I, Stephanie L Merhar M.D., hereby submit this original work as part of the requirements for the degree of Master of Science in Clinical and Translational Research.

It is entitled:
Pharmacokinetics of levetiracetam in neonates with seizures

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Pharmacokinetics of Levetiracetam in Neonates with Seizures

A thesis submitted to the
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Master of Science in Clinical and Translational Research

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by

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ABSTRACT

Objective:
To evaluate the pharmacokinetics and adverse events of intravenous levetiracetam in treating newborns with seizures.

Study design:
This was a prospective, open-label observational pharmacokinetic study in neonates ≥ 32 weeks gestational age and ≤ 30 days of age with seizures persisting despite treatment with phenobarbital. A loading dose of intravenous levetiracetam was given as per the prescribing physician, followed by additional doses based on clinical response. Blood samples were prospectively collected and analyzed for levetiracetam concentrations. Vital signs were monitored during levetiracetam treatment, and safety and efficacy data were collected.

Results:
Eighteen patients (median 39 weeks gestation and 2 postnatal days) were included. Initial loading doses ranged from 14.3-39.9 mg/kg. Median (range) clearance, volume of distribution, and elimination half-life were 1.2 ml/min/kg (0.5-2.9), 0.89 L/kg (0.4-1.3), and 8.9 hours (3.2-13.3), respectively. No adverse events related to levetiracetam were observed. Nine out of 18 patients required additional loading doses of levetiracetam to control their seizures.

Conclusions:
LEV clearance was lower, volume of distribution larger, and half life longer in neonates as compared to older children. Given the increased volume of distribution and lower clearance in neonates, we recommend a loading dose of at least 30 mg/kg, followed by maintenance dosing every 8-12 hours.
ACKNOWLEDGMENTS

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INTRODUCTION

Neonatal seizures are a common problem, affecting two to five per thousand infants in the first month of life (1-3). Recent animal and human studies have shown that seizures can harm the developing brain (4-6), reinforcing the current practice of treating electrographic seizures in newborns. Phenobarbital, introduced in 1914, is still the most common medication used to treat neonatal seizures (7). However, in the one randomized controlled trial on its use in neonates, phenobarbital was effective in eliminating neonatal seizures only 43% of the time (8). In addition, phenobarbital has been shown to cause neuronal apoptosis in rat pups (9,10), and children receiving phenobarbital for febrile seizure prophylaxis were found to have decreased cognitive ability and school achievement compared with those receiving placebo (11-13). For these reasons, there has been considerable interest in recent years in developing new antiepileptic medications for use in neonates.

Levetiracetam (Keppra®), introduced in 2000, is an anticonvulsant with a novel mechanism of action that has been studied extensively in adults and older children (14-18). Although its precise mechanism of action has not been fully established, levetiracetam is known to bind to synaptic vesicle protein 2A in the central nervous system and does not appear to directly affect the traditional excitatory and inhibitory neurotransmitter systems (18,19). Studies in adults have shown that levetiracetam has linear pharmacokinetics (18), which means that changes in dose result in proportional changes in drug concentration and exposure as measured by the area under the concentration-time curve (AUC). Levetiracetam is mainly excreted by the kidneys, and elimination correlates with creatinine clearance (18). The major metabolic pathway is enzymatic hydrolysis by a plasma esterase (18). The volume of distribution of levetiracetam is similar to total body water, and it has no documented clinically significant drug-drug interactions.
Pharmacokinetic, safety, and efficacy trials have been performed in children down to 1 month of age (14-16,20,21). Case series have suggested that levetiracetam may be safe and effective in the treatment of neonatal seizures (22-24), and a recent prospective study in 38 term and preterm infants showed no adverse effects of a slow titration of levetiracetam (25). The aim of our study was to determine the pharmacokinetics of levetiracetam and to gather preliminary safety and efficacy in neonates with seizures.

