Development of Intravenous Topiramate for Neuroprotection and Seizure Control in Neonates

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I would like to thank all the patients and volunteers that contributed to this work. Their willingness to participate in these studies will hopefully lead to better outcomes for babies with seizures. Without their participation none of this would be possible. I would also like to acknowledge my advisers and committee members for their support.

DEDICATION

This thesis is dedicated to my amazing husband and family who have provided me endless support. This thesis is also dedicated to the families and babies who have experienced neonatal seizures. I hope this research will lead to a more effective and safer treatment for this devastating disease.

ABSTRACT

Hypoxic-ischemic brain injury in newborns is a significant medical problem with a high mortality rate, grave neurological sequelae including impaired cognition, neonatal seizures, and serious treatment-related adverse effects that can cause further brain injury. A safer, more effective treatment for neonatal seizures combined with the potential for neuroprotection would represent a significant advancement in the care of babies with hypoxic-ischemic brain injury. Intravenous topiramate holds the promise of controlling seizures and providing neuroprotection in newborn babies, but its safety and dosing must first be established in adults and, possibly, older infants and children.

When designing a new treatment option for neonatal seizures it is important to understand the developmental differences in the pathophysiology of seizures in the immature brain compared to the adult brain. Developmental age-specific mechanisms exist that alter the generation of seizures, the effect of seizures on the brain, and the effectiveness and impact of antiseizure therapy. Differences in the expression and activity of excitatory and inhibitory pathways in the developing brain may explain why many of the traditional antiepileptic drugs used to treat neonatal seizures are ineffective.

The research presented in my dissertation is focused on defining the safety and pharmacokinetics of intravenous topiramate. Topiramate is an antiepileptic drug used in adults and children to treat epilepsy. Recent research has shown topiramate is highly

effective in controlling seizures and is neuroprotective in newborn laboratory animals in models of status epilepticus and cerebral ischemia. The proven safety and effectiveness of topiramate for seizures in older children and adults together with substantial laboratory evidence showing benefit in models of hypoxic-ischemic encephalopathy strongly suggest that topiramate would be useful in the treatment of neonatal seizures and in addition might provide neuroprotection resulting from hypoxic-ischemic insult.

The studies included in this thesis include an animal study, a Phase I study in patients with epilepsy and migraines, and a healthy volunteer pharmacokinetic and safety study. The goal of the animal study was to determine plasma topiramate concentrations in rat pups given doses previously shown to result in neuroprotective effects. In that study, the neuroprotective dose produced concentrations slightly above the proposed therapeutic range (5 to 20 µg/mL), while the non-neuroprotective dose produced concentrations approximately twice as high as the therapeutic range for topiramate when used to treat epilepsy. Results from this study now provide target concentrations for future neuroprotection studies.

Results for the two human studies included in this thesis provide previously unreported information about topiramate. In adults, topiramate plasma concentrations attained by intravenous infusion were very similar to oral administration. The determination that the oral absorption is approximately 100% indicates patients can be given the same dose when switched from intravenous to oral, or vice versa. The studies also revealed an

extended elimination half-life of topiramate indicating it can be given once or twice daily in some patients while maintaining targeted plasma concentrations.

Intravenous infusion of doses of 25 mg to 100 mg over 10 to 15 minutes appears to be safe. No serious adverse events were reported by subjects following intravenous or oral administration of topiramate. Subjects reported no local discomfort due to administration of the intravenous formulation. Reported side effects were generally mild and resolved by 4 hours regardless of route of medication. Topiramate is known to have cognitive and neurological adverse events. Onset of cognitive adverse events and ataxia occurred early post-infusion, demonstrating the intravenous infusion may have quick penetration into the brain. For the treatment of neonatal seizures, in which a fast onset of action is required, rapid penetration into the brain is beneficial. Ideally, a rapid reduction of the duration and frequency of seizures should minimize the long-term neurodevelopmental adverse outcomes that occur after neonatal seizures.

These studies provide pharmacokinetic and safety data needed to begin studies in younger patients. Results from these studies will inform the design of subsequent studies, including controlled clinical trials intended to determine the efficacy and safety of intravenous topiramate for neuroprotection and seizure control in neonates.

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CHAPTER 1

INTRODUCTION

1.1 NEONATAL SEIZURES

The risk for seizures following hypoxic-ischemic injury is significant during the neonatal period. Not only do current treatments for neonatal seizures have inadequate efficacy, they are also associated with well defined adverse effects. Recent research in basic developmental neuroscience suggests treatment of hypoxic-ischemic brain injury and neonatal seizures in newborns can be improved.. Infants with neonatal seizures have a high mortality rate and frequently have serious neurological sequelae including impaired cognition and developmental delay. Compounding the problem is the standard therapies for treating neonatal seizures have serious adverse effects including further brain injury. The absence of adequately controlled trials raises concerns about the efficacy of standard drug therapy, while the acute and chronic adverse effects, including neurotoxicity, expose the neonate to significant risks.

1.1.1 Definition

Neonatal seizures are defined as seizures occurring during the first 30 days of life. Seizures are generally seen in the intensive care unit and in critically ill neonates. Hypoxic-ischemic encephalopathy caused by birth asphyxia is the most common etiology for neonatal seizures.[1] Hypoxic-ischemic encephalopathy is brain injury that results due to asphyxia and lack of oxygen to the brain. Brain hypoxia and ischemia during birth are the primary physiological processes that lead to hypoxic-ischemic encephalopathy and can lead to neonatal seizures.

Additional risk factors for neonatal seizures include both preterm birth and low-weight infants. Other less common causes include inborn errors of metabolism, intracranial hemorrhaging, infections, and metabolic irregularities. In a study of 200 neonates who developed seizures, 99% of the cases had an identified etiology.[2] Birth asphyxia was the most common cause (35%), followed by infection (34%), metabolic disturbances (12.5%), and intracranial bleeding (9.5%).[2] These results are consistent with other reports.[3]

1.1.2 Incidence

Neonatal seizures, regardless of etiology, are a rare medical condition. They are defined as seizures occurring during the first month of life, regardless of gestational age, and are estimated to occur in fewer than 1% of live births.[2, 3] This estimate has varied from 0.5% in term babies to 22.2% in preterm babies.[1, 4-7] The large variability is due both to the wide range of gestational ages and difficulty in recognition of neonatal seizures. Lanska et al. completed a population-based, retrospective cohort study of neonatal seizures in all neonates born to residents of Fayette County, Kentucky, from 1985 to 1989.[7] Neonatal seizures occurred in 58 of 16,428 newborns (3.5/1,000 live births or 0.35%).[7] Saliba et al. completed a similar study among infants born between 1992 and 1994 in Harris County, Texas.[8] This study found 207 cases of clinical neonatal seizures among 116,048 live births (1.8/1,000 live births or 0.18%).[8] A prospective study, involving all the obstetric and neonatal units across the province of Newfoundland, Canada, was completed to determine the incidence, clinical features, etiologic distribution, and day of

onset in neonates with seizures.[1] From 1990 to 1994, the incidence of neonatal seizures was 2.6/1000 live births (0.26%) and hypoxic-ischemic encephalopathy was the presumed cause in 40% of cases.[1]

In another study, 35% of 200 infants with neonatal seizures were caused by birth asphyxia.[2] Based on this data, there are approximately 3,100 to 6,200 cases per year of neonatal seizures secondary to hypoxic-ischemic encephalopathy in the United States. Neonatal seizures are a short lived condition only affecting newborns during the first month of life. Therefore, the number of neonates with seizures at any particular time is far less than 200,000, and falls within the definition of a rare disorder. Classifying neonatal seizures as a rare medical disorder (see section 1.6) qualifies for incentives under the Orphan Drug Act including tax credits, market exclusivity, exemption of prescription drug user fees, formal protocol assistance, and Food and Drug Administration (FDA) funding through a grant program to foster development of new therapies.

1.1.3 Clinical course

The clinical presentation of neonatal seizures is different than seizures in older children or adults. They are frequently multifocal and behaviorally subtle. Neonatal seizures frequently recur without treatment. They often display electro-clinical dissociation, in which neonates display movements that mimic seizures or electrographic seizures

without a clinical manifestations and are recognized only with an EEG. Future studies are needed to determine if treatment of subclinical seizures improves outcomes.

Hypoxic-ischemic encephalopathy after birth asphyxia, usually occurs soon after birth, but it can occur unexpectedly and requires ~~a~~ rapid initiation of intensive care. Seizures caused by hypoxic-ischemic encephalopathy typically escalate in frequency and duration, but normally subside after a few days.[9, 10] Therefore, rapid diagnosis and treatment is essential.

Since newborns often display electro-clinical dissociation, diagnosis of neonatal seizures is best made in conjunction with an EEG. However, in clinical practice this is often impractical because EEGs are not always available. Therefore, treatment is frequently initiated before an EEG can be completed. Recently, there has been increased interest in using 1 or 2 channel EEGs to monitor and detect seizures. Data has shown that a full EEG still remains the gold standard for quantifying and detecting seizures.[11] Additional studies are required to determine the feasibility of using reduced channel EEGs.

1.1.4 Prognosis

Neonatal seizures cause significant morbidity and mortality. One of the first studies that reviewed long-term outcomes following neonatal seizures was the Collaborative Perinatal Project.[12] The study found a 35% mortality rate in infants who have had neonatal seizures. Furthermore, the study found 30% of survivors had long-term adverse

neurological outcomes at 7 years of age. These adverse outcomes included cerebral palsy in 13%, IQ less than 70 in 19%, and epilepsy (all types) in 20%. More recent studies have found lower mortality rates. Sheth et. al., in a study of 356 neonates with seizures, reported a mortality rate of 19%.[3] In a more recent study, the mortality rate was 7%, but adverse neurological outcomes remained similar to the decades earlier rate of 28%.[13] Decreased mortality rates following neonatal seizures are thought to be due to improved maternal and neonatal intensive care and enhanced resuscitation techniques. Unfortunately, these improved techniques have not decreased long-term morbidity following seizures.

Research in animal models has shown that seizures in the immature or neonatal brain cause less neuronal injury and death than seizures in the adult brain.[14, 15] Although the immature brain is more resistant to neuronal death, seizures in the developing brain cause more long-term harmful effects. These effects are though to be a result of reduced brain growth, changes in receptor expression, reduced deoxyribonucleic acid (DNA) synthesis, and altered neuronal connectivity.[16-19]

1.1.5 Pathophysiology

When designing a new treatment option for neonatal seizures it is important to understand the developmental differences in the pathophysiology of seizures in the immature brain compared to the adult brain. Developmental age-specific mechanisms

exist that alter the generation of seizures, the effect of seizures on the brain, and the effectiveness and impact of antiseizure therapy.[10]

Seizures at all ages are a result of an imbalance between excitatory and inhibitory neuronal pathways. In the neonatal brain, increased excitatory activity exists at baseline. Synapse and dendritic spine density peak during the first month of life. [20-22] Glutamate receptors, the main excitatory pathway, are also overexpressed in the immature brain increasing susceptibility to seizures.[23-26] Glutamate receptor subunit composition is developmentally regulated and results in increased receptor excitability in the neonatal brain.[25, 27-29] An overexpression of glutamate receptor subtypes in the developing cortex corresponds to the time of increased seizure susceptibility.

