

Injectable Levetiracetam Use In the Dog

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Introduction and Literature Review

Seizures are one of the most common neurologic diseases in dogs (Podell, Fenner et al. 1995). Many diseases can cause seizures in veterinary patients, and these causes are often divided into primary or idiopathic epilepsy; secondary or symptomatic epilepsy (caused by a structural, inflammatory or traumatic disorder of the brain); or reactive epilepsy (the result of metabolic disease or intoxication outside of the CNS) (Bateman and Parent 1999; Platt and Haag 2002; Zimmermann, Hulsmeier et al. 2009). Regardless of the underlying cause, short, isolated seizures may develop into the more serious conditions of status epilepticus or acute repetitive seizures.

Seizures are the result of excessive release of excitatory neurotransmitters, and inadequate release of inhibitory neurotransmitters, resulting in uncontrolled and synchronous electrical discharges that recruit larger groups of neurons within the brain (Huff and Fountain 2011). The majority of seizures are isolated events that stop without intervention. However, status epilepticus (SE) and acute repetitive seizures (ARS) are conditions that require emergency treatment. Failure to intervene or unsuccessful treatment may result in neuronal necrosis, hyperthermia, cardiac arrhythmias, metabolic acidosis, kidney damage, disseminated intravascular coagulation or cardiorespiratory failure, and predispose patients to further seizure episodes (Bateman and Parent 1999). Defining status epilepticus has evolved over the last several decades. Currently, most people define SE as a single seizure lasting 5 minutes or longer, or 2 or more seizures without regaining consciousness in between, but the traditional definition has been

seizure activity lasting for at least 30 minutes. Motivation for shortening this definition has been the need for urgent and aggressive treatment early in the process, and as a result of dramatic increases in mortality with increasing durations of seizure activity. Furthermore, between 30-60 minutes, irreversible neuronal damage begins, due to excitotoxicity cell injury (Huff and Fountain 2011). Mechanisms responsible for neuronal death caused by prolonged seizures have been intensively studied. The hippocampus has been shown to be most sensitive to neuron loss, which can have effects on memory and learning, and may predispose to additional seizures in the future. A cascade of events is thought to be responsible for this cell loss. It starts with excessive excitatory neurotransmission, activating voltage gated calcium channels and N-methyl-D-aspartate (NMDA) receptors. Intracellular calcium and other changes in ion concentrations lead to the generation of reactive oxygen species, uncouples mitochondrial oxidative phosphorylation, and activates degradative enzymes, such as proteases, lipases, and endonucleases. Furthermore, damage caused by prolonged seizures results in increased plasticity and synaptic reorganization, abnormal axonal generation, and alterations in the cortical network, lowering the seizure threshold in damaged areas (Holmes 2002).

Two larger retrospective studies evaluating SE and ARS have been published to date in veterinary medicine (Bateman and Parent 1999; Zimmermann, Hulsmeyer et al. 2009). While these were designed somewhat differently, many similar conclusions were reached: the prevalence of SE/ARS was found to be 0.44% and 0.7% in the two studies during the study periods (6 years and 7 years); overall mortality rates (mostly from

euthanasia) were 25% and 38%, with death occurring in a relatively small number of cases (2.1% or 5.1%); the underlying causes identified were idiopathic epilepsy (26.8% and 37.5%), symptomatic epilepsy (35.1% and 39.8%), and reactive epileptic seizures (6.7% and 22.7%), and low AED concentration or the cause was undetermined (5.7% and 25.8%, respectively; only evaluated in Bateman study); symptomatic epilepsy (inflammatory CNS disease, intracranial neoplasia or trauma) was found to carry a worse prognosis in both studies.

While there are guidelines for the emergency treatment of seizures, no studies have been reported in dogs evaluating the efficacy of various antiepileptic drugs (AEDs), and treatment recommendations for seizure emergencies is based on clinical experience and the results of human or rodent studies. One published algorithm (Podell 1998) recommends diazepam as first-line therapy. If that is unsuccessful in stopping seizures, phenobarbital is used next. In cases that remain refractory to these 2 medications, additional recommended medications are diazepam or midazolam continuous rate infusion, followed by general anesthesia with pentobarbital, propofol or inhalant anesthesia. Other treatment options include mannitol for elevated intracranial pressure, and ketamine has been used in some refractory cases (Serrano, Hughes et al. 2006). In addition to in-hospital therapy, at-home therapy with rectal or intra-nasal administration of diazepam has been shown to be effective (Podell 1995; Platt, Randell et al. 2000).

In humans, SE and ARS are also common reasons for presentation for emergency treatment. The number of cases of SE and ARS in adults is approximately 152,000, with 42,000 deaths annually in the United States (DeLorenzo, Hauser et al. 1996; Fountain

2000). Reported mortality rates in humans are variable, depending on the age of the patient and underlying cause, but range from 8% in children to 38% in the elderly, with an overall mortality rate of 22% (DeLorenzo, Hauser et al. 1996; Chin, Neville et al. 2004). These estimates of morbidity and mortality are roughly comparable to those reported in studies in dogs.

Despite high levels of morbidity and mortality, little prospective research has been performed to determine the optimum treatment regimen for people. To date, only 3 prospective, randomized, double-masked studies have been performed. The first (Leppik, Derivan et al. 1983) showed that lorazepam was superior to diazepam. Treiman et al (1998) showed that lorazepam was superior to phenytoin alone, and was comparable to phenobarbital treatment and combination diazepam and phenytoin treatment. In the third study, Alldredge et al (2001) found that lorazepam was superior to diazepam, and both were superior to placebo. Notwithstanding the lack of high quality evidence, general guidelines for the treatment of SE and ARS have been established. First-line therapy is almost always lorazepam (or another benzodiazepine); second-line therapy typically consists of either phenytoin or fosphenytoin. There is less agreement regarding third-line treatment, with commonly used drugs including levetiracetam (LEV), valproic acid, propofol, midazolam, phenobarbital, pentobarbital and ketamine. Rarely, general anesthesia, maintained with gas anesthetics is required. There have been a number of retrospective and prospective, open-label studies evaluating newer AEDs for the treatment of SE/ARS. The most commonly tested medications are valproic acid, levetiracetam and lacosamide, and other emerging drugs, including brivaracetam,

carbamazepine and topiramate (Conway, White et al. 2009; Shorvon 2011). Unfortunately, there have been no large, prospective, well-controlled studies demonstrating the efficacy of any of these drugs.

Levetiracetam (Keppra, UCB Pharma) was approved in the US in 1998 for the oral treatment of partial onset seizures in adults. A parenteral formulation was approved in 2006 for use as bridge therapy. Since that time, there have been increasing reports of the off-label use of LEV for the treatment of seizure emergencies. One of the earliest larger reports of its use in humans with SE showed a success rate of 100% (Knake, Gruener et al. 2008). However subsequent studies have had variable response rates. In adults, success rates have ranged from 44-71%, and LEV has been reported to cause few side effects (details in Table 1) (Eue, Grumbt et al. 2009; Gamez-Leyva, Aristin et al. 2009; Moddel, Bunten et al. 2009; Alvarez, Januel et al. 2011). A wide safety margin and minimal drug-drug interactions has led to LEV use in children, the elderly and critically ill patients with SE. A case series of critically ill children showed that 100% of children had cessation of seizure activity or significant reduction in seizures (Abend, Monk et al. 2009). Eighty seven and a half percent of elderly patients had cessation of seizures in one small case series (8 patients; (Fattouch, Di Bonaventura et al. 2010). Timing of LEV administration has only been evaluated in a single clinical study. It was found to be most effective when given early (after only benzodiazepine therapy or no therapy; 78.5% success rate), and significantly less effective if used later as add-on therapy [after benzodiazepines plus phenytoin, valproate or both; 46.1% response rate (Aiguabella, Falip et al. 2011)].

Levetiracetam has been shown to have a unique binding site and mechanism of action. It binds to synaptic vesicle protein 2A, and is thought to decrease abnormal nerve conduction through modification of the transport, docking, fusion, exocytosis and recycling of neurotransmitters. It has also been shown to alter ion flow through GABA channels, Na-dependent $\text{Cl}^-/\text{HCO}_3^-$ channels, high voltage Ca^{2+} channels and K^+ channels (Xu and Bajjalieh 2001; Lynch, Lambeng et al. 2004; De Smedt 2007).

Synergy between LEV and diazepam has been shown in both rodent models of status epilepticus, and in a clinical population of human patients with acute repetitive or prolonged seizures. In rats with electrical-stimulation induced SE, the combination of LEV and diazepam was superior to either drug alone, even when plasma concentrations were well below the therapeutic range of either drug alone (Mazarati, Baldwin et al. 2004). A retrospective evaluation of human patients with mental retardation and acute repetitive seizures (≥ 3 seizures in 1 hour) or prolonged seizures (a single seizure lasting ≥ 3 minutes) compared the efficacy of rectal diazepam, IV LEV and the combination of both medications. Combination therapy was found to be most effective, with 3/36 patients having recurrence of seizures within 4 hours (the primary endpoint of the study). Patients who received only diazepam had a recurrence rate of 6/24, compared to 10/28 patients in the LEV-only group (Modur, Milteer et al. 2010).

In addition to its antiepileptic effects, LEV has been shown to have neuroprotective effects following prolonged seizures in some studies in rodent models. Reported neuroprotective benefits include preventing mitochondrial dysfunction (Gibbs,

Walker et al. 2006), upregulation of endogenous antioxidants in the brain (Ueda, Doi et al. 2009), and prevention of hypoxia-induced neuronal death in cell culture (Sendrowski, Bockowski et al. 2011). However, other studies have failed to show any neuroprotection from LEV administration (Brandt, Glien et al. 2007; Gibbs and Cock 2007).

