Disposition of levetiracetam in healthy adult horses

by

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Abstract

Nine horses received 20 mg/kg of intravenous LEV (LEV_{IV}), 30 mg/kg of intragastric, immediate release LEV (LEV_{IR}) and extended release (LEV_{ER}), in a 3-way randomized crossover design. Serum samples were collected over 48 hours, and LEV concentrations determined by immunoassay. Mean $\pm$ SEM peak concentrations for LEV_{IR} and LEV_{ER} were 50.72 ± 3.53 and 53.58 ± 5.31 µg/mL, respectively. The mean y-intercept for IV administration was 64.54 ± 8.33 µg/mL. The terminal half-life was 6.38 ± 1.97, 7.07 ± 1.93 and 6.22 ± 1.35 hours for LEV_{IR}, LEV_{ER} and LEV_{IV}, respectively. Volume of distribution at steady state was 0.63 ± 0.02 L/kg. Total body clearance after IV administration was 1.24 ± 0.10 ml/kg/min. Bioavailability was excellent. Based on this study, a recommended dosing regimen of intravenous or oral LEV, of 32 mg/kg every 12 hours is likely to achieve and maintain therapeutic range with optimal kinetics throughout dosing interval.

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List of Abbreviations

AED   Anti-epileptic drug

ANOVA   Analysis of variance

AUC   Area under the curve

C_{avgss} Average plasma drug concentration at steady-state

CBC   Complete blood count

CI   Confidence interval

Cl   Total body clearance

C_{max} Peak plasma concentration

C_{maxss} Peak plasma concentration at steady-state

C_{min} Trough plasma concentration

C_{misss} Through plasma concentration at steady-state

CSF   Cerebro-spinal fluid

C_{ss} Plasma concentration at steady-state

C_0 Peak drug concentration extrapolated from y-intercept

F   Bioavailability

GABA	Gamma-aminobutyric acid
ER	Extended release
ILAE	International League Against Epilepsy
IM	Intramuscular
IR	Immediate release
IV	Intravenous
LEV	Levetiracetam
LEV_{ER}	Levetiracetam, extended release formulation
LEV_{IV}	Levetiracetam, intravenous formulation
LEV_{IR}	Levetiracetam, immediate release formulation
MAT	Mean absorption time
MRT	Mean residence time
PK	Pharmacokinetcs
PO	Per os
SEM	Standard error of the mean
SV2	Synaptic vesicle protein 2
T_{max}	Time to peak plasma concentration

$t_{1/2}$ Elimination half-life

Vd Volume of distribution

Vd_{ss} Volume of distribution at steady-state

Λz elimination rate constant

Chapter 1: Literature review

Section 1: Seizures in horses

A seizure is a non-specific paroxysmal cerebral dysrhythmic event which arises due to excessive discharges from cerebrocortical neurons. Epilepsy is defined as reoccurring seizures from a chronic underlying process.[1]

Given that the basic mechanism of neuronal excitability is the action potential, a hyperexcitable state can result from increased excitatory synaptic neurotransmission, decreased inhibitory neurotransmission, an alteration in voltage-gated ion channels, or an alteration of intra- or extra- cellular ion concentration in favor of membrane depolarization. Action potentials occur due to depolarization of the neuronal membrane, with membrane depolarization propagating down the axon to induce neurotransmitter release at the axon terminal. The action potential occurs in an all-or-none fashion as a result of local changes in membrane potential brought about by net positive inward ion fluxes. Membrane potential thus varies with activation of ligand-gated channels, whose conductance is affected by binding to neurotransmitters; or with activation of voltage-gated channels, whose conductance is affected by changes in transmembrane potential; or with changes in intracellular ion compartmentalization. Neurotransmitters are substances that are released by the presynaptic nerve terminal at a synapse and subsequently bind to specific postsynaptic receptors for that ligand. Ligand binding results in channel activation and passage of ions into or out of the cells.

The major neurotransmitters in the brain are glutamate, gamma-amino-butyric acid (GABA), acetylcholine (ACh), norepinephrine, dopamine, serotonin, and histamine.[2]

The commission on classification and terminology of the International League Against Epilepsy (ILAE) first created a comprehensive classification for seizures in man 25 years ago.[3] A standardized classification has numerous advantages, including the foundation for a coherent and systematic approach in the diagnosis and treatment of epilepsy, as well as for a common mode of communication among clinicians. According to the ILAE guidelines, seizures in man can be classified by type (Axis 2), as partial or generalized. Partial seizures are caused by a focal abnormal neuronal discharge resulting in localized motor signs or sensations. The first clinical signs of partial seizures are highly indicative of the epileptic focus localization. Partial seizures are further categorized as simple, if alertness and normal mentation are maintained, or complex, if impairment of consciousness is reported. A generalized seizure involves the entire cerebral cortex and results in generalized motor activity of the whole body, including convulsive seizures, nonconvulsive seizures and myoclonic seizures. Consciousness may be impaired and this impairment may be the initial manifestation. Generalized seizures can originate from both cerebral hemispheres from the onset (primary generalized seizures), or progress from partial seizures (secondary generalized seizures).[3] In addition, classification of seizures by etiology (Axis 3), a major determinant of clinical course and prognosis, has been described and can be further divided into four categories: idiopathic, symptomatic, provoked, and cryptogenic.[3,4]

Classification of seizures and epilepsy by etiology and type has been recently described in horses for the first time.[5,6] Lacombe et al. (2013), identified seizures with or without secondary generalization as the most common type among the 104 horses included in a 20-year retrospective study.[5] This finding is in agreement with studies in small animals and man.[7-10] In dogs, it has been reported that primary generalized seizures account for less than a third of the reported seizures, and that local seizures with or without secondary generalization appear to be the most common type of seizure observed.[5] Likewise, focal seizures were the most common type of seizure in cats[8], as well as in human epileptic patients based on large epidemiological and meta-analysis studies.[9,10] Lacombe et al. (2013), also investigated clinical variables as predictive factors for seizure type in the same group of horses, and reported significant associations between seizure type and gender, frequency of seizures, and presence of seizures during hospitalization. Mares were found to be more prone to generalized seizures then male horses. Estrogen and progesterone concentrations have been shown to be related to alterations in seizure threshold in women and female dogs[11], and a similar risk factor appears to apply to equine seizures. For a horse with recurrent seizures (i.e., epilepsy), the odds of exhibiting focal rather than generalized seizures has been reported to be approximately 4 times higher than in a similar horse with one episode.[5] This is in agreement with studies in human patients affected by epilepsy, which reported that focal seizures prevailed over generalized seizures.[9,10] Although it was commonly believed that dogs with epilepsy were more likely to exhibit generalized

seizures[12], it has been demonstrated that focal seizures were more frequently

associated with secondary/acquired epilepsy.[7] Furthermore, generalized seizures in

horses were more likely to be observed during hospitalization than were focal seizures

without generalization. This finding highlights the fact that detailed clinical observation

regarding the onset of seizure is required, because focal seizures may be subtle and

transient clinical signs may go undetected or be interpreted as the pre-ictal phase of an

impending seizure.[5] In addition, seizure type was not associated with its etiology,

reflecting the fact that the clinical presentation of seizure is independent of its etiology.[5]

Consistent with these findings, the distribution of the various types of seizures was not

associated with the underlying disease and the etiological classification in dogs and

cats.[7,8] Therefore, when applying the ILAE definitions, similarities in regard to seizure

phenomenology and type exist between horses, small animals and man.

According to its etiology, symptomatic (i.e., identified structural brain pathology;

35.6% of cases) and cryptogenic (i.e., unknown; 54.8% of cases) were reported to

occur more commonly than reactive (i.e., identified systemic disease with normal brain

function; 1.9% of cases) and idiopathic (i.e., suspected genetic predisposition; 2.7% of

cases) seizures in a referral-based equine population included in a retrospective

observational study.[6] In this study, symptomatic seizures were identified in the presence

of intracranial tumors, cholesterol granulomas and equine protozoal myeloencephalitis,

while juvenile idiopathic epilepsy was responsible for the cases diagnosed with

idiopathic seizures. The majority of the horses, however, exhibited recurrent seizures

associated with unremarkable neurodiagnostic examinations and were classified as cryptogenic epilepsy. Cryptogenic epilepsy is suspected to be symptomatic, but the etiology cannot be identified. As in man, epilepsies of unknown cause account for the majority of all cases of epilepsy,[13] demonstrating the need for future research in neurodiagnostic testing and brain imaging.

Foals have a lower seizure threshold and are more susceptible to conditions causing seizures when compared to adult horses.[1] The most common causes of seizures in foals under 2 weeks of age are hypoxic-ischemic encephalopathy, trauma, bacterial meningitis and juvenile idiopathic epilepsy in Egyptian Arabian foals.[14] The latter has been studied by Aleman et al. (2006), in a retrospective case series over a 20-year period, including 22 foals. The age of onset of affected foals ranged from 2 days to 6 months. Seizures were characterized by generalized tonic and clonic motor activity, staring, and loss of consciousness. The most common postictal signs were transient blindness and abnormal mental status. The interictal neurological examination was otherwise normal. Clinicopathologic data and imaging diagnostics were normal except in 4 foals that developed complications. Electroencephalography revealed epileptiform activity in 9 of the 13 foals. The long term prognosis was favorable with cessation of seizures by 1 year of age. The most common complication was head trauma and the most common concurrent disease was pneumonia.[14]

Although seizures are relatively uncommon in horses compared with other species, they have equine welfare and human safety implications. Seizures in horses are

challenging for the veterinarian to treat and manage and are very distressing and dangerous to the owner or caretaker of adult horses. Immediate control of seizure-like activity in the horse is a priority as prolonged or reoccurring seizures may result in permanent neurological damage or in further injury to the horse or to any human caretaker. The aim of pharmacotherapy is to maintain a seizure-free status by the use of long-acting anticonvulsant therapy, without unacceptable adverse effects.[1]

Section 2: Therapy of seizures in horses

Anticonvulsant therapy is used to prevent the spread of the seizure focus, raise the seizure threshold and decrease the electrical excitement of abnormal neurons without disrupting normal function.[15] Over the past 15 years, several new antiepileptic drugs (AEDs) have been successfully introduced into human medicine with the global aim of providing better control of seizures and a more favorable safety and tolerability profile over traditional AEDs.[16] However, in veterinary medicine, new information is almost exclusively limited to dogs and cats.[14] Treatment for seizure disorders in horses is currently limited to conventional first line AEDs such as phenobarbital and bromide salts.

Phenobarbital is the most commonly used anticonvulsant in horses.[15] Although it has been used to treat epilepsy for over 80 years, its mechanisms of action are not fully elucidated. Phenobarbital potentiates the actions of gamma-aminobutyric acid (GABA), the inhibitory neurotransmitter in the central nervous system. Neuronal stabilization by GABA in postsynaptic neurons occurs from increased intracellular chloride

conductance, which hyperpolarizes the membrane; the overall result is an increase in the seizure threshold and a decrease in the electrical activity of the seizure focus.[15] Its major reported side effect is drowsiness, which although not life threatening, may be considered unacceptable by the horse owner.[1] Phenobarbitol has serious abuse potential as a calming agent for fractious horses during competition, which although illegal, is anecdotally widely practiced. The risks associated with phenobarbitol have culminated in it being classified as a controlled substance (scheduled S4). The majority of the phenobarbital is metabolized by the liver and therefore its use in animals with liver disease is contraindicated.[17] Phenobarbital is a classic example of a drug that causes induction of hepatic P450 microsomal enzymes. Increased metabolic activity of these enzymes will lead to increased elimination of phenobarbital and other hepatically metabolized drugs.[17-19] In horses, interactions have been reported for tetracyclines, chloramphenicol and ivermectin.[1] Long term phenobarbital administration in horses has been associated with significant increases in hepatobiliary enzymes and further drug interactions.[20] Furthermore, phenobarbital may cause respiratory depression, bradycardia, hypotension, and hypothermia in neonatal foals.[20,21] Given the variability in half-life, clearance, and metabolism of phenobarbital in all species, including horses, therapeutic drug monitoring is mandatory to ensure adequate anticonvulsant concentrations are achieved and toxicity minimized.[1,15,17-19] This requires additional veterinary examinations and costs associated with testing.