METHODS

This study was conducted at Cincinnati Children's Hospital Medical Center and Good Samaritan Hospital between October 2008 and June 2010. The institutional review board at each hospital approved the protocol and written informed consent was obtained from the legal guardian of all subjects. An investigational new drug application (IND) was obtained from the FDA to perform this study.

Eligible neonates were ≤ 30 days old and ≥ 32 weeks gestational age, with seizures requiring treatment with levetiracetam. Levetiracetam is often used as a second line medication for the treatment of neonatal seizures in our institution. All subjects received at least 20 mg/kg of phenobarbital before receiving levetiracetam. Exclusion criteria included birth weight < 2000 g and known creatinine ≥ 2.0 mg/dl.

Levetiracetam injection (100 mg/ml, UCB Pharma) was used for the initial loading dose in all patients. Dosing was not specified in the protocol and was determined by the clinician prescribing the drug. Levetiracetam was infused over 15 minutes as per package insert. Blood sampling was conducted using a D-optimal sparse sampling design with three samples collected in each patient (26). Patients were divided into three groups to obtain informative time points
over the entire dosing interval. Group 1 had samples drawn at 2-15 minutes, 1-2 hours, and 12 hours post infusion; Group 2 had samples drawn at 2-15 minutes, 2-4 hours, and 18 hours post infusion, and Group 3 had samples drawn at 5-15 minutes, 4-8 hours, and 20-24 hours post infusion.

In order to prevent metabolism by esterases in the test tube, the plasma was separated by centrifugation directly after collection (27) and stored at -70°C until analysis. Levetiracetam concentrations were determined by the Bioanalytical Core Laboratory at Georgetown University using a validated liquid chromatography-electrospray tandem mass spectrometry assay (28). The lower limit of detection of the assay was 0.1 µg/mL for plasma. Within-run and between-run imprecision (n=10) for LEV controls ranged from 2.3%-4.7% and 3.4%-8.9%, respectively (29).

Non-linear mixed effects modeling (NONMEM, version 7.1, ICON Dev. Soln., Ellicott City, MD) with PDx-Pop® (version 4.10, 2007 ICON Dev. Soln., Ellicott City, MD) interfaced with Xpose® (version 4.0, release 6, update 1) was used to perform the pharmacokinetic analyses. Individual Bayesian pharmacokinetic parameter estimates were calculated using MW/PHARM (Version 3.60; MediWare, Groningen, The Netherlands) (30). The parameters of interest were clearance, elimination half life, volume of distribution, and area under the curve at 2, 4, 6, 12, and 24 hours. SAS (Version 9.2, SAS Institute Inc, Cary, NC) was used to analyze associations between demographic and pharmacokinetic parameters.

Safety assessments included a physical examination before and 24 hours after the loading dose of levetiracetam and monitoring of vital signs (heart rate, respiratory rate, oxygen saturation, and blood pressure) every 15 minutes for one hour after the loading dose and hourly for 24 hours. All infants in the study had a renal panel, glucose, and electrolytes drawn before receiving the initial dose of levetiracetam. Further labs were drawn for clinical reasons and were
recorded in the research record. Adverse events were reported by bedside nurse in response to an open question about whether any problems were seen after the loading dose. Study subjects' charts were reviewed at hospital discharge to identify any adverse events occurring during the 24 hours after the loading dose and during the remainder of the hospital stay.

Seizure frequency before and after the loading dose of levetiracetam was monitored clinically or documented using full channel EEG at the discretion of the clinicians caring for the patient. Further loading doses of levetiracetam were administered for the continuation of clinical and/or electrographic seizures as per the discretion of the clinical team. Efficacy was coded as a dichotomous variable: whether or not the infant required further loading doses of levetiracetam after the initial loading dose. Statistical analysis for efficacy data was performed using SAS (Version 9.2, SAS Institute Inc, Cary, NC).