Two important reasons for the increasing frequency of LEV use are a high safety profile, and favorable pharmacokinetics. Compared to all other AEDs, LEV has the widest safety margin. Doses as high as 1200mg/kg/day have been shown to cause only mild side effects in long-term oral dosing studies in dogs, including ataxia, sedation, vomiting and diarrhea (data on file UCB Pharma). Multiple canine studies at clinically relevant doses of 20-60mg/kg, both orally and parenterally, have shown that LEV is very well tolerated, with no side effects reported in purpose-bred research dogs (Dewey, Bailey et al. 2008; Patterson, Goel et al. 2008). Similar doses have been reported to be well tolerated in clinical canine patients (Volk, Matiasek et al. 2008).

Levetiracetam pharmacokinetics have been described as close to “ideal” (Perucca and Johannessen 2003). It has 100% bioavailability when given IM or PO (Patterson, Goel et al. 2008); minimal cytochrome P450 metabolism and liver enzyme induction; minimal protein binding; minimal drug-drug interactions; and reaches steady-state concentrations within 1-2 days, given its short half-life (3-4 hours in dogs). However, a recent publication (Moore, Munana et al. 2011) showed changes in LEV pharmacokinetics in dogs receiving typical oral doses of phenobarbital (2-3.3mg/kg twice daily) concurrently with a single oral dose of LEV at 16.7 – 27.8mg/kg. Maximum plasma LEV concentrations decreased from 32.29±6.76 to 18.22±8.97 µg/ml, and

elimination half-life decreased from 3.43 ± 0.47 to 1.73 ± 0.22 hours. They concluded that LEV dosage adjustments may be needed in dogs concurrently receiving PB. Another publication from this same group of researchers showed that repeated oral dosing of LEV resulted in only minor alterations in pharmacokinetics. The terminal half-life increased from 2.87 ± 0.21 hours to 3.59 ± 0.82 hours, and the total area under the concentration-versus-time curve increased from 268.52 ± 56.33 to 289.31 ± 51.68 h· μ g/ml, indicating minimal drug accumulation (Moore, Munana et al. 2010).

As part of their pharmacokinetic research, Patterson and colleagues (2008) investigated an approximately 20mg/kg dose of LEV given IV or IM. Additionally, these researchers intentionally extravasated half of the IV LEV dose in some patients to evaluate for evidence of phlebitis or cellulitis caused by administration of LEV. Pharmacokinetics were similar between IV and IM routes, and caused no necrosis or inflammation at IM injection sites. Furthermore, following intentional extravasation, no damage to the tissues was observed, and other than a slight delay in the time to reach maximum plasma concentration, no significant differences in pharmacokinetics were seen when compared to IV administration.

Other than pharmacokinetics and pre-clinical safety studies, little information is known about LEV use in dogs. The largest description of oral LEV use in clinical patients contained a retrospective component with 8 dogs, and a prospective component of 14 dogs, all of which were pharmaco-resistant to standard therapy with phenobarbital and potassium bromide. Five of eight dogs (62.5%) in the retrospective evaluation were

classified as responders. In the prospective, open-label, uncontrolled portion of the study, 9/14 dogs were classified as responders. After 4-8 months, however, 6/9 dogs had an increase in their seizure frequency (Volk, Matiasek et al. 2008). The only other report of LEV use in a clinical patient is a case report describing the successful use of oral LEV in a dog with refractory seizures secondary to organic aciduria (Platt, McGrotty et al. 2007). There are no reports to date evaluating injectable LEV in clinical patients.

Three projects will be discussed in this thesis. The objective of the first is to describe the prevalence and outcome of seizure emergencies at the University of Minnesota, College of Veterinary Medicine. Project 2 is a prospective, double-masked, placebo-controlled trial evaluating the safety and efficacy of levetiracetam (LEV) in client-owned dogs. Our hypothesis was that LEV would be well tolerated and superior to placebo in stopping seizures in these dogs. Finally, the objective of the third chapter was to describe the safety and pharmacokinetics of subcutaneously administered LEV.

Table 1. Summary of selected studies evaluating the efficacy of IV LEV for SE or ARS in people.

Authors	Patient population	# of patients	Responder Rate	LEV dose	Serious AE
Eue et al 2009	General patients	42	44%	1000-2000mg	None
Gamez-Leyva et al 2009	General patients	34	71%	500-1500mg	None
Alvarez et al 2011	Adult general patients	58	52%	1000-3000mg	Not reported
Abend et al 2009	Critically ill children	10	100%	6.5-31 mg/kg	None
Fattouch et al 2010	Elderly	9	88%	1500mg	None

Aiguabella et al 2011	General population	40	58%	1000-1500mg	None
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Retrospective analysis of status epilepticus and acute repetitive seizures at the University of Minnesota

Chapter Summary

Objective – To describe the prevalence, underlying etiology and outcome of dogs presenting to the University of Minnesota Veterinary Medical Center for status epilepticus (SE) or acute repetitive seizures (ARS).

Design – Retrospective analysis.

Animals – 110 client-owned dogs.

Procedures – The electronic medical record system was searched for dogs presenting to the U of M VMC for a presenting complaint containing “seiz” or “epilep”, between October 1st, 2008 and December 31st, 2010. Underlying etiologies were determined based on clinical testing and/or post-mortem examination.

Results – 110 dogs fulfilled the inclusion criteria, 33 with SE and 77 with ARS. Prevalence was found to be 1.2% of admissions to the ER Service during the study period. A cause for seizures was identified in 53.6% of patients (59/110). Idiopathic epilepsy was the most common diagnosis (n=32; 54%), followed by neoplasia (n=12;

20%), inflammatory (n=6; 10%), ischemic/thrombotic (n=3; 5%), toxin ingestion (n=2; 3%), trauma (n=2; 3%), and 1 (1.7%) dog each had metabolic (hypocalcemia) or post-anesthetic causes. Overall mortality rate was 34.5%, and dogs with SE, inflammatory CNS disease, smaller dogs, and dogs >10 years old were more likely to die or be euthanized. Dogs with idiopathic epilepsy were more likely to be discharged from the hospital.

Conclusions and Clinical Relevance – Seizure emergencies were common reasons for dogs to be presented to the U of M VMC. The mortality rate was high in these dogs, particularly those with inflammatory CNS disease.

Seizure emergencies are common reasons for presentation of dogs to veterinary hospitals. Two large retrospective studies have shown the prevalence of seizure emergencies to be 0.44-0.7% (Bateman and Parent 1999; Zimmermann, Hulsmeyer et al. 2009). Morbidity and mortality from seizures are high in both humans and animals. Mortality reported in the Bateman study was 25.3% and in the Zimmermann study was 38.5%. These are comparable to estimates in human medicine, where overall mortality is reported at 22%, ranging from 8% in children to 38% in the elderly (Chin, Neville et al. 2004). Underlying causes for seizures that have been associated with a poor outcome in dogs include inflammatory central nervous system diseases and cerebral neoplasia (Bateman and Parent 1999; Zimmermann, Hulsmeyer et al. 2009). Other parameters found to be associated with a poor outcome were loss of seizure control at 6 hours of

hospitalization and the development of partial status epilepticus in the Bateman study, while Zimmermann reported a better outcome for dogs with SE caused by intoxication compared to dogs with symptomatic epilepsy. Gender, breed, age at seizure onset, type of seizure activity and findings on CSF analysis were all evaluated. Breeds found to be overrepresented were English Foxhound, Pug, Teacup Poodle, Boston Terrier and Lakeland terrier in the Bateman study, and Golden Retrievers, Beagles and German Shepherd Dogs in the Zimmermann study. Female dogs were underrepresented in both studies, particularly intact female dogs.

The purpose of this study was to evaluate the number of cases of seizure emergencies presenting to the U of MN VMC over a 27 month period, the underlying etiologies causing seizures, and the outcomes of these cases.

Materials and Methods

Medical records of dogs presenting to the Emergency Service of the University of Minnesota Veterinary Medical Center between October 1st, 2008 and December 31st, 2010. The medical record database was searched for a presenting complaint containing “seiz” (to include seizure, seizures, and seizuring) or “epilep” (to include epilepsy, epileptic, and epilepticus). There were 299 dogs with a presenting complaint that fit the search criteria, with 110 of these dogs having documented status epilepticus (SE) and/or acute repetitive seizures (ARS; commonly referred to as cluster seizures in veterinary medicine).

Status epilepticus was defined as a single seizure lasting >5 minutes or 2 or more seizures without regaining consciousness in between. Acute repetitive seizures were defined as 3 or more seizures in the 12 hours prior to presentation to the ER (Bleck 1999; Lowenstein, Bleck et al. 1999; Beran 2008; Gilad, Izkovitz et al. 2008).

Information recorded for each case was breed, gender, age at the time of presentation, age at the time of seizure onset, body weight, survival to discharge, underlying etiology, whether the patient had ARS or SE, and whether the patient was hospitalized.

Underlying etiology was based on clinical diagnosis at the time of admission, or by retrospective evaluation of the tests performed and necropsy findings, when available. For statistical analysis, etiology was classified as idiopathic epilepsy (based on a characteristic age of onset, normal interictal interval, and lack of abnormalities on diagnostic testing), inflammatory CNS disease (defined as increased cellularity and/or increased protein concentration on CSF analysis; characteristic changes on MRI, and/or findings at necropsy), neoplastic (either primary intracranial neoplasia or metastatic/invasive from other primary tumor sites), ischemic/thrombotic, traumatic, toxic, metabolic, or post-anesthetic. In cases where insufficient testing was performed to make a confident diagnosis, the etiology was classified as unknown.