Potassium bromide is the oldest anticonvulsant drug, and was first used in 1857 to treat seizures in people. Although it is currently rarely used in humans because of its toxicity, potassium bromide has traditionally been used as a second anticonvulsant drug when seizures continue to occur in dogs and cats despite adequate plasma concentrations of phenobarbital.[15] In dogs, the long-half life and the lack of hepatotoxic effects make this drug an attractive option.[22] The mechanism of action is uncertain, but potassium bromide appears to stabilize neuronal cell membranes by interfering with chloride ion transport. It potentiates the effects of GABA by hyperpolarizing the membrane. It may act synergistically with other GABAergic drugs, such as phenobarbital, to raise the seizure threshold.[15,23] Appropriate dosage regimens and therapeutic serum concentrations have been reported for humans and dogs.[24-27] In other species, drug uptake and response are highly variable.[28] In dogs, it takes up to 3 months for potassium bromide to achieve steady state concentrations.[29] Therapeutic drug monitoring is also necessary to ensure serum concentrations are within the therapeutic range and below toxic concentrations.[26,27] Adverse effects are often dose dependent and include central nervous system effects, gastrointestinal tract problems, increased water consumption and urination, muscle pain and skin disorders.[24-27] Some side effects may relate to altered calcium ion uptake associated with the intracellular accumulation of bromide.[30] Possible effects on thyroid function have been evaluated in rats and dogs.[31,32] There are reports of the efficacy of this drug as part of combination therapy for the management of certain types of refractory epilepsy in children and

dogs.[22,24,25] The clinical efficacy of potassium bromide as an anticonvulsant in horses has not been evaluated.[28]

Other AEDs such as phenytoin and sodium pentobarbital have been considered as alternative therapy for horses with refractory seizures but not without significant side effects.[33,34] Anecdotal reports exist for the use of primidone for the treatment of seizures in foals, but its pharmacokinetic properties and clinical effects are unkown.[35,36] Diazepam, a benzodiazepinic antiepileptic drug, has been routinely used for short-term control of seizures in horses. However, because of its short half-life, repeated doses must be frequently administered. Its prolonged use can lead to respiratory depression or arrest in foals.[21] Therefore, diazepam is not a good choice for long-term control of seizures.[1,14,15]

An ideal AED for the therapy of seizures in horses is currently not available. An ideal AED is rapidly absorbed after oral ingestion, quickly achieves steady-state concentrations, demonstrates linear kinetics (plasma drug concentration correlates directly with dose so, thus, drug concentration is predictable allowing easier dose titration in relation to achieving seizure control), minimal or no protein binding, is minimally metabolized by the liver (thus minimizing the risk of drug interactions, liver disease and production of pharmacologically active metabolites), rapidly traverses the blood-brain barrier, small side effect profile, a long half-life allowing for dosing intervals sufficiently convenient to facilitate owner compliance, and most important effective in controlling epileptic events.[37,38]

Section 3: Levetiracetam, an alternative to traditional seizure therapy

Levetiracetam (LEV), ((S)-α-ethyl-2-oxo-1-pyrrolidine acetamide) (Figure 1), is the

(S)-enantiomer of the ethyl analogue of piracetam, and as a pyrrolidone derivate, it

shares a similar chemical structure to numerous nootropic drugs.[39] It is structurally

unrelated to other AEDs, with an empirical formula of $C_8H_{14}N_2O_2$ and a molecular

weight of 170.21. LEV is a white to off-white powder with a bitter taste and faint odor. It

is highly soluble in water (0.104g/mL), freely soluble in methanol (0.054 g/mL) and

chloroform (0.065 g/mL), soluble in ethanol (0.016 g/mL), sparingly soluble in

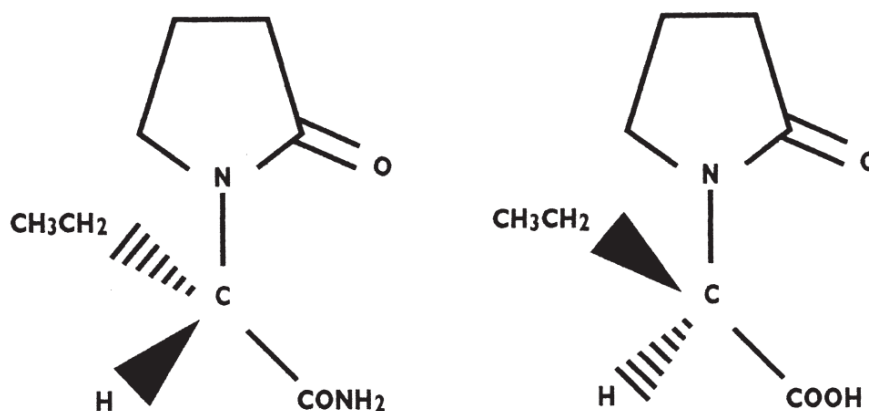acetonitrile (0.006 g/mL) and practically insoluble in n-hexane.[40]

Figure 1 – The chemical structure of levetiracetam ((S)-α-ethyl-2-oxo-1-pyrrolidine

acetamide) (left) and its primary pharmacologically inactive metabolite L057 (right).

Anti-epileptic activity of LEV

LEV is a broad-spectrum anti-epileptic agent licensed for human use as adjunctive therapy for the treatment of patients with partial seizures with or without secondary generalization, myoclonic epilepsy or primary generalized tonic-clonic epilepsy in the USA.[40] However, there is increasing evidence that LEV is efficacious as monotherapy for partial refractory seizures, and its use has been approved in the European Union as a monotherapy treatment for epilepsy in case of partial seizures.[41] Its significant clinical efficacy, highly favorable therapeutic index, and simple pharmacokinetic characteristics have established LEV as a very efficacious and safe AED in human medicine.[40-44] When compared with traditional and other new AEDs as a treatment for partial refractory epilepsy in human patients in a systematic review and meta-analysis, LEV and topiramate were the most efficacious in controlling seizure frequency.[42] In a European multicenter, double-blinded, randomized, placebo-controlled trial, LEV (500 mg or 1000 mg twice daily) was compared with placebo as add-on therapy in 324 human patients with uncontrolled simple or complex partial seizures, or both, with or without secondary generalization. After enrollment, three parallel groups were assessed during a baseline period of 8 to 12 weeks, followed by a 4-week titration interval and a 12-week evaluation period. LEV significantly decreased the seizure frequency compared with placebo. A reduction in seizure frequency of at least 50% occurred in 22.8% of patients in the 1000 mg group and 31.6% of patients in the 2000 mg group,

compared to 10.4% of patients in the placebo group. No difference in the adverse effects was reported between the LEV and placebo groups.[44]

In its first years of use in veterinary medicine, LEV has shown to be an effective add-on or solitary medication for seizure control in small animals. In dogs found to be pharmacoresistant to phenobarbital and/or potassium bromide, the addition of LEV achieved a 50% reduction in seizures in 5 out of 8 patients in a retrospective study, and in 8 out of 14 in a prospective study, as an add-on medication (10 mg/kg, q 8 hrs). In dogs that remained refractory, the dosage was increased to 20 mg/kg, q 8 hrs, for 2 months. After the increase, one other dog responded to LEV treatment. LEV was well tolerated by all dogs and sedation was the only side-effect reported in just one of treated dogs.[45] LEV efficacy over placebo as adjunctive treatment was not demonstrated by Munana et al. (2012) in a randomized, blinded trial involving 34 client-owned dogs with idiopathic epilepsy, refractory to phenobarbital and bromide, although the power of the study was limited. Administration of LEV to epileptic dogs was considered safe.[46] In a study including 12 client-owned cats suspected to have idiopathic epilepsy that was poorly controlled with phenobarbital, the addition of oral or IV LEV at 20 mg/kg q 8 hrs, achieved a greater than 50% reduction in seizures in 7 of 10 animals.[47] Hardy et al. (2012) performed a randomized, placebo-controlled, double-masked study including 19 client-owned dogs treated for status epilepticus or acute repetitive seizures with IV LEV (30 or 60 mg/kg) or placebo. The responder rate after LEV administration was 56% compared to 10% for placebo. Dogs in the placebo group

received significantly more boluses of diazepam than the LEV groups. No serious adverse effects were attributable to LEV administration.[48]

Mechanism of action of LEV

LEV's pharmacological profile has been presumed to relate to a distinctive mechanism of action when compared with traditional AEDs. LEV does not seem to act by means of any of the three main mechanisms currently accepted for the antiseizure action of established AEDs: (*i*) gamma-aminobutyratergic (GABAergic) facilitation, (*ii*) inhibition of sodium channels, or (*iii*) modulation of low-voltage activated calcium currents.[49] Previous studies revealed that LEV binds saturably, reversibly, and stereospecifically to an unidentified binding site in rat brain.[50] Screening of a large number of known AEDs and other neuroactive compounds failed to identify any with high affinity for the LEV-binding site, providing support for the novelty of the LEV-binding site.[50] Testing a series of LEV analogs revealed a strong correlation between their affinities for the brain binding site and their antiseizure potencies in the audiogenic mouse model of epilepsy.[50] This finding indicates a functional role for the unidentified brain binding site in the antiseizure actions of LEV.

Detection of a LEV-binding site in brain tissue provided the rationale to search for the LEV-binding molecule. Further characterization of the binding site led to its classification as an integral membrane protein of widespread distribution in brain, localized in neurons and enriched in the synaptic vesicle membrane fraction.[50,51] Among possible candidate proteins is the synaptic vesicle protein 2 (SV2).[52]

SV2, an integral membrane protein present on all synaptic vesicles, is a small gene family consisting of three isoforms, designated SV2A, SV2B, and SV2C. SV2A is the most widely distributed isoform, being nearly ubiquitous in the CNS, as well as being present in endocrine cells. SV2B is brain specific, with a wide but not ubiquitous distribution, and SV2C is a minor isoform in brain.[52] The brain distribution of the LEV-binding site, as revealed by autoradiography, matches the equivalent distribution of SV2A as determined by immunocytochemistry. Both SV2A -/- and SV2B -/- homozygous knockout (KO) mice have been reported, as well as double A/B KOs. SV2A, and SV2A/B -/- mice exhibit a severe seizure phenotype whereas the SV2B -/- mice do not. Studies of the SV2 KOs indicate that SV2 has a crucial role in the regulation of vesicle function, although not in vesicle biogenesis or synaptic morphology.[53]

Lynch et al. (2004), demonstrated by photoaffinity labeling of purified synaptic vesicles, that the synaptic vesicle protein SV2A is the brain binding site of LEV. There was an excellent correlation between the binding affinity of LEV and derivatives in brain and to heterologously expressed human SV2A in fibroblasts. These data have implications for the mechanism of action of LEV as an antiepileptic drug, suggesting that LEV acts by modulating the function of SV2A, and potentially for future research into the contribution of presynaptic mechanisms to seizure initiation and propagation in the brain.[52]

Pharmacokinetics of LEV in human patients

LEV (Keppra®) is available as film-coated tablets containing 250, 500, 750 and 1000 mg of levetiracetam for oral administration, although not all concentrations are available in every country. A new extended release version of LEV (Keppra XR®) has been approved for the treatment of epilepsy in humans allowing for effective once daily oral dosing.[54] A dose of 1000 mg of Keppra XR® given once daily compared to 500 mg of LEV immediate release given twice daily were found to be bioequivalent.[55] A liquid, clear, colorless, grape-flavored liquid (100 mg/mL) LEV formulation for oral ingestion and an intravenous formulation are also commercially available as generic forms. Currently, generic formulations for oral LEV are also commercially available as immediate-release (IR) and extended-release (ER) tablets. However, the prescription of generic formulations is not risk free. Chaluvadi et al. (2011) demonstrated that a compulsory switch from branded to generic LEV lead to poor clinical outcomes, with significant increased adverse effects and seizure frequency.[56]

The pharmacokinetic profile of LEV has been evaluated in healthy volunteers; adult, pediatric, and elderly human patients with epilepsy; and patients with renal and hepatic impairment. Overall, LEV has a very favorable pharmacokinetic profile, with rapid absorption following oral administration, excellent bioavailability, rapid achievement of steady-state concentrations, linear kinetics, and minimal plasma protein binding. In addition, LEV is not hepatically metabolized. There is no saturable absorption or metabolism and no auto-induction, as observed with gabapentin, phenytoin, and

carbamazepine. In clinical trials, the pharmacokinetics of LEV are comparable among healthy male and female subjects, adult patients with epilepsy, and Caucasians and Asians.[40]

Absorption

LEV is rapidly and almost completely (>95%) absorbed following oral administration of doses ranging from 250 mg to 5000 mg.[57] The extent of absorption is independent of dose. Peak plasma concentrations (C_{max}) are achieved in approximately 1hour and decline to baseline within 48 hours following oral ingestion. Following a single 1000 mg dose and repeated 1000 mg twice daily doses, C_{max} is typically 31 and 43 μg/mL, respectively. In healthy volunteers, C_{max} and the area under the plasma concentration-time curve (AUC) display dose linearity in the range of 500–5000 mg. Steady-state concentrations are generally attained after 2 days of repeated twice-daily dosing.

