# A seven-day study of the pharmacokinetics of intravenous levetiracetam in neonates: marked changes in pharmacokinetics occur during the first week of life

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**INTRODUCTION:** Levetiracetam (LEV) is increasingly used in the treatment of neonatal seizures. The aim of this study was to determine pharmacokinetics in neonates with seizures and to obtain preliminary safety and efficacy data.

**METHODS:** Eighteen term neonates with seizures persisting after 20mg/kg of phenobarbital received intravenous LEV for 1 wk. LEV was administered as a 20 or 40 mg/kg bolus followed by 5–10 mg/kg/d. Pharmacokinetic data were analyzed using a nonlinear mixed-effects population approach. Continuous electroencephalogram monitoring allowed preliminary assessment of the efficacy of LEV in this population.

**RESULTS:** LEV clearance (CL) increased from a mean of 0.7 ml/min/kg (SD 0.27 ml/min/kg) on day 1 to 1.33 ml/min/kg (SD 0.35 ml/min/kg) by day 7. Mean half-life was 18.5 h (SD 7.1 h) on day 1 of the study and decreased to 9.1 h (SD 2.0 h) by day 7. The mean volume of distribution was 1.01 l/kg (SD 0.13 l/kg). No study-related serious adverse events were observed.

**DISCUSSION:** CL of LEV in neonates was higher than expected on the basis of immature renal function in term infants and increased significantly during the first week of life. More frequent dosing of LEV is needed in term infants to maintain serum concentrations in the range seen in children and adults.

The standard treatments for neonatal seizures are inadequate (1). Current treatment relies on medications in use between 1914 (phenobarbital) and 1938 (phenytoin). When used individually, each of these agents produces seizure cessation in <50% of infants treated (1,2). When used in combination, the seizure cessation rate is still <60% (3). Acute side effects of phenobarbital and phenytoin include hypotension, suppression of respiratory drive, cardiac arrhythmia, and sedation. Chronic exposure to phenobarbital may be associated with decreased cognitive ability (4–6). Studies on animals suggest that these agents may cause accelerated neuronal apoptosis when used in immature subjects (7).

Levetiracetam (LEV) ((-)-(S)-α-ethyl-2-oxo-1-pyrrolidine acetamide) is a very promising medication for the treatment of neonatal seizures. An intravenous preparation of LEV is available, allowing its use in neonates with seizures, who frequently cannot be fed. LEV is a chemically novel anticonvulsant agent that has been in clinical use for almost a decade in adults and older children with good efficacy, an excellent safety profile, and near ideal pharmacokinetic characteristics. Studies on animals have shown that LEV does not cause neuronal apoptosis in the immature brain and shows promise as a neuroprotective agent (8,9).

Promising data are emerging from recent studies regarding the efficacy of LEV in neonates (9–13). Khan *et al.* reported a 32% rate of complete cessation of clinical and electrographic seizure activity following a LEV load of 10–50 mg/kg in a series of 21 patients (14). Abend *et al.* reported complete seizure cessation in 30% of patients 24 h following a LEV load of 10–20 mg/kg (15). These preliminary efficacy data are encouraging, particularly considering that LEV was mostly administered as a second- or third-line agent in patients with pharmacoresistant seizures. However, the tendency of neonatal seizures to resolve spontaneously over time makes the interpretation of these data difficult.

There is evidence that off-label use of LEV for neonatal seizures is becoming commonplace (16). This is despite a paucity of basic data on the pharmacokinetics, safety, and efficacy of this drug in this population in the first few days of life. These data are needed to allow the rationale use of this anti-epileptic drug in neonates. We therefore conducted a pharmacokinetic study of LEV and a preliminary study of its safety and efficacy in neonates with seizures.

## RESULTS

### Patient Baseline Characteristics

A total of 18 patients received treatment with intravenous LEV, six at the first dosing level and 12 at the higher dose. At enrollment all subjects had a corrected gestational age between 37 and 41 wk and weight between 2.5 and 4.7 kg. In eight subjects, the underlying etiology of seizures was hypoxic ischemic encephalopathy (HIE). Plasma creatinine did not exceed 0.09 mmol/l in any subject. Five of these subjects received hypothermia treatment during the study period, (body cooling to 33.5 °C for 72 h). Patient demographics are detailed in **Table 1**.

