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STANDARD ARTICLE

Population pharmacokinetics of extended-release levetiracetam in epileptic dogs when administered alone, with phenobarbital or zonisamide

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Background: Extended-release levetiracetam (LEV-XR) has gained acceptance as an antiepileptic drug in dogs. No studies have evaluated its disposition in dogs with epilepsy.

Hypothesis/Objectives: To evaluate the pharmacokinetics of LEV-XR in epileptic dogs when administered alone or with phenobarbital or zonisamide.

Animals: Eighteen client-owned dogs on steady-state maintenance treatment with LEV-XR (Group L, n = 6), LEV-XR and phenobarbital (Group LP, n = 6), or LEV-XR and zonisamide (Group LZ, n = 6).

Methods: Pharmacokinetic study. Blood samples were collected at 0, 2, 4, 8, and 12 hours after LEV-XR was administered with food. Plasma LEV concentrations were determined by high-pressure liquid chromatography. A population pharmacokinetic approach and nonlinear mixed effects modeling were used to analyze the data.

Results: Treatment group accounted for most of the interindividual variation. The LP group had lower C_{MAX} (13.38 μg/mL) compared to the L group (33.01 μg/mL) and LZ group (34.13 μg/mL), lower AUC (134.86 versus 352.95 and 452.76 hours·μg/mL, respectively), and higher CL/F (0.17 versus 0.08 and 0.07 L/kg/hr, respectively). The half-life that defined the terminal slope of the plasma concentration versus time curve (~5 hours) was similar to values previously reported for healthy dogs.

Conclusions and Clinical Importance: Considerable variation exists in the pharmacokinetics of LEV-XR in dogs with epilepsy being treated with a common dose regimen. Concurrent administration of phenobarbital contributed significantly to the variation. Other factors evaluated, including co-administration of zonisamide, were not shown to contribute to the variability. Drug monitoring may be beneficial to determine the most appropriate dose of LEV-XR in individual dogs.

KEYWORDS

antiepileptic drug, canine, drug disposition, drug interactions, seizures

1 | INTRODUCTION

Epilepsy is the most common chronic neurological disorder in dogs,[1] with an estimated prevalence of 0.6-0.75% in the general dog population.[2,3] Approximately half of dogs with epilepsy are diagnosed with idiopathic epilepsy, a clinical syndrome characterized by ≥2

Abbreviations: AED, antiepileptic drug; AUC, area-under-the-curve for the plasma-concentration versus time profile; CL/F, clearance; C_{MAX}, maximum plasma concentration; CV, coefficient of variation; HPLC, high-performance liquid chromatography; LEV, levetiracetam; LEV-XR, extended-release levetiracetam; NLME, nonlinear mixed effects; T_{MAX}, time to maximum concentration; V/F, volume of distribution

unprovoked seizures at least 24 hours apart for which no underlying cause can be identified other than a confirmed or suspected genetic predisposition.[4] Antiepileptic drugs (AED) are the mainstay of treatment, and most dogs require lifelong treatment. Consequently, there is a continuous effort to introduce AED protocols into veterinary practice that will maximize seizure control while minimizing adverse medication-related effects.

Levetiracetam (LEV) is a second-generation AED that was approved for use in humans in 1999, and is being utilized with increasing frequency in veterinary medicine, both as a first line treatment and as add-on treatment. The drug possesses several favorable pharmacokinetic properties in dogs, including high PO bioavailability (100%), lack of hepatic metabolism and a wide therapeutic index.[5] However, the elimination half-life of 3-4 hours in dogs requires an 8-hour dosing interval, and this frequency of administration is prohibitive for many pet owners. More recently, an extended-release formulation of levetiracetam (LEV-XR) became available, and this drug has gained acceptance as an AED in dogs. The pharmacokinetics of LEV-XR have been evaluated in 2 single dose studies involving healthy dogs, both of which supported q12h dosing to maintain blood concentrations within the reference range for humans (5-45 μg/mL).[6,7] No studies have evaluated the disposition of LEV-XR when administered as maintenance treatment to dogs with epilepsy, either with or without other AEDs. The objective of our study was to evaluate the pharmacokinetics of LEV-XR in epileptic dogs when administered alone, or concurrently with either phenobarbital or zonisamide.