In multiple dose-ranging studies, LEV exhibits predictable, linear and dose-proportional steady-state pharmacokinetics, with steady-state concentrations occurring within 2 days of initiation of administration.[37,40] Thus, after administration of LEV 500 mg twice daily, mean trough plasma concentrations of 35 (range 18–59) μmol/L were attained with a mean C_{max} of 100 (range 59–147) μmol/L. For LEV 1000 mg twice daily, the corresponding values for trough and C_{max} were 70 (range 29–218) μmol/L and 188 (range 135–235) μmol/L, respectively. For LEV 1500 mg twice daily, the corresponding values for trough and C_{max} were 94 (range 41–200) μmol/L and 265 (range 212–370) μmol/L, respectively. More recently, two studies have reported that LEV dose and blood

concentrations are linearly related.[58,59] The first study was cross-sectional in design and was undertaken as part of a 1-year post-marketing surveillance of LEV in 71 patients with focal and generalized epilepsies.[58] The second, more extensive study involved the pooled analysis of blood concentration data from 590 patients who had participated in various phase III randomized controlled trials of levetiracetam.[59]

The effect of food on the absorption of LEV was evaluated in an unblended, 3-way-crossover study of 10 healthy volunteers.[60] After an overnight fast, subjects received a single dose of LEV 500 mg administered either as an intact tablet with 120 mL water or crushed and mixed with 4 ounces of apple sauce or 120 mL of a common enteral feeding formulation. In these healthy volunteers, the overall rate and extent of absorption of oral LEV were not significantly impaired after crushing and mixing of the tablet with either a food vehicle or a typical enteral nutrition feeding formulation. The data suggested that peak serum concentrations of LEV may be slightly reduced after mixing with enteral feeding formulations (C_{max} reduced in 27%), although the difference was not significant compared with control values. Thus, crushed or whole LEV tablets can be ingested without regard to meal times.

Rouits et al. (2009) compared the relative bioavailability of LEV extended-release (ER) tablets with immediate-release (IR) tablets following single and multiple dosing, the food effect, and the dose-proportionality from 1000 to 3000 mg. After a single dose, LEV ER and IR were bioequivalent with respect to area under the curve (AUC) and C_{max}. At steady state, the AUC were also bioequivalent. In the dose-proportionality trial, the AUC

and C_{max} increased linearly with the dose. Therefore, LEV ER 1000 mg SID was demonstrated to be bioequivalent to LEV IR 500 mg BID without food effect and with EX absorption dose-proportionally from 1000 to 3000 mg.[55]

Distribution and plasma protein binding

The volume of distribution (V_d) of LEV is 0.5–0.7 L/kg, a value close to the V_d of total body (intracellular and extracellular) water.[40] Steady-state plasma concentrations are reached in 24-48 hours of initiation of therapy. In rats, mice and rabbits, LEV rapidly distributes into tissues with concentrations approximating that in the blood, with exception of lower concentrations in the lens (0.38 mg/mL) and adipose tissue (0.28 mg/mL), and higher concentrations in the kidneys (1.39 mg/mL). Studies conducted in rats indicate that LEV readily crosses the blood-brain barrier to enter both the brain extracellular and cerebrospinal fluid compartments.[61,62] The temporal pharmacokinetic interrelationship of levetiracetam in blood and cerebrospinal fluid (CSF) was studied after acute intraperitoneal administration of levetiracetam (20, 40 and 80 mg/kg), using an animal model that permits concurrent blood and CSF sampling in freely moving rats.[61] After administration, levetiracetam rapidly appeared in both serum (time to maximum concentration (T_{max}) mean range 0.25 - 0.50 h) and CSF (T_{max} mean range 1.33-1.92 h), suggesting ready penetration of the blood CSF barrier. Both serum and CSF levetiracetam concentrations rose essentially linearly and dose-dependently, suggesting that transport across the blood-CSF barrier is not rate limiting over the levetiracetam concentration range observed in this study. However, while apparent

elimination half-life ($t_{1/2}$) values for both serum and CSF were dose-independent (mean value range 1.8-2.8 and 4.4-4.9 h, respectively), $t_{1/2}$ values for CSF were significantly larger suggesting drug accumulation within the CSF compartment. Furthermore, Loscher et al (1998) have demonstrated that LEV has antiepileptogenic activity in rats that persists for weeks after the elimination of the drug from the plasma.[63] LEV uptake is independent of multidrug transporters P-glycoprotein or the multidrug resistant family protein (MRP) such as MRP1 and MRP2.[64] LEV does not display brain-region specificity, as indicated by its comparable distribution in the extracellular fluid of the hippocampus and frontal cortex.[62]

In humans, LEV is largely unbound (<10%) to plasma proteins; thus, the risk for protein-binding interactions being clinically significant is negligible.[37]

Metabolism

LEV is minimally metabolized.[37] After 24 hours, 93% of the administered dose is excreted, with 66% of an administered dose found unchanged in urine and 27% excreted as inactive metabolites.[65] Results of radiolabeled studies show that LEV has one major (24% of dose) pharmacologically inactive, acidic metabolite (i.e., L057; Figure 1) and two minor inactive metabolites (approximately 3% of dose). Other unknown components account for only 0.6% of the dose. The metabolic pathway of the two minor inactive metabolites has not been determined yet, although the clinical relevancy of these metabolites is likely to be negligible. Production of the L057

metabolite was not supported by hepatic-dependent enzymes.[37] No enantiomeric interconversion was observed for either LEV or its primary metabolite.

The effect of LEV on the activity of hepatic cytochrome P450 (CYP) enzymes was investigated using *in vitro* human liver microsomal markers.[66] CYP isoenzymes are involved in the oxidative metabolism of multiple endogenous and exogenous compounds. Most clinically significant pharmacokinetic drug-drug interactions involve the induction or inhibition of CYP enzymes. LEV and its primary metabolite L057 were evaluated for their potential inhibitory effect on 11 different drug-metabolizing enzymes (CYP3A4, CYP1A2, CYP2C19, CYP2E1, CYP2C9,CYP2D6, epoxide hydrolase, and the uridine 5-diphosphoglucuronyltransferases (UGTs) UGT*1, UGT1*6, UGT1*1, and UGT). At concentrations exceeding 5 times the plasma therapeutic concentration, enzyme activities were unaffected. These results suggest that LEV is unlikely to produce clinically relevant interactions through the induction or inhibition of CYP-mediated reactions.

The primary site for the hydrolysis of LEV appears to be the blood.[66] An *in vitro* study comparing human whole blood and liver homogenate clearly shows that LEV is hydrolyzed to L057, but that liver homogenate is responsible for very little hydrolysis. Thus, after 6 hours of incubation (LEV 200 µmol/L), liver homogenate hydrolysis represented only 0.6% of that seen by whole blood. Further characterization of the metabolism of LEV has revealed that its hydrolysis is inhibited by paraoxon, a broad spectrum inhibitor of B-esterases, but not by other B-esterase-type inhibitors specific for

cholinesterases and/or carboxylesterase (physostigmine, metoclopramide) or A-esterase inhibitors (EDTA, chloromercuribenzoate). As this inhibition profile resembles that observed for the hydrolysis of tazarotene, it can be concluded that a type B esterase, distinct from the classical cholinesterases and carboxylesterases, is involved in the metabolism of LEV.[67] LEV auto induction does not appear to be a feature of LEV hydrolysis.[68]

Excretion and elimination

The major route of excretion for LEV is through urine. Sixty-six percent of an administered dose is eliminated unchanged and 27% is excreted in urine as inactive metabolites.[65] Excretion via the fecal route accounted for only 0.3% of the administered dose. LEV is cleared fairly rapidly, with approximately 93% of drug excreted within 24 hours following oral administration. Renal clearance of LEV occurs at a rate of 40 mL/min/1.73 m^2 (0.6 mL/min/kg), indicating excretion by glomerular filtration and partial subsequent tubular reabsorption. Renal clearance of the primary metabolite L057 is approximately 4.2 mL/min/kg, indicating active tubular secretion in addition to glomerular filtration. In healthy, young volunteers, the elimination half-life of LEV ranges from 6 to 8 hours, allowing a twice-daily regimen, and does not vary by dosage, route, or frequency of administration.[69] As previously stated, steady-state plasma concentrations are reached after 48 hr. Importantly, the elimination half-life of LEV is increased to 10 to 11 hours in elderly patients, and also is increased in patients with

renal impairment and in patients with severe hepatic impairment and concomitant renal impairment (hepatorenal syndrome).[70,71]

Adverse effects of LEV

Sharief et al (1996), investigated the tolerability of LEV as an experimental drug (ucb L059) as an add-on therapy, in a single-blind, ascending-dose pilot study in 17 patients with refractory epilepsy. Ucb L059 was well tolerated; only mild or moderate adverse effects were reported, including drowsiness, memory impairment, depression, and mood changes. No clinically significant changes in laboratory or safety evaluation was detected during treatment.[43] In a multicenter, double-blind, responder-selected study evaluating the efficacy and tolerability of 3000 mg/day of LEV monotherapy in patients with refractory partial seizures, the incidence of adverse effects compared with the placebo was comparable (placebo 53%, LEV 55%). Adverse effects that were more common in the LEV group and had an incidence of greater than 5% included asthenia, infection and somnolence. Accidental injury and headache occurred more often with placebo. No clinically meaningful changes were noted in physical and neurological examinations or electrocardiography results. In addition, there was little or no change from baseline in laboratory values during the entire study.[41] Shorvon et al (2000) reported that the administration of LEV as an add-on therapy in patients with refractory partial seizures did not affect plasma concentrations of concomitant antiepileptic drugs or alter vital signs or laboratory parameters. No significant difference in the incidence of adverse effects was observed between treatment groups (70.8% for the 1000 mg/day

group; 75.5% for the 2000 mg/day group), or between the LEV and placebo groups (73.2% placebo group). The most commonly reported adverse effect in the LEV group was asthenia, headache, and somnolence[44] corroborating with Ben-Menachem et al. (2000).[41]

Because LEV has a highly favorable therapeutic index and is associated with few significant adverse effects, routine monitoring of LEV drug concentrations seems not to be necessary in human patients, as dose can be safely titrated with therapeutic response.[40]

Drug interactions with LEV

Theoretically, LEV can be expected to have a very low potential for drug interaction since it is neither protein bound in blood nor metabolized in the liver.[40] Nevertheless, the interaction potential of LEV has been extensively investigated in studies conducted *in vitro*, in healthy volunteers and in patients with epilepsy. To date, no clinically relevant pharmacokinetic interactions between AEDs and LEV have been identified. Similarly, LEV does not interact with digoxin, warfarin and human low-dose contraceptive pills; however, adverse pharmacodynamic interactions with carbamazepine and topiramate have been demonstrated.

Pharmacokinetics of LEV in small animals

Dewey et al. (2008) determined the plasma pharmacokinetics of LEV after a single intravenous dose (60 mg/kg) in normal dogs using a high-performance liquid chromatography assay validated for canine plasma.[72] There were no obvious adverse

effects associated with intravenous (IV) bolus administration of LEV in any of the dogs. Plasma LEV concentrations remained above or within the reported therapeutic range for humans (5–45 mg/mL) for all dogs, for all time periods evaluated. Mean values for pharmacokinetic parameters included the following: maximum plasma concentration, 254 mg/mL; half-life, 4.0 hours; volume of distribution at steady state, 0.48 L/kg; clearance, 1.4 mL/kg/min; and median residence time, 6.0 hours. In this study, LEV was well tolerated.

Patterson et al. (2008) investigated the safety and pharmacokinetics of LEV administered intramuscularly (IM), IV, and orally to dogs.[73] Six hound dogs received 19.5-22.6 mg/kg of LEV IM, IV and orally with a wash-out period in between. All dogs received 500 mg LEV orally and 5 mL of 100 mg/mL LEV IM. Three dogs received 500 mg of LEV IV and three dogs received 250 mg LEV IV with 250 mg given perivascularly to approximate extravasation. Safety was assessed using a pain scale at time of IM administration and histopathological examination 24 hours to 5 days after injection. Intravenous LEV half-life was 180 ± 18 min. Bioavailability of IM LEV was 100%. Mean time to C_{max} after IM was 40 ± 16 min. The mean C_{max} IM was 30.3 +/- 3 μg/mL compared to the C_0 of 37 ± 5 μg/mL for IV. Mean inflammation score (0-4 scale) for IM LEV was 0.28 and for saline 0.62. Extravasation did not cause tissue damage. In this study, parenteral LEV was well tolerated and appeared safe following IM and IV injections in dogs.