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**Table 1.** Patient demographics

| Subject number | Gender | Weight (kg) | Gestational age (wk) | Postnatal age[a](d) | Etiology of seizures | Hypothermia treatment |
|---|---|---|---|---|---|---|
| 101 | Male | 3.315 | 38 | 2 | Unknown[b] | No |
| 102 | Female | 3.26 | 41 | 4 | Brain malformation (polymicrogyria, hypoplastic brainstem and cerebellar vermis, Dandy–Walker malformation) | No |
| 103 | Male | 3.035 | 38 | 2 | IVH | No |
| 104 | Female | 3.275 | 38 | 2 | IVH | No |
| 105 | Male | 3.685 | 40 | 3 | HIE (laminar necrosis bilateral perirolandic cortex) | No |
| 201 | Male | 2.62 | 36 | 5 | Unknown | No |
| 202 | Female | 4.65 | 39 | 2 | HIE | Yes |
| 203 | Female | 2.745 | 37 | 5 | Unknown | No |
| 204 | Male | 3.235 | 37 | 1 | HIE | Yes |
| 205 | Female | 3.48 | 39 | 3 | Stroke | No |
| 206 | Male | 2.55 | 40 | 1 | HIE | Yes |
| 207 | Male | 3.02 | 39 | 3 | HIE | No |
| 208 | Male | 3.514 | 40 | 1 | Stroke | No |
| 401 | Female | 3.48 | 40 | 1 | HIE | Yes |
| 402 | Female | 2.93 | 37 | 1 | HIE | Yes |
| 403 | Female | 3.275 | 41 | 1 | HIE | No |
| 404 | Female | 3.36 | 39 | 3 | Birth trauma (skull fracture, intracranial hemorrhage, and right parietal stroke) | No |
| 405 | Male | 2.95 | 40 | 4 | Unknown | No |

IVH, intraventricular hemorrhage; HIE, hypoxic ischemic encephalopathy.

[a]Postnatal age at start of therapy.

[b]All subjects with unknown etiology had investigations including brain magnetic resonance imaging, sepsis screen with lumbar puncture, and extensive metabolic investigations.

### Pharmacokinetic Results

The final pharmacokinetic data set contained 149 plasma concentration values from the 18 subjects. All subjects had five or more evaluable serum levels. Urine collections were completed in 18 subjects.

Figure 1 shows serum drug concentration vs. time curves for all subjects. The mean (+SD) LEV concentrations 1 h after the initial doses of 20 and 40 mg/kg were 18.2 ± 5.9 and 33.0 ± 9.8 μg/ml, respectively. The concentrations of predose LEV before the sixth dose were 1.4 + 0.5 and 2.0 + 1.4 μg/ml for the 5 and 10 mg/kg dose levels, respectively. Figure 2 shows goodness of fit for the final model, with population predicted LEV concentrations vs. measured LEV concentrations.

Postnatal age was found to be a significant covariate for LEV clearance (CL) (change in objective function, −63.3). Serum creatinine also appeared to be inversely related to CL but this association did not meet the model development criteria for inclusion. Sex, seizure onset day, dosing arm, recent phenobarbital concentration, and hypothermia were not found to be significant covariates for LEV CL. Given the limited number of subjects, intersubject variability could be determined for CL but not for volume of distribution. The population typical parameter estimates were well within the 95% bootstrap confidence intervals for the individual parameters: volume of distribution, 0.979 (0.88–1.11) l/kg; CL on fifth day of life, 0.97 (0.850–1.13) ml/min/kg; and age effect (θ3) on CL ($(Age/5)^{\theta 3}$), 0.399 (0.286–0.531)

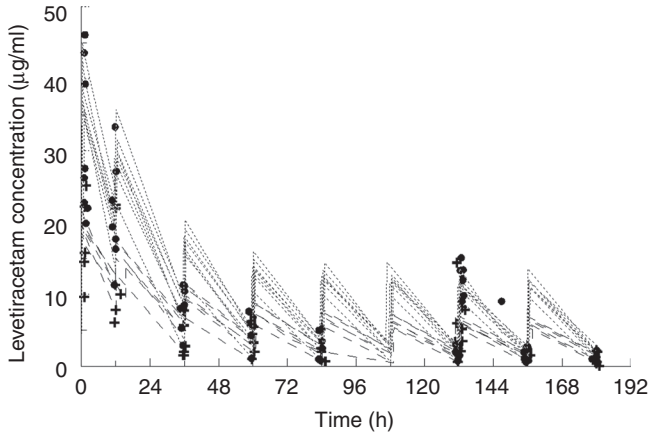


**Figure 1.** Serum drug concentration vs. time curves for all subjects.

The empiric Bayesian estimates for individual subject pharmacokinetic parameters are summarized in Table 2. The mean volume of distribution seen in neonates was 1.01 l/kg (bootstrap 95% confidence interval 0.88–1.11 l/kg), greater than previously reported in older children and greater than total body water. CL was much greater than the predicted 0.1–0.3 ml/min/kg on the basis of kidney immaturity. During the week-