Glutamate receptors include ligand-gated ion channels and metabotropic receptors. Glutamate receptor ligand-gated ion channels are permeable to sodium, potassium, and sometimes calcium. Glutamate receptors are categorized based on the specific ligands that activate the receptor and include: *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate. The NMDA subtype is a heterometric receptor and is developmentally regulated (NR1 and NR2). In the neonatal brain, the main NR2 subunit variant is the NR2B and results in a longer current decay time compared to the adult NR2A subunit variant.[10] Immature NMDA receptors also have decreased magnesium sensitivity causing increased receptor excitability.[30]

Neonatal seizure therapy targeting NMDA receptors is limited due to its normal function in learning, brain development, and memory.[10, 28]

The AMPA glutamate receptor is responsible for most fast excitatory synaptic transmission. They are heteromeric and composed of four subunits (GluR1, GluR2, GluR3, or GluR4). In the developing brain, AMPA receptors lack the GluR2 subunit and are calcium permeable.[25, 29] Therefore, AMPA receptor composition enhances receptor excitability during the neonatal period. The AMPA receptor provides a developmental age specific target for the treatment of neonatal seizures.

Expression and activity of gama-aminobutyric acid (GABA) receptors, the main inhibitory pathway in the brain, are also developmentally regulated. Gama-aminobutyric acid binding and expression are both lower in the immature brain compared to the adult brain.[26, 31] Gama-aminobutyric acid receptor activity is also controlled by subunit expression and composition. In the neonatal brain, the α_4 and α_2 subunits are overexpressed and the α_1 subunit is underexpressed compared to adult brains.[32] Overexpression of the α_4 subunit decreases GABA receptor sensitivity to benzodiazepines.[32]

Another difference in the neonatal brain is that GABA receptor activation can result in depolarization not hyperpolarization of the membrane potential. This is a result of a reversed chloride gradient in the developing brain. The chloride gradient is reversed due

to underexpression of the calcium exporter, KCC2, and overexpression of the calcium importer, NKCC1.[33]

Differences in the expression and activity of the excitatory and inhibitory pathways in the developing brain may explain why many of the traditional antiepileptic drugs are ineffective in treating neonatal seizures. For example, phenobarbital and benzodiazepines, act on the GABA pathway. Drug development for neonatal seizure treatment needs to take into account age-specific differences in brain physiology.

1.1.6 Current treatment of neonatal seizures

Treatment of neonatal seizures has not significantly changed over the last few decades. For newborns at high risk for seizures, the standard practice is visual monitoring and an EEG if clinical activity is observed. If there is a treatable underlying etiology (for example hypoglycemia), the baby is given appropriate therapy. Otherwise, intravenous antiepileptic drugs including phenobarbital, phenytoin, and benzodiazepines are administered.[34] Most surveys demonstrate phenobarbital is the most commonly prescribed first-line treatment followed by phenytoin.[9] Midazolam and lidocaine are also used as second-line medications, but have little efficacy in stopping refractory neonatal seizures. Although widely used, there is very little data demonstrating the benefit of traditional antiepileptic drugs for the treatment of neonatal seizures, as most studies have been small and uncontrolled.

In an open-labeled design, Painter et al. studied 59 neonates with seizures confirmed by EEG that were randomly assigned to either phenytoin or phenobarbital.[35] Phenobarbital and phenytoin were given intravenously over 5-15 minutes once daily. Dosing of phenytoin and phenobarbital was designed to achieve unbound peak concentrations of 3 µg/mL and 25 µg/mL, respectively. Treatment was stopped after 7 days unless seizures continued. Complete seizures cessation was evaluated and assessed by EEG. Therapy was considered a failure if the neonate had an episode of electrical seizures lasting longer than 2.5 minutes or a total of 2.5 minutes of seizure activity during any 5-minute period. Thirty neonates were assigned to phenobarbital and 29 to phenytoin. The study included both premature and full-term babies. Gestational age, race, and cause of seizures were similar between treatment groups. Seizure cessation was similar between groups with phenobarbital and phenytoin stopping seizures in 43% and 45%, respectively, of patients.[35] Since there was no placebo-controlled group in this study, the actual efficacy of both drugs is unknown. Spontaneous seizure cessation rate without treatment is unknown. Therefore, the actual efficacy of phenobarbital and phenytoin is likely less than 43% and 45%. Regardless, a response rate of less than 50% illustrates the need for improved treatment of neonatal seizures. In a more recent study, only 4 out of 14 infants responded to phenobarbital therapy.[36]

Further complicating the use of phenobarbital and phenytoin in neonates is the growing body of evidence showing these drugs may be harmful to the developing brain. In utero exposure to antiepileptic drugs has long been recognized to cause adverse effects such as

developmental delay, fetal malformations, and microcephaly.[32-35] Antiepileptic drugs are also associated with impaired cognition when these drugs are given to infants and young children. Phenobarbital exposure during infancy can cause cognitive impairment that lasts into adulthood.[37-40]

Several recent reports suggest certain antiepileptic drugs can cause accelerated preprogrammed cell death (apoptosis) in laboratory animal pups that are comparable in development to human neonates. This neurotoxicity, occurs in the absence of any hypoxic-ischemic insult or seizures. Glier et al. investigated the neurotoxic properties of single does of phenytoin (10-50 mg/kg) and phenobarbital (20-100 mg/kg) in the brains of postnatal day 7 rats.[41] Phenytoin produced increased apoptosis at doses of 20 mg/kg and phenobarbital at doses of 40 mg/kg and above. Similar results were found in a study by Bittigau et al. that found the threshold for increased apoptosis was 20 mg/kg for phenytoin, 40 mg/kg for phenobarbital, and 5 mg/kg for diazepam.[42] The proapoptotic doses found in these studies are similar to doses used to control seizures in rodent models of epilepsy.[43, 44] Furthermore, the doses used in these animal studies likely result in plasma drug concentrations which are attained in human neonates when given standard phenobarbital or phenytoin therapy. These studies support the hypothesis that phenobarbital and phenytoin cause cognitive impairment, in part, by enhancing apoptotic neuronal death. When taken together, research suggests the use of phenobarbital and phenytoin in neonates may result in lasting cognitive impairment.

In addition to these long-term adverse effects, phenobarbital and phenytoin also have acute short term risks associated with their use. Intravenous administration of phenobarbital and phenytoin can cause central nervous system depression, hypotension, bradycardia, respiratory depression, and cardiac arrhythmias. Phenobarbital and phenytoin are both potent inducers of metabolic enzymes, which may complicate the treatment of other medical problems common in newborns with seizures (sepsis, hypoglycemia, jaundice, hemorrhage, congenital heart defects, immaturity, and anemia).

Another challenge in the treatment of neonatal seizures is determining the duration of therapy. Most clinicians agree chronic therapy is not needed, but there is currently no consensus among clinicians on how long to treat after neonatal seizures. Studies investigating the optimal duration of therapy after neonatal seizures are warranted.

1.1.7 New treatment options for neonatal seizures

The development of new approaches to treating neonatal seizures should involve either new or older drugs that exert their effect through mechanisms of action different from the standard medications . Currently approved drugs being investigated for potential use in neonatal seizures include topiramate, bumetanide, and levetiracetam. Silverstein and Ferriero reported the results of a survey of 55 child neurologists regarding their choice of a second-line, add-on antiepileptic drug in the treatment of neonatal seizures.[45] The leading add-on candidates were topiramate (recommended by 55%) and levetiracetam (recommended by 47%). Among those recommending topiramate, 70% perceived

treatment to be beneficial and 63% perceived no side effects. Among those recommending levetiracetam, 58% perceived treatment to be beneficial and 92% perceived no side effects.

Bumetanide is a loop diuretic and is FDA-approved for the treatment of edema associated with heart failure, hepatic disease, and renal disease.[46] Bumetanide blocks the NKCC1 transporter, which is overexpressed in the neonatal brain. This alters the intracellular chloride concentration gradient and is thought to suppress seizure activity.[33] Bumetanide is commercially available in both intravenous and oral formulations. It has been well studied for its diuretic properties in this age group.[47-50] In animal models, bumetanide in combination with phenobarbital was more effective than phenobarbital alone in controlling seizures.[51] Bumetanide antagonizes the chloride transporter resulting in a more negative GABA equilibrium potential which may enhance the efficacy of phenobarbital. In neonatal rats phenobarbital was effective in stopping or decreasing seizures in 30% of neonatal rats.[51] The combination of phenobarbital and bumetanide stopped seizures in 70% and decreased the frequency and duration in the remaining 30%.[51] Soul and colleagues are undertaking a Phase II NIH-funded study in neonates to determine dosing and refine methods to detect seizures. Although bumetanide may reduce seizures when given in addition to phenobarbital, phenobarbital use continues to raise concerns regarding adverse effects on the developing brain. Therefore, a safer and more effective treatment option that decreases phenobarbital use would be beneficial.

Levetiracetam is not approved for the treatment of neonatal seizures, but many pediatric neurologists and neonatologists are using it as a second-line option.[45] Levetiracetam is FDA approved for adjunctive therapy in the treatment of partial seizures in adults and children 4 years of age and older, myoclonic seizures in adults and adolescents 12 year of age and older, and primary generalized tonic-clonic seizures in adults and children 6 years of age and older.[52] Both intravenous and oral formulations are commercially available. Due to the availability of an intravenous formulation, levetiracetam is becoming used as second-line therapy when traditional antiepileptic drugs fail. To date, levetiracetam's efficacy and safety has not been adequately studied in a controlled clinical trial. An initial pharmacokinetic and safety study of intravenous levetiracetam in neonates is currently being conducted at University of California, San Diego. The precise mechanism of levetiracetam antiseizure effects is unknown, but it may be due to its binding to the synaptic vesicle protein SV2A.[52] Recently, a small pilot study in 6 neonates was completed where levetiracetam was administered orally to neonates (increasing dose 10mg/kg per day over 3 days).[53] No severe adverse events were observed and all six patients became seizure free within 6 days.[53] Five patients remained seizure free after 3 months with continued levetiracetam treatment.[53]

1.1.8 Therapeutic hypothermia

Hypothermia is increasingly employed for hypoxic-ischemic encephalopathy. As a result, many neonates with seizures are getting hypothermia therapy. Hypothermia appears to be a safe and effective intervention. Studies have shown both a decrease in

mortality and improved neurological outcomes after moderate hypothermia in neonates with hypoxic-ischemic encephalopathy.[54-56] In a study of 325 infants less than 6 hours of age and a gestational age of at least 36 weeks with perinatal asphyxial encephalopathy, intensive care plus cooling of the body to 33.5 ºC for 72 hours was compared to intensive care alone.[54] At 18 months of age, infants in the cooled group had an increased rate of survival without a neurological abnormality (relative risk, 1.57; 95% CI, 1.16 to 2.12; P=0.003).[54] Among survivors, cooling resulted in decreased rates of cerebral palsy (relative risk, 0.67; 95% CI, 0.47 to 0.96; P=0.03).[54] In a similar study in infants with a gestational age of at least 36 weeks and less than six hours of age with moderate or severe encephalopathy, whole-body cooling to temperature of 33.5 ºC for 72 hours reduced death or moderate or severe disability (risk ratio, 0.72; 95 percent confidence interval, 0.54 to 0.95; P=0.01).[55]