2 | MATERIALS AND METHODS

2.1 | Animals

Eighteen client-owned dogs with epilepsy were enrolled. All dogs had a presumptive diagnosis of idiopathic epilepsy based on at minimum a tier 1 level of confidence as outlined by the International Veterinary Epilepsy Task Force.[8] Six dogs were recruited into each of three groups based on their established maintenance AED treatment regimen: LEV-XR only (L group), LEV-XR in combination with phenobarbital (LP group), and LEV-XR in combination with zonisamide (LZ group). To be eligible for the study, all administered AEDs had to be at steady-state conditions, with repeated administration and no change in dose for a minimum amount of time equal to 5 half-lives for the drug. All dogs were being given generic formulations of the AEDs, representing various manufacturers. No other drugs were being administered aside from monthly parasitic preventatives. Owners were required to provide informed consent before the dog's participation in the study. Nine dogs presented to NC State Veterinary Hospital for study participation, whereas the remaining 9 dogs presented to a regional veterinary hospital for sample collection according to a standardized study protocol. This study was approved by the Institutional Animal Care and Use Committee at NC State University.

2.2 | Sample collection

Owners were instructed to withhold food from their dogs overnight before participation in the study. Dogs presented to the hospital on the morning of the study and were admitted for the day. Blood samples were taken from each dog at five time points throughout the day: immediately before administration of the morning dose of LEV-XR (0-hour sample), and at 2, 4, 8, and 12 hours after LEV-XR administration. At each sampling point, ~6 mL of blood was collected from either the jugular, cephalic, or saphenous vein and placed in a sodium heparin tube (BD Vacutainer sodium heparin tubes; Becton, Dickinson and Company, Franklin Lakes, NJ). Dogs were fed their regular diet at the time the morning dose of LEV-XR was administered, and were observed to consume their food. Other prescribed AEDs were administered in accordance with their established treatment schedule. Water was available throughout the study. Blood samples were centrifuged after collection, and plasma harvested and frozen. Samples collected at outside sites were shipped to the investigators frozen and on ice by overnight delivery service. All samples were stored at −80°C until assayed.

2.3 | Drug analysis

Plasma LEV, phenobarbital, and zonisamide concentrations were measured using high-pressure liquid chromatography (HPLC) methods developed and validated in the author's (MGP) laboratory. The HPLC system components[9–11] and the method for analyzing LEV concentrations[9] have been described previously. Plasma phenobarbital and zonisamide concentrations were determined on 0-hour samples. Phenobarbital concentrations were measured using an identical assay previously validated for cats.[12] A partial validation was used to adapt the assay for the study in dogs. Blank plasma from untreated dogs was fortified with phenobarbital to prepare quality control samples and a range of concentrations for a calibration curve. The calibration curve ranged from 1 to 60 μg/mL. The lower limit of quantification of 1 μg/mL for the phenobarbital assay represented the lowest concentration on the linear calibration curve that could be back-calculated to within 15% of the nominal concentration. For the zonisamide assay, a pure analytical reference standard was obtained from a supplier (Zonisamide reference standard, >99% pure; TOCRIS Biosciences, Bristol, UK, www.tocris.com). The reference standard powder was dissolved in acetone for preparation of calibration curve standards. Blank (control) plasma from pooled untreated dogs was fortified (spiked) with concentrations to make a calibration curve of five standards ranging from 0.5 to 100 μg/mL, as well as a zero sample to check for background noise and interfering peaks. After extraction with solid-phase extraction cartridges, the eluent was dried, reconstituted with mobile phase, and 20 μL injected into the system. The mobile phase consisted of 30% acetonitrile and 70% buffer solution at a flow of 1 mL/min. Separation was achieved with a reverse-phase C-8 column and detection of peaks with ultraviolet (UV) absorbance at 215 nm. The calibration curve was linear from the highest concentration down to the lower limit of quantification of 0.5 μg/mL (r^2 > 0.999). All samples incurred from the study were well above the lower limit of quantification for these assays.