long treatment period, CL doubled, to reach the CL seen in older children. For comparison, pharmacokinetic parameters reported in other pediatric studies are summarized in **Table 3** (17–21). Although 10 of 12 subjects treated at the higher dosing level had trough LEV concentrations >6 μg/ml at 36 h, no subjects had trough LEV concentrations >6 μg/ml by the end of the week of treatment. Before the seventh dose, LEV trough levels averaged 1.7 μg/ml (SD 1.0) on 5 mg/kg/d maintenance and 2.4 μg/ml (SD 1.3) on 10 mg/kg/d maintenance.

Substantial LEV metabolite concentrations were seen in both plasma and urine with a large range of values across the subjects (see **Tables 4** and **5**). Plasma and urinary UCB L057/LEV ratios were higher in subjects receiving the higher dosing regimen. The urinary UCB L057/LEV ratio showed substantial increases between the 0–12 as compared with the 12–36 h collection, suggesting increased hydrolysis. The urinary UCB L057/LEV ratio was not predictive of LEV CL. The plasma UCB L057/LEV ratio also failed to predict LEV CL and was stable throughout the therapy. Overall, these data suggest that both renal CL and hydrolysis pathways are maturing during this time frame.

### Efficacy in Seizure Cessation: Preliminary Analysis

As a simple measure of LEV efficacy, 6 of the 18 subjects studied in this trial required no additional anti-epileptic drugs after LEV was commenced because of cessation of both clinical and electrographic seizures. Five of the responders were among the 12 subjects who received the higher dose of LEV (42%). There was only one responder among the six subjects who received the lower LEV dose. (An additional of three subjects had an initial response to LEV; with temporary cessation of seizures documented by electroencephalograms (EEG) as the loading dose was given but later had recurrence of seizures). Detailed analysis of EEG data from this study demonstrating the effect of LEV on EEG-confirmed neonatal seizures is under way and will be reported separately.

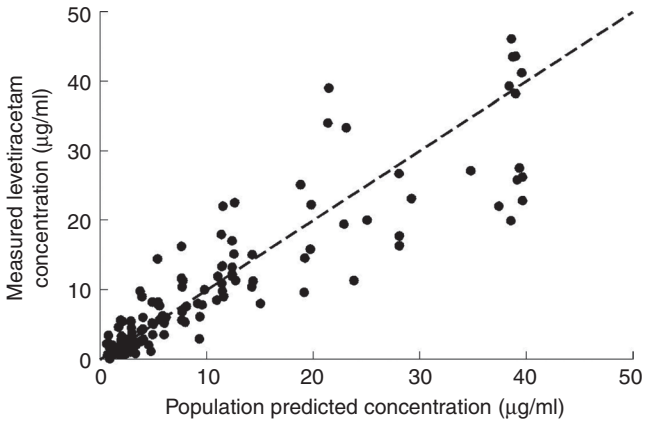


**Figure 2.** Goodness-of-fit plot. Population predicted vs. measured levetiracetam drug concentrations.

### Adverse Events and Safety Monitoring

Patients were assessed daily for possible adverse clinical events related to LEV treatment. LEV was well tolerated. Only one serious adverse event occurred in this study. One subject with a brain malformation and a high phenobarbital level required intubation while on treatment with LEV. LEV was not thought to be causal of this event.

Mild adverse events which resolved spontaneously and were possibly related to treatment with LEV included mild sedation in two subjects, feeding difficulty in three subjects, mild apnea and bradycardia in one subject, and decreased urine output responding to furosemide in one subject. In no case was LEV thought causative of an adverse event.

Blood tests (complete blood count, chemistry with electrolytes, creatinine, urea, and alanine aminotransferase) were performed on day 3 and day 7 of treatment in all but one subject, whose day 7 monitoring chemistry and full blood count were not performed; however, a serum creatinine was measured.

### Hematology

Three subjects developed mildly low platelet counts (range 125–132) while on treatment with LEV. All subjects had HIE. In two of the three subjects, the platelet count normalized by day 7 while on treatment with LEV.