Hypothermia can be induced by either head cooling or whole body cooling. The current standard is to cool babies to a core body temperature of 33.5 ºC for 72 hours. It is unknown whether head cooling or whole body cooling is superior nor is the precise mechanism of hypothermia known. One hypothesis is that hypothermia decreases oxygen consumption in the brain, cerebral energy utilization, excitatory amino acid accumulation, cytokine levels, apoptosis, and reduces blood brain barrier permeability.[57-61]

Hypothermia affects the drug disposition although the phenomena is incompletely understood.[62] Many babies treated with hypothermia are on multiple drugs. Therefore, the influence of cooling on pharmacokinetics needs to be considered. A meta-analysis of 21 studies found the cooling can significantly alter disposition for some commonly used medications. Mild to moderate hypothermia decreases the systemic clearance of drugs between 7% to 22% per degree below 37º C.[62] A significant mechanism by which therapeutic hypothermia alters drug disposition is changes in cytochrome P450-mediated metabolic system. One mechanism is decreased substrate binding to cytochrome P450.[63-65] Hypothermia also decreases the rate of redox reactions, alters nicotinamide adenine dinucleotide phosphate P450 reductase activity, changes cytochrome b5 activity, and modifies lipid membrane fluidity.[62]

Therefore, special attention to dosing of drugs used to treat neonatal seizure during therapeutic hypothermia is needed. Dosing adjustments may be required to maximize drug efficacy and safety during cooling. Frequent monitoring of drug levels and outcomes are needed during cooling and rewarming. More studies in neonates are needed to fully characterize the influence of cooling on drug pharmacokinetics and dynamics.

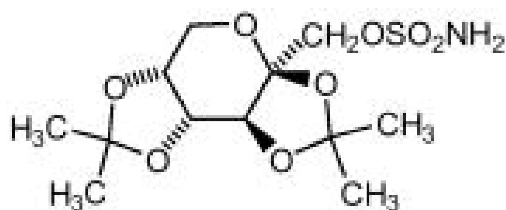
1.2 TOPIRAMATE

1.2.1 Physical-chemical properties

Topiramate is 2,3:4,5-Di-O-isopropylidene-ß-D-fructopyranose sulfamate (Table 1). It has a molecular formula of $C_{12}H_{21}NO_8S$ and a molecular weight of 339.36. Topiramate

is a white crystalline powder with a bitter taste.[66] It is most soluble in alkaline solutions containing sodium phosphate or sodium hydroxide with a pH of 9 to 10.[66] Topiramate is freely soluble in acetone, chloroform, dimethylsulfoxide, and ethanol. Topiramate's water solubility is 9.8 mg/mL.[66]

Figure 1: Chemical structure of topiramate



1.2.2 Pharmacology

Topiramate has a broad range of activity, although its precise antiseizure mechanism of action is unknown. It blocks voltage-gated sodium channels, augments GABA at certain subtypes of $GABA_A$ receptors, inhibits α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate glutamate ionotropic receptors, and is a weak inhibitor of some isoenzymes of carbonic anhydrase (CA-II and CA-IV). Topiramate's neuroprotective effect in the immature brain is thought to be due to glutamate receptor blockage.[67]

1.2.3 Pharmacokinetics

The pharmacokinetic profile can affect the clinical utility of a drug. Newer antiepileptic drugs, like topiramate, tend to have fewer drug interactions and a more favorable pharmacokinetic profile than older antiepileptic drugs.

Absorption

Absorption is rapid with peak plasma concentrations occurring 2 hours after oral administration and is linear with dose.[68-71] Administration of topiramate with food decreases the rate but not the extent of absorption. Food decreases the mean maximum concentration by approximately 10% and delays the mean time to maximum concentration by approximately 2 hours.[71] Due to the lack of an intravenous formulation, the absolute bioavailability of topiramate was previously unknown.

Distribution

Topiramate is only 9-17% bound to plasma proteins.[69, 72] Therefore is unlikely to displace or be displaced by highly protein bound drugs. The apparent volume of distribution is 0.6-0.8 L/kg which is similar to the distribution of total body water.[69]

Metabolism

Twenty to 30% of a dose undergoes metabolism when administered in the absence of enzyme inducers.[73] Multiple metabolites are formed by hydroxylation, hydrolysis, and glucuronidation.[74] When topiramate is administered with an enzyme inducer, up to 50%

of a dose undergoes metabolism.[70, 75, 76] The exact enzymes involved in the biotransformation of topiramate are unknown.

Excretion

In the absence of enzyme induction, renal clearance is the major route of elimination. The clearance of topiramate can be significantly reduced in patients with renal dysfunction. In a study of the effects of renal dysfunction on topiramate clearance, the clearance was reduced by 42% in patients with a creatinine clearance 30-69 ml/min and 54% in patients with a creatinine clearance less than 30 ml/min.[77]

Half-life

When administered alone, topiramate has a half-life of 20-30 hours.[69, 71, 78] , whereas the presence of an enzyme inducer decreases the half-life to 12-15 hours.[70, 79, 80] Due to its half-life, twice daily topiramate dosing is generally recommended in the treatment of epilepsy. Oral topiramate clearance (CL/F) is approximately 1-2 L/hr when given alone.[69, 71, 73, 81]

Drug interactions

The potential for drug interactions is a concern when administering multiple antiepileptic drugs. Topiramate is not thought to significantly alter the metabolism of other antiepileptic drugs, although its metabolism can be changed by enzyme inducing medications. Many of the traditional antiepileptic drugs alter topiramate metabolism.

Topiramate oral clearance can increase 2-fold when given with enzyme inducing medications.[79, 80] Coadminstration with carbamazepine or phenobarbital may lower concentrations by 40%.[75] Topiramate inhibits CYP2C19.[74]

Pharmacokinetic differences in infants

The pharmacokinetics of topiramate in infants and children differs from adults. Therefore, infants and children require higher doses per weight to attain therapeutic drug concentrations. Dahlin et al. in a study of 91 infants and children found that the clearance of those age 0-8 years was 1.5-fold higher than the clearance of those 9-17 years of age when treated with topiramate alone or in combination with a non-enzyme inducer and 2-fold higher when treated with an enzyme inducer.[82] In a retrospective, case-matched study comparing seventy children age 1-17 years and 70 adult controls age 18-65 years matched for comedication and sex, average topiramate clearance was 42% higher in children than adults in those without enzyme inducers and 50-100% higher in those taking enzyme inducers.[83] Apparent topiramate clearance in infants age 24-30 months with infantile spasms was 2-fold higher than the normally reported value in adults.[84]

There is only one published study reporting topiramate pharmacokinetics in neonates. Filippi et al. investigated the effect of hypothermia on topiramate pharmacokinetics in asphyxiated neonates treated with prolonged whole-body hypothermia. Thirteen full-term newborns were treated with either mild (33–34 ºC) or deep (30–33 ºC) whole body hypothermia for 72 hours and all received oral topiramate (5 mg/kg once a day) for 3

days. Topiramate concentrations were measured on dried blood spots. Plasma topiramate concentrations were evaluated for the first nine infants, after 48 h of hypothermia. Topiramate concentrations were measured before the administration of the third dose of topiramate and at 48.5, 49, 49.5, 50, 52, 54, 56, 60, 64, 68, and 72 hours. For the remaining 4 patients, topiramate concentrations were measured at the beginning of hypothermia, before the first dose of topiramate and from 0.5 to 72 hours after dosing for a total of 28 measurements. Results showed that the time to maximum concentration was delayed and apparent total body clearance was lower in infants undergoing deep hypothermia compared to mild hypothermia, although the differences were not statistically significant.[85] This suggests slower absorption and elimination during hypothermia. Topiramate maximum and minimum concentrations, half-life, and area under the curve were greater than those observed in normothermic infants.[85] Topiramate pharmacokinetics were not significantly different between babies on deep versus mild hypothermia.[85] One possible reason for a lack of a statistically significant difference was the small number of patients. Another limitation of the study is that calculations of pharmacokinetics were done assuming patients were at steady-state. As these patients are changing both medically and physically during this period of time, steady-state is unlikely. The delay in absorption in the deep hypothermic group may have been due to slower gastric emptying and decreased blood flow to the gastrointestinal tract. No adverse effects were attributable to topiramate were identified. This indicates neonates clear topiramate and that once daily dosing of topiramate should be possible. Further studies are necessary to characterize the relationship between hypothermia and

pharmacokinetic processes. Because neonatal seizure studies will likely include patients on hypothermia, the possible effects of cooling on topiramate pharmacokinetics must be incorporated into the study designs.

1.2.4 Indications

Topiramate is FDA-approved as monotherapy in patients greater than 10 years of age with partial onset or primary generalized tonic-clonic seizures.[66] It is also approved as adjunctive therapy for adults and pediatric patients (2 to 16 years of age) with partial onset seizures or primary generalized tonic-clonic seizures, and in patients greater than 2 years of age with seizures associated with Lennox-Gastaut Syndrome.[66] Topiramate is also approved in adults for the prophylaxis of migraine headaches.[66]

1.2.5 Neuroprotection

There is an increasing interest in using topiramate for neuroprotection as a result of promising laboratory studies. Neurotoxicity can result from seizures as well as ischemia. During seizures, oxygen and glucose deprivation to mitochondria can cause neuronal cell death. Cerebral ischemia causes increased extracellular levels of glutamate resulting in an influx of sodium and calcium into the neuron , which eventually also leads to neuronal cell death.[86] Topiramate is thought to prevent neuronal cell death primarily through activity at AMPA receptors.[67] The AMPA glutamate receptor is responsible for most fast

excitatory synaptic transmission. In the developing brain AMPA receptors are calcium permeable and inhibition causes a decrease in calcium influx.[25, 29]

Neuroprotection is studied in animal models by inducing a seizure or ischemic event. Subsequently, the proposed neuroprotective agent or placebo is given and effects of the injury can be assessed. Ischemic injury can be induced by multiple methods including occlusion of the carotid artery, injection of thrombus, or provoking seizures (through kainite administration or electrical stimulation). The proposed neuroprotective agent can be given prior to or post insult. However as it is difficult to predict when ischemic events are going to occur, finding a neuroprotective agent that is effective when given after insult is essential. The outcome measures in neuroprotection studies can include pathophysiological and/or behavioral assessments. One pathophysiological outcome commonly used is percent reduction of infarct volume compared to control. A neuroprotective agent should decrease the size of brain injury and infarct volume. After ischemia, damage can be assessed by using a silver or 2,3,5-triphenyltetrazolium chloride stain to determine of the percentage of infarct volume. Many studies also include behavioral measures such as animal maze performance.