2.4 | Description of pharmacokinetic model

Plasma drug concentrations versus time were plotted on linear and semilogarithmic graphs for visual inspection and assessment of the best model for pharmacokinetic analysis. Analysis of the curves and pharmacokinetic modeling then were performed using a commercial pharmacokinetic program (Phoenix WinNonlin, Certara, St. Louis, MO). Compartmental analysis of the data was performed using a weighting factor of 1/(predicted Y),^{2} where Y is the LEV plasma concentration. The primary parameters were calculated using the following formula:

$$C = \frac{k_{01} FD}{V(k_{01} - k_{10})} \left[e^{-k} 10^{t} - e^{-k} 01^{t}\right] \quad (1)$$

Where C is the plasma LEV concentration, t is time after administration, k_{01} is the non-IV absorption rate assuming first-order absorption, k_{10} is the elimination rate constant, V is the apparent volume of distribution, F is the fraction of drug absorbed, and D is the non-IV dose. Secondary parameters calculated from the model included the peak concentration (C_{MAX}), time to peak concentration (T_{MAX}), area under the plasma-concentration versus time profile (AUC), PO clearance (CL/F) and the respective absorption and elimination half-lives ($t½$). The model used steady-state conditions with the LEV dose in each group administered to steady state with an interval of every 12 hours.

2.5 | Population pharmacokinetics

Initial standard 2-stage analysis generated estimates for parameters in the model. These values were used as initial estimates for the population pharmacokinetic analysis. One dog in the LZ group was given a very high dose of LEV-XR (>200 mg/kg) and was excluded from the analysis. Because the study subjects were clinical patients, sampling times were at more sparse intervals than typically obtained from research animals. Therefore, to analyze these data with a sparse sampling design, a population pharmacokinetic analysis with nonlinear mixed effects (NLME) modeling[13,14] was performed using commercial software (Phoenix NLME, Certara, St. Louis, MO).

Various models and different error structures were tested to determine the best fit base model. The model was parameterized according to Equation (1), and run with a quasi-random parametric expectation maximization (EM) algorithm using steady-state conditions with a dose administered every 12 hours. Final model selection was based on goodness-of-fit plots, statistical significance between models using −2LL (twice the negative log likelihood), Akaike information criterion (a goodness-of-fit measure based on the log likelihood adjusted for the number of parameters [degrees of freedom] in the fit), and coefficient of variation (CV%) of parameter estimates. The model measured the fixed effects (typical values) for primary pharmacokinetic parameters and random effects attributed to interindividual (intersubject) variability. Secondary parameter estimates were obtained using standard compartmental equations.

After the final base model was obtained for the population, an examination of covariates was performed to determine if there were factors that could explain the variability in the primary parameters (k_{01}, k_{10}, and volume of distribution). The covariates examined were treatment group (L, LP, and LZ), LEV dose per dog (500, 750, 1000, and 1500 mg), body weight (kg), age (years), and sex (neutered male or spayed female). Generic manufacturer was investigated as a covariate, but the number of different manufacturers resulted in small sample sizes that precluded valid analysis. Treatment, LEV dose per dog, and sex were considered as categorical covariates, and weight and age as continuous covariates. Diagnostic box plots were used to explore the variation (η, eta) for categorical covariates. Diagnostic scatter plots of continuous covariates versus random effects (η, eta) were used to explore their potential significance.

The covariates (treatment group, LEV dose per dog, body weight, age, and sex) were tested in a simple stepwise approach with forward inclusion and backward elimination. The effects of the covariate on a parameter were evaluated based on improvement in the −2LL. Results were considered statistically significant if the decrease was associated with a P value of <.01. If a significant covariate was identified, a backward elimination step was used to assess the significance of the covariate, and an increase in the −2LL with a P value of <.001. After this covariate was considered significant, the covariate remained in the final model. The predictive accuracy of the final model was tested using the visual predictive check with 10 observations simulated for each subject stratified by the categorical covariate. The visual predictive check was examined to compare observed quantiles with quantiles predicted by the model.

Additional details on the population pharmacokinetic analysis are provided in Supporting Information.