Three subjects developed white-cell counts below the normal range for their institution. In these cases the leukopenia was very mild (range 6.6–7 × 1,000 cells/mm$^3$).

In eight subjects, the level of hemoglobin dropped while on treatment to below the normal range. In four of the eight subjects, this resolved by day 7 while still receiving treatment. One subject had low hemoglobin before treatment (13.8 gm/dl), and during the first week of life the level of hemoglobin dropped further to 10.8 gm/dl. In three subjects mildly low levels of hemoglobin persisted (range 13–14 gm/dl).

### Chemistry

Serum creatinine did not increase during treatment. Small increases in blood urea were seen in three subjects. In two subjects, with HIE and abnormal baseline pretreatment alanine aminotransferase levels, alanine aminotransferase increased on treatment with LEV. In both the cases, alanine aminotransferase

**Table 2.** Pharmacokinetic results

| | Mean | SD | Median | Minimum | Maximum | BS 95% CI |
|---|---|---|---|---|---|---|
| Vd (l/kg) | 1.01 | 0.13 | 0.98 | 0.81 | 1.24 | 0.88–1.11 |
| CL day 1 (ml/min/kg) | 0.71 | 0.27 | 0.65 | 0.38 | 1.42 | 0.69–0.92 |
| CL day 7 (ml/min/kg) | 1.31 | 0.35 | 1.33 | 0.88 | 2.37 | 1.04–1.45 |
| T½ day 1 (h) | 18.5 | 7.1 | 15.6 | 8.8 | 32.7 | 12.6–16.2 |
| T ½ day 7 (h) | 9.1 | 2.0 | 9.0 | 5.3 | 12.7 | 8.2–10.3 |

BS, bootstrap; CI, confidence interval; CL, clearance; T½, half life; Vd, volume of distribution.

**Table 3.** Comparison of pharmacokinetic parameters from previous studies

| Reference | Age | CL, ml/min/kg (CL/F [a] for oral dosing studies) | T½ (h) | V/F [b], l/kg |
|---|---|---|---|---|
| This neonatal study (i.v. dosing) | Day 1 | 0.71 ± 0.27 | 18.5 ± 7.1 | 1.01 ± 0.13 |
| This neonatal study | Day 7 | 1.31 ± 0.35 | 9.1 ± 2 | 1.01 ± 0.13 |
| Merhar *et al.* (17) | 0–30 d | 1.21 (0.47–2.89) | 8.9 (3.2–13.3) | 0.89 (0.37–1.26) |
| Glauser *et al.* (18) (oral dosing) | 2–46 mo | 1.46 ± 0.42 | 5.3 ± 1.3 | |
| Pellock *et al.* (19) (oral dosing) | 6–12 y | 1.43 ± 0.36 | 6.0 ± 1.1 | 0.72 ± 0.12 |
| Fountain *et al.* (20) (oral dosing) | 4–12 y | 1.10 ± 0.16 | 4.9 ± 0.4 | |
| Chhun *et al.* (21) (oral dosing) | 4–16 y | 1.24 ± 0.29 | 6.8 ± 1.5 | 0.72 ± 0.12 |

All statistics expressed as mean ± SD in all studies except Merhar *et al.,* in which median and range were given.

[a]CL/F = apparent clearance after oral administration.

[b]V/F = apparent volume of distribution.

returned to below the pretreatment baseline level by day 7 of LEV treatment. Mild abnormalities of serum electrolytes were seen with no consistent pattern. One subject developed treatment-emergent hyponatremia. This infant was critically ill with HIE and was receiving hypothermia and multiple medications. The hyponatremia (126 mmol/l) resolved to normal by day 4 of treatment and remained normal at day 7. Three subjects had hypokalemia while receiving LEV treatment. In two cases this was mild, (potassium = 3 mmol/l, 3.8 mmol/l) and serum potassium normalized spontaneously by day 7. A third patient received treatment with potassium chloride for a serum potassium level of 2.7 mmol/l. Transiently raised serum potassium levels were seen in five subjects and mild hyperchloremia was seen in six subjects; in three chloride normalized by day 7 of treatment. Mild hypercalcemia was seen in five subjects (maximum calcium 2.8 mmol/l), and mild hypocalcemia was seen in two subjects (minimum calcium 1.9 mmol/l).

Mildly low serum bicarbonate developed in three subjects (minimum serum bicarbonate 16 mmol/l). In one subject serum bicarbonate increased while on treatment to 35 mmol/l on day 3, then normalized spontaneously.

