F is the fraction absorbed for a non-IV dose, and D is the dose. In this model, it was assumed that k_{01} was much greater than k_{10} or that there was no flip-flop effect caused by slow absorption after oral administration. Without an accompanying IV dose pharmacokinetic analysis, this assumption was only speculative. However, because the LEV administered was an immediate-release formulation that has been found to be rapidly absorbed in other studies,[10,11] we believe that this assumption was justified. Secondary parameters were calculated from these primary parameters. For example, the AUC from time zero to infinity was calculated as AUC = dose/($V \bullet k_{10}$).

Plasma concentrations measured for samples collected on the last day of the multiple dosing study were analyzed in a manner identical to that used for the first-day samples, except that the dosing input was changed to indicate that the last dose followed 6 days of dosing every 8 hours, allowing for accumulation. Therefore, the time points for analysis on day 7 started on hour 144 and continued for 24 hours (hour 168).

Dosing simulation—Pharmacokinetic parameters derived from the first-day samples were used to develop a simulation of repeated dosing for the time period used in the study (ie, every 8 hours for 6 days). Calculated concentrations were then compared with the actual concentrations measured on the last day of dosing. Simulations were performed with a commercial pharmacokinetic software program.[h]

Statistical analysis—Descriptive statistics were calculated, and data are reported as mean ± SD. A paired *t* test was used to compare pharmacokinetic parameters obtained after administration of the first and last doses. Values of $P < 0.05$ were considered significant.

Results

Levetiracetam was well tolerated by all dogs in the study. One dog vomited after the first dose of the drug was administered on day 1, but no other adverse effects were observed during the study. The plasma LEV concentrations on day 1 and day 7 were graphed (**Figure 1**). After administration of the first and last doses of LEV, absorption was rapid, with peak concentration occurring 0.62 hours after administration of the first dose and approximately 1 hour after administration of the last dose. The mean ± SD terminal half-life was 2.87 ± 0.21 hours on day 1 and 3.59 ± 0.82 hours on day 7. Minimal accumulation occurred, with peak concentrations of 59.91 ± 11.54 µg/mL on day 1 and 52.41 ± 10.08 µg/mL on day 7 and only a small increase in AUC from day 1 (268.52 ± 56.33 h•µg/mL) to day 7 (289.31 ± 51.68 h•µg/mL). The ratio of the AUC values indicated an accumulation ratio of 1.08. Pharmacokinetic parameters measured after the last dose of LEV were not significantly different from those values obtained after the initial dosing. Mean morning trough LEV concentration (18.42 ± 5.16 µg/mL) was significantly ($P = 0.01$) different from mean afternoon trough concentration

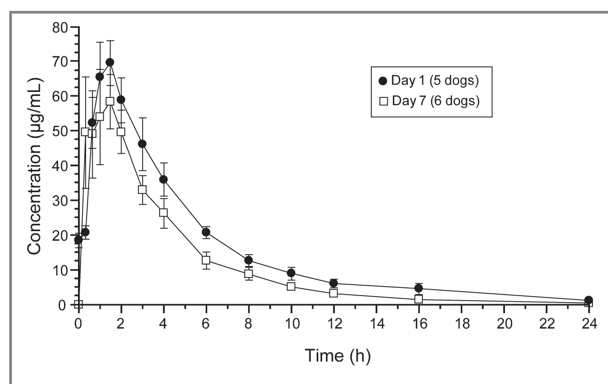

Figure 1—Mean plasma LEV concentrations in dogs following administration of a single dose (mean dose, 21.7 mg/kg; range, 20.8 to 22.7 mg/kg), PO, and after administration of the last dose (on day 7) following administration for 6 days at a dosage of 20.8 to 22.7 mg/kg, PO, every 8 hours. Error bars represent SD.

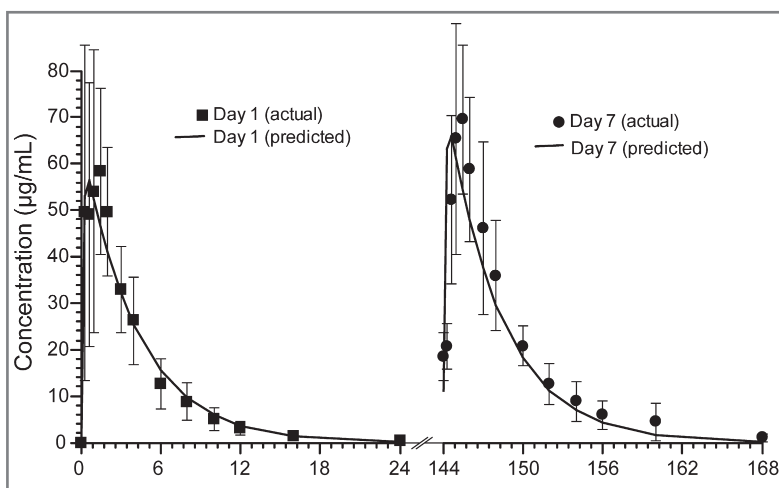

Figure 2—Actual mean plasma LEV concentrations in dogs after administration of the last dose (on day 7) following administration for 6 days at a dosage of 20.8 to 22.7 mg/kg, PO, every 8 hours, and concentrations predicted by a simulation of repeated dosing based on pharmacokinetic parameters derived after administration of a single dose. Error bars represent SD.