3 | RESULTS

3.1 | Dog demographics

Breeds represented in the study included mixed breed (n = 6), Labrador retriever (n = 3), Australian shepherd (n = 2), and one each of Basset hound, Golden retriever, Pembroke Welsh corgi, Vizsla, Curly-coated retriever, and English springer spaniel. There were 11 spayed females and 6 neutered males, with a median body weight of 25.7 kg (range, 7.8-45.5 kg). Dogs were 3-12 years of age (median, 6 years) with a duration of epilepsy of 0.5-0.6 years (median, 1 year).

3.2 | AED administration

The mean PO dose of phenobarbital for dogs in the LP group was 2.86 mg/kg q12h (SD, 1.40), with a mean serum phenobarbital concentration of 28.48 μg/mL (SD, 11.67). The mean PO dose of zonisamide for dogs in the LZ group was 7.82 mg/kg q12h (SD, 2.22), with a mean serum zonisamide concentration of 55.09 μg/mL (SD, 33.63). Generic LEV-XR formulations from 4 different manufacturers were prescribed for study dogs (Apotex Corporation, Weston, Florida; Qualitest Pharmaceuticals, Huntsville, Alabama; Lupin Pharmaceuticals, Incorporated, Baltimore, Maryland; BluePoint Laboratories, Columbus. Ohio). The LEV dose per dog administered at each dosing interval was 500 mg in 8 dogs, 750 mg in 7 dogs, 1000 mg in 1 dog, and 1500 mg in 1 dog. The mean PO dose of levetiracetam was 31.86 mg/kg for group L dogs, 30.91 mg/kg in the LP group, and 23.52 mg/kg in the LZ group. To consider these differences, the LEV

concentration was normalized to the mean study dose (29.4 mg/kg) for the pharmacokinetic analyses.

3.3 | Pharmacokinetic analysis using population model

The pharmacokinetic model used for the analysis was a 1-compartment model with first-order absorption and elimination. The spaghetti plot of LEV concentrations fit to the model for all dogs in the study is shown in Figure 1, with the fitted line shown for each dog overlaid on the actual data points. Three of six dogs in the LP group had LEV concentrations <5 μg/mL at 2 of the sampling time points in the study, which is considered the minimum therapeutic concentration in humans. One dog in the L group had an 0-hour sample of <5 μg/mL.

Analysis of box plots and scatter plots determined that the inclusion of body weight, LEV dose per dog, age, or sex as a covariate did not improve the model based on the criteria for improvement in the −2LL. Therefore, these variables were not included in the final model. Visual examination of the box plots for the covariate of LEV dose per dog identified a possible effect on the primary parameter of volume of distribution for the 1000 and 1500 mg dose. However, with only one dog in each of these categories there was insufficient evidence to conclude that there was an effect, and the covariate of LEV dose per dog was not included in the final model. Visual examination of the box plots of η (eta) for the covariate of treatment group showed that among the primary parameters of k_{01}, k_{10}, and volume of distribution, volume of distribution was the parameter most likely affected by the covariate. Treatment group was added as a categorical variable and tested against the base model, demonstrating that this factor accounted for most of the intersubject variation (η) for the parameter of volume of distribution (Figure 2). The volume of distribution in this model actually is apparent volume of distribution per fraction absorbed, or *V*/*F* for a PO dose, and is the primary parameter in this model most affected by extent of absorption, *F*. An improvement in the model was observed, with a statistically significant decrease in the −2LL ($P < .01$). A backward elimination step confirmed the significance of the covariate, and an increase in the −2LL ($P < .001$). A spaghetti plot of dogs grouped by treatment group, after accounting for random effects and including treatment group as a covariate, is depicted in Figure 3.