All blood test abnormalities were reviewed by an independent data safety monitoring board. Mild abnormalities in blood count and serum chemistries were thought consistent with what would have been expected in this patient population of sick neonates. No serious or consistent treatment-emergent laboratory abnormalities were observed.

**Table 4.** Plasma LEV and UCB L057 metabolite levels

| | | Plasma levels, trough before second dose, at 12 h | | | | | Plasma levels, trough before seventh dose, at 132 h | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | Median | Minimum | Maximum | Mean | SD | Median | Minimum | Maximum |
| Low dose | LEV level (μg/ml) | 13.29 | 7.17 | 10.65 | 6.13 | 22.5 | 1.91 | 1.18 | 1.80 | 0.62 | 3.42 |
| | Metabolite UCB L057[a] (μg/ml) | 1.03 | 0.60 | 0.99 | 0.37 | 2.00 | 0.15 | 0.07 | 0.15 | 0.07 | 0.24 |
| | Metabolite/LEV ratio | 0.08 | 0.02 | 0.07 | 0.06 | 0.1 | 0.09 | 0.03 | 0.09 | 0.07 | 0.12 |
| High dose | LEV level (μg/ml) | 24.35 | 8.52 | 23.1 | 11.3 | 39.0 | 2.44 | 1.28 | 2.25 | 0.70 | 5.40 |
| | Metabolite UCB L057 (μg/ml) | 11.49 | 8.09 | 13.4 | 2.0 | 23.8 | 1.23 | 1.01 | 1.2 | 0.07 | 2.8 |
| | Metabolite/LEV ratio | 0.49 | 0.32 | 0.49 | 0.07 | 0.89 | 0.46 | 0.36 | 0.36 | 0.05 | 1.15 |

LEV, levetiracetam.

[a]UCB L057 is the name of the main acid metabolite of levetiracetam.

**Table 5.** Urine LEV and UCB L057 metabolite levels

| | | Urine levels at 0–12 h collection | | | | | Urine levels at 12–36 h collection | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | Median | Minimum | Maximum | Mean | SD | Median | Minimum | Maximum |
| Low dose | LEV level (μg/ml) | 67.83 | 15.80 | 67.25 | 45.50 | 86.50 | 51.33 | 13.91 | 47.40 | 39.00 | 76.75 |
| | Metabolite UCB L057[a] level (μg/ml) | 7.65 | 3.20 | 7.75 | 2.09 | 11.60 | 9.23 | 2.90 | 8.68 | 5.77 | 13.90 |
| | Metabolite/LEV ratio | 0.11 | 0.06 | 0.10 | 0.05 | 0.20 | 0.19 | 0.08 | 0.16 | 0.11 | 0.32 |
| High dose | LEV level (μg/ml) | 158.54 | 79.61 | 134.25 | 74.00 | 289.00 | 139.9 | 84.09 | 114.75 | 61.00 | 337.00 |
| | Metabolite UCB L057 level (μg/ml) | 101.61 | 83.15 | 96.60 | 5.32 | 308.00 | 137.90 | 96.76 | 128.85 | 10.6 | 324.6 |
| | Metabolite/LEV ratio | 0.81 | 0.77 | 0.75 | 0.07 | 2.83 | 1.37 | 1.4 | 1.12 | 0.12 | 5.32 |

LEV, levetiracetam.

[a]UCB L057 is the name of the main acid metabolite of levetiracetam.

## DISCUSSION

About two-thirds of LEV is eliminated as unchanged drug in the urine while the remaining third is hydrolyzed to UCB L057, also excreted in the urine. Based on glomerular function immaturity in neonates (22), we expected LEV CL in this population would be between 15 and 45% of that of older populations. Conducting this study in sick neonates, we were obliged to err on the side of caution and use a conservative estimation of CL in our dosing selection.

CL of LEV in neonates was higher than predicted and increased significantly during the first week of life into the range seen in older children (**Tables 2** and **3**) and exceeded values reported in adults (23). This study illustrates the importance of performing pharmacokinetic studies in neonates and the inaccuracy of our best predictions extrapolating to neonates from pharmacokinetic data in older subjects.

Our data are in agreement with data from the concurrently performed neonatal study by Merhar *et al.* (17) and further advance those data. Because of the week-long duration of our study we were able to detect the dramatic change in pharmacokinetics of LEV within the first week of life. Identifying this change reduces interindividual variability at any given time point. Our analysis of UCB L057 metabolite levels is not previously reported in neonates.