Most recently, Carnes et al. (2011) determined the pharmacokinetics of LEV after oral and intravenous administration of a single dose to clinically normal cats.[74] In a randomized crossover study, LEV (20 mg/kg) was administered orally and IV to 10 healthy purpose-bred cats. Plasma LEV concentrations were determined via high-performance liquid chromatography. Mean ± SD peak concentration was 25.54 ± 7.97 μg/mL. Half-life was 2.95 ± 0.95 hours and 2.86 ± 0.65 hours for oral and IV administration, respectively. Mean volume of distribution at steady state was 0.52 ± 0.09 L/kg, and mean clearance was 2.0 ± 0.60 mL/kg/min. Mean oral bioavailability was 102 ± 39%. Plasma drug concentrations were maintained in the therapeutic range reported for humans (5 to 45 μg/mL) for at least 9 hours after administration in 7 of 10 cats. Only mild, transient hypersalivation was evident in some cats after oral administration.

Section 4: Pharmacokinetic analysis using noncompartmental models

A model is a mathematical formula that emulates the function of a physical process. To estimate pharmacokinetic (PK) parameters from plasma concentration versus time profiles, compartmental and non-compartmental approaches may be used. The compartmental approach represents the body as a system of one or more compartments that usually have no physiological or anatomical meaning.[75,76] Rate constants describe the transfer of molecules between the compartments and out of compartments (elimination). The approach relies on nonlinear regression analysis to fit an exponential equation to the data. By contrast, the non-compartmental method (using

statistical moment analysis) is based on the area under the compound

concentration versus time curve (AUC) and the mean residence time (MRT) and can be

applied practically to any PK data.[77] Non-compartmental analysis (NCA) requires that

fewer assumptions be made than for compartmental analysis in modeling

concentration versus time data.[78,79] However, the limitation of NCA, unlike

compartmental analysis, is that it lacks the ability to predict PK profiles when there are

alterations in a dosing regimen, since it cannot predict a compound concentration at any

time. Thus, the most appropriate method to use will depend on the purpose of the

analysis and the nature of the data.

   In order to accurately characterize the blood/plasma concentration versus time

profile of a compound, one needs to sample blood/plasma over seven or more (at the

very minimum, five) time points for IV administration.[78] This is because most

compounds have an early distribution phase prior to a terminal elimination phase. As a

result, at least 2 points in the initial phase (usually within the first 15 min after injection)

are recommended for a reliable estimation of the initial blood/plasma concentration

extrapolated to time zero (C_0). Although this concentration after IV dosing is imaginary,

since no compound is in the plasma at the time of injection, C_0 is useful for calculating

the AUC from time zero to the first sampling time point (for non-compartmental analysis

methods, the first 2 time points are extrapolated back to time zero on a semi-logarithmic

scale). At least 3 time points during the terminal phase are required for a reliable

estimation of the terminal half-life. In addition, the 3 or more time points chosen for

estimation of the terminal half-life should span at least 2 half-lives. Important PK

parameters estimated from plasma concentration versus time curves following oral (and

other extravascular routes of) administration include: (i) C_{max}, which is the highest

compound concentration observed after a non-parenteral route of administration,

and T_{max}, which is the time at which C_{max} is observed, (ii) terminal half-life ($t_{1/2}$), which

can be affected by the rate of compound absorption and disposition (distribution and

elimination), and (iii) bioavailability (F), the fraction of an extravascularly administered

dose that reaches the systemic circulation. Thus, as with IV administration, seven (at

least 5) time points are recommended after oral or other non-parenteral routes of

administration in order to adequately capture the entire concentration versus time

profile. At least one time point prior to and 3 time points after T_{max} during the terminal

elimination phase is recommended for the estimation of the $t_{1/2}$. The data point

at T_{max} should not be included in an estimation of the terminal half-life.

The AUC estimated for compound plasma concentrations (or alternatively blood or

serum concentrations; while plasma and serum are derived from whole blood, serum is

obtained from coagulated blood such that it differs from plasma in not having fibrin and

related coagulation factors) is the primary measure of overall compound exposure

following IV or extravascular administration.[78] The units of AUC are

concentration × time (e.g., h*µg/mL). The AUC is commonly estimated by the linear or

log trapezoidal methods or a combination thereof. The linear trapezoidal method for

estimating AUC over 2 adjacent time points, t_1 and t_2 ($AUC_{t_1 - t_2}$, the area of a

trapezoid between t_1 and t_2), should be performed on a linear scale as follows:

$$AUC_{t1-t2} = [(t_2 - t_1) \times (C_2 + C_1)]/2$$

The log trapezoidal method uses the following equation:

$$AUCt1\text{-}t2 = [(t_2 - t_1) \times (C_2 + C_1)]/\ln(C_2/C_1)$$

The linear trapezoidal rule is most reliable for slowly ascending and declining curves,

but is error prone if there is a sharp bending in the curve. The log trapezoidal rule is

usually more reliable for an exponentially declining curve and is error prone in an

ascending curve or near a peak. As a result, the choice of a combination of linear and

log trapezoidal methods is available in commercially available software (*e.g.*,

Phoenix® WinNonlin®, Certera). The latter software uses the log trapezoidal rule

after C_{max} or after C_0 for IV bolus administration (if $C_0 > C_{max}$); otherwise the linear

trapezoidal rule is used. Following a single IV dose, the AUC from time zero to the first

sampling time point (AUC_{0-t1}) uses C_0 as the concentration at time zero, whereas

following a single non-parenteral (e.g., PO) dose, the concentration at time zero is

generally zero. To estimate the AUC over an extended time period, the areas of the

individual trapezoids are added. To estimate the AUC from the last sampling time point

(t_{last}), assuming that the concentration (Ct_{last}) is not zero, to infinity the following equation is used:

$$AUCt_{last-\infty} = Ct_{last}/\lambda_z$$

where λ_z is the terminal rate constant, usually obtained from nonlinear regression analysis of at least the last 3 data points on the compound concentration versus time curve, plotted on a semi-logarithmic scale. The value used for Ct_{last} is either the measured concentration at t_{last} or that predicted from the regression line fitted to the last 3 data points. The latter is more reliable in cases where the compound concentration determined at t_{last} is near the lower limit of quantification of the bioanalytical assay and/or the correlation coefficient (r) for the regression analysis is poor. The AUC from the time of dosing and extrapolated to infinity ($AUC_{0-\infty}$) is equal to the sum of the $AUC_{0-tlast}$ and $AUCt_{last-\infty}$. For the estimate of the $AUC_{0-\infty}$ to be reliable, the percentage of the $AUC_{0-\infty}$ that is extrapolated from t_{last} to infinity should not, as a rule, exceed 15%.

The mean residence time (MRT) is the arithmetic mean of the duration that a compound resides in the body before being eliminated and can be calculated as $AUC_{0-\infty}/AUMC_{0-\infty}$, where $AUMC_{0-\infty}$ is the area under the first moment curve (the AUC of a plot of the product of concentration (C) × time (t) versus t). The unit of MRT is time (min or

h). $AUMC_{0-tlast}$ can also be estimated with the trapezoidal rule as described above.

To extrapolate the AUMC from the last time point to infinity:

$$AUMCt_{last-\infty} = [(Ct_{last} \times t)/\lambda_z] + (Ct_{last}/\lambda_z^2)$$

$AUMC_{0-\infty}$ is equal to the sum of $AUMC_{0-tlast}$ and $AUMCt_{last-\infty}$. The units for AUMC are concentration $\times$ time^2 (e.g. ng/min^2/mL).

The systemic clearance of a compound (Cl, also referred to as the total body clearance, (Cl$_T$) can be calculated from the plasma concentration versus time curve determined following IV dosing ($AUC^{iv}_{0-\infty}$):

$$Cl = Dose^{iv}/AUC^{iv}_{0-\infty}$$

where Dose^{iv} is the IV dose administered. The units of Cl are expressed as volume/time (e.g. mL/min or L/min (or h)) and are usually normalized to kg of body weight (e.g. mL/min/kg).

The simplest method to estimate the volume of distribution at steady-state (V_{ss}) is to use moment analysis; V_{ss} equals the product of Cl$_s$ and MRT, determined following IV administration:

$$Vd_{ss} = Cl \times MRT_{iv}$$

The units of V are usually expressed as mL/kg or L/kg, when normalized to kg body weight. As mentioned previously, Cl and Vd_{ss} can only be estimated from IV administration data and not from concentrations determined following non-parenteral administration. Vd_{ss} is used to assess the extent of distribution of a compound from the plasma to the tissues.

The bioavailability (F) of a compound is the fraction of an extravascularly administered dose that reaches the systemic circulation. Absolute bioavailability (F) is determined by calculating the ratio of the dose-normalized AUCs following extravascular and IV administration. Oral bioavailability (F^{po}) is given as an example below:

$$F^{po} = (AUC^{po}_{0-\infty} \times Dose^{iv})/(AUC^{iv}_{0-\infty} \times Dose^{po})$$

This equation assumes the same total clearance of the drug after extravascular and intravascular doses. Bioavailability is model independent, requiring only first order drug elimination. Bioavailability ranges from F = 0, in which case none of the extravascular drug reaches the systemic circulation, to F = 1 for drugs that are completely absorbed. Incomplete bioavailability (F<1) can occur with any extravascular administration route, but is usually lowest with oral drug administration.

Relative bioavailability (F_{rel}) between 2 routes of administration, dosage forms or formulations is similarly calculated as the ratio of the dose-normalized AUC of the test form or formulation to the dose-normalized AUC of the reference form or formulation. Since F is a fraction (between 0 and 1), it has no units and is often expressed as a percentage (%F = F × 100%).

The mean absorption time (MAT) following oral administration of a compound (MAT^{po}) can be estimated from the MRT since the MRT of a compound after p.o. administration (MRT^{po}) includes the time required for absorption and the MRT after IV administration (MRT^{iv}):

$$MAT^{po} = MRT^{po} - MRT^{iv}$$

The half-life ($t_{1/2}$) of a compound is the time (units in min or h) it takes for the plasma concentration or the amount of compound in the body to decrease by 50%. For compounds with plasma concentration versus time profiles that decline in a mono-phasic manner (1 compartment model), half-life is readily determined and the relationship between $t_{1/2}$, Cl and Vd is represented as:

$$t_{1/2} = 0.693 \times (Vd/Cl)$$

As Cl increases, $t_{1/2}$ decreases; as Vd increases, $t_{1/2}$ increases. Thus, $t_{1/2}$ is a secondary parameter that is a function of the Cl and Vd of the drug. For compounds which exhibit multi-exponential (*e.g.*, bi- or tri-phasic) patterns of decline (2- and 3-compartment models, respectively), two or more half-lives may be calculated. The terminal or elimination $t_{1/2}$ of a compound refers to the time it takes for its concentration in plasma to decrease by half during the terminal log-linear phase (represented as a straight line on a semi-logarithmic plot) of the plasma concentration versus time profile. This may be estimated by curve-fitting, in which at least 3 data points during the terminal phase are used (over which the time interval is greater than at least twice the estimated $t_{1/2}$). The slope ($-\lambda_Z/2.303$) of the terminal phase of the Log plasma concentration versus time curve is used to determine the terminal $t_{1/2}$:

$$t_{1/2} = 0.693/\lambda_Z$$

Section 5: Levetiracetam concentration determination

High-performance liquid chromatography has been commonly used for measuring LEV concentrations. Recently, a homogeneous immunoassay for LEV measurement in serum and plasma was introduced by ARK Diagnostics, Inc. This immunoassay was established on the Siemens ADVIA 1200 automated chemistry analyzer and validated.[80] The intraday precision was assessed by 10 replicates of two concentrations of quality control materials in a batch; whereas interday precision was estimated by assaying the

same materials one set per day for 20 days. Linearity was evaluated by serially diluting

the highest calibrator and a high patient specimen run in triplicate, whereas the lower

limit of quantification was confirmed by 10 measurements of a low-level specimen

diluted from a calibrator and another from a diluted patient specimen. This method was

compared with a commercial high-performance liquid chromatography method

(Chromsystems) using 63 specimens from human patients who were on LEV therapy.