Statistical Analysis

A commercially available software package was used for statistical analysis (SAS v9.2). Data were tested for normality by the Kolmogorv-Smirnov test. All data were

found to be non-normally distributed, therefore they are presented as median (range). The Mann-Whitney *U*-test was used to make comparisons between age and body weight. A $p < 0.05$ was considered significant. Proportions were compared by use of the Fisher's exact test, and the Bonferroni correction ($p < 0.05/n$, where n = the number of levels being tested) was applied when testing within levels of individual variables was performed.

Results

Signalment – During the study period, 110 dogs were presented to the ER for seizure emergencies, 33 for SE and 77 for ARS. This equates to 1.2 cases of SE, and 2.9 cases of ARS per month during the study. Forty seven different breeds were included in this study, with Labrador retrievers being most common ($n=18$). The other breeds with ≥ 3 dogs included were mixed breed ($n=15$), golden retriever ($n=5$), Boston terrier ($n=4$), German shepherd dog ($n=4$), boxer ($n=3$), cocker spaniel ($n=3$), miniature dachshund ($n=3$), pit bull ($n=3$), pug ($n=3$), and Shetland sheep dog ($n=3$). Sex distribution of 110 dogs was 43.6% spayed female ($n=48$), 7.6% intact female ($n=8$), 43.6% castrated male ($n=48$) and 5.5% intact male ($n=6$). The median age at the time of presentation for all dogs was 6.5 years (range 0.25 – 15 years), and the median age at seizure onset was 5 years (range 0.25 – 15 years; Table 2).

Clinical Findings – The prevalence of ARS or SE for all admissions to the ER during the study period was 1.24%. Of the 110 dogs presented for seizure emergencies, 81 were hospitalized in the intensive care unit. Sixteen dogs were euthanized

immediately after presentation, 11 dogs were treated on an outpatient basis at the owners' request, and 2 dogs died en route to the hospital.

Etiology for seizures was identified in 54.5% of patients (60/110). Idiopathic epilepsy was the most common diagnosis (n=32; 54%), followed by neoplasia (n=12; 20%), inflammatory (n=7; 12%), ischemic/thrombotic (n=3; 5%), toxin ingestion (n=2; 3%), trauma (n=2; 3%), and 1 (1.7%) dog each had metabolic (hypocalcemia) or post-anesthetic causes for their seizures.

Inflammatory CNS disease was confirmed at necropsy on 5 of the 7 dogs (2 dogs had compatible imaging and CSF changes, but no necropsy performed). Histopathology revealed 2 dogs had granulomatous meningoencephalitis, 2 dogs had necrotizing lymphohistiocytic meningoencephalitis ("pug dog encephalitis"), and the fifth dog had mixed inflammatory infiltrates (lymphoplasmacytic and eosinophilic meningoencephalitis).

Overall mortality in this group of dogs was 34.5% (38/110). Six dogs died, while 32 dogs were euthanized at the owners' request. Mortality associated with each of the underlying etiologies is shown in Table 3. When the Bonferroni correction was applied, inflammatory CNS disease was significantly more likely to be associated with death or euthanasia compared to the other etiologies ($p=0.0004$). Dogs diagnosed with idiopathic epilepsy were significantly more likely to survive ($p=0.0001$).

Neither the median age at the time of presentation nor the median age at seizure onset were significantly different between dogs that survived compared with those that did not ($p<0.78$ and $p<0.29$, respectively). Body weight was significantly higher in dogs

that survived compared to those that did not ($p < 0.0081$). Dogs with inflammatory CNS disease (median=7.8kg, range 1.7-9.6kg) weighed significantly less than dogs with other underlying etiologies (median=22kg, range 1.3-70kg; $p < 0.004$). Mortality rate was significantly higher for geriatric dogs (defined as ≥ 10 years of age [$p < 0.0401$]). 46.4% of geriatric dogs died or were euthanized, compared to 30.5% of dogs < 10 years old. In-hospital mortality was significantly more likely for dogs with SE (67%, 22/33 dogs died/euthanized) compared to dogs with ARS (20.7%, 16/77 dogs died/euthanized [$p < 0.0001$]).

Discussion

The prevalence of seizure emergencies was 1.2% of admissions to the ER during the study period. This prevalence is comparable to previous studies that showed prevalence of 0.44-0.7% of total hospital admissions, and in our hospital constitutes approximately one case per week.

Mortality associated with SE/ARS of 34.5% in this study is also comparable to the Bateman and Zimmermann studies, which showed mortality rates of 25.3 and 38.5%, respectively. As was demonstrated in those studies, we found inflammatory CNS disease to be associated with a poor outcome, and the etiology most likely to cause death (all 3 dogs that died with a known etiology had underlying inflammatory CNS disease). Mortality for dogs with SE/ARS caused by inflammatory CNS disease was 100% in both

this study and the Bateman et al study, suggesting that dogs with this etiology presenting for SE/ARS have a grave prognosis.

Inflammatory CNS disease has been hypothesized to have a poor prognosis due to the high risk of herniation due to inflammatory cell infiltrates and edema formation and subsequent intracranial pressure changes. Infiltration of inflammatory cells into areas of the brainstem that control cardiovascular and respiratory function may also lead to cardiopulmonary arrest (Bateman 1999).

Smaller dogs in this study were found to have a poorer outcome. The most likely explanation for this finding is due to smaller breeds of dogs being predisposed to inflammatory CNS disease, which has been associated with poor outcome in this and previous studies (Bateman and Parent 1999; Zimmermann, Hulsmeyer et al. 2009). All of the dogs with inflammatory CNS disease weighed less than 10kg in this study, weighed less than dogs without inflammatory CNS disease, and included 6 different breeds (dachshund, shih tzu, pug, chihuahua, rat terrier and shiba inu), many of which are reported to be at increased risk for inflammatory CNS disease (Talarico and Schatzberg 2010). The reason for the poorer outcome in older dogs (>10 years old) is not clear from the medical records. Many of the older dogs (21/28) did not have a thorough workup, and were classified as “unknown” etiology, and were either treated symptomatically or euthanized due to perceived poor prognosis. Of the 7 dogs that had a definitive diagnosis, 5 were diagnosed with intracranial neoplasia, and 2 had previously diagnosed idiopathic epilepsy. Geriatric humans with SE/ARS also have a higher mortality rate.

Towne et al (Epilepsia 1994) showed that for people >60 years old, mortality was 38%, and for people >80 years old, mortality rose to 50%.

The retrospective nature of this study imparted some limitations. Medical records were not always uniformly recorded, making extraction of all relevant information for each case impossible. This may have also led to a failure to identify and include cases that met the inclusion criteria. In many cases, the exact number and duration of seizures was frequently not recorded, or clients were unsure of the information. Nearly one half of the cases included did not have adequate workup to allow for a confident diagnosis. Many animals did not have long-term follow-up information in the medical record, so this was not analyzed.

In conclusion, the results of this study indicate that seizure emergencies present to the University of Minnesota Veterinary Medical Center ER on average once weekly. Prevalence and mortality rate are comparable to other previously published studies, and inflammatory CNS disease carries a grave prognosis.

Table 2. Selected baseline characteristics of dogs presented to the University of MN for SE/ARS.

All Dogs	
Parameter	median
Age at presentation	6.54
Age Seizure onset	5
Weight	22.8
Gender	% of Total
Female intact	7.3 (n=8)
Female spayed	43.6 (n=48)
Male intact	5.5 (n=6)
Male castrated	43.6 (n=48)

Table 3. Selected baseline characteristics of survivors vs non-survivors, and overall mortality rates, mortality rates based on underlying etiology, and mortality rates based on seizure type. †significant difference (Bonferroni corrected $p < 0.0056$).

	Survivors	Non-survivors	p-value		
Age at presentation	7.0 (0.25 – 15)	6.5 (0.25 – 15)	0.78		
Age Seizure onset	5.0 (0.25 – 15)	6.0 (0.25 – 15)	0.29		
Weight (median[range])	22.9 (1.3 – 70.0)	10.8 (1.7 – 54.3)	0.0081†		
Etiology					
Etiology category	N	Lived	Died/euth	% mortality	p-value
Inflammatory	7	0	3/4	100	0.00004†
Idiopathic epilepsy	32	30	0/2	6.25	0.0001†
Neoplastic	12	11	0/1	8.3	0.0545
Ischemic/thrombotic	3	1	0/2	66.6	0.27
Toxin	2	1	0/1	50	1.0
Trauma	2	1	0/1	50	1.0
Hypocalcemia	1	1	0/0	0	1.0
Post-anesthesia	1	1	0/0	0	1.0
Undetermined	51	26	3/22	49.0	0.0046
Totals	110	72	6/32 = 38 (34.5% mortality)		
Seizure type and outcome					
Seizure type	Lived	Died/euth	% mortality		
SE	11	3/19	67%		
ARS	61	3/13	20.7% ($p < 0.0001$)		

Double-masked, placebo-controlled study of intravenous levetiracetam for the treatment of status epilepticus and acute repetitive seizures in dogs

Chapter Summary

Background: Status epilepticus (SE) and acute repetitive seizures (ARS) are common canine neurologic emergencies. No evidence-based studies are available to guide therapy in veterinary patients. Parenteral levetiracetam has many favorable properties for the emergency treatment of seizures, but its safety and efficacy in dogs for SE and ARS are unknown.

Hypothesis: Intravenous levetiracetam is superior to placebo in controlling seizures in dogs with SE or ARS after treatment with IV diazepam.

Animals: Nineteen client-owned dogs admitted for SE or ARS.

Methods: Randomized, placebo-controlled, double-masked study. Dogs with SE or ARS were randomized to receive IV levetiracetam (30 or 60 mg/kg using an adaptive dose-escalation approach) or placebo, in addition to standard of care therapy. They were monitored for at least 24 hours after admission for additional seizures.