Several factors may account for the greater-than-predicted LEV CL in the neonate. LEV has low plasma protein binding and its renal CL is substantially less than glomerular filtration in adults. These characteristics suggest that LEV undergoes net renal tubular reabsorption in adults. Therefore, one potential explanation for the higher-than-expected CL in newborns is that in addition to immature glomerular function, infants may have reduced capacity for tubular reabsorption of LEV. Altered hydrolysis to UCB L057 may also contribute. The newborn expression of the specific esterase responsible for LEV hydrolysis is unknown. Our analysis of UCB L057 metabolite levels demonstrates that the activity of this enzyme accounts for up to 30% of overall LEV CL by 36 h of life. (In children and older adults it accounts for approximately one-third of LEV CL.) The stable UCB L057 metabolite/ LEV ratios in serum over the course of the week indicates that both elimination pathways increase in function during the first week of life.

All 18 subjects in our study were comedicated with phenobarbital and 16 received maintenance therapy with phenobarbital. LEV is metabolized by a β-esterase present in serum and liver (24). Because metabolism of LEV is not mediated by hepatic cytochrome 450 isoenzymes, increased CL associated with comedication was not expected. However, in a pediatric study examining age effects and drug interactions with LEV, a significant 30% increase in LEV CL in subjects comedicated with enzyme-inducing anti-epileptic drugs such as phenobarbital, carbamazepine, and phenytoin was reported (25). The authors postulate that this effect may be mediated by comedication inducing the enzymatic hydrolysis of LEV and note other drugs in which this process has been documented. We therefore analyzed phenobarbital levels available in 15 subjects. The median concentration of phenobarbital was 34 (range 18–49) mg/dl. Phenobarbital concentration was not a significant covariate for LEV CL during univariate analysis (1.98 drop in model objective function from the base model).However, as all of the subjects were on phenobarbital, it would be difficult for this study to detect differential effects of phenobarbital levels on LEV CL; therefore, this remains a possible partial explanation for the greater-than-expected LEV CL seen.

Loading with LEV 40 mg/kg followed by 10mg/kg once daily dosing results in a large drop-off in serum concentrations over the first week of life with more than 50% infants expected to have trough concentrations below 5 μg/ml. With our improved knowledge of the pharmacokinetics of LEV in neonates we can now construct a dosing guideline to maintain desired trough concentrations. In adults on standard therapeutic doses of LEV, trough concentrations are typically in the range 6–20 μg/ml. Given the intractability of the seizures in many neonates and the safety profile of LEV, we suggest that the upper end of this range could be applied in the neonate.

**Figure 3** compares median predicted LEV serum concentrations at different maintenance dose frequencies: the 10 mg/kg daily maintenance dose used in this study as compared with 10 mg/kg maintenance dosing every 12 h and every 8 h.

**Figure 4** shows the expected distribution of LEV serum concentrations (5th to 95th percentiles) with a loading dose of 40 mg/kg followed by a 10 mg/kg maintenance dose every 8 h. These figures demonstrate that 8-hourly maintenance dosing is required to ensure that 95% of infants maintain trough concentrations greater than 10 μg/ml. This dosing regimen is predicted to maintain trough levels above 20 μg/ml for the first 3 d of treatment, when seizures are most active.

Although this would be our recommended dosing regimen at the present time, the optimal serum level to aim for in the setting of neonatal status epilepticus is unclear. LEV has an extremely high therapeutic index, >148, in rodents (26). No deaths, organ failure, or other irreversible toxicity was seen

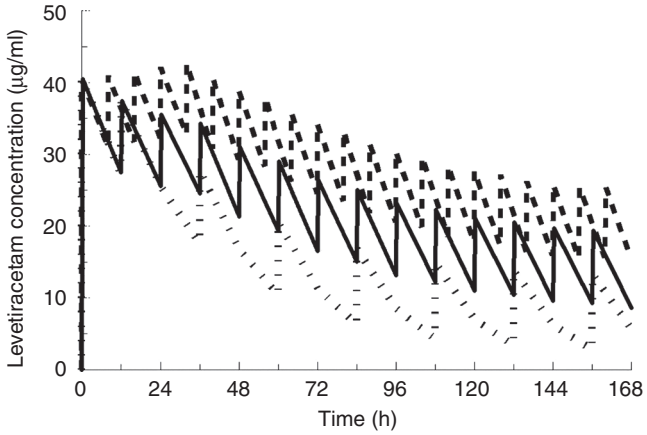


**Figure 3.** Simulations of proposed dosing. Comparison of expected median levetiracetam serum concentrations with a loading dose of 40 mg/kg followed by 10 mg/kg administered every 8 h (upper line), 12 h (middle line), or 24 h (lower line).