Neuroprotection has been reported for topiramate in models of status epilepticus and cerebral ischemia. Yang et al. investigated topiramate neuroprotection after cerebral ischemia in rats.[86] Rats were embolized and after 2 hours were given intraperitoneal saline, low-dose topiramate (20 mg/kg), or high-dose topiramate (40 mg/kg).[86]

Treatment with high-dose topiramate showed significantly more neuroprotection than low-dose topiramate, indicating a dose-dependent neuroprotective effect.[86] Lee et al. studied the protective effects of intraperitoneal topiramate after global ischemia in gerbils.[87] Although topiramate at a dose of 50 mg/kg failed to reduce neuronal damage, 100 mg/kg and 200 mg/kg significantly decreased neuronal damage.[87] After status epilepticus, topiramate decreased rat hippocampal neuronal injury at intraperitoneal doses of 20, 40, and 80 mg/kg.[88] Follett et al. studied the protective effect of intraperitoneal topiramate doses of 10, 30, and 50 mg/kg in a rat model of periventricular leukomalacia (white matter injury).[67] Only the 30 mg/kg topiramate dose had a significant attenuation of lesion severity and motor deficits.[67] In several models, topiramate appears to have a dose-dependent effect on reducing neuronal damage when administered after injury. Table 1 lists the major neuroprotection studies involving topiramate.

Table 1: Topiramate neuroprotection studies

Study	Injury	Animal	Dose (mg/kg)	Result
Lee et al.[87]	global ischemia	gerbils	50, 100, or 200	50 mg/kg had no effect, 100 & 200 mg/kg reduced neuronal damage
Yang et al.[86]	cerebral ischemia	rats	20 or 40	reduced infarct volume & deficit scores, dose dependent effect
Niebauer et al[88]	status epilepticus	rats	20, 40, or 80	reduced degeneration at all doses
Follett et al.[67]	periventricular leukomalacia	rat pups	10, 30, or 50	30 mg/kg, but not 50 mg/kg, had a protective effect on lesions and attenuated motor deficits
Koh et al.[89]	hypoxic induced seizures	rat pups	30 & NBQX	repeated dose prevents susceptibility to hippocampal injury
Cha et al.[90]	status epilepticus & neonatal seizures	rats	80 mg/kg/day	after 4 weeks of treatment no difference in cell loss

Neuroprotection of other antiepileptic drugs

Multiple antiepileptic drugs have been evaluated for neuroprotection using in-vitro and animal models. Barbiturates and benzodiazepines both have mechanisms of action that includes potentiating the effects of GABA. These drug classes have failed to show neuroprotective effects in models of brain ischemia.[91] Barbiturates and benzodiazepines also decrease brain perfusion, which could have detrimental effects when given during

brain ischemia.[92] Therefore, barbiturates and benzodiazepines do not make good choices for use as neuroprotectants.

Phenytoin, fosphenytoin, and other hydantoins block voltage-dependent sodium channels. Kaptanoglu et al. studied the neuroprotective effects of phenytoin (1, 10, or 30 mg/kg) following spinal cord injuries in rats.[93] Significant ultrastructural neuroprotection was observed with 30 mg/kg.[93] Phenytoin was also studied in an animal model of global ischemia and protected neural injury only when given prior to the ischemic onset.[94] This class of drugs also reduces brain blood flow and metabolism, which are unwanted effects in ischemia.[92] Phenytoin has also been shown to increase neuronal apoptosis in neonatal animals.[42] Therefore, phenytoin is not a candidate for neuroprotection.

Carbamazepine blocks voltage-dependent sodium channels. Carbamazepine reduced neuronal death in an animal model after focal irreversible brain ischemia when given within 30 minutes of ischemic onset.[95] Furthermore, carbamazepine has been shown to reduce cerebral blood flow and increase apoptosis making it not a good option for neuroprotection in neonates.[41,77] Therefore, carbamazepine is not a viable option for studying neuroprotection in neonates. Safety of carbamazepine has not been evaluated in neonates. In addition, intravenous formulation of carbamazepine is not commercially available.

Valproate has been shown in a rat model of status epilepticus to reduce neuronal damage in the hippocampus and dentate hilus.[96] However, valproate also increases apoptosis and decreased cerebral blood flow.[41, 97] In addition, valproate is not recommended for use in neonates as the incidence of fatal hepatic toxicity is highest in that age group.

Lamotrigine was investigated in a rat model of focal ischemia and reduced the infarct volume by 38% when given 1 hour after the onset.[98] It has also been found to reduce neuronal death after global ischemia in animal models when given prior to injury.[92] More studies are needed to determine if lamotrigine is useful as a neuroprotectant.

Levetiracetam was studied in a rat model of ischemic injury and decreased infarct volume by 20-30%, when given 30 minutes prior to onset and continued for 24 hours as a continuous infusion.[99] Costa et al. investigated, but did not find, neuroprotection following in-vitro ischemia on striatal neurons.[100] Wang et al. studied the effect of levetiracetam after closed head injury in a murine model and determined that low-dose (18 mg/kg every 12 hours for 3 days) and high-dose (54 mg/kg every 12 hours for 3 days) improved functional and histological outcomes.[101]

Apoptosis

Neurons die after seizures and cerebral ischemia through both necrotic and apoptotic processes.[92] Apoptosis, or preprogrammed cell death, occurs due to the exposure of antiepileptic drugs themselves, separate from the effects of the initial injury. Increased

neuronal apoptosis after antiepileptic drug exposure is a possible mechanism for long-term cognitive impairments which have been observed after phenobarbital and phenytoin use in infants.[38-41] The neurotoxic and apoptotic effects after exposure to antiepileptic drugs are thought to be limited to the developing and immature brain.[67] The mechanism of toxicity is thought to be a result of depression of synaptic neurotransmission. Increased apoptosis has been reported after exposure to both GABA receptors agonists and voltage-gated sodium channels antagonists.[41, 42] Unlike other antiepileptic drugs that primarily work by other mechanisms of action, increased apoptosis in neonatal animal models has not been observed with the pure AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f) quinoxaline-2,3-dione (NBQX).

Apoptosis is a typical part of the development and health of multicellular organisms. Cell death occurs in response to numerous signals and stimuli. The ability of a stimulus to induce apoptosis depends on the stage of cell cycle, the age of the cell, severity of stimulus, and expression of apoptotic proteins. Distinct changes in cells occur after signaling to begin apoptosis. Caspases are activated early in the apoptosis cascade resulting in degradation of cell components that are vital for normal cell function, such as structural proteins and DNA repair enzymes. The caspases also activate enzymes that breakdown DNA. As cells components are degraded, their morphology changes and they are removed by macrophages.

Glier et al. investigated whether topiramate causes apoptotic neurodegeneration seen with other antiepileptic drugs in the developing rat brain.[41] The neurotoxic effect of topiramate (5-80 mg/kg) was compared to phenytoin (10-50 mg/kg), phenobarbital (20-100 mg/kg), and valproate (5-400 mg/kg).[41] Topiramate slightly increased apoptotic neuronal death at doses of 50 mg/kg and above, but these doses are much higher than reported doses used to control seizures in infant rodent models (5-20 mg/kg).[41] The separation between topiramate antiseizure and neurotoxic dose was much larger than it was for phenytoin, valproate, and phenobarbital.[41] The degree of apoptosis was up to 4-fold higher after phenytoin, valproate, and phenobarbital at antiseizure doses as compared to topiramate.

1.2.6 Topiramate use in infants & neonates

Although topiramate has not been well studied in neonates, there are a number of reports describing its pharmacokinetics, efficacy, and safety in infants under 2 years of age. Mikaeloff et al. studied the pharmacokinetics of topiramate in 22 children age 6 months to 4 years with epilepsy.[102] This study showed the apparent oral clearance of topiramate was higher in infants taking enzyme inducing AEDs (85.4 +/- 34.0 ml/h/kg) compared with those taking non-inducing AEDs (46.5 +/- 12.8 ml/h/kg).[102] Numerous studies have evaluated topiramate in the treatment of infantile spasms. Zou et al. studied topiramate safety and efficacy in 54 infants (age 24-36 months) with infantile spasms.[103] The maximum dosage was 26 mg/kg per day and minimum dosage was 1.56 mg/kg per day. They found that 81.4% of patients had a reduction of seizure frequency from baseline

greater than 30% and 57.4% were seizure free for more than 24 months.[103] In an open-labeled chart review of infants less than 24 months of age (range from 1 month to 19 months), 28 patients with refractory epilepsy on topiramate were evaluated.[104] Seven of 8 subjects with infantile spasms responded to topiramate treatment (at least a 50% reduction in seizures), and 50% of subjects with other seizure types responded.[104] Topiramate was well tolerated with side effects occurring in 5 patients leading to discontinuation in only 2 of the patients.[104] Valencia et al. studied the efficacy and tolerability of topiramate in 13 infants younger than 2 years of age (range 4-24 months).[105] Topiramate had a greater than 50% reduction in seizures in 8 of the subjects.[105] In a study of topiramate in 47 children age 6-60 months with refractory epilepsy, 60% of subjects had at least a 50% reduction in seizure frequency.[106] Grosso et al. studied the efficacy of topiramate in an open-labeled prospective study in infants less than 2 years.[107] They enrolled 59 children in the study: 22 had localization-related, 23 generalized, 6 Dravet's syndrome, and 8 unclassifiable epilepsy. The median follow-up period was 11 months (range 3-27 months). Mean topiramate dose was 5.2 mg/kg per day (range 1.6-8.9 mg/kg per day). Topiramate was effective (at least 50% decrease in seizure frequency) in 47% of patients, including 13% who were seizure-free. Topiramate was more effective in localization-related epilepsy (48% responded) than in generalized epilepsy (32% responded). Topiramate was well tolerated. The most frequently reported adverse effects were drowsiness, irritability, hyperthermia, and anorexia.

Unfortunately, most of these studies were open-label and included children with refractory epilepsy in which topiramate was used as a second-line antiepileptic drug. A recent study investigated topiramate pharmacokinetics, safety, and tolerability in infants age 1-24 months.[108] This was a double-blind, placebo-controlled, parallel-group study in 149 patients with refractory partial onset seizures. There were three assigned fixed-dosage levels within the trial: 5, 15 and 25 mg/kg/day. The blinded phase was followed by a 1 year open-label treatment phase in which topiramate at doses up to 60 mg/kg/day were used. Median percentage reduction from baseline of daily seizure rate was not significantly different (p=0.97) between topiramate 25 mg/kg/day (20.4%) and placebo (13.1%). [108] Investigators concluded that topiramate (5-25 mg/kg/day) was not effective for infants age 1-24 months as adjunctive treatment for refractory partial onset seizures.[108] Treatment-emergent adverse effects such as fever, diarrhea, vomiting, anorexia, weight decrease, somnolence, and viral infection occurred more frequently with topiramate than with placebo.

The only published report of topiramate use in neonates was a recent pilot study of oral topiramate in 13 newborns being treated with mild or deep whole body hypothermia for hypoxic-ischemic encephalopathy.[85]

1.3 STABLE-LABELED ISOTOPES

A key element of studies presented in this thesis is the use of stable-labeled intravenous topiramate formulations which allow rigorous characterization of oral and intravenous

pharmacokinetics without interrupting maintenance therapy. Stable-labeled isotopes of drugs are molecules with a greater molecular weight but otherwise identical to the common, unlabeled drug. A compound labeled with a stable isotope has one or more atoms replaced by a nonradioactive isotope e.g. C^{13} instead of C^{12} or N^{15} instead of N^{14}. The substitution gives a greater molecular weight to the labeled compound compared to the original molecule, which can be detected using mass spectrometry.