Results: The responder rate (defined as dogs with no additional seizures after administration of the study medication) after levetiracetam was 56% compared to 10% for placebo (p=0.06). Dogs in the placebo group required significantly more boluses of diazepam compared to the levetiracetam group (p<0.03). Seizure etiologies identified were idiopathic epilepsy (n=10), inflammatory CNS disease (n=4), intracranial neoplasia (n=2), hepatic encephalopathy (n=1) and 2 dogs had no cause determined. No serious adverse effects were attributable to levetiracetam administration.

Conclusions and Clinical Importance: Levetiracetam was safe and potentially effective for the treatment of SE and ARS in these client owned dogs. Larger, controlled clinical trials are needed to confirm this preliminary observation.

Abbreviations:

AED – antiepileptic drug

ARS – acute repetitive seizures

CNS – central nervous system

CSF – cerebrospinal fluid

LEV – levetiracetam

MRI – magnetic resonance imaging

PB – Phenobarbital

SE – Status epilepticus

Seizure emergencies such as status epilepticus (SE) and acute repetitive seizures (ARS) are common reasons for presentation to veterinary emergency clinics (Saito, Munana et al. 2001; Platt and Haag 2002). The definitions of SE and ARS have evolved over the last several decades. The duration of seizure activity for a patient to be considered in SE most frequently is defined as seizure activity lasting 5 minutes or longer. ARS is less well defined, but has been described as 2 or more seizures in a 5-12 hour period, differing from the patient's usual seizure behavior (Bleck 1999; Beran 2008; Gilad, Izkovitz et al. 2008). ARS also is commonly referred to as cluster seizures in veterinary medicine. Morbidity and mortality from SE and ARS is high in both humans and dogs. In the largest studies to date in veterinary medicine, the mortality rate was 25.3 to 38.5% (Bateman and Parent 1999; Zimmermann, Hulsmeier et al. 2009) . In humans, SE-associated mortality estimates typically range from 3 to 40% (Chin, Neville et al. 2004), depending on the population evaluated, but overall mortality is approximately 22% (DeLorenzo, Hauser et al. 1996), similar to that seen in dogs. The optimal treatment for SE in humans and dogs is unknown. Despite the large number of cases, few well-controlled studies have been performed evaluating the efficacy of available medications in humans (Leppik, Derivan et al. 1983; Treiman, Meyers et al. 1998; Alldredge, Gelb et al. 2001). To our knowledge, no published studies exist in dogs.

An injectable formulation of levetiracetam^a (LEV) was approved in 2006 for use as bridge therapy in patients unable to take oral medications. Since then, its off-label use in humans has been reported (Fattouch, Di Bonaventura et al. 2010; Alvarez, Januel et al.

2011) as has its use in dogs for the treatment of refractory SE, but no prospective, blinded studies have been conducted. The mechanism of action of LEV is not completely understood, but it is thought to act by binding to the synaptic vesicle 2a protein on the presynaptic terminal, and modulating synaptic vesicle fusion and neurotransmitter release (Xu and Bajjalieh 2001; Lynch, Lambeng et al. 2004). There are a number of other purported mechanisms that may inhibit epileptic activity, including indirect effects on GABAergic neurotransmission, inhibiting the Na^+ -dependent $\text{Cl}^-/\text{HCO}_3^-$ exchanger, and modulation of K^+ and high-voltage Ca^{2+} channels (De Smedt 2007). Levetiracetam has a favorable pharmacokinetic profile. It is relatively rapidly and extensively absorbed via PO and IM routes; readily crosses the blood-brain barrier; is minimally protein-bound (<10%); is primarily eliminated by renal excretion (66% unchanged in the urine, 24% is metabolized by enzymatic hydrolysis); has no known drug interactions; and, follows linear kinetics. A number of studies have shown that LEV is safe in healthy dogs at dosages up to 60 mg/kg IV (Patterson, Goel et al. 2008), but no reports of parenteral LEV use in clinical cases have been published to date. The major limitation to chronic LEV treatment in veterinary patients is that it has a relatively short plasma half-life of 3-4 hours (Dewey, Bailey et al. 2008; Patterson, Goel et al. 2008). However, in rats, LEV half-life in the brain and CSF is 1.5-2 times longer than the plasma half-life, and it is thought to maintain higher concentrations in the brain in humans (Doheny, Ratnaraj et al. 1999; Tong and Patsalos 2001).

The primary aim of this pilot study was to evaluate the efficacy and safety of IV LEV compared to placebo in client-owned dogs with SE or ARS. We hypothesized that LEV

treatment would be superior to placebo in stopping seizure activity in dogs with SE/ARS after treatment with IV diazepam.

Materials and Methods

Dogs

Nineteen client-owned dogs with SE or ARS presented to the Veterinary Medical Center (VMC) of the University of Minnesota (UMN) between October 2007 and March 2010 were included in the study. This study was approved by the UMN Institutional Animal Care and Use Committee (#0905A65361). Informed consent was obtained from all owners before enrollment in the study. Dogs were eligible for inclusion if they had SE or ARS. Status epilepticus was defined as a single seizure lasting longer than 5 minutes or 2 or more seizures without completely regaining consciousness between seizures. Acute repetitive seizures were defined as 3 or more seizures in a 12-hour period in the 24 hours before presentation. Dogs were excluded if they were azotemic with a urine specific gravity < 1.030, hypoglycemic (blood glucose < 60 mg/dl) or hypocalcemic (total Ca < 9.3 mg/dl or ionized Ca < 5.1 mg/dl).

Study Design

This was a randomized, placebo-controlled, double-masked study. Dogs that presented to the UMN VMC for SE or ARS were eligible for inclusion in the study. Animals were enrolled only if they had another seizure while hospitalized, or were actively seizing on

presentation. At the time of an additional seizure in the hospital, dogs were treated with IV diazepam^b (0.5 – 1 mg/kg) as soon as possible after the in-hospital seizure, and either LEV or placebo also was administered IV no longer than 2 hours later. LEV and placebo were stored in sequentially numbered vials with no other labeling.

The LEV therapy was based on adaptive dose-escalation approach. Dogs were randomized to receive LEV or placebo in permuted blocks of 4. By protocol, the first 10 dogs received 30 mg/kg of LEV (n=5) or an equivalent volume of 0.9% saline^c (n=5). Because no adverse effects were seen at the planned interim analysis of the first 10 patients, and a higher dosage (60 mg/kg) was found to be well tolerated in healthy dogs¹⁹, the dosage was increased to 60 mg/kg LEV (n=4) or an equivalent volume of 0.9% saline (n=5) for the last 9 dogs. The solution was administered undiluted as a slow bolus over 5 minutes. Blood samples were collected in EDTA tubes at 15, 45 and 180 min after administration of the study solution. Subsequently, plasma was harvested and stored at -20°C until analysis for plasma LEV concentrations. If seizures continued or recurred anytime after administration of the study solution, dogs were treated at the clinicians' discretion, according to hospital guidelines for the emergency treatment of seizures, which included recommended treatment with a diazepam constant rate infusion (CRI), followed by IV phenobarbital^d (PB) if needed, and later with IV propofol or IV pentobarbital if seizures continued.

The primary endpoint was whether dogs were “responders” or not. A responder was defined as a dog that had no additional seizures after administration of the study solution for the next 24 hours. Secondary endpoints were the number of seizures until 24 hours seizure-free, the number of hours until 24 hours seizure-free, the number of episodes of SE in hospital, the percent all-cause mortality while hospitalized, length of hospitalization, number of bolus injections of diazepam given, percentage of dogs receiving a CRI of diazepam, duration of diazepam CRI, percentage of dogs receiving IV PB, dose of IV PB, percentage of dogs receiving either propofol or pentobarbital treatment, hours of propofol or pentobarbital treatment, and proportion of dogs that experienced vomiting, diarrhea, ataxia, or decreased alertness.

Before this study, power analysis based on estimated variances indicated that 46 patients would be necessary to detect a 40% difference in responder rate with 80% power. 20 dogs were planned to be included in this pilot study.

Drug and Pharmacokinetic Analyses

Plasma LEV concentration was measured by high-performance liquid chromatography using a previously described method (Dewey, Bailey et al. 2008; Patterson, Goel et al. 2008). Pharmacokinetic parameters were determined by non-compartmental analysis using commercially available software (WinNonLin, Version 5.2)^e. Clearance, distribution volume, and elimination half life calculations were done by standard computation procedures assuming first order elimination.

Statistical Analyses

Statistical analysis was performed using a commercially available software program (SAS, version 9.2)^f. Data was analyzed for normality with the Kolmogorov-Smirnov test, and all data were found to be non-parametric. Fisher's exact test was used to compare the number of responders vs non-responders, the percentage of all-cause mortality, the percentage of dogs receiving diazepam CRI, the percentage of dogs receiving propofol or pentobarbital therapy, and the percentage of dogs that experienced vomiting, diarrhea, ataxia or decreased alertness. The Wilcoxon rank sum test was used to compare the median number of seizures until 24 hours seizure-free, the number of hours until 24 hours seizure-free, the number of episodes of SE, the length of hospitalization, the number of bolus injections of diazepam given, the duration of diazepam CRI, and the duration of propofol or pentobarbital treatment between the LEV and placebo groups.

Results

Study Population

Nineteen dogs were included in this study. Twenty cases were planned, but due to budgetary reasons the 20th case was not enrolled. Twelve additional dogs initially were eligible and owner consent was obtained, but failed to have subsequent seizure activity in the hospital, and therefore were not enrolled. For the 19 enrolled cases, there were 10

female (7 spayed and 3 intact) and 9 male (7 castrated and 2 intact) dogs. The most common breeds were Boston Terrier and Golden Retriever, with 3 patients of each breed.