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Table 1—Previously reported pharmacokinetic parameters for LEV after PO, IM, and IV administration of single doses to dogs.

	Isoherranen et al[7]	UCB Pharma[i]	Patterson et al[8]			Dewey et al[6]
Variable	IV	PO	IV	IM	PO	IV
Dose (mg/kg)	20	54	20	20	20	60
Clearance (mL/kg/min)	1.5	1.9	2.1	2.3	2.1	1.5
Half-life (h)	3.6	3.3	3.0	3.0	3.0	4.0
VD (L/kg)	0.45	0.54	0.53	ND	ND	0.48
Absorption (%)	NA	100	NA	100	ND	ND

NA = Not applicable. ND = Not determined.VD = Volume of distribution.

(12.57 ± 4.34 µg/mL), indicating diurnal variation in LEV plasma concentrations.

Values for plasma LEV concentration calculated from our dosing simulation were plotted graphically and compared with the actual concentrations measured on day 7 (**Figure 2**). Calculated values closely approximated values obtained on day 7 of the study.

Discussion

Results of the present study indicated that the pharmacokinetics of LEV in dogs were unchanged after 6 days of oral administration. Oral administration of LEV every 8 hours at a dose of 20 mg/kg consistently produced plasma drug concentrations within the proposed therapeutic range of 5 to 45 µg/mL. The slight increase in the AUC after 6 days reflected a predictable accumulation to steady state over time. This degree of accumulation may be expected with multiple dosing because after administration of each dose, there is some residual drug in the body that will supplement the next dose. Approximately 10 to 12 hours after administration of a single oral dose of the drug, plasma concentrations were less than the proposed therapeutic range, indicating that administration every 8 hours is probably important with this drug. The degree of accumulation measured in the present study (accumulation ratio, 1.08) was close to the value of 1.17 that was predicted on the basis of calculation of the accumulation ratio with the following equation: accumulation ratio $= 1/(1 - e^{-k_{10}\tau})$, where τ is the dosing interval (8 hours) and k_{10} is the elimination rate. The close match between actual and predicted accumulation ratios helps explain the close match between plasma LEV concentrations calculated from our dosing simulation on the basis of day 1 pharmacokinetic values and actual concentrations measured on day 7.

The pharmacokinetics of LEV observed in the present study are similar to those observed in other studies[10,11,i] following PO, IM, or IV administration of a single dose in dogs (**Table 1**). We suggest that these data indicate that the pharmacokinetics of LEV are probably not dose dependent. Additionally, elimination half-life and clearance data were similar and independent of route of administration, suggesting that the route of administration does not affect the pharmacokinetics of LEV.

In the present study, the diurnal difference in plasma drug concentrations that was observed was unexpected, with morning trough concentrations significantly higher than afternoon trough concentrations. This difference was particularly striking in 1 dog, in which afternoon plasma LEV concentration on day 7 was approximately 50% of the morning plasma LEV concentration. Additionally, another dog had an afternoon trough concentration that was close to the lower limit of the proposed therapeutic range for LEV. It is possible that diurnal changes in disposition of LEV may be an important contributor to breakthrough seizures in some patients. The reason for diurnal changes in plasma drug concentrations is not known; however, possible explanations include diurnal differences in glomerular filtration rate that exist in many species, including dogs,[13] and differences in renal tubular reabsorption of the drug throughout the day. Because LEV is a relatively pH-insensitive drug and because changes in renal tubular drug reabsorption are usually dependent on fluctuations in urine pH, a difference in renal tubular reabsorption of LEV seems unlikely. Diurnal changes in glomerular filtration rate are a more likely explanation for the differences in plasma drug concentrations observed.

Establishing a therapeutic range for LEV concentration in dogs is relevant when dosage recommendations are made. Additionally, because LEV may be administered in conjunction with other AEDs (eg, phenobarbital), pharmacokinetic studies that investigate common AEDs used in combination for the treatment of patients with refractory epilepsy may be helpful.

a. Keppra (250-mg tablet), UCB Pharma, Smyrna, Ga.
b. Quaternary solvent delivery system, Agilent 1100 series, Agilent Technologies, Wilmington, Del.
c. Agilent 1100 series autosampler, Agilent Technologies, Wilmington, Del.
d. Agilent variable wavelength detector, Agilent 1100 Series, Agilent Technologies, Wilmington, Del.
e. Agilent chemstation software, Agilent 1100 Series, Agilent Technologies, Wilmington, Del.
f. Zorbax Rx (C8, 4.6 × 150 mm, 5 µm), Agilent Technologies Inc, Wilmington, Del.
g. Solid-phase extraction cartridges, Varian Bond Elut CN-E (3-mL cartridges), Varian Inc, Lake Forest, Calif.
h. WinNonlin, version 5.0.1, Pharsight Corp, Mountain View, Calif.
i. UCB Pharma, Smyrna, Ga: Unpublished data, 1999.

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