Stable-labeled isotopes are free of the risks associated with radioactive isotopes. If labeled in the correct position, it does not change the chemical, pharmacological, or pharmacokinetic behavior of the molecule This permits investigation of drug disposition without requiring additional doses or interfering with unlabeled medication in the body.

Pairing orally administered drug with intravenously administered, stable-labeled isotopes of the same drug is a valuable tool for pharmacokinetic and bioavailability studies.[109] Stable-isotope and unlabeled drug can be simultaneously administered and resulting plasma concentrations can be measured using sensitive chromatographic mass spectrometric techniques. This method permits measurement of the bioavailability, distribution volume, and elimination half-life under steady-state conditions. For antiepileptic drugs, it allows rigorous characterization of pharmacokinetics under steady-state conditions. The alternative is the interruption of drug therapy for several days or more in order to characterize elimination half-life, but such an interruption exposes the patient to an increase risk of seizures.

The Epilepsy Research Group at the University of Minnesota has previously made and administered stable-isotope formulations of phenytoin, fosphenytoin, lamotrigine, and carbamazepine to determine pharmacokinetics in patients under steady-state conditions. These studies demonstrate that stable-isotopes of antiepileptic drugs can be effectively and safely used in patients with epilepsy to rigorously characterize pharmacokinetics under steady-state conditions without interrupting therapy.[110, 111]

Malik et al. studied stable-labeled isotopes of phenytoin and phenobarbital in 9 infants with neonatal seizures.[112] The use of stable-labeled isotopes allowed determination of absorption, clearance, and elimination in neonates in which steady-state had not been achieved.[112] Investigators concluded that a nonradioactive labeled isotope can be particularly useful in neonates when volumes of distribution and pharmacokinetic processes are rapidly changing.[112]

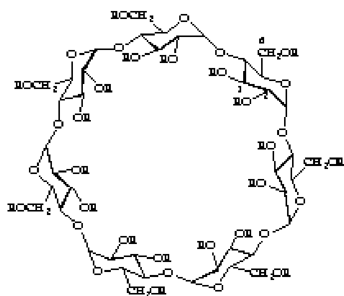
1.4 INTRAVENOUS TOPIRAMATE FORMULATION

Intravenous topiramate was formulated as a 10 mg/ml solution dissolved in a 10% sulfobutyl cyclodextrin (Captisol®). The formulation was manufactured at the University of Iowa, Division of Pharmaceutical Sciences under good manufacturing practices.

1.4.1 Cyclodextrin formulation

Cyclodextrins are complex molecules that have a hydrophilic exterior and a lipophilic interior cavity (see Figure 2). Cyclodextrins are pharmacologically inert, but can improve drug solubility and stability by attracting poorly water soluble compounds into the cavity while the cyclodextrin-drug combination remains in solution. Drug rapidly dissociates from the cyclodextrin once the solution is injected into the bloodstream. Captisol® (Cydex) is a polyanionic β-cyclodextrin derivative that has an improved safety profile over other cyclodextrins. It has been shown to be safe in humans and has received FDA approval for use in the parental formulations of voriconazole (VFEND®), aripiprazole (Abilify®), and ziprasidone (GEODON®).

Figure 2: Structure of Captisol®

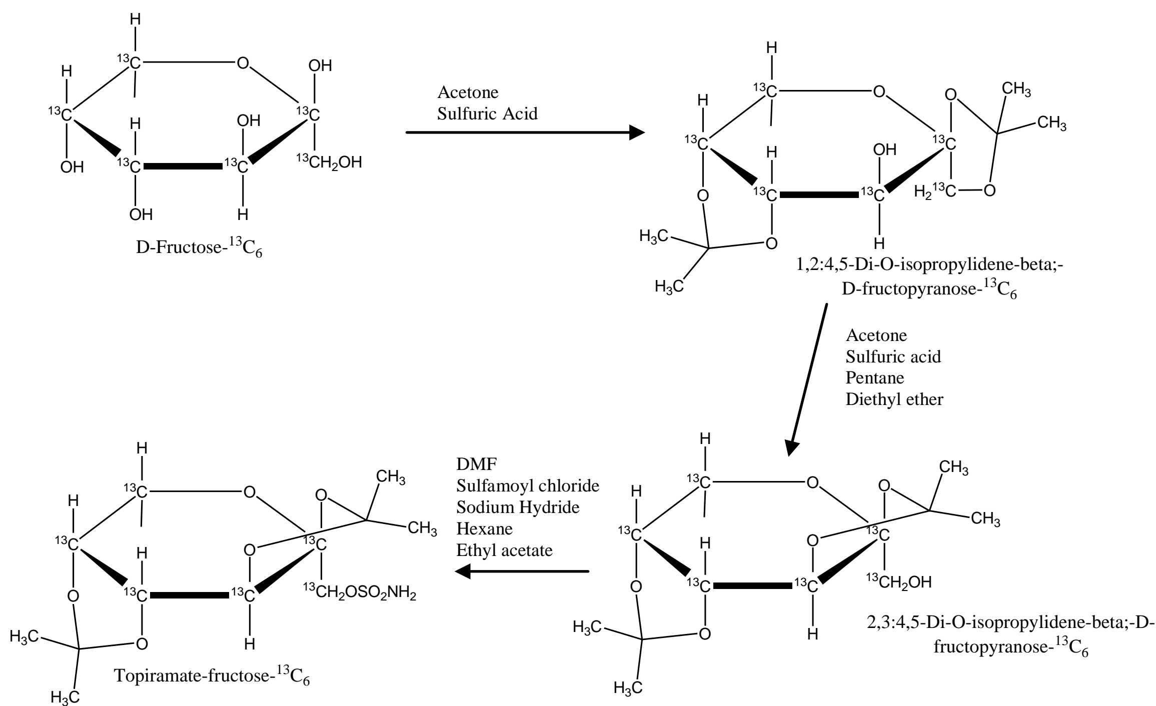


1.4.2 Synthesis of stable-labeled topiramate

Topiramate was synthesized by combining D-fructose with acetone under acidic conditions to yield diisopropylidenefructopyranose. Sodium hydride is then added. After stirring, sulfamoyl chloride is added to yield topiramate. A ^{13}C -labeled isotope was used

in this formulation. The synthesis of a $[^{13}C]_4$ stable labeled isotope was accomplished by using 1,3-$^{13}C_2$-acetone in place of acetone. Synthesis of stable-labeled topiramate was completed by Isotech Laboratories Inc. (Champaign, Illinois) (see Figure 3)

Figure 3: Synthesis of $[^{13}C]_6$-topiramate

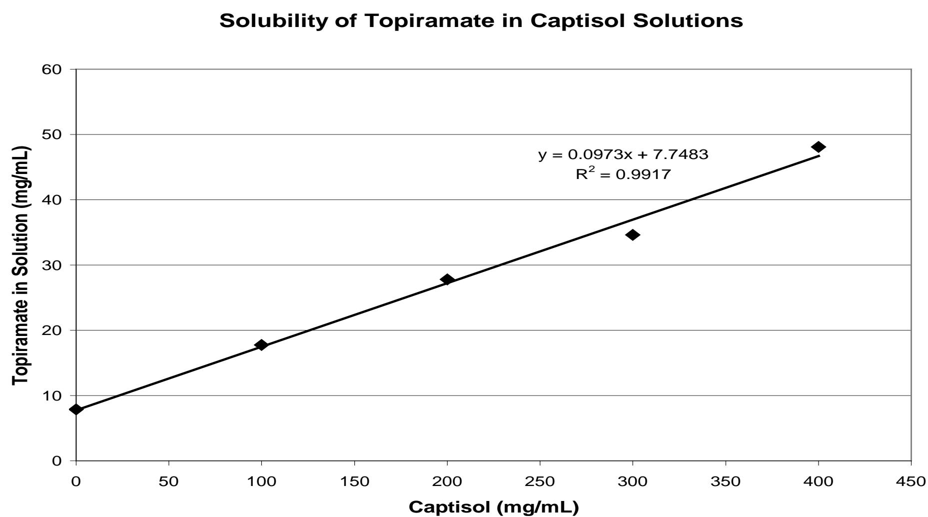


1.4.3 Solubility

Topiramate is poorly soluble in water (9.8 mg/ml), with little stability. The solubility and stability of topiramate were improved with the addition of a cyclodextrin. There is a linear relationship between topiramate solubility and Captisol® concentration (Figure 4). These results indicate 10 mg/ml of topiramate can be dissolved in 10% w/v Captisol®

solution. This formulation is concentrated enough to limit the injection volume but does not require amounts of Captisol® larger than those in currently approved products.

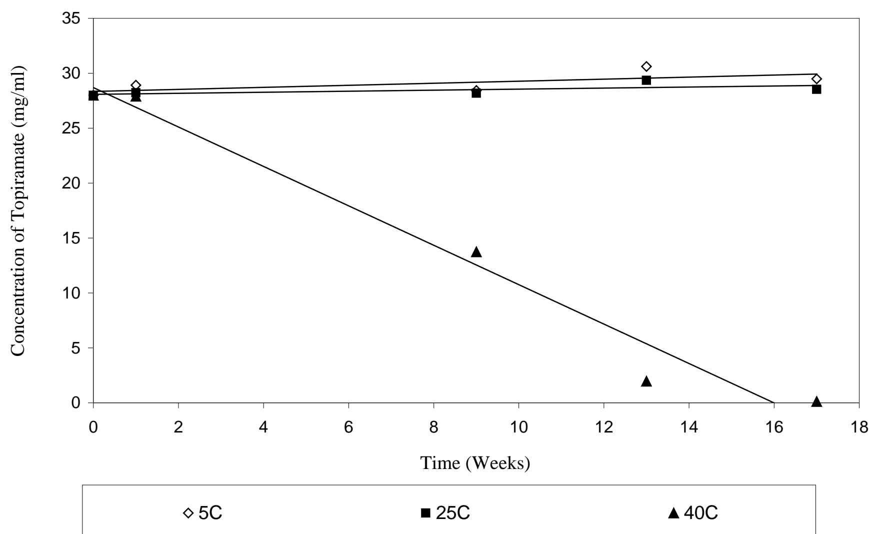
Figure 4: Phase solubility of topiramate in Captisol®



1.4.4 Stability

Stability studies were carried out to determine shelf life and the optimal conditions for storage. The stability of a 10 mg/ml topiramate in a 10% Captisol® solution was examined at various temperatures. Without refrigeration, topiramate solution is not stable (Figure 5). The 10 mg/ml topiramate in 10% Captisol solution has remained stable for 3 years at 25˚ C.