There were 2 Labrador Retrievers, and 1 of each of the following breeds: American Staffordshire terrier, Basset hound, Boxer, English setter, Great Pyrenees, Jack Russell terrier, Maltese, Miniature Schnauzer, Old English sheepdog, Standard Poodle, and mixed breed.

There was no significant difference between the LEV and placebo groups in median age, body weight, number of seizures in the 12 hours before presentation, historical seizure onset, at-home diazepam therapy before presentation or previous PO AED therapy (Table 4).

Idiopathic epilepsy was the most common diagnosis in both groups (LEV n=6; placebo n=4). Idiopathic epilepsy was defined as seizure onset from 1-6 years of age, normal neurological status between seizures, normal biochemistry results and no abnormalities detected on imaging of the brain and in CSF analysis (when available) (Thomas 2010). Two dogs in the placebo group had brain tumors confirmed later (confirmed glioma in 1 dog based on post-mortem examination, presumed glioma based on MRI findings in the other dog). Three dogs in the placebo group and 1 dog in the LEV group had inflammatory CNS disease (1 granulomatous meningoencephalitis, 2 lymphoplasmacytic meningitis, and 1 neuronal necrosis). One dog in the LEV group had hepatic cirrhosis and hepatic encephalopathy. No etiology was determined in 2 dogs, 1 in each group. The dog in the LEV group was a 9-year old, spayed female Labrador retriever that had acute onset of seizures and a history of pleural effusion of unknown etiology. No

additional diagnostic tests were performed, the dog had no more seizures, and was discharged from the hospital. No follow-up information was available. The dog in the placebo group was a 6-year old spayed female standard poodle that had no previous seizure history. Additional diagnostic tests were declined by the owners, who elected humane euthanasia after 21 hours of hospitalization. The dog had no additional motor seizures, but continued to vocalize and have abnormal mentation, and necropsy was declined after euthanasia.

Primary and Secondary Outcomes

In this study, 5/9 (56%) dogs in the LEV group responded to therapy, compared to only 1/10 (10%) in the placebo group ($p = 0.06$). There was no obvious difference when comparing the number of responders for the 30 and 60 mg/kg LEV doses: 3/5 (60%) and 2/4 (50%), respectively. In all 19 cases, LEV or placebo was administered within 30 minutes after the seizure that completed eligibility for final enrollment in this study. Administration of LEV or placebo occurred a median of 3 hours (range, 0.75-8.25 hours) after admission to the hospital. Survival to discharge was not significantly different between the 2 treatment groups. In the LEV group, 2/9 (22%) dogs died ($n=1$) or were euthanized ($n=1$), and in the placebo group, 4/10 (40%) dogs were euthanized ($p = 0.6$). Dogs in the placebo group received significantly more IV boluses of diazepam (median, 2; range 1-6) than the dogs in the LEV group (median, 0; range, 0-2; $p < 0.03$). 18/19

dogs received PB while hospitalized, but there was no difference in the median dose between the 2 treatment groups. No dogs received pentobarbital or propofol treatment.

All dogs that were euthanized were considered stable but continued to have seizure activity, or were moderately sedated due to administration of more than one AED or both, and usually were euthanized for financial reasons. There were no significant differences between groups for any of the other secondary endpoints (Table 5).

Regardless of treatment group, 5/6 (83.3%) dogs that were classified as responders were diagnosed with idiopathic epilepsy. No diagnosis was determined in the 6th dog due to lack of diagnostic evaluation permitted by the owners. In contrast, 5/13 (38.5%) dogs in the non-responder group were diagnosed with idiopathic epilepsy. There were no significant differences between the median age, weight, number of seizures in the 12 hours before presentation, proportion of dogs receiving previous AED therapy, initial diazepam dose or serum PB or serum KBr concentrations of responders compared to non-responders (Table 6).

Adverse Effects

Ataxia was seen in 3/9 LEV-treated and 1/10 placebo-treated dogs, and decreased alertness was seen in 4/9 LEV-treated and 4/10 placebo-treated dogs. All dogs with ataxia also had decreased alertness. All but 1 of the dogs with ataxia, decreased alertness or both also received PB IV after the study injection. The 1 dog that was not treated with

PB was in the LEV group, and had been treated with diazepam CRI, and was in continuous SE for 5 hours despite therapy, until euthanasia. Of the 8 dogs that had decreased alertness, 2 had been treated with long-term PO PB before presentation, 3 had inflammatory CNS disease, and 1 had hepatic encephalopathy.

One dog in each group had both vomiting and diarrhea. The dog in the LEV group was in liver failure and had received a lactulose and neomycin-containing enema before developing diarrhea. The 1 dog in the placebo group was diagnosed with a presumed glioma based on MRI findings, and the cause for its vomiting and diarrhea was not apparent.

The only dog to die in this study was in the LEV group. Death occurred approximately 46 hours after administration of 30 mg/kg LEV. After LEV therapy, this dog also received PB (4 mg/kg/d IV) and diazepam CRI (1.2 mg/kg/h) for 21 hours. The cause of death based on postmortem examination was lymphoplasmacytic meningitis, with vascular and cerebrocortical necrosis.

Pharmacokinetics

Mean plasma LEV concentration-time data are shown in Figure 1. At all time points, LEV concentrations after both the 30 and 60 mg/kg doses were within or exceeded the proposed human therapeutic range of 5-45 µg/ml (Patsalos 2000; Bazil 2002). For the dogs that received the 30 mg/kg dose, the mean \pm standard deviation plasma LEV

concentrations were 57.0 ± 17.8 , 39.0 ± 14.1 and 21.4 ± 5.0 $\mu\text{g/ml}$ at 15, 45 and 180 minutes, respectively. For the dogs that received the 60 mg/kg dose, the mean plasma LEV concentrations were 141.4 ± 56.3 , 118.6 ± 61.6 and 61.7 ± 68.0 $\mu\text{g/ml}$ at 15, 45 and 180 minutes, respectively. Plasma LEV concentrations were dose proportional and the mean CL, VD, and half-life estimates for the 30 mg/kg and 60 mg/kg doses were similar: CL = 2.75 ml/min/kg vs 2 ml/min/kg, Vd = 0.5 L/Kg vs 0.4 L/Kg, and half-life = 2.2 hrs vs 2.3 hrs.

Discussion

The treatment of seizure emergencies in dogs is based on the results of clinical experience and uncontrolled case series and trials. Our study represents the first randomized, double-masked, placebo-controlled trial for the treatment of SE or ARS in a clinical population. Despite the small sample size, IV LEV in addition to IV diazepam treatment showed a trend toward superiority over placebo and IV diazepam for the treatment of SE or ARS in dogs.

LEV was well tolerated by the dogs in this study. It was not possible to separate ataxia and decreased alertness as a result of seizure activity and the administration of other drugs commonly known to cause these effects from the potential effects of LEV, therefore some contribution from LEV is possible. The only dog that died was in the LEV group. However death was unlikely to have been related to LEV administration, because it occurred 46 hours after administration (10-12 half-lives had elapsed by that point).

Furthermore, this dog was diagnosed with inflammatory and necrotizing CNS disease, a seizure etiology commonly associated with death.

There were no serious adverse effects attributable to LEV therapy, and rapid infusion of large doses of undiluted LEV were well tolerated. Based on these results in clinical patients and the very wide therapeutic index of LEV, clinicians may consider IV LEV as adjunctive therapy for the treatment of SE or ARS in dogs, because it appears unlikely to lead to or result in any clinically relevant adverse events, and it is potentially effective.

The pharmacokinetics of parenteral LEV have been evaluated in healthy dogs (Patterson, Goel et al. 2008). LEV doses used in these studies were approximately 20 mg/kg (Patterson, Goel et al. 2008) and 60 mg/kg (Dewey, Bailey et al. 2008) with maximum plasma concentrations of 30.3 $\mu\text{g/ml}$ and 254 $\mu\text{g/ml}$, respectively. These concentrations cannot be directly compared because LEV infusion rates and initial blood sampling times were different in the 2 studies [LEV infused over 2 minutes, and 2 minutes post-injection sampling time (Dewey, Bailey et al. 2008), and LEV infused over 5 minutes, and 15 minutes post-injection sampling time (Patterson, Goel et al. 2008)]. The pharmacokinetic parameters obtained in our study are consistent with the results from the studies in healthy animals, except for the half-life of 2.2-2.3 hours in clinical patients, compared to 3-4 hours in healthy dogs. Concurrent, chronic PB administration appears to decrease the plasma half-life of LEV administered PO from 3.43 h to 1.73 h in experimental dogs (Moore, Munana et al. 2011). Five of the 9 patients in the LEV group

had been receiving long-term PB therapy PO before presentation for SE or ARS. Despite this shortened half-life, plasma LEV concentrations are predicted to be within the proposed therapeutic range for approximately 9 hours when a 60 mg/kg dose is administered.

An evidenced-based standard of care for SE and ARS has not been established in veterinary medicine. Benzodiazepines are most commonly used as a bolus for first-line therapy or, for refractory cases, as a CRI (Indrieri 1989). Phenobarbital typically is the second drug of choice, followed by oral or rectal KBR or IV NaBr. Other treatments that have been recommended for treatment of seizure emergencies in dogs that do not respond to initial treatment include pentobarbital, propofol, phenytoin, isoflurane, and ketamine (Serrano, Hughes et al. 2006), but there is not any published data to help differentiate which is most effective.