RESULTS

A total of 21 infants who received levetiracetam for clinical and/or electrographic seizure control were screened for the study from October 2008 to May 2010, and 19 of these infants were enrolled in the study. The 2 patients who were not enrolled received levetiracetam before consent could be obtained from the parents. One of the 19 subjects was excluded due to a lab error resulting in only 2 usable samples. The resulting pharmacokinetic data consisted of 54 levetiracetam concentrations from 18 subjects. One patient was enrolled in the study at day of life 30 but did not receive levetiracetam until day of life 32. This was not discovered until all samples had been drawn, and the decision was made to include this patient in the final analysis. The patient characteristics are summarized in table 1.

A loading dose of IV levetiracetam was administered at the discretion of the prescribing physician. Loading doses ranged from 14.4-39.9 mg/kg. Further loading doses were
administered for the continuation of clinical and/or electrographic seizures as directed by the clinical team caring for the patient. A maintenance dosing regimen of levetiracetam was initiated in most cases, and was given IV during the study period in 17/18 patients. The first maintenance dose was given 8-12 hours after the loading dose(s).

**Pharmacokinetic modeling:**

A two compartment model with first order elimination provided the best fit to the data. The most significant covariates determined in the univariate analysis were weight, postmenstrual age, serum creatinine, and creatinine clearance. After multivariate analysis, only weight and creatinine clearance remained in the final model. Some unexplained variability remained in the model (32-43%), which may be due to the small number of subjects with only 3 levetiracetam concentrations per subject.

The parameter estimates from the base model were entered into MWPHARM to determine the pharmacokinetic parameters for each individual subject, shown in table 2. The median (range) Cmax as predicted by the model was 39.8 (14.8-91.9) mg/L. The highest measured concentration measured in the study patients was 87.6 mg/L, 1 hour after a 30 mg/kg dose. There was a significant linear relationship between serum creatinine and half life (Figure 1, Pearson correlation r = 0.67, p = 0.0002). Linear relationships were also found between serum creatinine and levetiracetam clearance (r = -0.53, p = 0.02), and creatinine clearance and levetiracetam clearance (r = 0.66, p = 0.003).

**Safety and tolerability:**

Levetiracetam was well tolerated in this population. No changes in vital signs or laboratory parameters were observed. Several infants were noted to be somnolent in the 24 hours after levetiracetam administration. However, the majority of these infants were critically
ill and had received both phenobarbital and levetiracetam. The somnolence was recorded as possibly related to levetiracetam administration.

One patient with overwhelming HSV infection died after withdrawal of support 24 hours after receiving levetiracetam. One patient with severe HIE died after withdrawal of support 10 hours after receiving levetiracetam. These deaths were deemed not related to levetiracetam administration.

**Efficacy:**

This was designed as a pharmacokinetic study and was not sufficiently powered to determine efficacy. Seizure frequency before and after the loading dose of levetiracetam was monitored at the discretion of the clinicians caring for the patient. Nine of the 18 patients were monitored by continuous full channel EEG during the initial loading dose of levetiracetam and for at least 6 hours afterwards. Seven out of the 10 patients who received a 20 mg/kg loading dose received a second loading dose of IV levetiracetam within 12 hours of the first due to continued electrographic and/or clinical seizures. During the study period, clinical practice in our institution changed to using a higher loading dose of levetiracetam (30 mg/kg instead of 20 mg/kg). This higher loading dose seemed to be more effective in stopping initial seizures (figure 2). A simple logistic regression model was constructed modeling the initial dose in mg/kg on efficacy (whether the patient required a second dose of levetiracetam acutely to control continued seizures) and the area under the ROC curve was reported. This simple model had an area under the ROC curve of 0.77 (p = 0.10). Overall, after receiving levetiracetam, 15 out of the 18 infants in the study (83%) did not require a third-line medication for seizure control.

**DISCUSSION**
The main objective of this study was to determine the pharmacokinetics of IV levetiracetam in neonates with seizures. We found that levetiracetam pharmacokinetics were different in this population than in adults and older children. The safety profile appeared to be favorable with no serious adverse effects noted. Two deaths occurring in this critically ill study population were deemed to be unrelated to levetiracetam treatment. We also found that levetiracetam was effective for acute seizure control, with only 3 of the 18 infants requiring a third line drug beyond phenobarbital and levetiracetam.