The assay cycle was 10 minutes with a theoretical throughput of 800 per hour. The

intra- (n = 10) and interday (n = 20) coefficients of variation were 8.1% or less for the

two concentrations tested. The manufacturer-claimed analytical measurable range (2.0-

100.0 µg/mL) was confirmed by serial dilution and lower limit of quantification

experiments. Among the 63 patient samples studies, four showed LEV levels below 2.0

µg/mL by both methods. Deming regression using the remaining 59 paired patient

results by ARK immunoassay and the high-performance liquid chromatography method

showed a correlation coefficient of 0.9962, a linear regression slope of 0.98, and an

intercept of 0.61 with a mean bias of 0.04%. In this study, the ARK immunoassay was

suitable for clinical use of monitoring LEV concentrations in serum/plasma on an

automated chemistry analyzer (Siemens ADVIA 1200).

Section 6: Protein binding

Binding of drugs to plasma proteins is one of many factors that influences drug

pharmacokinetics.[81] Binding of a drug to plasma protein reduces free drug available to

penetrate from the blood circulation into tissues to reach the therapeutic target or the kidney for elimination. The drug–plasma protein complex cannot permeate through cell membranes by passive transcellular or paracellular permeation. Only free drug passes through membranes to reach tissues, and only free drug molecules are available for liver metabolism and renal excretion. Therefore, it is generally accepted that the effect of a drug is related to the exposure of a patient to the unbound concentration of the drug at its action site rather than its total concentration. Drugs can bind to a variety of blood constituents, including albumin, α1-acid glycoprotein, lipoproteins, red blood cells, leukocytes, platelets and α-, β- and γ-globulins. The interaction of a drug with plasma proteins is electrostatic, hydrophobic, satiable and reversible. Drug–plasma protein complexes in the plasma serve as drug reservoirs for the free drug concentration. As the drug is removed from the body by various elimination processes, drug–plasma protein complexes prolong the duration of drug action.

There are two complementary factors of plasma protein binding: (i) degree of binding at equilibrium, which is expressed as percent bound or percent unbound in plasma, or equilibrium dissociation constant K_d; and (ii) rate of association and dissociation, which is expressed as association and dissociation rate constants K_a and K_d, respectively. These factors affect the absorption, distribution, metabolism and elimination of a drug.[81] For example, if the drug is highly bound (i.e. low percent unbound) and tightly bound (i.e. slow dissociation) to plasma proteins, the effect of the binding on the pharmacokinetics of the drug can be 'restrictive' to drug retention in plasma and drug

distribution into target tissue; can decrease metabolism and clearance but increase half-life and drug brain penetration; therefore, the requirement for higher loading but lower maintenance doses. By contrast, if the drug has high percentage binding and a fast dissociation rate, low percentage binding and a slow dissociation rate, or low percentage binding but a fast dissociation rate then the effect of the binding on its pharmacokinetic can be 'nonrestrictive' or 'permissive'. High percentage binding can be restrictive or permissive to liver extraction.

There are numerous methods available for measuring plasma protein binding including ultrafiltration, ultracentrifugation, equilibrium dialysis, high-performance frontal analysis, solid-phase microextraction, charcoal adsorption and *in vivo* microdialysis.[81,82]

Ultrafiltration uses either individual sample vials or a 96-well ultrafiltration device to separate free drug from its binding fraction of plasma protein.[81] An aliquot of a plasma sample is loaded into the upper chamber of an ultrafiltration apparatus that has a membrane with a certain molecular weight cut-off. The device is centrifuged and the solution is ultrafiltered through the membrane by the force of the centrifugation. Unbound drug moves with the liquid through the membrane into the receiver chamber, whereas drug bound to plasma protein remains in the loading chamber. Ultrafiltration is conducted under the assumptions that: (i) the drug does not bind to the membrane; (ii) there is no leakage of plasma protein through the membrane; (iii) the equilibrium constant does not change as the protein is gradually concentrated during the separation process; and (iv) the membrane is equally permeable to the drug and water. After

centrifugation at 2000 g for 30 min, the filtrate is collected. The concentration of test

drug in the receiver is quantitated, and the fraction unbound is calculated as this

ultrafiltrate concentration is divided by the total initial concentration.

The following equations can be used to determine the fraction bound (FB) and %

protein binding:

$$FB = (C_{BD} - C_{BF}) / C_{BD}$$

$$Fu = (1 - FB)$$

$$\% \text{ protein binding} = (1 - Fu) \times 100$$

where FB denotes the fraction bound, C_{BD} the concentration of drug in buffer; C_{BF}

the concentration of drug in filtrate after ultrafiltration of drug in buffer; C_{SD} the

concentration of drug in serum (or plasma); C_{SF} the concentration of drug in filtrate after

ultrafiltration of drug in serum (or plasma); and Fu is the free fraction of drug.[75]

Although ultrafiltration is a simple and rapid technique that is especially applicable to

unstable drugs, the major disadvantage of this technique is the nonspecific binding of

drugs to filter membrane that is composed of cellulose acetate and a plastic device. It is

thought that the nonspecific binding can sometimes affect 20–30% of tested drugs.[81]

Section 7: Justification of the study

Pharmacokinetics in veterinary medicine have been used to describe the processes of drug absorption, distribution and elimination (metabolism and excretion) in animals. Drugs administered intravascularly distribute to sites where they produce their intended effects. The rate and extent of access to these sites of action (therapeutic or toxic) in the presence of competing events such as metabolism, excretion and distribution to other tissues, or presence of other drugs will determine the therapeutic outcome. If a drug is administered extravascularly, the accessibility of the drug to the systemic circulation, or systemic availability, will also influence the clinical outcome. This process is especially complex when the extravascular route is oral, as the drug must gain access to the portal circulation from the enteric environment and 'survive' passage through the gut wall and liver with their respective ability to metabolize and inactivate drugs. Knowledge of the pharmacokinetic and pharmacodynamic parameters that describe drug disposition and effects in animals, as well as the inter-individual variability associated with these parameters and the pathophysiological factors that contribute to this variability, are critical to the design of appropriate dosage regimens in animals.

The goal of drug administration consists of achieving the desired effect while minimizing the risks of toxicity. The complex processes that determine the circulation of drugs through the body are influenced by many different factors, a number of which arise from the physiology of the subject receiving the drug. In addition, pathologic processes can alter drug disposition by modifying the physiological functions that

influence the circulation of drugs in the body. Altered pharmacokinetics may ultimately result in therapeutic failure or altered tissue distribution. Consequently, knowledge of the factors that modify the disposition of drugs and the extent of this modification is critical to properly describe the kinetic relationships between drugs and patients. Potential sources of variability encountered in the clinical setting are age, weight, gender, breed, disease status, concomitant use of other drugs, altered physiological functions (such as renal or hepatic), hydration status, cardiac output, fever, nutritional status, genetic polymorphisms, etc. Customarily, dosage regimens are determined from studies conducted in a small number of generally healthy individuals which cannot account for all of the possible clinical factors that may be present in a patient and the relationship of these factors to drug disposition and effect.

Dosage regimens for humans and other species cannot be extrapolated to horses due to physiological differences between species; therefore the need to study species-specific levetiracetam pharmacokinetics is highlighted here. Pharmacokinetic studies in healthy horses provide general principles of the drug's disposition.

The purpose of this study was to investigate the pharmacokinetics of levetiracetam in healthy adult horses, after a single dose IV (LEV$_{IV}$) and intragastric administration of levetiracetam immediate (LEV$_{IR}$) and extended release (LEV$_{ER}$) formulations to determine a treatment regimen that would achieve and maintain therapeutic serum concentrations described in humans and small animals (5 to 45 ug/ml) (Bazil, 2002), throughout a dosing interval. In order to maximize the chances of successful seizure

control, the target mean drug plasma concentration was established at 35 μg/mL (75%

of the therapeutic range). A second purpose was to compare the pharmacokinetics

between the crushed extended release and the immediate release formulations, in case

the latter is withdrawn from the commercial market due to the greater acceptance of the

extended release formulation among human patients.

Chapter 2: Disposition of levetiracetam in healthy adult horses

Section 1: Material and methods

Animals

Nine light breed adult horses (8 geldings and 1 mare) ranging in age from 9 to 14 years old, and body weight from 441 kg to 598 kg (mean 539 kg) were studied. Horses had not received any medication for 15 days prior to the beginning of the study. All horses were determined to be clinically healthy based upon physical examination, complete blood count (CBC), fibrinogen concentration, biochemical profile and urine dipstick. On the day prior to each period of the study, each horse was weighed and an indwelling intravenous catheter was aseptically placed in a jugular vein. Horses were kept in a stall throughout the sampling periods and had free access to coastal bermuda hay, excluding one hour before and immediately after LEV administration. At the conclusion of each study period, the indwelling catheter was removed. All procedures were approved by the Institutional Animal Care and Use Committee at Auburn University.

Study Design

Horses were randomly assigned to one of the three treatment groups in a prospective, 3-way crossover design separated by a 7-day minimum washout period. Randomization was performed utilizing a random number table generator. All horses initially received either a single dose of oral levetiracetam immediate release - LEV_{IR} (Keppra® 500 mg, UCB Pharma SA, Belgium) of approximately 30 mg/kg, oral

levetiracetam extended release - LEV$_{ER}$ (Levetiracetam Extended Release Tablets 500 mg, Watson Laboratories, Inc., CA) of 30 mg/kg, or intravenous levetiracetam - LEV$_{IV}$ (Levetiracetam Injection 100 mg/mL, Sun Pharmaceutical Ind. Ltd, India) of 20 mg/kg, in the first period. Alternative formulations were administrated during in the second and third periods to complete the crossover design. Based on the individual body weight, the calculated dose for the oral formulations was rounded to the nearest 500 mg whole tablet. Tablets for both oral formulations were crushed and dissolved in 500 mL of water and immediately administered via nasogastric tube, followed by approximately 1.5 liters of water. For the IV administration, the calculated dose of the commercial IV preparation of LEV was administered as a slow bolus over 5 minutes, through a temporary jugular vein catheter.

Baseline (time 0) serum samples were collected from all horses prior to drug administration. After LEV$_{IV}$ administration, blood samples were collected from a separate intravenous catheter in the opposite jugular vein at 5, 10, 20, 30, 45, 60, 90 minutes and 2, 3, 4, 6, 8, 12, 18, 24, 36 and 48 hours. Following intragastric administration of LEV, the nasogastric tube was removed and blood samples collected at 10, 20, 30, 45, 60, 90 minutes and 2, 3, 4, 6, 8, 12, 18, 24, 36 and 48 hours through an intravenous catheter. Five mL of blood was collected and discarded prior to collection of each sample. After each sample was collected, indwelling catheters were flushed with 5 mL of heparinized sodium chloride 0.9% USP (10 IU/mL, Hospira, Deerfield, IL). All samples were collected into plain glass tubes (Monoject; Tyco,

Mansfield, MA, USA), allowed to clot at room temperature, and centrifuged at 1200 rpm for approximately 15 minutes. The serum was harvested and stored at -80°C until assayed.

Evaluation for adverse effects

Heart rate, respiratory rate and rectal temperature were monitored before drug administration and at each time point at which blood samples were obtained for 48 hours after LEV administration. The horses were also observed for evidence of adverse drug reactions. A biochemical profile was performed prior to beginning each phase.

Sample analysis and protein binding

Frozen archived serum samples previously collected from 20 clinically healthy horses were used to validate the ARK Diagnostic (Sunnyvale, CA) Levetiracetam® Immunoassay on a Siemens (New York, NY) Dimension Xpand Plus® general chemistry analyzer. Serum samples were thawed at 21°C and then mixed to assure homogeneity. Analysis of the serum samples was performed and LEV was detected in equine serum using the previously validated immunoassay. Commercially available kits for LEV were used for system calibration (ARK Diagnostic Levetiracetam Calibrator Kit®, Sunnyvale, CA) and for sample quality control (ARK Diagnostic Levetiracetam Control Kit®, Sunnyvale, CA). The upper limit of quantitation was 150 μg/mL, lower limit of quantitation was 2 μg/mL. Coefficient of variation values were less than 5% throughout the range of quantitation.

In order to obtain LEV protein binding, the free drug concentration was determined by ultrafiltration of pooled samples obtained at C_{max} for each formulation at 37°C using Centrifree® (Amicon, Bedford, MA) systems. The fraction bound to plasma was determined as 1 – (free concentration/total concentration).