Guidelines for the treatment of SE or ARS in humans are based on only a few well-controlled studies. The best evidence exists for the use of lorazepam as initial therapy. It has been shown to be superior to phenytoin and placebo, and comparable to diazepam, PB and combination therapy of phenytoin and diazepam (Leppik, Derivan et al. 1983; Treiman, Meyers et al. 1998; Alldredge, Gelb et al. 2001). If seizures continue despite benzodiazepine therapy, administration of IV phenytoin or fosphenytoin is commonly recommended. If necessary, IV valproic acid or LEV are recommended as third-line therapy, but no studies have proven their efficacy. If a patient fails to respond to these drugs, treatment with PB, or induction of anesthesia with pentobarbital, propofol or

midazolam, the last 2 given as a CRI, is recommended (Vernau and LeCouteur 2009). Ketamine also is used in some refractory cases (Steffen and Grasmueck 2000; Serrano, Hughes et al. 2006).

Definitions of SE and ARS are not uniformly accepted. SE is commonly defined as a single seizure lasting from 5-30 minutes, depending on the publication. The definition of ARS is even more variable than that of SE. Most commonly, a range of increased seizure activity compared to normal for the patient over 5-12 hours has been used in human studies to define ARS (Bleck 1999; Beran 2008; Gilad, Izkovitz et al. 2008). However, a duration of increased seizure activity of 24 hours has been used previously (Cranford, Leppik et al. 1979). In the current study, ARS was defined as 3 or more seizures in a 12-hour period in the 24 hours before presentation. The goals for the definition used were to maximize the number of eligible cases, without including dogs that were unlikely to continue to seizure. We also acknowledge that in veterinary medicine, owners may live several hours away from a referral institution, and that dogs may have spent time at the referring veterinarian's clinic before presentation, prolonging the time from seizure activity to presentation to the University of Minnesota.

The outcome of dogs with SE has been evaluated in 2 large studies. Both studies found that symptomatic epilepsy (i.e. seizures caused by a lesion in the brain, most commonly inflammatory CNS disease or brain tumor) is associated with higher mortality (Bateman and Parent 1999; Zimmermann, Hulsmeyer et al. 2009). In our study, the placebo group contained 5 dogs with symptomatic epilepsy, compared with only 1 dog in the LEV group ($p=0.14$), which may have confounded the statistical results for this

limited number of patients. Three of the 5 dogs in the placebo group were euthanized, and the 1 dog in the LEV group died.

No significant differences were detected between the dogs that were classified as responders, compared to those that did not respond (Table 3). Dogs in the non-responder group had higher PB concentrations, but this difference was not significant ($p=0.07$). Additionally, dogs appeared less likely to respond if they presented with SE. Only 1/6 dogs in the responder group presented for SE, compared to 6/9 dogs in the non-responder group presented for SE ($p=0.33$). Phenobarbital concentrations were not always determined at the time of presentation, and dogs in the non-responder group may have received additional injectable or PO PB administration before blood collection, due to persistent seizure activity. Partial SE has been correlated with poor outcome in 1 previous study (Bateman and Parent 1999), and with larger patient numbers, the presence of SE may have been associated with a lower response rate. Dogs with idiopathic epilepsy were more often classified as responders (5/6 responders [83.3%] were diagnosed with idiopathic epilepsy vs 5/13 [38.5%] of non-responders). Dogs with idiopathic epilepsy were shown to have better survival than dogs with symptomatic epilepsy in a large retrospective analysis of SE (Zimmermann, Hulsmeyer et al. 2009), and our results further support these findings.

A secondary goal of this study was to further establish the dog as a viable clinical model of human SE and ARS. The canine model is gaining support for many reasons. It is a naturally-occurring condition, not an artificially-induced model, and the body size of dogs is closer to that of humans, compared to rodent models, making pharmacokinetic

data potentially more relevant for developing dosing regimens (Leppik, Patterson et al. 2009). Another benefit of the dog model is the ability to obtain informed consent from pet owners. In human SE, it is impossible to obtain consent from a person during a seizure, although patient representatives can provide consent, if available. Exceptions from informed consent for emergency research are possible, but must meet strict criteria. One of these criteria is evidence of a benefit of the therapeutic intervention in animal studies (FDA §50.24). Evidence from studies in dogs could provide a vital intermediate step in transitioning from research in rodent models to applications in humans.

Despite the prospective nature of this study, it had some limitations. Not all cases were managed by the same clinician and therefore there was some variation in treatments received (other than LEV); there was a relatively small number of cases with resulting low power (power calculations performed after completion of the study, assuming 56% responder rate for dogs in the LEV group and 10% responder rate in the placebo group, showed the need for 16 dogs in each group to achieve 80% power); despite randomization, there was likely a clinically important difference in the seizure etiology between groups; in 2 cases, a final diagnosis was not determined due to client or patient limitations; and finally, EEG data could not be collected, making it impossible to know if there was true cessation of abnormal CNS electrical activity or simply cessation of motor activity (so-called non-convulsive SE, a common sequela to convulsive SE in humans (DeLorenzo, Waterhouse et al. 1998)). Similarly, the small sample size prevented adequate exploration of dose (or concentration)-response relationships.

LEV doses below 100 mg/kg IV have been shown to be well tolerated in healthy dogs (data on file, UCB Pharma). Future areas for research include determining the maximum safe dosage of IV LEV for clinically affected dogs, evaluating timing of therapy relative to use of benzodiazepines or other AEDs, determining efficacy and safety of single versus multiple doses of LEV, and the most effective dosing interval.

The results of this study suggest that LEV is a safe, potentially effective drug for the treatment of SE or ARS in dogs. Additional studies involving a larger number of dogs and use of various LEV dosing regimens are indicated.

Table 4. Comparison of selected pre-treatment characteristics between the 2 treatment groups. Data presented as median (range).

	LEV group	Placebo group	p-value
Age (years)	3 (1.5 – 12.0)	5.05 (0.2 – 11.5)	0.68
Weight (kg)	30.0 (5.2 - 43.0)	11.7 (3.4 - 42.0)	0.11
Seizure onset (mos prior)	10 (0.1 - 54)	3.5 (0.1 - 90)	0.84
# seizures in 12h before presentation	3 (2 - 9)	5 (1-15)	0.34
Previous oral AED therapy	4/9	5/10	0.66
At home/rDVM	1/9	4/10	0.30

diazepam therapy before presentation			
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LEV, levetiracetam; AED, antiepileptic drug; rDVM, referring veterinarian.

Table 5. Comparison of primary and secondary endpoints between treatment groups.

Data presented as median (range) or proportion.

	LEV group	Placebo group	p-value
Primary Endpoints			
Responder	5/9	1/10	0.06
Secondary Endpoints			
In-hospital mortality	2/9	4/10	0.6
# seizure until 24h seizure free	0 (0-8)	1.5 (0-2)	0.3
# hrs until 24h seizure free	24 (24-48)	30.5 (24-36)	0.2
# episodes of SE	0 (0-2)	0 (0-0)	0.2
Duration of hospitalization (hrs)	28 (5-72)	31.5 (12-48)	0.9
Duration of hospitalization	28 (22-72; n=7)	40 (31-48; n=6)	0.4

(hrs) survivors only			
Initial diazepam dose (mg/kg)	0.50 (0.35 – 1.24)	0.49 (0.28 – 1.14)	0.5
# diazepam boluses	1(0-2)	2 (1-6)	0.03
Received diazepam CRI	3/9	1/10	0.3
Duration of CRI (hrs)	10 (4-21; n=3)	16 (16-16; n=1)	1.0
# seizures 1st 24h	0 (0-8)	1 (0-4)	0.7
Received IV PB	8/9	10/10	0.5
PB dose 1st 24h (mg/kg)	4.8 (0 - 15.8)	10.1 (3.4 - 18.0)	0.08

CRI, continuous rate infusion; PB, phenobarbital.

Table 6. Comparison of selected parameters between dogs classified as responders or non-responders. Data presented as median (range) or proportion.

	Responders	Non-responders	p-value
Age (y)	3.55 (3-9)	6 (0.2 – 12)	0.8
Weight (kg)	30.6 (21.3 – 40.9)	10.2 (3.4 – 35.5)	0.1
# seizures in 12h	3 (2-9)	3 (1-15)	0.9

before presentation			
Idiopathic epilepsy	5/6	5/13	0.1
Previous AED therapy	4/6	5/13	0.4
Initial diazepam dose (mg/kg)	0.50 (0.28 - 1.14)	0.50 (0.35 - 1.24)	0.9
PB level (µg/ml)	22.0 (17 – 22.6; n=3)	29.7 (25.1 – 31; n=5)	0.08
KBr level (mg/ml)	0.6 (0.5 – 0.8; n=3)	1.35 (1.0 – 1.7; n=2)	0.2
Status epilepticus	1/6	6/13	0.3