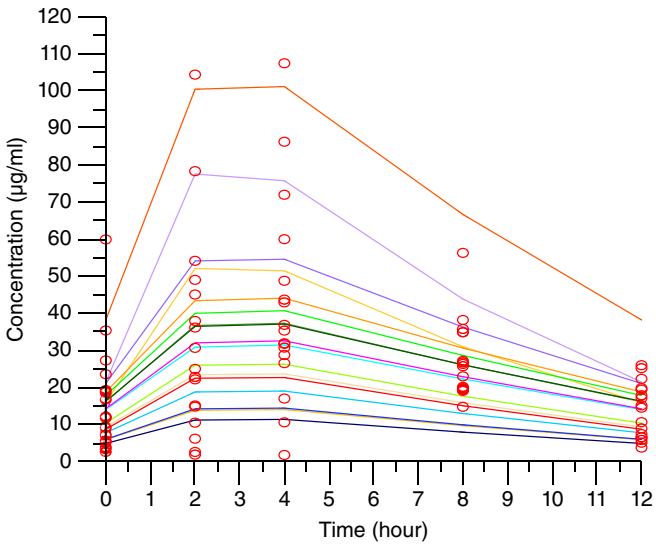

FIGURE 1 Plot of levetiracetam concentrations for individual dogs, fit to the model, before the addition of covariates. Each line represents an individual dog. Each open circle represents an actual data point. The fitted line shown for each dog is overlaid on the actual data points

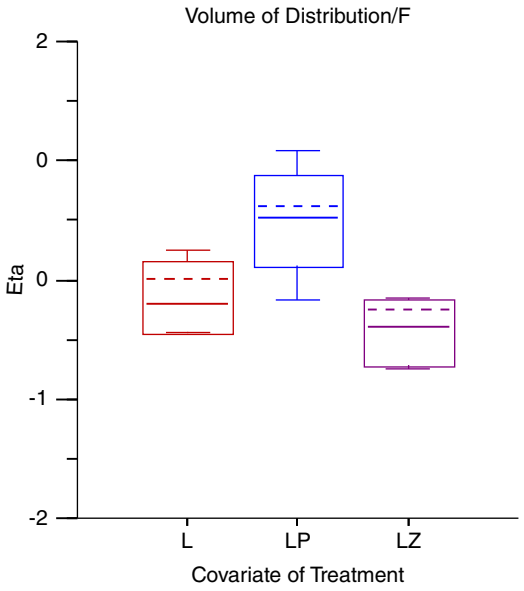

FIGURE 2 Box plot of covariate of treatment versus eta (random variable) for the primary parameter of volume of distribution. The solid and dashed lines within the box represent the mean and median, respectively. The boxes represent the 25th to 75th percentiles, and the whiskers on the plot are the 5th to 95th percentiles ($P < .01$)

Pharmacokinetic parameters for the final population model that included the covariate of treatment group are listed in Table 1. Primary and secondary parameters are included for the population as a whole and sorted by treatment group. The LP group had lower C_{MAX} (13.38 μg/mL) compared to the L group (33.01 μg/mL) and LZ group (34.13 μg/mL), lower AUC (134.86 versus 352.95 and 452.76 hours·μg/mL, respectively), and higher CL/F (0.17 versus 0.08 and 0.07 L/kg/hr, respectively). The half-life that defined the terminal slope of the plasma-concentration versus time curve (~5 hours) was similar to values previously reported for healthy dogs.

4 | DISCUSSION

We used a population pharmacokinetic approach and NLME modeling to evaluate the disposition of LEV-XR in dogs with naturally occurring epilepsy, and found substantial variation in the pharmacokinetics of LEV-XR in dogs being treated with a common dose regimen, with variability in concentration-time profiles across individuals. Furthermore, the model identified the effect of treatment group as the most significant contributor to the variation among the factors evaluated (LEV dose per dog, age, sex, body weight, and treatment group).

Population pharmacokinetic modeling can be utilized to identify the source of variation in a population, and is particularly useful for