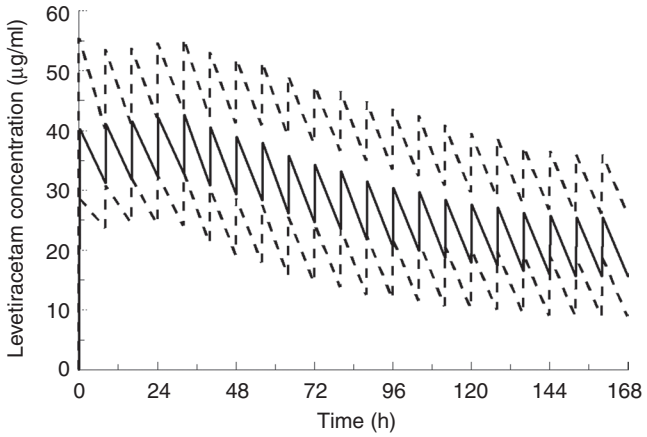


**Figure 4.** Expected distribution of levetiracetam serum concentrations with a loading dose of 40 mg/kg followed by 10 mg/kg every 8 h. 95th percentile (upper line), median (middle line), and 5th percentile (lower line).

after long-term oral treatment up to doses of 1,800 mg/kg/d in the rat, 960 mg/kg/d in the mouse, and 1,200 mg/kg/d in the dog (27). Ten years of experience have shown LEV to be extremely safe in humans also. Dose escalation studies should be performed and this safety margin should be exploited if additional efficacy would be obtained by using higher doses. A recent pediatric study suggested that there may be additional efficacy of extremely high doses of LEV. Subjects with refractory status epilepticus received a mean dose of 228 ± 48 mg/kg/d. The higher doses were effective in relieving status epilepticus where standard doses had been ineffective. These high doses were well tolerated; the authors report no significant short-term side effects, including behavioral side effects (28).

This study has several limitations. We have studied a small number of subjects. The subject population was also narrowly designed to include term infants during the first few days of life with relatively normal renal function for age. Given the dynamic nature of LEV CL in our study population, preterm and older term infants or those with some renal dysfunction are likely to have different LEV CL and possibly altered dosing requirements.

Our preliminary efficacy data are encouraging. It should be remembered that these data reflect the response rate to LEV when used as a second-line agent in subjects refractory to phenobarbital as a first-line agent. As such, this response rate of 42% seen in the higher dose cohort compares favorably with efficacy data for phenytoin and phenobarbital when used as second-line agents. Painter *et al.* found that of 4 of 17 (24%) of neonates with seizures refractory to phenobarbital responded to the phenytoin as the next anti-epileptic drug, and 5 of 16 (31%) neonates with seizures refractory to phenytoin responded to phenobarbital as the next anti-epileptic drug (3). However, the tendency of neonatal seizures to resolve spontaneously over time makes interpretation of these very preliminary data difficult.

In summary, this study has achieved a more accurate knowledge of the pharmacokinetics of LEV in neonates. LEV was well tolerated in this study of sick neonates. Further safety, efficacy, and dose escalation studies are now needed to determine the target concentration for efficacy and related optimal dose.

## METHODS

### Study Design

The trial was an open-label pharmacokinetic and preliminary safety study with LEV added on to phenobarbital treatment. Between August 2007 and February 2009, eligible neonates admitted in three participating neonatal intensive care units were recruited to this study. The sites were the University of California San Diego Medical Center, Sharp Mary Birch Hospital San Diego, and Auckland City Hospital, Auckland, New Zealand. The institutional review board at each center approved the protocol and informed consent was obtained from the parents in each case. The study was registered with the Clinical Trials Registry (NCT00461409).

### Study Entry Criteria

Subjects were inpatients in the three participating neonatal intensive care units. Eligibility required the study subjects to be less than 14 d of age with a corrected gestational age between 37 wk and 44 wk and weight of at least 2.5 kg. To receive the study drug, subjects had to be experiencing clinical or electrographic seizures that persisted after receiving a 20 mg/kg loading dose of phenobarbital. Subjects were excluded from the study if they had a serum creatinine of >1.2 mg/dl at the time of enrollment, if they were anuric or if seizures were because of a biochemical abnormality such as hypoglycemia or hypocalcemia, which once rectified resulted in seizure cessation. Patients were also excluded from this study if death of the patient seemed imminent.