Figure 5: Stability of a 10mg/ml topiramate in 10% Captisol®



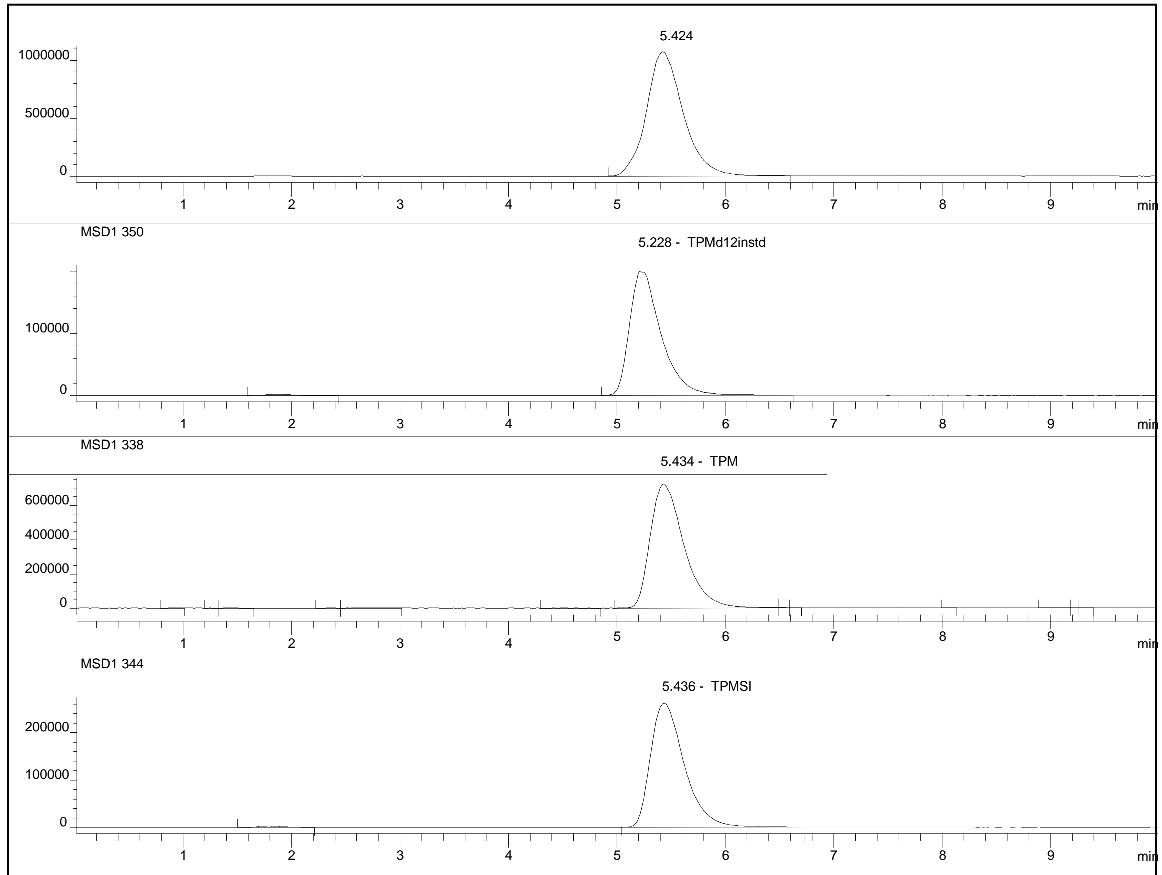
1.5 ANALYTICAL METHODS

1.5.1 Assay

The analytical method was designed to measure topiramate and stable-labeled topiramate in plasma using liquid chromatograph mass spectrometry. The analytes were separated using a Zorbax C18 column and a mobile phase consisting of an ammonium acetate buffer and methanol. The quantization was performed using selective ion monitoring negative mode, with topiramate-d10 as the internal standard. The data was generated using Agilent ChemStation software. Figure 6 is an example chromatograph of topiramate, stable-labeled topiramate, and internal standard.

Plasma samples were run with a 7-concentration standard curve (run in triplicate) and nine quality control samples (low, med, and high run in triplicates). The calibration curve was linear in the concentration range of 0.1-20 µg/mL. The limit of detection was 0.5 ng/mL, while and the limit of quantification was 0.04 µg/mL. The precision for topiramate and stable-labeled topiramate ranged from 2-5% and 3-5%, respectively, with accuracy values of topiramate and stable-labeled topiramate between 97.6-102.5% and 95.2-106%, respectively. The recovery for topiramate in spike plasma ranged from 94-105% and 90-106% for stable-labeled topiramate.

Figure 6: Chromatograph of topiramate, stable-labeled topiramate, and internal standard



1.6 ORPHAN DRUG DEVELOPMENT

Neonatal seizures are a short-lived condition, only affecting newborns during the first month of life. The incidence of neonatal seizures is less than 1% of live births (see section 1.1.3). Approximately 10,000 to 20,000 babies per year in the U.S. have neonatal seizures, thus making the condition a rare disorder.

Rare diseases are frequently chronic, life-threatening, and debilitating diseases that impact patients' lives significantly. Drug development for these conditions have been limited by challenges in availability of subjects for clinical trials, the lack of understanding of the underlying mechanism of the disease, funding for research in rare conditions, and a lack of financial incentives to bring drugs to market. The passage in 1983 of the Orphan Drug Act has help overcome many of these challenges by providing incentives for the development of orphan drugs.

An orphan drug is defined as a vaccine, diagnostic, or preventive drug for a disease or condition affecting fewer than 200,000 people in the United States. A drug can also be considered an orphan drug if the indication affects more than 200,000 people in the United States and there is no reasonable expectation that the cost of developing the drug will be recovered from sales. Currently, there are an estimated 5,000 to 6,000 known rare diseases.[113] Approximately 25 million people in the U.S. are affected by a rare disease.[113] Rare diseases are not so rare when considering the total number of affected individuals.

The Orphan Drug Act offers sponsors incentives to facilitate research in orphan conditions. Orphan products are given seven years of market exclusivity upon FDA approval. Through this market exclusivity, the FDA will not approve any identical drug used for the same purpose. This exclusivity is given even if the drug cannot be patented. Another incentive for sponsors developing orphan products are tax credits equaling 50% of clinical research expenses undertaken by a sponsor to generate data for approval of the

product. Formal protocol assistance from the Center for Drug Evaluation and Research at the FDA is given when requested. Through this assistance, the sponsor and the FDA work together to establish necessary trials to get the drug to market as soon as possible. Another incentive to researching orphan conditions is a grant program which supports the investigation of rare disease treatment. The grants support early clinical trials and are primarily awarded to academic researchers. This program fund about 15-20 new grants per year and provides $200,000 per year for up to 3 years for phase I studies and $400,000 per year for 4 years for phase II trials.

A sponsor must apply to the Office of Orphan Product Development at the FDA to receive orphan drug designation. A request for designation can be made at any stage of drug development, although it must be occur before new drug application submission. Pharmaceutical companies may seek orphan status for a drug which is already FDA approved, if efficacy and safety can be shown in the rare disease. Although orphan products must be demonstrated to be as safe and effective as other products, sponsors performing clinical trials with orphan drugs have challenges not seen with trials of other non-orphan products. Many of the difficulties in designing studies are due to the small population size affected by the condition. Patients are often geographically dispersed and trials usually require multiple clinical sites. Many times randomized, double-blind, placebo-controlled trials are not possible in the setting of rare diseases. Due to the smaller number of subjects enrolled in these studies, the drug may need to show a more robust treatment effect than would be needed with trials enrolling a larger number of

subjects. Another challenge when studying rare disorders is that often the disease is not well characterized i.e. the epidemiology, clinical course, and underlying pathophysiology may not be completely known.

Rare diseases and orphan drugs are now on the political agenda. There are well organized patient advocacy groups which have been essential in the development of orphan drugs. Pharmaceutical companies committed to the development of orphan drug products have emerged. Public funding is also available for research, through the Office of Orphan Product Development at the FDA. Although this area has made great progress, there are some remaining challenges. Funding for rare diseases and orphan drug development is inadequate. There needs to be increased awareness of rare diseases. The pharmaceutical industry views the incentives of the Orphan Drug Act as inadequate. There also needs to be timely access to orphan treatments for patients with these conditions.

1.7 MY ROLE IN PROJECTS

Grant applications

Co-wrote four grant applications for the studies included in this thesis

- New Therapies Grant from the Epilepsy Research Foundation (funded)
- FDA Office of Orphan Product Development (funded)
- Parents Against Childhood Epilepsy (not funded)
- Academic Health Center at the University of Minnesota (not funded)

Investigation drug application

- Prepared and submitted an investigational drug application (IND) for use of intravenous topiramate in patients with epilepsy and migraines
- Prepared and submitted an updated IND for the use of intravenous topiramate in healthy adult volunteers

Development of formulation

- Coordinated and oversaw manufacturing of intravenous topiramate at the University of Iowa
- Prepared an injectable topiramate formulation for animal studies
- Coordinated and analyzed the stability results to determine shelf life and the optimal conditions for storage for the intravenous topiramate formulation

Determination of neuroprotective topiramate concentrations in neonatal rat pups

- Formulated injectable topiramate solution
- Quantification of topiramate plasma concentrations
- Pharmacokinetic analysis of a rat pup concentrations.

Phase 1 study of intravenous topiramate in patients with epilepsy and migraines

- Prepared and submitted an investigational drug application for use of intravenous topiramate in patients with epilepsy and migraines

- Co-wrote grant application to FDA Office of Orphan Product Development
- Designed study and wrote the protocol
- Prepared, submitted, and had approved an IRB application
- Served as study coordinator (recruited, screened, enrolled, and consented subjects)
- Supervised and managed the study
- Analyzed the pharmacokinetic and safety data

Two-way crossover study of oral and intravenous topiramate in adult healthy volunteers

- Prepared and submitted an updated investigational drug application for the use of intravenous topiramate in healthy adult volunteers
- Co-wrote grant application to Epilepsy Foundation
- Designed the study and wrote the protocol
- Prepared, submitted, and had approved an IRB application
- Supervised and managed the study
- Analyzed the pharmacokinetic and safety data

Design a Clinical Trial to Determine Safety and Efficacy of IV TPM for Neonatal Seizures

- Using data from both the animal and human studies as well as information from the literature on neonatal seizures, I created several study designs that could be used to conduct prospective, randomized, controlled clinical trials of intravenous topiramate in neonates with seizures

CHAPTER 2

DETERMINATION OF NEUROPROTECTIVE TOPIRAMATE CONCENTRATIONS IN NEONATAL RAT PUPS

2.1 BACKGROUND

Neonatal seizures and hypoxic-ischemic encephalopathy are both conditions characterized by ischemia-reperfusion problems in which periods of reduced or absent cerebral blood flow are followed by restoration of the cerebral circulation. Babies with hypoxic-ischemic encephalopathy experience multisystem malfunction with reduced cardiac output from myocardial depression, abnormal lung mechanics, and impaired function of the gastrointestinal tract, liver and kidneys.

Use of a neuroprotectant that can be given after an ischemic event could significantly improve outcomes in babies born with neonatal seizures and hypoxic-ischemic encephalopathy. The prevalent form of brain damage after hypoxic-ischemic insult is white matter injury, or periventricular leukomalacia. Primary objectives for a neuroprotection trial in this population would be prevention or reduction of periventricular leukomalacia. Secondary goals of neuroprotection would be the reduction of gray matter injuries, prevention of seizures, and prevention of strokes. An ideal candidate drug should be neuroprotective even when given after injury. Drug pharmacology is complex in the neonatal period. Hepatic immaturity is common, and comedications are commonly administered. Hypothermia is an added difficulties in drug management. In the many neonates, bowel motility is decreased and erratic and therefore, an intravenous drug is required.