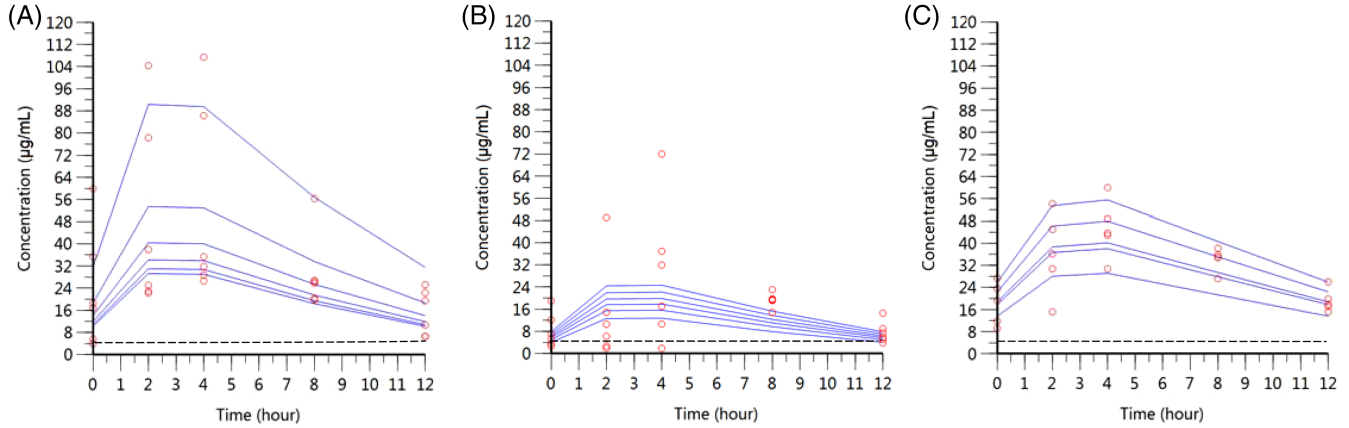

FIGURE 3 Population model plots of fitted curves for all individual dogs (solid line) with observed data points (open circles) after the addition of treatment as a covariate. (A) LEV-XR alone; (B) LEV-XR and phenobarbital; (C) LEV-XR and zonisamide. Minimum reference range in humans in noted by (------)

clinical studies when only sparse sampling is possible.[13,14] Data are considered sparse when insufficient numbers of samples are collected in an individual to perform the relevant pharmacokinetic analysis for the individual.[14] Our study was unique among pharmacokinetic AED studies in dogs, in that a population pharmacokinetic approach was used to identify the source of variability in LEV concentrations within the study population. When the covariate of treatment group (L, LP, LZ) was included in the model, it accounted for a significant variation in the pharmacokinetics. Furthermore, improvement in the model with the addition of the covariate was observed in the concentration versus time plots of individual dogs. Body weight also was explored as a possible covariate while considering that dog size can affect drug absorption or clearance, but it was not shown to be significant in this model. Similarly, the covariates of age and sex did not produce significant reduction in variability when included in the model. Finally, the LEV dose per dog was evaluated as a possible covariate, under the premise that the tablet size or number of tablets might have an effect on drug dissolution. It was not determined to be a significant source of variability in the study, and was not included in the final model. However, 2 of the 4 categories for this covariate (1000 mg and 1500 mg) included only one dog, limiting the conclusions that can be drawn from this analysis. Indeed, a limitation of our study is small sample size, which limits the analysis of some potential covariates.

Concurrent administration of phenobarbital accounted for much of the variability in LEV-XR pharmacokinetics in dogs with epilepsy. In contrast, coadministration of zonisamide was not shown to contribute to the variability. The LP group had lower peak concentration (C_{MAX}) and AUC than did the other 2 treatment groups. The values obtained for C_{MAX} and AUC in the L and LZ groups are comparable to those obtained in a previous study evaluating the disposition of LEV-XR in healthy dogs after single PO dosing at 30 mg/kg.[7]

Phenobarbital coadministration also produced an increase in PO clearance (CL/F) and apparent volume of distribution per fraction absorbed (*V*/*F*) for LEV compared to the other treatments. Because it was not possible to deliver an accompanying IV dose to these dogs to

TABLE 1 Final parameters for the population analysis of levetiracetam concentrations with LEV-XR administered alone, with phenobarbital, or with zonisamide. Administration of LEV-XR was repeated every 12 hours to achieve steady-state conditions before sampling