Pharmacokinetic analysis

Serum LEV concentrations were analyzed using noncompartmental analysis with log-linear regression of the terminal plasma concentration decline (WinNonlin Professional® Pharsight Corp., Mountain View, CA, USA). The area under the curve (AUC) was determined by the trapezoidal method from time 0 extrapolated to infinity. For IV administration, peak plasma concentrations were extrapolated from the y-intercept (C_0), whereas for intragastric administration, the actual peak plasma concentration (C_{max}) and the time to achieve peak plasma concentration (T_{max}) were reported. Mean volume of distribution at steady state (Vd_{ss}) was determined for LEV_{IV}. Total body clearance (Cl) was determined for LEV_{IV} and clearance divided by bioavailability (Cl/F) for LEV_{IR} and LEV_{ER}. In addition, the elimination rate constant ($λz$), mean absorption time (MAT), mean residence time (MRT) and terminal half-life (t ½) were determined for each horse and each route. Bioavailability (F) after intragastric administration was determined by the use of the equation (AUC after oral administration * Dose IV)/(AUC after IV administration * Dose intragastric) x 100. The time that the drug concentration remained in the therapeutic range defined for humans (5 to 45 µg/mL) was determined. Recommended single IV and oral doses were calculated by the

use of the equations (Cl*C_{ss}*dosing interval) and (Cl/F*C_{ss}*dosing interval), respectively, where C_{ss} is desired plasma concentration at steady state. Estimates of steady-state C_{max} and C_{min} based upon 8, 12 and 24 hour dosing regimens were made by superimposition methods.[83,84] Simulations were calculated using sampling time intervals. Fluctuation was calculated by the use of the equation (C_{max}-C_{min}/C_{avg}).

Statistical analysis

Descriptive statistics (mean ± standard error of the mean and 95% confidence intervals) for each parameter were generated for normally distributed data; except for $t_{1/2}$ which was reported as the harmonic mean and its comparable measure of standard deviation (SD), pseudo-SD.

Pharmacokinetic parameters obtained for each intragastric formulation were compared using analysis of variance (ANOVA). Statistical significance was set at $P \leq$ 0.05.

Bioequivalence was assessed based on LN-transformed values by an analysis of variance model including the effects of formulation, period, sequence, and subject. The 90% confidence intervals (CI) for the ratio % (LEV_{IR}/LEV_{ER}) of the least squares means of AUCs and C_{max} parameters were constructed. These 90% confidence intervals were obtained by exponentiation of the 90% confidence intervals for the difference between the least squares means based upon a LN scale.[85] Power was to detect a 20% difference.

Section 2: Results

Adverse effects

All horses appeared to tolerate LEV well; adverse effects were apparently limited to yawning in 1 horse, transient low head carriage in 5 horses, and lip leaking in 6 horses. These observations were not related to any specific LEV formulation. No significant sedative effects, abnormal heart rate, respiratory rate or rectal temperature or any other abnormalities were observed in any of the 9 horses.

Pharmacokinetics after IV administration of a single dose of LEV

Serum concentrations of LEV were above or within the therapeutic range defined for humans for at least 12 hours in 8 of 9 horses. Mean LEV concentration time curve after IV administration is shown in Figure 2. C_0 was 64.54 ± 8.33 μg/mL, λz was 0.11 ± 0.008 1/hr, AUC was 17119 ± 1256 h*μg/mL, AUC corrected by dose was 856 ± 63 h*μg/mL, $t_{1/2}$ was 6.22 ± 1.35 hours, MRT was 8.79 ± 0.55 hours, Vd_{ss} was 0.63 ± 0.02 L/kg, and Cl was 1.24 ± 0.10 mL/min/kg (Table 1). Individual values are reported in Appendix 1.

Pharmacokinetics after intragastric administration of a single dose of LEV$_{IR}$

Serum concentrations of LEV were above or within the therapeutic range defined for humans for at least 12 hours in 9 of 9 horses. Mean LEV$_{IR}$ concentration time curve after intragastric administration is shown in Figure 3. C_{max} was 50.72 ± 3.53 μg/mL, T_{max} was 39.44 ± 10.36 min, $t_{1/2}$ was 6.38 ± 1.97 hours, MRT was 9.61 ± 0.85 hours, F was

$96 \pm 0.07\%$, AUC was 24396 ± 1404 h*μg/mL, AUC corrected by dose was 813 ± 47 h*μg/mL, and Cl/F was 1.28 ± 0.08 mL/min/kg (Table 2). Individual values are reported in Appendix 2.

Pharmacokinetics after intragastric administration of a single dose of LEV$_{ER}$

Serum concentrations of LEV were above or within the therapeutic range defined for humans for at least 12 hours in 9 of 9 horses. Mean LEV$_{ER}$ concentration time curve after intragastric administration is shown in Figure 2. C_{max} was 53.58 ± 5.31 ug/mL, T_{max} was 38.33 ± 4.41 min, $t_{1/2}$ was 7.07 ± 1.93 hours, MRT was 10.58 ± 0.94 hours, F was $98 \pm 0.09\%$, AUC was 25260 ± 2130 h*μg/mL, AUC corrected by dose was 842 ± 71 h*μg/mL, and Cl/F was 1.28 ± 0.11 mL/min/kg (Table 2). Individual values are reported in Appendix 3.

Statistical analysis

Statistical differences could not be detected among C_{max}, T_{max}, AUC, Cl/F, and F obtained after intragastric administration of LEV$_{IR}$ and LEV$_{ER}$. MRT ($P = 0.005$) and $t_{1/2}$ ($P = 0.047$) were statistically greater for LEV$_{ER}$. MAT was evaluated for each horse and route; however, due to a rapid drug absorption phase, the negative MAT values were nonsensical.

When assessing bioequivalence between the oral formulations, the obtained ratio of the AUC geometric means (LEV$_{ER}$/LEV$_{IR}$) and the confidence interval were 1.02 (0.92

– 1.09), with a power of 0.997. For C_{max}, the obtained ratio and confidence interval were 1.06 (0.81 – 1.23), with a power of 0.827.

LEV protein binding

The mean LEV fraction bound to plasma protein was $4.5 \pm 4.4\%$.

Calculation of recommended repeated dosing regimen

Based on an observed Cl of 0.0746 L/hr/kg, and desired C_{ss} of 35 mg/L, and dosing intervals of 8, 12 and 24 hours for LEV_{IV}, recommended doses of 20.9, 31.3 and 62.7 mg/kg were obtained, respectively. Based on an observed Cl/F of 0.0769 L/hr/kg, and desired C_{ss} of 35 mg/L for either crushed tablets of LEV_{IR} and LEV_{EX}, and dosing intervals of 8, 12 and 24 hours, recommended doses of 21.5, 32.3 and 64.6 mg/kg were obtained, respectively.

Predicted trough drug plasma concentrations at steady state (C_{minss}), peak drug concentrations at steady state (C_{maxss}), average drug concentration (C_{avg}) and fluctuation ratios after each recommended dosing regimen for LEV_{IV} and oral LEV are shown in Table 3.

Figure 2 – Mean ± SEM plasma LEV concentrations at various times after IV

administration of a single dose of LEV (20 mg/kg) to 9 horses. Time of LEV

administration was designated as time 0.
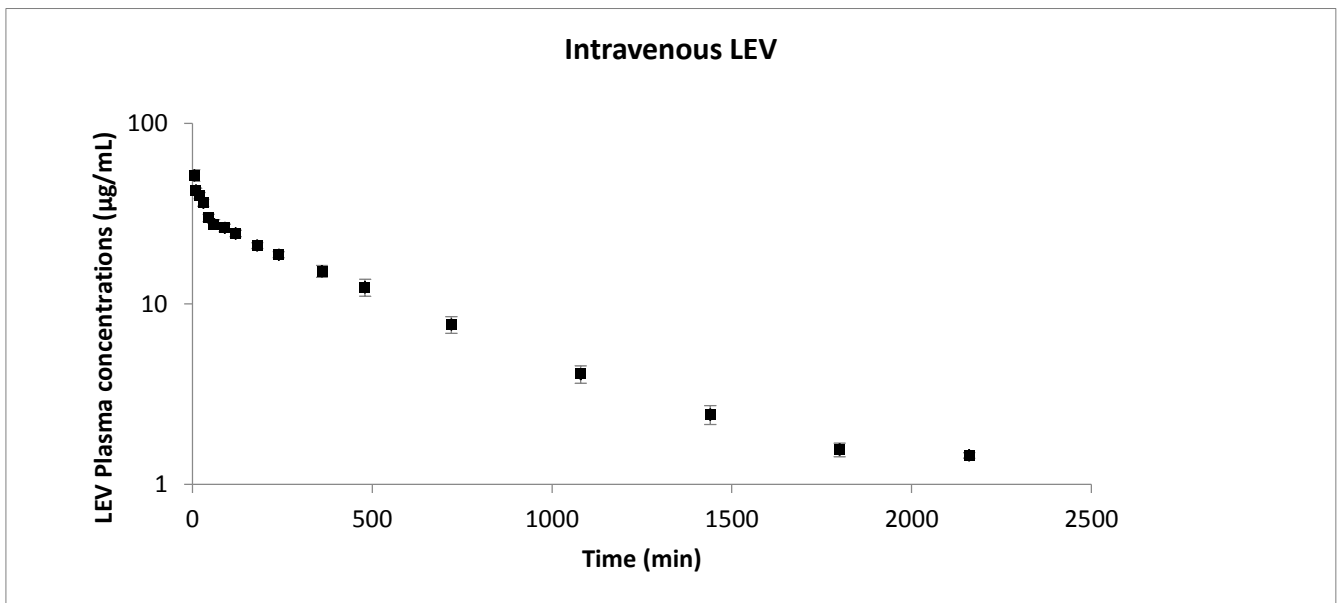
Figure 3 – Mean ± SEM plasma LEV concentrations at various times after intragastric administration of a single 30 mg/kg dose of LEV_{IR} and LEV_{ER} to 9 horses. Time of LEV administration was designated as time 0.
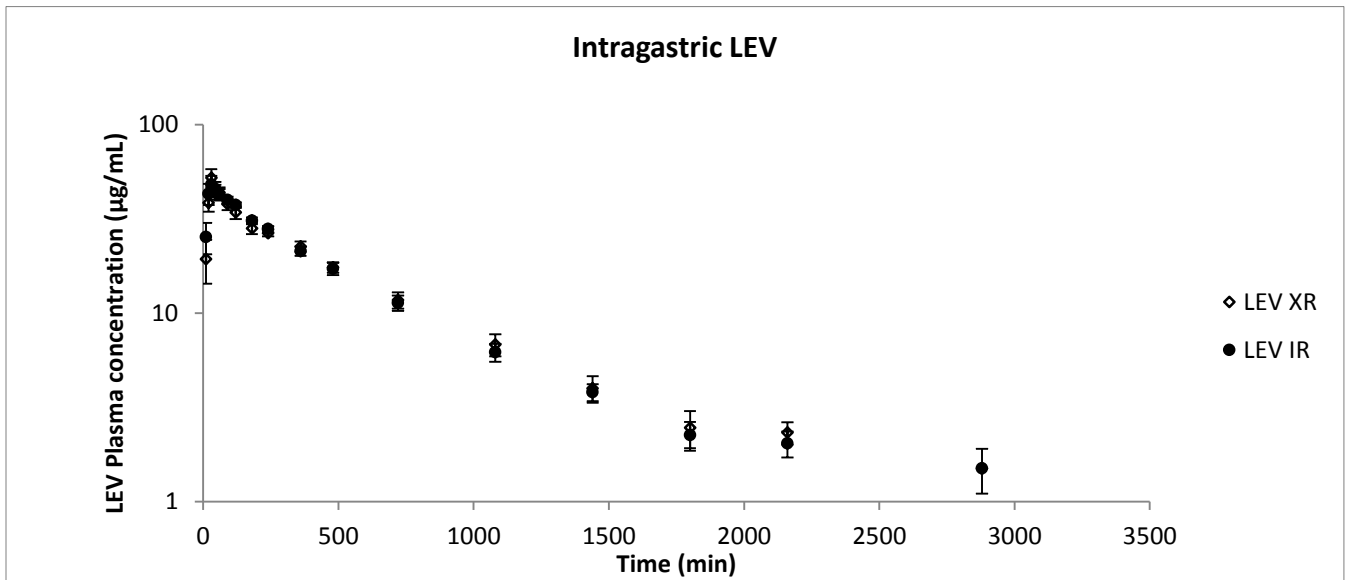
Table 1 – Pharmacokinetics parameters (mean $\pm$ SEM [95% confidence interval]) of

LEV_{IV} in serum after administration of a single dose (20 mg/kg) to 9 horses.