Although several putative neuroprotectants have been studied, none have demonstrated significant benefits in humans. Topiramate neuroprotection has been reported in experimental models of status epilepticus and cerebral ischemia.[67, 86-90] It has also been shown to reduce damage and improve functional abilities in immature animal models of white matter injury induced by hypoxia, ischemia, or hypoglycemia. In a newborn rodent model of hypoxic-ischemia, intraperitoneal injection of topiramate at 30mg/kg, but not 50mg/kg, significantly protected against periventricular leukomalacia.[67] However, the plasma concentrations attained and pharmacokinetics associated with these doses were not determined. Research investigating topiramate as a neuroprotectant in other laboratory animals and humans requires defining the drug concentrations associated with neuroprotective doses.

In other neonatal rodent hypoxic-ischemic models, the same 30 mg/kg dose of topiramate protected against neuronal injury, the development of acute seizures, and chronic epilepsy.[89, 114] Detailed information describing the plasma concentrations and pharmacokinetic characteristics of topiramate following intraperitoneal administration in newborn rat pups is needed to help translate these findings to other animal models of white matter neuroprotection, and, subsequently, to human trials.

2.2 STUDY AIMS

The aim of the study was to determine plasma topiramate concentrations and pharmacokinetics in postnatal day 7 rat pups following 30mg/kg and 50mg/kg

intraperitoneal doses. Results from this pilot study will inform the design of subsequent studies intended to determine the safety, tolerability, and efficacy of topiramate for neuroprotection in human infants.

2.3 STUDY METHODS

Animals

Animal experiments were conducted in accordance with the Animal Welfare Act for the care and use of laboratory animals and the project was approved by the University of Pennsylvania Institutional Animal Care and Use Committee. The experiments were carried out on newborn postnatal day 7 to 10 Long Evans and Sprague-Dawley rats, weighing 15-29 grams. Postnatal day 7 to 10 rats are thought to be developmentally similar to full-term human neonates. The rats were housed at Abramson Research Center rat research facility at Children's Hospital of Philadelphia with standard environmental conditions including twelve hour light and dark cycles. Animals were allowed free access to food and water for the duration of the experiments. The animals were sacrificed by rapid decapitation and one milliliter of core blood was collected at the specified time points.

Drug

Topiramate was formulated as a 5mg/ml solution dissolved in phosphate-buffered saline (pH 7.4). Pharmaceutical grade topiramate was purchased from Toronto Chemical.

Experimental Design

Each 7 to 10 day old neonatal rat pup was weighed and administered an intraperitoneal dose of either 30 mg/kg or 50 mg/kg topiramate. Each rat was sacrificed by decapitation at a specified time (15, 30, 60, 90, 120, 240, 360 or 480 minutes) after the injection. An average of six animals per dosing group was used for each sample time. This sacrifice design allowed the collection of serial plasma topiramate concentrations following the 30 mg/kg and 50mg/kg injections. Blood samples were obtained by exsanguinating the animal and collected in citrated tubes. Whole blood was centrifuged and plasma samples were immediately frozen at -80˚C until analysis.

Topiramate Assay

Plasma topiramate concentrations were quantified using a liquid chromatography mass-spectroscopy method (see section 1.5.1 Assay)

Topiramate pharmacokinetic analysis

Topiramate concentration-time data were analyzed by noncompartmental modeling using WinNonLin® (version 5.2, Pharsight Corporation, Palo Alto, CA). The analysis utilized sparse sampling methods. The concentration-time curve was calculated by taking the mean value for each time point. Using the concentration-time data, the area under the plasma topiramate concentration-time curves ($AUC_{0-\alpha}$) were determined by the log-linear trapezoidal method with the tail area calculated from C_{last}/k; the maximum plasma concentration (C_{max}) was obtained from the mean of the observed highest topiramate

levels, the terminal half-life ($t_{1/2}$) was calculated as 0.693/k, and k was the slope of the terminal regression line. Concentrations were weighted using $1/y^2$. The pharmacokinetic parameters were used to carry out simulations to estimate steady-state topiramate concentrations associated with neuroprotective and non-neuroprotective doses. Steady-state concentrations were estimated for the single-dose data assuming linear pharmacokinetics and using the following equation:

$$Css_{average} = \frac{Dose}{CL * \tau}$$

2.4 RESULTS

A total of 109 post-natal day 7 to 10 rats were used in this experiment. Sixty-two pups received an intraperitoneal topiramate dose of 30 mg/kg and forty-seven pups received a 50 mg/kg dose. Seventy-two pups were Sprague-Dawley and 37 were Long Evans. The rats weighed between 15-29 grams at time of dosing. The concentration-time profiles of topiramate after 30 mg/kg and 50 mg/kg intraperitoneal dosing are shown in Figure 7 and Figure 8, respectively. The data indicate both the 30 mg/kg and 50 mg/kg doses result in a wide variability of concentrations.

Figure 7: Topiramate Concentrations after 30 mg/kg in Post-Natal Day 7 to 10 Rat Pups

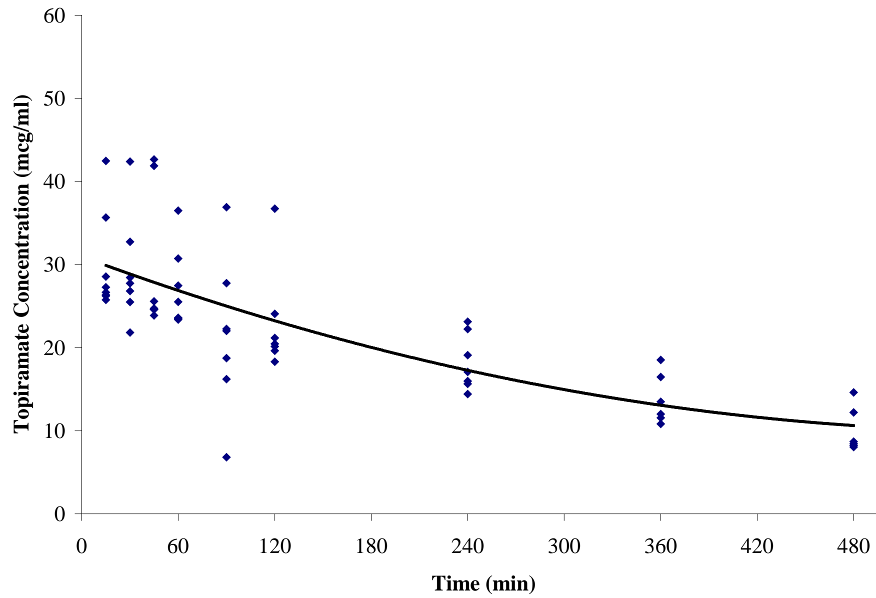
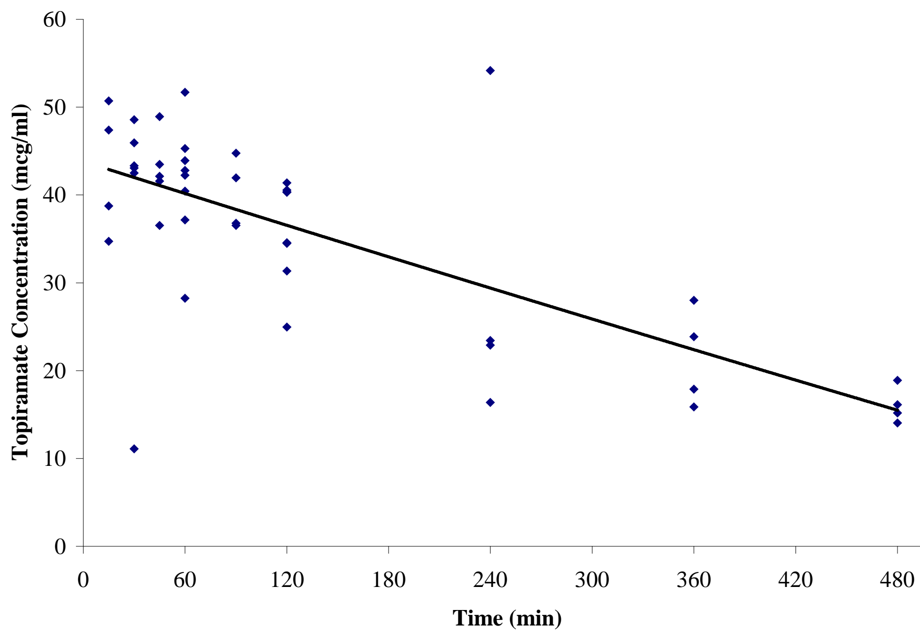


Figure 8: Topiramate Concentrations 50 mg/kg in Post-Natal Day 7 to 10 Rat Pups



The results of the noncompartmental pharmacokinetic analysis are summarized in Table 2. Maximum plasma concentrations were observed after 15 minutes, indicating topiramate was rapidly absorbed after intraperitoneal dosing. Maximum concentrations observed following the 30 mg/kg and 50 mg/kg intraperitoneal dosing were 29.9 µg/mL and 42.9 µg/mL, respectively. The volume of distribution, clearance, and half-life for the 30 mg/kg and 50 mg/kg groups were similar.

Table 2: Pharmacokinetic parameters after intraperitoneal topiramate

Parameter	30 mg/kg	50 mg/kg
C_{max} (µg/mL)	29.9	42.9
V_d (ml)	18.9	18.5
CL (ml/hr)	2.82	2.78
$t_{1/2}$ (min)	278.8	277.8
T_{max} (min)	15	15
k (1/min)	0.0036	0.0036

Simulation of dosing 30 mg/kg and 50 mg/kg every 12 hours produced respective steady-state average concentrations of 17.8 µg/mL and 28.0 µg/mL (Table 3).

Table 3: Steady-State prediction after 30mg/kg and 50 mg/kg intraperitoneal topiramate

Parameter	30 mg/kg IP	50 mg/kg IP
$Css_{average}$ (µg/mL)	17.8	28.0
Css_{min} (µg/mL)	5.9	8.5
Css_{max} (µg/mL)	35.8	51.4

2.5 DISCUSSION AND CONCLUSIONS

Hypoxic-ischemia is considered to be the primary cause of periventricular leukomalacia which is considered to lead to much of the long-term neurodevelopmental sequelae. Glutamate levels increase during hypoxic-ischemia and cause excessive glutamate receptor activation leading to neuronal death.[115-118] Kainate and AMPA receptor antagonists may provide clinical benefit by preventing white matter injury. Medications that are neuroprotective and decrease the morbidity associated with periventricular leukomalacia are needed.