		LEV-XR alone			LEV-XR + phenobarbital			LEV-XR + Zonisamide			Overall (all groups)		
Parameter	**Units**	**Value**	**Ω^2**	**CV%**	**Value**	**Ω^2**	**CV%**	**Value**	**Ω^2**	**CV%**	**Value**	**Ω^2**	**CV%**
AUC	hours·µg/mL	352.95	—	—	134.86	—	—	452.76	—	—	388.72	—	—
Clearance	L/kg/hr	0.08	—	—	0.17	—	—	0.07	—	—	0.08	—	—
C_{MAX}	mg/L	33.01	—	—	13.38	—	—	34.13	—	—	32.99	—	—
k_{01} half-life	hr	4.45	—	—	3.72	—	—	5.60	—	—	5.01	—	—
k_{10} half-life	hr	1.31	—	—	1.57	—	—	1.57	—	—	1.37	—	—
T_{MAX}	hr	3.28	—	—	3.38	—	—	4.01	—	—	3.53	—	—
θk_{01}	1/hr	0.16	0.030	17.304	0.19	0.020	14.228	0.12	0.0004	2.117	0.138	0.058	24.39
θk_{10}	1/hr	0.53	0.0013	3.5870	0.44	0.0009	2.9850	0.44	0.0002	1.322	0.505	0.001	3.32
θV	L/kg	0.15	0.505	80.946	0.39	0.589	89.405	0.15	0.029	17.264	0.151	0.221	49.66

Abbreviations: LEV-XR, extended-release levetiracetam; CV, coefficient of variation; Ω^2, variance of the random source error; AUC, area-under-the-curve for the plasma-concentration versus time profile; C_{MAX}, peak concentration; θk_{01}, theta (typical value) for absorption rate and associated half-life; θk_{10}, theta for elimination rate and associated half-life; θV, theta for volume of distribution; T_{MAX}, time to peak concentration. Clearance (CL) and *V* as shown in the table represent V/F and CL/F because they are calculated as per fraction absorbed for an oral dose. Values for Ω^2 and CV% are not available for secondary parameters (shown by "—") because random effects are only measured for primary parameters.

allow for an assessment of *V* and CL independent of fraction absorbed, it cannot be determined what factor is responsible for these differences. However, it seems most likely that the addition of phenobarbital affected the fraction of drug absorbed. Previous studies have identified a similar interaction between phenobarbital and the immediate release formulation of LEV in both healthy dogs[10] and dogs with epilepsy,[11] with significant differences reported for volume of distribution, clearance, AUC and C_{MAX}. Phenobarbital administration may lead to induction of oxidative metabolism of LEV in dogs.[9] Although the location of the oxidative enzymes responsible for the increase in LEV metabolism is not known, it may be the liver or intestine, or both. An increase in the presystemic metabolism of LEV at these sites would result in a smaller fraction of drug absorbed, which in turn would lead to an increase in PO drug clearance (CL/*F*) and *V*/*F* and a decrease in C_{MAX} and AUC.[9] Because an accompanying IV dose was not administered to allow determination of the fraction absorbed (*F*), any change in F will result in an apparent change in CL/*F* and *V*/*F*, independent of any alteration in systemic CL or *V*.

Extended-release formulations are designed for drugs with short half-lives to prolong drug exposure by delaying the rate of release into the gastrointestinal tract, and thus allow for a longer dose interval. Consequently, an extended-release drug formulation should have a longer T_{MAX} with less fluctuation in maximum and minimum concentrations compared to the immediate release formulation.[15] The T_{MAX} for LEV in our study population ranged from 3 to 4 hours, which is slightly longer than the reported T_{MAX} of 1-3 hours for immediate release LEV in the dog.[5,7,9–11] Two previous studies evaluating the pharmacokinetics of LEV-XR in healthy dogs reported a T_{MAX} range of 3-8 hours.[6,7] One of these studies evaluated the effect of food on LEV-XR absorption, and determined that T_{MAX} was prolonged in dogs given the PO dose of medication with food compared to dogs that were fasted, with mean values of 6.6 and 3.4 hours, respectively.[6] The authors concluded that administering the drug with food produced longer exposure to the extended-release formulation, because the presence of food has been shown to affect drug release from the stomach.[16] The dogs in our study were fed at the time of dosing, but had values for T_{MAX} similar to those reported in the previous study for the fasted dogs. The second study reported a T_{MAX} for LEV-XR of 5-8 hours after it was administered to healthy dogs in a fasting state.[7] Thus, the effect of food on the absorption of LEV-XR is unclear. Differences in generic formulations of LEV-XR can affect the fraction of drug absorbed in dogs,[7] and generic formulations might differ in their rate of absorption, thereby playing a role in the difference in T_{MAX} between the present study and previous reports. Although 4 different generic formulations were administered, over half of the dogs were being treated with drug from a single manufacturer (Apotex Corporation, Weston, Florida). The pharmacokinetics of this specific generic formulation have not been evaluated previously in dogs. All generic formulations are bioequivalent to the brand-name formulation in humans, but there are no assurances of bioequivalence in dogs. An attempt was made to investigate generic drug manufacturer as a covariate in this pharmacokinetic model, but the number of different manufacturers and the unequal distribution among manufacturers resulted in small sample size that limited the analysis. Other possible causes for the variation in T_{MAX} among studies include differences in amount or composition of the diets being fed that can affect gastric emptying, bile flow or gastrointestinal pH; the potential that the tablet was chewed or otherwise disrupted in some dogs; a drug interaction; or, a manifestation of a difference between healthy dogs and dogs with epilepsy.