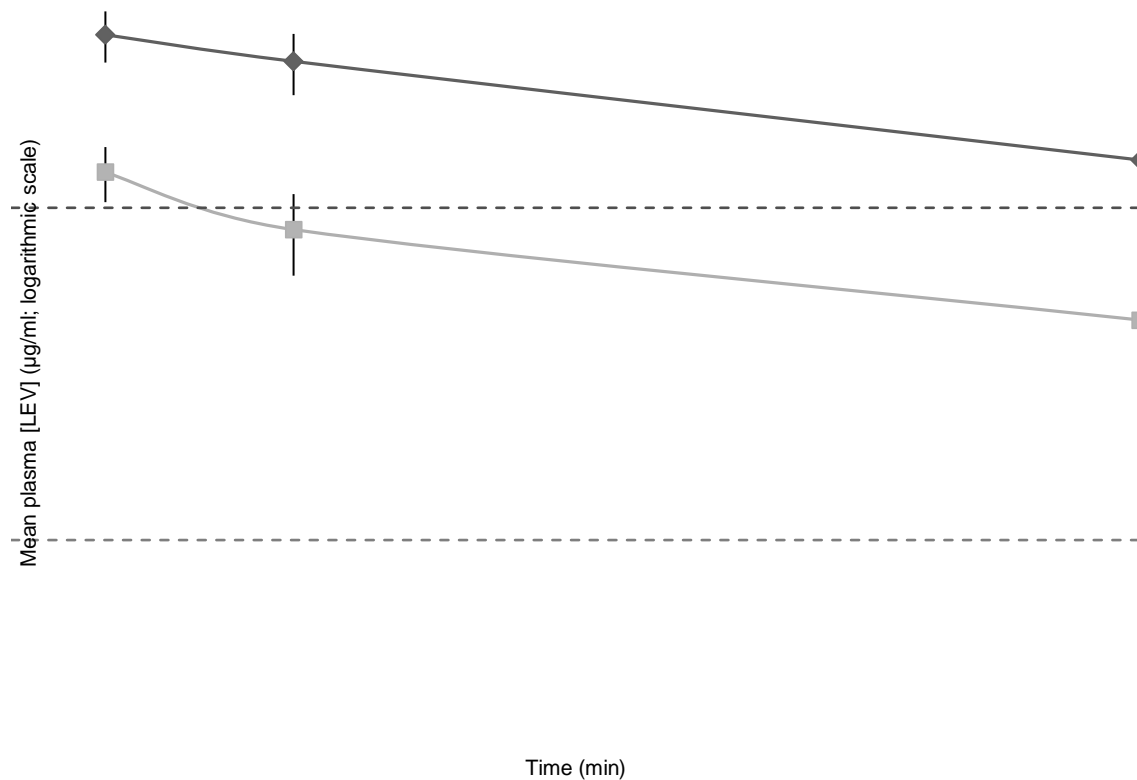


Figure 1. Mean (\pm SD) plasma concentration of LEV at 15, 45 and 180 minutes at doses of 30 and 60mg/kg. Dashed lines indicate the proposed therapeutic range of LEV (5-45 μ /ml). Diamonds: 60mg/kg dose; squares: 30mg/kg dose.

Footnotes

- a. Keppra, UCB Pharma, Brussels, Belgium
- b. Diazepam, Hospira, Inc., Lake Forest, IL
- c. 0.9% NaCl, Hospira, Inc., Lake Forest, IL
- d. Phenobarbital, Baxter Healthcare Inc., Deerfield, IL
- e. WinNonlin, version 5.2, Pharsight Corporation, Mountain View, CA
- f. SAS version 9.2, SAS Institute Inc., Cary, NC

Subcutaneous administration of levetiracetam in healthy dogs

Chapter Summary

Objective – To determine the plasma concentrations and tolerability of subcutaneously administered levetiracetam (LEV) in healthy dogs.

Animals – 4 purpose-bred dogs.

Procedures – Dogs received a single subcutaneous LEV injection (60mg/kg undiluted). Plasma LEV concentrations were determined at 15, 120 and 420 minutes after injection by high-performance liquid chromatography. Dogs were monitored for adverse effects and the injection sites were inspected and palpated for 72 hours after injection

Results – Mean \pm SD plasma LEV concentrations were 65.2 ± 29.5 , 114.5 ± 10.5 and 84.9 ± 20.6 $\mu\text{g/ml}$ at 15, 120 and 420 minutes, respectively. No adverse effects were noted in any of the dogs at any time following LEV administration.

Conclusions and Clinical Relevance – Administration of SQ LEV was well tolerated, and exceeded the suggested therapeutic range (5-45 $\mu\text{g/ml}$) within 15 minutes of administration, and remained above the range for at least 7 hours. These data indicate that SQ LEV administration may be an alternative for the at-home treatment of cluster seizures in dogs, and prospective studies in epileptic dogs are warranted.

Abbreviations

AED – Antiepileptic drug

CSF- Cerebrospinal fluid

GABA – gamma amino butyric acid

LEV – Levetiracetam

PB – Phenobarbital

Introduction

A parenteral formulation of levetiracetam^a (LEV) was approved by the FDA in 2006 for use as bridge therapy for human patients who are unable to take oral medications. Since that time, it has been used extensively in human patients off-label for the treatment of seizure emergencies (status epilepticus and acute repetitive seizures (Trinka and Dohesberger 2009)), and its use has been studied for similar cases in dogs^b. Previous canine studies have shown that parenteral LEV at doses up to 60mg/kg is well tolerated in healthy dogs, has 100% bioavailability and does not cause tissue injury or necrosis with IM administration or when part of an IV dose was intentionally extravasated (Bateman and Parent 1999; Dewey 2008).

Levetiracetam has been shown to have a unique mechanism of action compared to other antiepileptic drugs. It has been shown to bind to the synaptic vesicle 2A protein in the presynaptic nerve terminal. The exact mechanism by which it inhibits abnormal nerve

conduction has not been fully elucidated, but there are a number of other purported mechanisms, including modulating neurotransmitter release (Meehan, Yang et al.) indirect effects on GABAergic neurotransmission, inhibiting the Na dependent $\text{Cl}^-/\text{HCO}_3^-$ exchanger, and affecting K^+ and high-voltage Ca^{2+} channels (De Smedt 2007).

Currently, rectal diazepam is the most commonly prescribed at-home treatment of acute repetitive seizures, commonly referred to as cluster seizures in veterinary medicine. It has been shown to decrease the number and severity of seizures, and decrease the number and cost of emergency room visits (Podell 1995). There are many limitations to rectal diazepam use: it must be stored in light-proof, glass vials, requiring pet owners to draw up the medication following the start of a seizure; there is significant first pass metabolism, requiring a doubling of the dose when given rectally; it is metabolized by the cytochromeP450 enzyme system, and in patients receiving phenobarbital, the dose must be doubled again; it is a schedule IV controlled substance, with significant human abuse potential; and many owners find rectal administration unpleasant (Wagner, Sams et al. 1998). Intranasal administration has been evaluated, and eliminates some of the complications from rectal administration, but may add a significant risk of bite wounds to the owner while attempting to administer a medication into the nose of a seizing dog (Platt, Randell et al. 2000).

A potential alternative or adjunct to rectal diazepam is SQ levetiracetam. Subcutaneous injection is commonly done by owners for other diseases (insulin for diabetic animals, SQ fluids in renal failure), and could be safely administered during a seizure. To our

knowledge, the pharmacokinetics of SQ LEV have not been reported. Therefore, the purpose of this study was to evaluate the safety and resulting plasma levels of a single dose of SQ LEV in healthy dogs.

Materials and Methods

Animals

Four purpose-bred hound dogs were used in this study. There were 2 female and 2 male dogs, all of which were sexually intact, and weighed between 20-25kg. All protocols were approved by the Institutional Animal Care and Use Committee of the University of Minnesota (IACUC # 0909A72693).

LEV Administration

Dogs were given a single SQ injection of 60mg/kg LEV (undiluted) between the shoulder blades. Blood was collected at 15, 120 and 420 minutes via jugular venipuncture. Blood was immediately transferred to EDTA-anticoagulated tubes, plasma was harvested, and stored at -20°C until further analysis. Dogs were monitored hourly for 7 hours for sedation, ataxia, vomiting and diarrhea. The injection site was also inspected and palpated at 1,2,4,7,24, and 72 hours after injection, and pain/discomfort was assessed using a previously described scale (Patterson, Goel et al. 2008).

Plasma LEV concentrations

Plasma LEV concentration was measured by high-performance liquid chromatography using a previously described method (Patterson, Goel et al. 2008). Mean and standard deviation were calculated with a commercial computer software program (SAS, version 9.2)^c.

Results

Safety

None of the dogs became sedated, ataxic, showed pain on palpation of the injection site at any time, had any discernable injection site reaction, or had gastrointestinal signs.

Plasma LEV concentrations

The mean \pm standard deviation plasma LEV concentration was 65.2 ± 29.5 , 114.5 ± 10.5 and $84.9 \pm 20.6\mu\text{g/ml}$ at 15, 120 and 420 minutes, respectively (Figure 2).

Discussion

Levetiracetam has a highly desirable pharmacokinetic profile in dogs when given IV or IM: it is 100% bioavailable (Patterson, Goel et al. 2008), no drug-drug interactions, minimal to no hepatic metabolism, linear kinetics and is minimally protein bound

(Perucca and Johannessen 2003). To our knowledge, this is the first study to evaluate the SQ pharmacokinetics of LEV in dogs.

Subcutaneous administration of 60mg/kg LEV was well tolerated and rapidly achieved plasma concentrations that exceed the reported therapeutic range of 5-45µg/ml (REF), and plasma concentrations remain above therapeutic concentrations for at least 7 hours. These results are comparable to the plasma concentrations found in a previous study evaluating 60mg/kg given IV, which found that LEV concentrations exceeded or were within the therapeutic range for 8 hours (the duration of the study), however this study reported approximate plasma LEV concentrations of 125, 90 and 35µg/ml at the 15, 120 and 420 minute time points, respectively, indicating more rapid onset but also more rapid elimination of the drug when compared to SQ administration (Dewey, Bailey et al. 2008).