The pharmacokinetics of levetiracetam in adults and older children have been well characterized. The half-life is 6-8 hours in adults (18) and 5-7 hours in older children (17,20). In the only study that evaluated the pharmacokinetics of levetiracetam in neonates, the half life was found to be 16-18 hours in newborn twins whose mother had received an oral dose of levetiracetam 45 minutes prior to delivery (31). We found the median half-life of levetiracetam to be 8.9 hours, which might be expected based on the lower clearance of levetiracetam in neonates. The volume of distribution, the hypothetical volume in which an amount of drug would need to be uniformly distributed to produce the observed blood concentration, is 0.5-0.7 L/kg in adults (32) and 0.6-0.7 L/kg in older children (17,20). The median volume of distribution in our study was 0.89 L/kg. Because neonates have higher body water content than adults and older children (as much as 85% total body water in preterm infants and 78% in full term infants as compared to 60% in adults) (33) and levetiracetam distributes in parallel with total body water, we would expect the volume of distribution to be higher in neonates than in older children and adults.

Total body clearance of levetiracetam in adults is 0.96 ml/min/kg (18) and in children is 1.43-1.46 ml/min/kg (17,20). We found the clearance in neonates to be 1.21 ml/min/kg,
intermediate between adults and older children. Weight-adjusted clearance is often higher in children than in adults, but models which allometrically scale clearance based on surface area or the 3/4 power model often predict no difference in the size scaled clearance between these two populations (34). Using the 3/4 power model, the standard clearance is 67.2 ml/min and the clearance of neonates (with a mean weight of 3.3 kg, as in this study) is predicted to be 6.8 ml/min. The clearance of the neonates in this study was found to be 3 ml/min in the NONMEM two compartment model and 4.2 ml/min in the MWPharm two compartment model, lower than would be predicted using the 3/4 power model. Allometric scaling is able to correct for growth, but does not correct for maturational differences in the population (34). Since clearance was found to be lower in neonates than would be predicted using allometric scaling, we conclude that the lower clearance of levetiracetam in this population is due to the lower GFR and possibly to lower plasma esterase activity. In support of this conclusion, a significant linear relationship was found between serum creatinine and levetiracetam clearance.

Based on the higher volume of distribution in neonates, the loading dose on a mg/kg basis should be higher than in older children and adults. We found that a loading dose of 30 mg/kg was more effective in controlling seizures than a loading dose of 20 mg/kg in our study population. Due to the reduced clearance in neonates, the dosing interval may need to be extended, at least until the GFR matures. Therefore, a twice daily dosing schedule may be more appropriate than a three times daily schedule in neonates over the first several weeks of life. In infants with reduced creatinine clearance, the dosing interval may need to be extended even further.

Routine therapeutic drug monitoring has not been recommended for levetiracetam in the past, as drug levels have not been found to correlate well with therapeutic efficacy (35,36). A
reference range of 12-46 mg/L has been proposed for adults with epilepsy (37), and most clinical labs suggest similar reference ranges. Toxic levels have not been well established. The highest concentration measured in our study was 87.6 mg/L and the highest Cmax predicted by the model was 91.9 mg/L. No adverse effects were seen beyond mild somnolence even at these high levels. Therapeutic drug monitoring may be useful in infants with impaired renal function, but our study does not provide the data to support routine monitoring.