The elimination or disappearance half-life determined in our study was approximately 1.5 hours for all treatment groups. However, this value can be misinterpreted because of the "flip-flop" phenomenon.[17] Flip-flop occurs when the terminal slope of the plasma-concentration versus time profile is determined by the rate of absorption, rather than the rate of elimination. Ordinarily, the terminal slope of the curve represents the elimination phase of the drug, but when absorption is prolonged from an extended-release PO formulation, absorption rate is slower than elimination rate. In this situation, PO drug absorption, not elimination, becomes the rate-limiting step to define the plasma-concentration versus time profile. A half-life of 2-5 hours has been reported with PO administration of immediate release LEV,[5,7,9,10] and values of 4-5 hours have been reported for LEV-XR.[6,7] The half-life in our study (represented by absorption because of the flip-flop situation) was 5 hours in the overall model, and the range of values in the different treatment groups was similar to those reported in previous studies with LEV-XR. Values for AUC also are similar between our study and previous studies on both the extended-release and immediate release formulations of LEV in dogs, suggesting that the overall drug exposure was consistent among studies. These findings suggest that LEV-XR does experience slower absorption in dogs with epilepsy compared to the immediate release formulation, which is the rate-limiting step to prolong plasma drug concentrations with q12h dosing.

A reference range for LEV concentrations has not been established in dogs, although the reference range in humans of 5-45 μg/mL often is cited for use in veterinary medicine. However, data from our study indicate that some dogs will not maintain blood concentrations at a minimum concentration of 5 μg/mL with q12h dosing. Dogs concurrently receiving phenobarbital are at the greatest risk of concentrations <5 μg/mL using currently recommended dosages. Three of 6 dogs in the LP group had LEV concentrations <5 μg/mL at 2 of the sampling time points in the study, whereas 1 dog in the L group had an 0-hour sample of <5 μg/mL. Furthermore, if we aim for a higher minimum concentration of 10 μg/mL, then 59% of dogs (10 of 17) in our study would fall below this targeted concentration at ≥1 time points. This includes 100% of dogs (6 of 6) in the LP group, 50% of dogs (3 of 6) in the L group, and 20% of dogs (1 of 5) in the LZ group. The majority of these low concentrations occurred at 0- or 12-hour sampling points, immediately before the next scheduled dosing of medication. This observation suggests that a higher dose of LEV-XR administered q12h may be needed in some dogs to maintain concentrations considered being therapeutic for human patients, and that monitoring should be utilized to optimize dosage.

In conclusion, we utilized a population pharmacokinetic model and identified considerable variation in pharmacokinetics and plasma drug concentrations in dogs with epilepsy treated with similar doses of LEV-XR, and determined that much of the variation could be attributed to concurrent administration of phenobarbital. In contrast, coadministration of zonisamide did not contribute to variability in LEV-XR pharmacokinetics in the population. These findings warrant

consideration when utilizing LEV-XR as a treatment for epilepsy in dogs. Drug monitoring may be indicated to determine the most appropriate dose for an individual dog.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

This study was approved by the IACUC at NC State University (protocol # 14-111-O).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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