PK Parameter	$LEV_{IV} \pm$ SEM (95% CI)
y-intercept (µg/mL)	64.54 $\pm$ 8.33 (6.90-122.17)
Λz (1/hr)	0.111 $\pm$ 0.008 (0.0009-0.0027)
AUC (h*µg/mL)	17119 $\pm$ 1256 (8426-25814)
AUC/Dose (h*µg/mL)	856 $\pm$ 63 (421-1290)
$t_{1/2}$ (h)	6.22 $\pm$ 1.35 (3.36-9.60)
MRT (h)	8.79 $\pm$ 0.55 (5.00-12.61)
Cl (mL/kg/min)	1.24 $\pm$ 0.10 (0.50-1.98)
Vd_{ss}	0.63 $\pm$ 0.02(0.46-0.79)
PK, pharmacokinetic; SEM, standard error of the mean; Λz, slope; AUC, area under the plasma concentration vs. time curve from time zero extrapolated to infinity; $t_{1/2}$, terminal half-life; MRT, mean residence time, Cl, total body clearance; Vdss, volume of distribution at steady-state.	

Table 2 – Pharmacokinetic parameters (mean $\pm$ SEM [95% confidence interval]) of LEV$_{IR}$ and LEV$_{ER}$ in serum after administration of a single dose (30 mg/kg) to 9 horses.

PK parameter	LEV$_{IR}$ $\pm$ SEM (95%CI)	LEV$_{ER}$ $\pm$ SEM (95%CI)	*P* value
C$_{max}$ (µg/mL)	50.72 $\pm$ 3.53 (26.29-75.16)	53.58 $\pm$ 5.31 (16.82-90.33)	0.456
T$_{max}$ (h)	39.44 $\pm$ 10.36 (32.20-111.09)	38.33 $\pm$ 4.41 (7.83-68.84)	0.988
t$_{1/2}$ (h)	6.38 $\pm$ 1.97* (1.91-11.91)	7.07 $\pm$ 1.93* (1.44-13.95)	0.047[a]
MRT (h)	9.61 $\pm$ 0.85 (3.76-15.50)	10.58 $\pm$ 0.94 (4.06-17.11)	0.005[a]
F (%)	96 $\pm$ 0.07 (0.89-1.03)	98 $\pm$ 0.09 (0.90-1.07)	NA
AUC (h*µg/mL)	24396 $\pm$ 1404 (14680-34111)	25260 $\pm$ 2130 (10527-39993)	0.421
AUC/Dose (h*µg/mL)	813 $\pm$ 47 (489-1137)	842 $\pm$ 71 (350-1333)	0.421
Cl/F (mL/kg/min)	1.28 $\pm$ 0.08 (0.73-1.83)	1.28 $\pm$ 0.11 (0.50-2.07)	0.421

[a]statistically different parameter among compared groups; C$_{max}$, observed maximum plasma concentration; AUC, area under the plasma concentration vs. time curve from time zero extrapolated to infinity; t$_{1/2}$, terminal half-life; MRT, mean residence time; F, bioavailability; T$_{max}$, time of observed maximum concentration; *P*-value obtained based on natural log-transformed values and an analysis of variance model including the effects of formulation, period, sequence and subject.

Table 3 - Estimates of steady-state C_{max} (C_{maxss}), C_{min} (C_{minss}), (C_{avgss}) and fluctuation ratio

(FR) based upon 8, 12, and 24 hour dosing regimens.

LEV_{IV}	C_{maxss}	C_{minss}	C_{avgss}	FR	C_{maxss}	C_{minss}	C_{avgss}	FR	C_{maxss}	C_{minss}	C_{avgss}	FR
Dose (mg/kg)	20.9	20.9	20.9	20.9	31.3	31.3	31.3	31.3	62.7	62.7	62.7	62.7
Interval (hr)	8	8	8	8	12	12	12	12	24	24	24	24
Mean	74.5	21.7	37.2	1.46	96.9	16.5	37.2	2.22	169.6	8.0	37.2	4.44
SD	15.1	6.5	8.2	0.33	20.1	5.1	8.2	0.47	35.8	2.9	8.2	0.89
Hi 95% C	84.4	25.9	42.6	1.68	110.1	19.9	42.5	2.53	193.0	9.9	42.6	5.02
Lo 95% C	64.7	17.4	31.9	1.24	83.8	13.2	31.8	1.91	146.2	6.1	31.9	3.86
Oral LE	C_{maxss}	C_{minss}	C_{avgss}	FR	C_{maxss}	C_{minss}	C_{avgss}	FR	C_{maxss}	C_{minss}	C_{avgss}	FR
Dose (mg/kg)	21.5	21.5	21.5	21.5	32.3	32.3	32.3	32.3	64.6	64.6	64.6	64.6
Interval (hr)	8	8	8	8	12	12	12	12	24	24	24	24
Mean	59.5	23.5	36.4	1.01	72.7	18.3	35.6	1.55	121.2	9.5	36.5	3.13
SD	13.1	7.2	7.2	0.28	18.7	6.6	7.6	0.42	29.8	4.5	7.2	0.79
Hi 95% C	65.5	26.8	39.7	1.14	81.4	21.3	39.2	1.74	135.0	11.5	39.8	3.49
Lo 95% C	53.4	20.2	33.1	0.88	64.1	15.2	32.1	1.35	107.4	7.4	33.1	2.77

SD, standard deviation; Hi 95% CI, upper limit of the 95% confidence interval; Lo 95% CI, lower li of the 95% confidence interval.

Chapter 3: Discussions and conclusions

Results of the present study indicate that the administration of LEV to healthy adult horses after a single 20 mg/kg IV or a 30 mg/kg oral dose of either LEV_{IR} or LEV_{ER} was apparently well tolerated and consistently produced mean serum drug concentrations within the proposed therapeutic range of 5-45 µg/ml for at least 12 hours after the administration of the IV and 18 hours after the administration of the oral formulations, respectively.

In order to assure accurate delivery of the calculated dose of the oral formulations to each horse, intragastric administration via nasogastric tube was performed instead of oral administration. In an attempt to reproduce a routine oral treatment, where the tablets are likely to be chewed by the animal, both immediate and extended release tablets were crushed. Also, to allow comparison of the pharmacokinetics between the crushed extended release and the immediate release formulations, in case the latter is withdrawn from the commercial market due to the greater acceptance of the extended release formulation among human patients. Because the extended release mechanism of the LEV_{ER} was altered by crushing the tablets, and the tablet dose of LEV_{IR} and LEV_{ER} were the same, the resulting LEV concentration profile for both oral formulations were very similar (Figure 3). In addition, despite the differences in pharmacokinetic parameters that occurred when comparing the brand (LEV_{IR}) with the generic (LEV_{ER}) formulation (i.e., shorter MRT and t1/2), these findings were not considered to be clinically significant.

The difference observed in the $t_{1/2}$ and MRT between the oral formulations could be explained by partially retained extended release mechanism of the crushed LEV_{XR} tablets. However, a retained LEV_{ER} extended release mechanism is not supported by comparison of T_{max}, and bioequivalence between LEV_{ER} and LEV_{IR}. Based on a previous report that food did not impair the extent of absorption of oral LEV in human patients,[60] the horses in this study were continuously offered hay except for the time they were restrained in stocks for 1 hour before and after administration of each dose. Also, as the stomach transit time for solid food in adult horses can take over 10 hours,[85] it is impractical to fast horses for drugs that require once or twice daily dosing.

Treatment options for seizure disorders in horses are limited, which mandates a need for identification and pharmacokinetic description of new AEDs for use in horses. Phenobarbital is currently the standard treatment for long-term seizure management in horses.[1] Although generally effective, phenobarbital has well described adverse effects, including marked sedation, respiratory depression, bradycardia, hypotension and induction of microsomal hepatic enzymes.[1,18,86] Phenobarbital is primarily metabolized by the liver, which may preclude its use in animals with hepatic dysfunction and generally increases the risk of drug interactions or hepatic disease.[87,88] Furthermore, given the variability in half-life, clearance, and metabolism of phenobarbital, therapeutic monitoring is essential to ensure adequate anticonvulsant concentrations are achieved and toxicity minimized.[1] Other AEDs such as potassium bromide, phenytoin and sodium pentobarbital have been considered as alternative therapy for horses with refractory

seizures but not without significant side effects.[27,32,33] Anedoctal reports exist for the use

of primidone for the treatment of seizures in foals, but its pharmacokinetic properties

and clinical effects are unknown.[34,35] Nevertheless, the clinical efficacy of these drugs

has not been evaluated in horses. Diazepam, a benzodiazepinic antiepileptic drug, has

been routinely used for short-term control of seizures in horses.[1] However, because of

its short half-life, repeated doses must be frequently administered and its prolonged use

can lead to respiratory depression or arrest.[14] Therefore, diazepam is not a good choice

for long-term control of seizures.

In the pharmacokinetic profile rating of AED for human patients by Patsalos (2004),

LEV was ranked first with "ideal" pharmacokinetics characteristics because of its rapid

and complete absorption after oral administration, minimal protein binding, no hepatic

metabolism, linear kinetics and no drug-drug interaction.[40] In our study, the C_{max} of

LEV_{IR} and LEV_{ER} were close to the LEV_{IV} C_o, and were reached within the first 40

minutes after intragastric administration. The disposition of LEV observed in the present

study was similar to that observed in humans but differed from that in cats and dogs.

The $t_{1/2}$ in horses for LEV_{IV} (6.22 ± 1.36 hours) is comparable to that for IV

administration in humans (7.16 ± 1.13 hours) and is considerably longer than that in

dogs (3.6 ± 0.8 hours) and cats (2.86 ± 0.65 hours).[74,89,90] Differences in $t_{1/2}$ in horses

and small animal species probably reflect differences in Cl and Vd_{ss} among these

species. LEV Cl is lower in horses compared with dogs (1.5 ± 0.3 mL/min/kg) and cats

(2.0 ± 0.6 mL/min/kg), while the Vd_{ss} is higher in horses than in dogs (0.52 ± 0.09 L/kg)

and cats (0.45 ± 0.13 L/kg). The longer half-life of LEV in horses allows LEV to be

dosed less often than the every 6-8 hours recommended for dogs or cats.[74,90]

A therapeutic range for serum concentrations of LEV has not been established in

dogs, cats or horses. In humans, the therapeutic range is not firmly established, but has

been reported as 5 to 45 µg/ml, on the basis of a typical dosing regimen of 500 mg to

1,500 mg every 12 hours with successful control of seizures.[91] For the purposes of the

present study, the reported therapeutic range for humans was used as a basis for

determining the desired plasma concentrations of LEV in horses. Mean plasma

concentrations in all horses were above 5 µg/ml for at least 12 hours after

administration of a single dose of 20 mg/kg LEV_{IV} and 18 hours after administration of a

single dose of 30 mg/kg LER_{IR} or LEV_{ER}. Based on the obtained LEV pharmacokinetic

parameters, and on a desired mean plasma drug concentration of 35 µg/ml between

dosing intervals, specific dose recommendations were predicted for multiple dosing

regimens with different dosing intervals. A dosage of 32 mg/kg every 12 hours, IV or

PO, is likely to achieve and maintain therapeutic range with optimal drug kinetics at

steady state, i.e., minimal drug accumulation and fluctuation between dosing.

The dose of 63 mg/kg every 24 hrs, IV or PO might be adequate for some horses

and offer a more convenient dosing regimen. However, the high peak plasma drug

concentration and fluctuation predicted with once daily dosing, could predispose these

patients to clinical signs of sedation and potential adverse effects. Moreover, for some

horses, a trough concentration in the low part of the human therapeutic range may be

inadequate to prevent seizures, and they may require further titration and individual

therapeutic drug monitoring, where trough (rather than peak) concentrations should be

targeted. On the other hand, the pharmacodynamic response to LEV may outlast its

presence in plasma, perhaps because of a longer maintenance of concentrations in the

cerebrospinal fluid (CSF) as has been determined in rats, for which the half-life in the

CSF is approximately twice that of the plasma.[61] Furthermore, LEV has

antiepileptogenic activity in rats that persists long after the elimination of the drug from

the plasma.[92] In humans, CSF $t_{1/2}$ values are more than three times that of blood and,

thus, the prolonged action of LEV in the brain could be explained by its prolonged efflux

from the brain compartment.[40] These studies reinforce the importance of LEV drug

monitoring and drug regimen tailoring for each patient treated for seizures.