### Intervention

Subjects were recruited in two ways. Patients recognized to be at high risk of developing neonatal seizures, for example neonates with HIE, were recruited prospectively. Other subjects were recruited at the time of presentation with seizures. Following recruitment and consent, patients were monitored by a three-channel continuous EEG with amplitude-integrated EEG to detect seizures (29). A neurologist skilled in neonatal EEG interpretation monitored the recording for the first hour and then at least every 8 h thereafter. If the EEG confirmed persistence of seizures half an hour after receiving phenobarbital, an i.v. LEV loading dose was administered over 15 min. LEV maintenance dosing was given starting 12 h after the initial infusion and continued every 24 h for a total of 1 wk.

If EEG-confirmed seizures persisted 1 h after completion of the LEV loading dose, patients received further medication following the local hospital protocol, typically further phenobarbital or fosphenytoin. The study protocol did not measure phenobarbital levels and did not require phenobarbital maintenance treatment.

The first cohort of babies ($n$ = 6) was treated with a 20 mg/kg initial load followed by 5 mg/kg/d as a single daily dose (qd). Following planned interim analysis of the first cohort, the dose was escalated. The second cohort ($n$ = 12) received 40 mg/kg as a load followed by 10 mg/kg/d qd.

The dosing selected for this trial was based on LEV pharmacokinetics in older populations and took into account expected developmental differences in term newborns. Distribution of LEV is characterized by low protein binding and a volume of distribution that approaches total body water (0.7 l/kg). Given the high total body water content in infants, it was expected that infant LEV volume of distribution would be slightly larger than in adults. LEV is cleared from the body by the kidney and by hydrolysis to UCB L057. Based on immature glomerular filtration in neonates and resulting renal function only 20% that of older children (22), LEV CL in infants was expected to be between 15 and 45% of an older population with the degree of hydrolysis present in neonates an important unknown variable. With these considerations, trough concentrations on the initial (20mg/kg load, then 5mg/kg qd) and second (40mg/kg load, then 10mg/kg qd) dose levels were expected to be at the low end and middle end of the range typically seen with therapeutic doses in adults (~35–120 µmol/l or 6–20 µg/ml).

Serial determinations of LEV concentrations were performed to enable pharmacokinetic analyses. Blood samples were collected before therapy, at predose troughs five times during the first week of therapy, and 1 h post peak levels following the first and seventh dose, to measure

peak and trough serum concentrations of LEV and its major metabolite UCB L057. Urine was collected in two aliquots: all urine output for the first 12 h after LEV therapy was commenced, and all urine output between 12 and 36 h. Concentrations of serum and urine LEV and UCB L057 were measured by a liquid chromatography tandem mass spectrometry method developed for this project (30) that permitted the simultaneous measurement of parent drug and metabolite.

Pharmacokinetic analysis was performed using the computer program NONMEM ver. 6.2 (ICON, Ellicott City, MD). NONMEM employs nonlinear mixed-effects modeling to analyze data composed of repeated measurements in nonlinear systems. This approach analyses all of the patient data together and determines the set of pharmacokinetic parameters and within and between-subject variability that describe the observed data. A one-compartment model was used and a population pharmacokinetic model was developed using the first-order conditional estimation subroutine with interaction. Patient weight was included in the model before the assessment of other covariates. Postnatal age, serum creatinine, sex, seizure onset day, dose level, phenobarbital concentration, and hypothermia were assessed as potential covariates on CL. An initial univariate screen was performed for each covariate and those that marginally improved the model (reduction in the objective function >4) were included in a backward-elimination multivariate analysis. A covariate that improved the final model by a reduction >6.6 was retained. After assessment of covariates, various residual error models (proportional, additive, and combined) were assessed. A bootstrap of the final model using 1,000 bootstrap data sets was performed to generate 95% confidence intervals of the parameters estimates using Wings for NONMEM (31). Empiric Bayesian estimates of individual subjects' pharmacokinetic parameters were generated using the population model parameters as the priors.

During the treatment phase, each patient was clinically reviewed daily. Follow-up continued by phone review at 3 and 6 d after intravenous administration of the study drug was completed. A follow-up visit was conducted 1 wk after completion of the treatment phase of the study.

Safety monitoring included measurement of complete blood count, serum creatinine, electrolytes, and liver enzymes at baseline, between 48 and 72 h of treatment and at completion of 7 d of treatment.

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