Infants in this study were closely monitored for 24 hours after the loading dose of levetiracetam to assess for adverse events. Two subjects died after withdrawal of support due to their severe underlying medical conditions during the 24 hours following levetiracetam administration, but these deaths were not considered related to levetiracetam. Somnolence was the only other adverse event recorded in subjects 24 hours after the dose that could possibly be related to levetiracetam. The somnolence did not interfere with feeding or cause subjects to require increased respiratory support. In older children, the main adverse events seen with levetiracetam administration are somnolence, nervousness, dizziness, and irritability. In adults and older children, small but statistically significant changes in hematologic parameters (RBC count, mean hemoglobin, and WBC count) have been seen. These hematologic changes have not been considered clinically significant by other investigators; therefore, we did not monitor complete blood counts as part of the study. However, many of the infants in the study had complete blood counts before and after receiving levetiracetam, and no changes were seen.

Although this study was not powered to determine efficacy, levetiracetam was considered to be effective for acute seizure control in this population. In addition, as shown in figure 2, a higher initial loading dose of 30 mg/kg appeared to be more effective than a loading dose of 20 mg/kg. When this relationship was examined with a simple logistic regression model, a trend
was found, but the study did not have sufficient power to detect statistical significance. Two recent papers (24,25) have also suggested that levetiracetam is safe and effective in neonates. These studies used lower doses (10 mg/kg BID), did not use continuous monitoring, and did not include a control group.

This is the first pharmacokinetic study of levetiracetam in the neonatal population. We were able to use a minimal sampling strategy to obtain pharmacokinetic parameters among infants with variable seizure etiologies. The limitations of our study include the small sample size with high interindividual variability. Although we intended to study preterm infants and infants later in the first month of life, most of the patients in the study were full term and treated with levetiracetam in the first two days of life. The pharmacokinetics of levetiracetam were different in the neonatal population as compared to older children and adults, and in this small study, levetiracetam was found to be safe and effective in the neonatal population. Future investigations are needed to evaluate higher loading doses of levetiracetam in neonates, such as the 50 mg/kg loading dose currently being used in older children with status epilepticus (38,39), and to obtain more data on the steady state pharmacokinetics of levetiracetam in neonates. Although levetiracetam appears to be effective in infants who have failed phenobarbital, more information needs to be gathered on the efficacy of levetiracetam as a first line drug.
Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n=18</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: Male</td>
<td>10</td>
<td>56</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>44</td>
</tr>
<tr>
<td>Race: African-American</td>
<td>7</td>
<td>39</td>
</tr>
<tr>
<td>Caucasian</td>
<td>11</td>
<td>61</td>
</tr>
<tr>
<td>Cause of seizures: HIE(^1)</td>
<td>6</td>
<td>33</td>
</tr>
<tr>
<td>Other(^2)</td>
<td>12</td>
<td>67</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA at birth (weeks)</td>
<td>38+6</td>
<td>35+2 - 41</td>
</tr>
<tr>
<td>Postnatal age (days)</td>
<td>2</td>
<td>0-32</td>
</tr>
<tr>
<td>Weight at time of dosing (kg)</td>
<td>3.5</td>
<td>2 – 4.4</td>
</tr>
<tr>
<td>Creatinine at time of dosing</td>
<td>0.7</td>
<td>0.2-1.6</td>
</tr>
</tbody>
</table>

1 HIE = hypoxic-ischemic encephalopathy

2 Other causes of seizures included ornithine transcarbamylase deficiency, hemimegalencephaly, cortical dysplasia, perinatal stroke, herpes simplex virus, meningitis, birth trauma, kernicterus, and unknown (4 patients)
Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance (ml/min/kg)</td>
<td>1.21</td>
<td>0.47-2.89</td>
</tr>
<tr>
<td>Volume of distribution (L/kg)</td>
<td>0.89</td>
<td>0.37-1.26</td>
</tr>
<tr>
<td>Half-life (hours)</td>
<td>8.9</td>
<td>3.2-13.3</td>
</tr>
</tbody>
</table>
Figure 2

Efficacy

- 10 patients got 20 mg/kg
  - 7/10 required 2nd loading dose of LEV acutely
  - 3/10 responded

- 8 patients got ≥30 mg/kg
  - 2/8 required 2nd loading dose and responded
  - 6/8 responded

- 4/7 responded
- 3/7 refractory
BIBLIOGRAPHY