In order to minimize plasma drug fluctuation between dosing, LEV could be

administered every 8 hours at 21 mg/kg. There will also be greater accumulation when

compared to dosing intervals of 12 and 24 hours.

Establishing a therapeutic range for LEV concentration in horses is relevant when

dosage recommendations are made. Even though seizures in horses are uncommon,

they remain a therapeutic, welfare and safety challenge for both veterinarians and horse

owners. On the basis of the present study, LEV should be considered as an alternative

to standard AEDs for seizure control in horses. LEV in particular has a highly desirable

pharmacokinetic profile in horses, being rapidly and well absorbed in face of continuous

feeding, exhibits linear kinetics, is not significantly bound to plasma proteins, is not

metabolized by hepatic enzymes and undergoes elimination with a mean half-life of approximately 7 hours. To our knowledge, there are no data on toxic effects of LEV in horses and additional studies evaluating LEV pharmacodynamics should be performed experimentally before using this drug routinely on clinical patients.

Ultimately, case series and clinical trials are encouraged to correlate the pharmacokinetics with the pharmacodynamics in prevention of seizures to improve dosage information for clinicians. However, due to the relatively low caseload of adult horses with seizures or epilepsy admitted to referral hospitals, it would take a very long time for these studies to build enough statistical power. Therefore, based on the pharmacokinetic evidence presented here, a recommended dosing regimen of intravenous or oral LEV, of 32 mg/kg every 12 hours is likely to achieve and maintain therapeutic range throughout the dosing interval, with optimal equilibrium kinetics. Because an assay is available to measure LEV in horses, monitoring is recommended to establish a dosing regimen and the individual patient therapeutic range.

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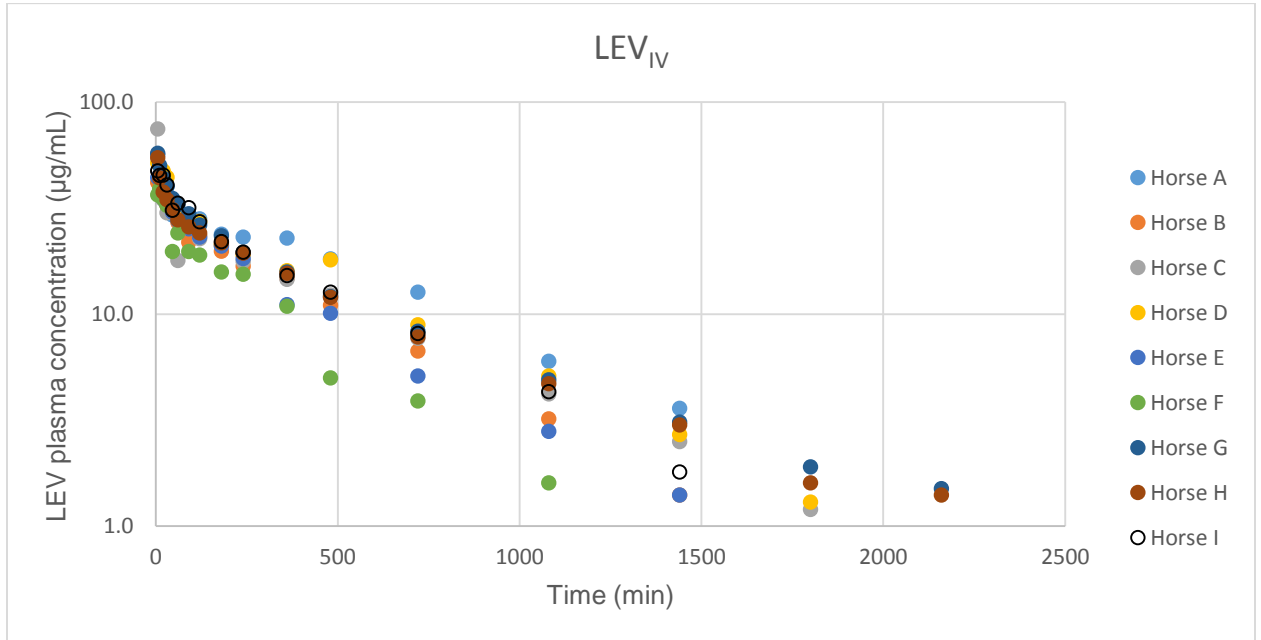
Appendices

Appendix 1A – Individual plasma drug concentration after administration of LEV$_{IV}$ of eight healthy adult horses.

Time	Horse A	Horse B	Horse C	Horse D	Horse E	Horse F	Horse G	Horse H	Horse I
5	56.4	41.8	74.7	51.7	44.1	36.5	57.4	54.7	47.4
10	40.1	36.6	45.8	44.0	38.0	38.8	50.2	43.9	45.2
20	41.9	36.9	34.7	47.4	35.3	35.0	43.8	37.7	45.3
30	40.0	32.7	30.0	44.2	32.9	32.5	40.1	34.6	40.6
45	29.3	30.0	30.3	33.3	30.3	19.7	35.1	31.1	30.9
60	28.2	27.1	17.9	30.7	27.6	24.1	32.8	27.8	33.4
90	25.6	21.8	29.0	29.1	25.3	19.7	29.7	25.8	31.8
120	28.1	23.5	22.7	27.4	23.1	19.0	26.3	24.1	27.3
180	23.8	19.8	21.1	21.2	20.9	15.8	23.4	21.7	22.0
240	23.1	16.8	17.7	19.0	18.3	15.4	19.5	19.4	19.6
360	22.8	14.8	14.6	16.0	11.1	10.9	15.8	15.6	15.2
480	18.2	11.0	12.3	18.0	10.1	5.0	12.1	12.0	12.7
720	12.7	6.7	7.7	8.9	5.1	3.9	8.3	7.8	8.1
1080	6.0	3.2	4.2	5.1	2.8	1.6	4.9	4.7	4.3
1440	3.6	1.4	2.5	2.7	1.4	-	3.1	3.0	1.8
1800	1.8	-	1.2	1.3	-	-	1.9	1.6	-
2160	-	-	-	-	-	-	1.5	1.4	-
2880	-	-	-	-	-	-	-	-	-

Appendix 1B – Semi-logarithmic graphic of individual plasma drug concentration

over time, after administration of LEV$_{IV}$ of eight healthy adult horses.

Appendix 2A – Individual plasma drug concentration after administration of LEV_{IR}

of eight healthy adult horses.

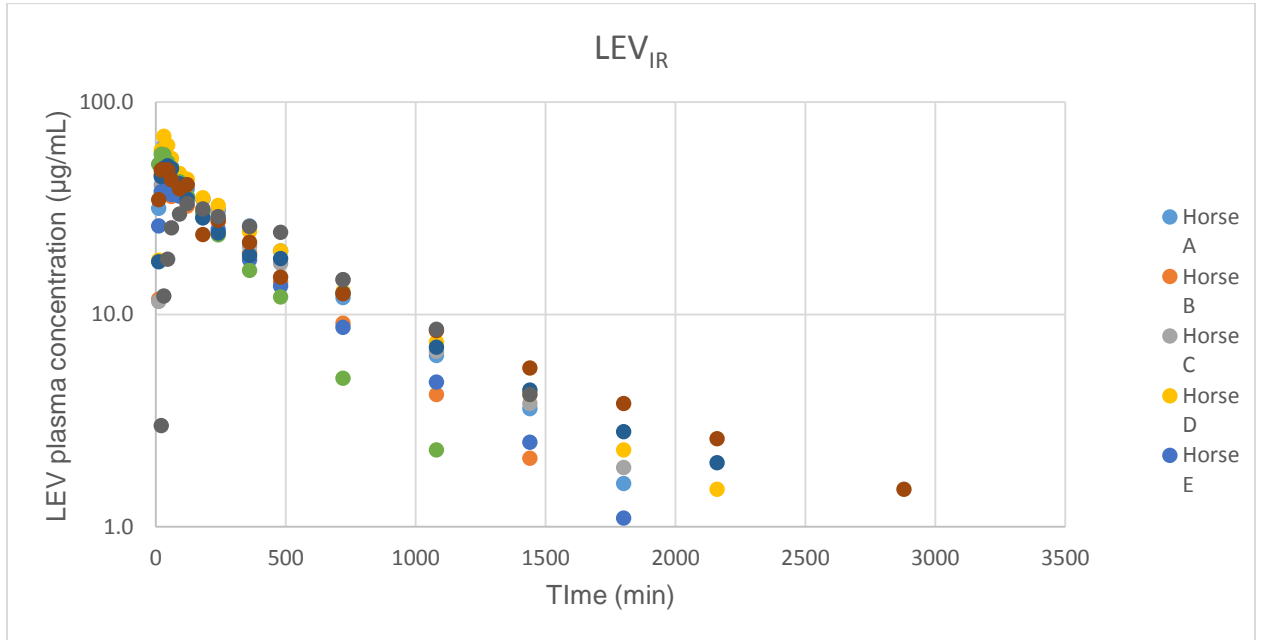| Time	Horse A	Horse B	Horse C	Horse D	Horse E	Horse F	Horse G	Horse H	Horse I
10	31.6	11.8	11.5	18.0	26.1	51.1	17.7	34.7	3.0
20	50.3	45.7	40.7	59.4	37.8	56.9	44.6	47.9	12.2
30	62.2	46.3	45.8	69.1	44.8	56.6	48.8	47.9	18.2
45	53.2	40.3	44.5	62.7	40.4	52.1	50.2	47.9	25.6
60	49.7	35.9	40.7	54.3	36.7	48.9	48.6	43.0	29.7
90	42.5	37.2	44.6	46.1	36.0	42.1	41.3	39.1	33.3
120	38.9	32.4	41.0	43.3	36.1	36.1	35.0	40.8	31.4
180	34.9	30.4	34.9	35.4	28.5	30.3	28.6	23.8	28.9
240	30.8	27.3	31.0	32.6	25.1	23.7	24.2	27.8	25.9
360	26.1	19.5	20.9	24.6	18.1	16.1	19.0	21.9	24.4
480	19.9	14.2	17.4	19.9	13.6	12.1	18.3	15.0	14.6
720	12.0	9.1	14.6	12.8	8.7	5.0	12.6	12.5	8.5
1080	6.4	4.2	6.7	7.4	4.8	2.3	7.0	8.4	4.2
1440	3.6	2.1	3.8	4.2	2.5	-	4.4	5.6	-
1800	1.6	-	1.9	2.3	1.1	-	2.8	3.8	-
2160	-	-	-	1.5	-	-	2.0	2.6	-
2880	-	-	-	-	-	-	-	1.5	-

Appendix 2B – Semi-logarithmic graphic of individual plasma drug concentration

over time, after administration of LEV$_{IR}$ of eight healthy adult horses.

Appendix 3A – Individual plasma drug concentration after administration of LEV$_{ER}$ of eight healthy adult horses.

Time	Horse A	Horse B	Horse C	Horse D	Horse E	Horse F	Horse G	Horse H	Horse I
10	58.3	10.6	27.6	50.5	9.9	24.5	12.1	29.8	6.7
20	81.3	31.3	46.1	40.0	36.1	42.5	38.4	39.2	14.0
30	52.7	34.8	52.9	62.9	54.5	44.2	59.5	60.1	20.2
45	54.6	33.2	50.7	50.5	36.2	41.2	62.1	46.6	22.7
60	47.1	37.1	45.9	47.6	45.3	39.3	51.3	44.8	27.1
90	44.2	35.5	40.3	37.5	27.7	33.6	47.4	45.5	26.1
120	32.1	32.0	35.6	35.9	35.2	19.2	42.0	37.7	25.8
180	29.6	28.6	31.5	29.9	21.7	20.7	34.2	34.7	20.2
240	25.6	24.9	27.1	26.9	27.0	21.3	29.7	30.6	22.2
360	19.9	19.2	23.4	28.8	20.6	14.8	22.5	26.1	22.2
480	14.0	14.6	17.3	20.3	15.6	10.9	17.4	21.9	19.5
720	7.7	8.4	11.0	16.2	10.1	6.4	9.3	15.5	14.5
1080	4.2	3.8	5.7	10.1	5.2	2.8	6.4	10.4	9.1
1440	1.8	1.9	3.3	6.2	2.8	1.3	3.7	6.9	5.6
1800	1.9	-	1.6	3.7	1.3	-	1.8	4.6	2.2
2160	-	-	-	2.3	-	-	1.7	3.5	-
2880	-	-	-	-	-	-	1.1	1.9	-

Appendix 3B – Semi-logarithmic graphic of individual plasma drug concentration over time, after administration of LEV$_{ER}$ of eight healthy adult horses.