Rectal administration of diazepam is the current recommendation for at-home therapy of seizures. The pharmacokinetics of diazepam and its metabolites are well studied, and they have been shown to rapidly reach peak concentrations after 10-15 minutes. The half-life of diazepam is quite short (approximately 15 minutes), but for some of its metabolites, the half-lives exceed 5 hours (Mealey and Boothe 1995; Papich and Alcorn 1995). Diazepam pharmacokinetics are significantly altered in patients receiving chronic phenobarbital (PB) therapy. There is a decrease in the maximum concentration achieved by >50%. Also prior PB therapy resulted in a more rapid rise to, but shorter duration of time spent in the therapeutic range, presumably due to increased hepatic metabolism (Wagner, Sams et al. 1998). These require a doubling of the

administered dose of diazepam for dogs on PB. Most reports describe no drug interactions with LEV. However, a recent publication in dogs showed that concurrent PB and LEV administration resulted in a shorter LEV plasma half-life from 3.43 to 1.73 hours and a lower peak concentration from 32.39 to 18.22 μ g/ml, in purpose-bred research dogs (Moore, Munana et al. 2011). This study evaluated an approximately 20mg/kg oral dose of LEV. These changes in pharmacokinetics are not expected to have significant effects when LEV is given SQ at the dose used in this study. Even if the maximum dose and plasma half-life were reduced by approximately 50%, it would still be expected to be above or within the therapeutic range for at least 3.5-4 hours.

Limitations to this study include: a full PK profile was not obtained. The pharmacokinetics of LEV given by the IM and IV routes has been shown to be linear, and no difference was seen when half of the IV dose was intentionally extravasated (Patterson, Goel et al. 2008). While plasma levels rapidly exceeded the therapeutic range, LEV is water soluble, and how rapidly the cerebrospinal fluid (CSF) concentration achieves comparable levels as in plasma, has not been well established in dogs. In rats, the time to achieve maximum CSF concentration is 4-5 times as long as for plasma. However, the half-life of LEV in the CSF is approximately twice as long (Doheny, Ratnaraj et al. 1999). Furthermore, the therapeutic range is extrapolated from human medicine, and has not been established in dogs. Histopathology was not performed on the tissues at the site of injection, but no overt pain was appreciated on palpation of the area. Furthermore, LEV has been shown to not cause tissue damage, even when intentionally extravasated (Patterson, Goel et al. 2008). To the authors' knowledge, no

study in dogs to date has shown any significant side effects from LEV at clinically relevant doses. Combined with the data presented here, SQ LEV is expected to be safe, and very unlikely to cause harm to canine patients.

Future areas for research could include: determining what is the maximum safe dose of SQ LEV; evaluating repeated dosing and determining the most effective dosing interval; and determining the most effective combination of at home AEDs. In human patients (Modur, Milteer et al. 2010) and in rodent models (Mazarati, Baldwin et al. 2004; Donato Di Paola, Gareri et al. 2007), levetiracetam has been shown to be synergistic with diazepam for the treatment of seizures. Therefore, a combination of SQ LEV and rectal diazepam may potentially work better than either alone in canine patients. Further study of SQ LEV in clinical patients with naturally occurring seizures, as sole rescue therapy and in combination with diazepam is warranted based on these results.

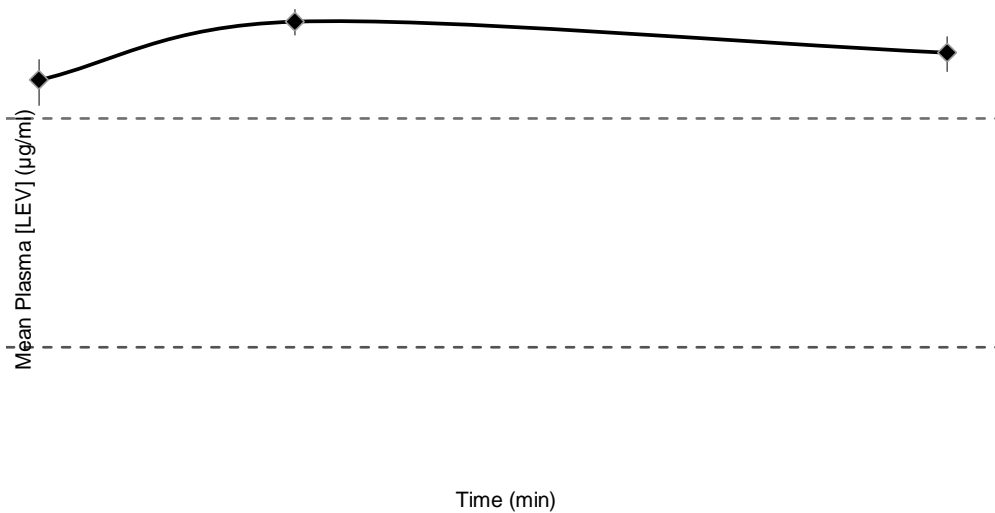


Figure 2. Semi-logarithmic plot of mean \pm SD plasma LEV concentration. Dashed lines indicate the therapeutic range of 5-45 μ g/ml.

Footnotes

- a. Keppra, UCB Pharma, Brussels, Belgium
- b. Patterson EE. The emerging role of parenteral levetiracetam for the emergency treatment of seizures. Abstract, American College of Veterinary Internal Medicine Forum, 2011.
- c. SAS version 9.2, SAS Institute Inc., Cary, NC

Conclusion

This thesis encompasses three distinct, but related studies. The first, a retrospective analysis of canine seizure emergencies at the U of MN VMC; second a prospective, double-masked, controlled clinical trial evaluating the safety and efficacy of Levetiracetam (LEV) for the treatment of seizure emergencies; and finally an evaluation of the pharmacokinetics of subcutaneously administered LEV in healthy dogs.

A retrospective analysis of the electronic database found 110 canine cases of SE (n=33) or ARS (n=77) that presented to the U of M VMC Emergency Service over the 27 month study period, corresponding to 1.24% of ER admissions during that time, and equating to approximately one case per week. Idiopathic epilepsy was the most common diagnosis, followed by intracranial neoplasia, inflammatory CNS disease, ischemic/thrombotic, intoxication, trauma, hypocalcemia and post-anesthetic. Overall mortality was 34.5% in this group of dogs, with dogs with SE more likely to die or be euthanized compared to dogs with ARS (p=0.0001). These findings are similar to previously reported mortality rates in dogs of 25.3-35.8% (Bateman and Parent 1999; Zimmermann, Hulsmeier et al. 2009) and humans of 27-31% (DeLorenzo, Kirmani et al. 2009). Dogs diagnosed with idiopathic epilepsy were more likely to survive (p=0.0001), and dogs with inflammatory CNS disease more likely to die or be euthanized (p=0.0013). Also associated with a poor outcome were age >10 years (p=0.0401) and smaller body weight (p<0.0081).

Many of these findings are similar to those that have been reported in previous retrospective studies, including prevalence, mortality rates, underlying etiologies, and

poor outcome associated with inflammatory CNS disease. Results not reported before in the veterinary literature are worse outcome with SE compared to ARS, older age, and smaller body weight.

Levetiracetam is a recently approved medication for the treatment of seizures in people, and exerts its antiepileptic effects through a novel mechanism. It has the widest safety margin of any current AED, and has a highly favorable pharmacokinetic profile. Our first study evaluating IV LEV was a prospective, double-masked, placebo controlled study, performed in clinical patients presented to the University of Minnesota Veterinary Medical Center for SE or ARS. We hypothesized that LEV would be superior to placebo in stopping seizures, and would cause no significant side effects. In 19 dogs (9 LEV treated and 10 placebo treated dogs), our results showed that LEV showed a strong trend toward being superior to placebo ($p < 0.057$). No significant adverse effects were attributable to LEV, though it was difficult to separate potential side effects caused by LEV from those caused by other AEDs or seizure activity. A major limitation was the different etiologies between the two treatment groups. Though not statistically significant, in the placebo group, 5/10 dogs had either inflammatory CNS disease or intracranial neoplasia, compared to only 1/9 in the LEV group. This was the first study of injectable LEV in clinical patients in veterinary medicine. Future areas for research could include: determining the maximum safe dose of IV LEV for clinical dogs; evaluating timing of therapy relative to use of benzodiazepines or other AEDs; determining efficacy of single versus multiple doses of LEV, and the most effective dosing interval.

Our final study evaluated the safety and pharmacokinetics of subcutaneously administered LEV in healthy dogs. A previous study (Patterson, Goel et al. 2008) demonstrated that when a portion of a dose of LEV was intentionally extravasated, pharmacokinetics were essentially unchanged from IV administration. Results from our SQ administration showed that by as early as 15 minutes, plasma LEV concentrations were above the proposed therapeutic range of 5-45 μ g/ml, and remained above this range for 7 hours (the duration of the study). No adverse effects or injection site reactions were seen in any of the dogs. These findings warrant further investigation in clinical patients for the at-home treatment of acute repetitive seizures, either as monotherapy or in combination with rectal or nasal diazepam.

There has been increasing interest in the use of dogs as a model for human SE/ARS. Benefits of the canine model include naturally occurring seizure disorders, in contrast to the induced rodent models used most frequently, larger body size making antiepileptic drug pharmacokinetics and drug dosing regimens more accurate, and the ability to obtain informed consent from dog owners (Leppik, Patterson et al. 2009). Evaluating different antiepileptic drugs such as valproic acid, levetiracetam, lacosamide, brivaracetam, and topiramate, and larger numbers of dogs in multi-center trials could provide benefits to both humans and dogs in a field that has seen very few prospective, blinded studies.

In summary, SE and ARS were found to be common reasons for presentation to the U of M VMC, and carry a high mortality rate. Levetiracetam when administered intravenously was found to be potentially effective and well tolerated in clinical patients,

and to rapidly achieve therapeutic concentrations in healthy dogs when given SQ, and maintain those concentrations for at least 7 hours without causing side effects. Taken together, these findings demonstrate that further study into the efficacy of LEV for seizure disorders in dogs is warranted, and that the U of M VMC is an excellent site for performing these clinical trials.

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