Topiramate, a medication approved for use in epilepsy and migraines, is neuroprotective at certain doses in newborn animal hypoxic-ischemic models. Topiramate antagonizes both AMPA and kainite receptors. In a neonatal rat pup model of hypoxic-ischemia, intraperitoneal injection of topiramate at 30mg/kg (but not 50mg/kg) given every 12 hours for 4 doses significantly protected against periventricular leukomalacia.[67] However, plasma topiramate concentrations were not measured. Since interspecies differences in pharmacokinetics are known, it is essential to characterize concentration time profiles associated with doses. These concentrations or exposures then become the target to be attained when studying other species including humans. Therefore, determining target topiramate concentrations associated with neuroprotection is needed to design topiramate dosing regimens in other animal and human studies.

Following an intraperitoneal injection, topiramate exhibits rapid absorption attaining maximum concentrations slightly higher than the steady-state concentrations reported when the drug is used orally to treat epilepsy i.e. between 5-20 μg/mL.[119] In this study, the neuroprotective dose (30 mg/kg) produced maximum concentrations (mean 29.9 μg/mL) slightly above this range, while non-neuroprotective doses (50 mg/kg) produced peak concentrations approximately twice as high as the therapeutic range for epilepsy. These concentrations are also slightly higher than those in neonates given 5 mg/kg/day while being treated with hypothermia for hypoxic-ischemic encephalopathy.[85]

During the study by Follett et al., rat pups were given topiramate every 12 hours for a total of 4 doses.[67] Based on the half-life estimated (4-5 hours), these animals were at steady-state. In the current study, neuroprotective doses of topiramate in neonatal rat pups resulted in average steady-state plasma concentrations of 17.8 μg/mL. Assuming the unbound fraction of plasma topiramate in rats is similar to humans, 30 mg/kg topiramate every 12 hours would result in a steady-state rat extracellular fluid concentration of 15.1 μg/mL, similar to central spinal fluid concentrations demonstrated in epilepsy patients treated with topiramate.[120, 121]

The limitations of this study include the large variability in topiramate concentrations. This study utilized a sparse sampling strategy because of limited blood sampling in neonatal rats. A study design utilizing multiple samples in an animal would allow a more accurate determination of topiramate pharmacokinetics. When considering the

translation of these results, it is also possible that neuroprotective concentrations may differ greatly between the rat pup model and human infants. Further research is also needed to determine which exposure parameter is best related to the neuroprotective effect of topiramate (C_{max}, C_{min} AUC, or $Css_{average}$).

These results indicate that doses shown to be neuroprotective in rat pup models result in peak topiramate concentrations in the range of 20-40 μg/mL. Future studies of topiramate in other laboratory animals should utilize dosing regimens that attain concentrations in this range. Further, the observation that plasma concentrations determined in this study are similar to those observed during standard treatment for epilepsy. This increases the likelihood that use of topiramate as neuroprotectant in newborn babies will be safe.

The long-term research aim of this study is to understand, develop, and refine dosing strategies for neuroprotective medications in neonates with seizures and hypoxic-ischemic encephalopathy. The central hypothesis is therapeutic topiramate concentrations can provide neuroprotection to neonates undergoing hypoxic-ischemic injury. The goal of the present study was to determine plasma topiramate concentrations in postnatal 7 to 10 day rat pups given doses previously shown to result in neuroprotective effects. Results from this study provide target concentrations that will inform the design of dosing strategies in future topiramate neuroprotection studies.

CHAPTER 3

A PHASE 1 STUDY OF INTRAVENOUS TOPIRAMATE SAFETY AND PHARMACOKINETICS IN PATIENTS WITH EPILEPSY AND MIGRAINES

3.1 INTRODUCTION

The aim of this first-in-humans, Phase I study is to provide initial safety and pharmacokinetic data essential to further develop intravenous topiramate as an orphan product for the treatment of neonatal seizures in newborns with hypoxic-ischemic encephalopathy. The results from this study will set the stage for future studies of intravenous topiramate in newborns with hypoxic-ischemic encephalopathy and seizures.

Topiramate is an antiepileptic drug used in adults and children to treat epilepsy. Recent research has shown topiramate is highly effective in controlling seizures and is neuroprotective in newborn laboratory animals in models of status epilepticus and cerebral ischemia. The proven safety and effectiveness of topiramate for seizures in older children and adults together with substantial laboratory evidence showing benefit in models of hypoxic-ischemic encephalopathy strongly suggests topiramate would be useful in the treatment of neonatal seizures resulting from hypoxic-ischemic encephalopathy and provide neuroprotection. Prior to studies of investigational drugs in newborns, the safety and pharmacokinetics must first be studied in adults.

This investigation is a pharmacokinetic and safety study of an intravenous topiramate formulation in adult patients already taking oral topiramate. A small (25 mg) dose of a stable-labeled intravenous topiramate was given to patients at the same time as his or her usual morning dose. Infusion site, systemic, and neurological adverse effects were monitored for up to 96 hours after dosing. Plasma topiramate concentrations from both

oral and intravenous topirmate doses were measured without interrupting patients' maintenance regimen. The advantage of the intravenous stable-isotope approach is that patients can be studied without interrupting maintenance therapy. Reliable estimates of topiramate bioavailability, half-life, distribution volume, and clearance can be determined.

The safety of a small, intravenous topiramate dose is needed prior to initiating, a safety and bioequivalence study comparing the investigational intravenous formulation with commercially available oral tablets. Administering intravenous topiramate in adults already taking oral topiramate is the safest population for a first-in-human study. Results from this pilot study will inform the design of subsequent studies intended to determine the efficacy and safety of intravenous topirmate for neuroprotection and seizure control in neonates.

3.2 STUDY AIMS

The aims of this study are to determine, the pharmacokinetics of orally and intravenously administered topiramate and the safety of an investigational intravenous topiramate formulation. The study was done in adult patients on maintenance topiramate therapy in order to minimize the risk of a first-in-human study of intravenous topiramate.

The Specific Aims of this project are to:

Aim #1: Characterize topiramate pharmacokinetics following oral and intravenous administration in adult patients on maintenance topiramate therapy

Hypothesis 1: Topiramate pharmacokinetics are similar after intravenous and oral administration. The absorption of oral topiramate is close to 100%.

Alternative Hypothesis 1: The absorption of orally administered topiramate is significantly less than 100%.

Aim #2: Determine initial safety of intravenous topiramate

Hypothesis 2: Intravenous administration of topiramate is safe.

Alternative Hypothesis 2: Intravenous administration of topiramate results in infusion site, systemic, or neurological adverse effects.

Exploratory Aim: Determine if sex, age, or other covariates affect topiramate absorption, distribution, or elimination

3.3 METHODS

3.3.1 Study Procedures

The study was approved by the Institutional Review Board at the University of Minnesota. Subjects were admitted to the General Clinical Research Center at the University of Minnesota on the morning of the study. After admission, patients

underwent a brief physical and neurological examination including an electroencephalogram. Routine blood tests including sodium, potassium, glucose, serum creatinine, chloride, urea nitrogen, hemoglobin, albumin, carbon dioxide, ALT, AST, alkaline phosphate, bilirubin, and total plasma protein were obtained. Subjects were confined to the clinical research unit for 12 hours following dosing. They returned 24, 48, 72, and 96 hours after dosing for additional blood draws and safety assessments including blood pressure, pulse, and self reported adverse events.

3.3.2 Selection and withdrawal of subjects

Twenty (20) patients 18 years of age and older were enrolled in the study. Patients were recruited primarily from the epilepsy and migraine clinics at Fairview University Medical Center, MINCEP Epilepsy Care, and other neurology clinics in the Minneapolis-St. Paul metropolitan area. Prospective subjects were identified from participating clinics and eligibility was reviewed. The names of patients indicating interest in the study were forwarded to investigators. Patients were contacted to describe the study and screened for inclusion and exclusion criteria. Subjects signed the consent form the morning of the study. The original signed consent forms are kept on file.

3.3.3 Inclusion and exclusion criteria

To be eligible for enrollment, subjects must have met all of the following inclusion criteria:

- Patients taking topiramate
- Persons 18 years of age and older

Subjects who had any of the following were not eligible for enrollment in this study:

- Patients who are pregnant
- Patients who are breast feeding
- Patients with significant medical problems who may not tolerate intravenous administration

3.3.4 Prior and concomitant medications

Use of previous concomitant medications were allowed during the study. If a new concomitant medication is deemed necessary, its use was determined on a case-by-case basis by the investigator who will consider the reason for administration and the expected effect on the safety and pharmacokinetic evaluations. The dose and reason for use of each concomitant medication were recorded in the subjects' study documentation.

3.3.5 Study drug, dose, and rational

All subjects were on maintenance topiramate therapy for management of epilepsy or migraine headaches. Subjects received a single 25 mg intravenous dose of the stable-labeled topiramate, followed by their usual morning oral dose. Based on an estimated apparent volume of distribution of 0.6-0.8 L/kg, a 25 mg intravenous dose will produce a

maximum concentration of approximately 0.45 to 0.6 mg/L.[68] Steady-state topiramate concentrations typically range from 5-20 mg/L.[122, 123] The predicted increase in the peak topiramate concentrations due to the intravenous doses is 2-20%. Therefore, toxicological effects due to increased plasma topiramate concentrations were minimized.

The volume of intravenous injection was measured precisely. The syringe was weighed before and after administration, as a double check on the dose administered. On the morning of the study, each person took his/her normal dose of topiramate. Concurrently, a 25 mg dose (2.5 ml) of intravenous topiramate was administered intravenously over 10 minutes with a syringe pump. A physician was present during and for at least 15 minutes following the intravenous administration.

3.3.6 Pharmacokinetic sampling

An indwelling catheter was placed in the left or right arm for blood sampling. Ten milliliter blood samples were collected for determination of plasma topiramate pre-dose, 5 ± 1, 15± 2 , 30 ± 3 minutes, and 1, 2, 4, 6, 10, 12, 24, 48, 72, and 96 hours after administration of the intravenous dose. The actual blood collection times for the 1 through 12 hour samples were allowed to deviate by ± 10 minutes from the specified times and up to 2 hours from 24 to 96 hour samples. In all cases, the actual time of the blood draws were recorded. Missed samples were also recorded.

3.3.8 Safety monitoring

A physician was present during and for at least 15 minutes following intravenous and oral drug administration and was on call throughout the study. Safety assessments include close monitoring of adverse events, vital signs, and monitored 12-lead electrocardiograms as well as clinical laboratory tests (sodium, potassium, glucose, serum creatinine, chloride, urea nitrogen, hemoglobin, albumin, carbon dioxide, ALT, AST, alkaline phosphate, bilirubin, and total plasma protein). An adverse event was defined as any reaction, side effect, or other untoward event, regardless of relationship to study drug, that occurs anytime after the subject has signed the consent form. This could be a clinically significant adverse change in clinical status, a treatment-emergent sign or symptom, a new illness, or a clinically relevant abnormal laboratory finding. All adverse events were recorded with the following minimum information: the specific event or condition, whether the event was a worsening of an existing medical condition, the dates of occurrence, severity, relationship to study medication, specific countermeasures (example: concomitant medications/procedures), and outcome. The investigators assessed the relationship of the adverse event to the study drug as: not related, unlikely, possibly, probably, or definitely related.

The intensity of an adverse event was assessed by a study physician using the following guidelines:

Mild: Awareness of signs, symptoms, or events that are otherwise easily tolerated

Moderate: Discomfort enough to cause interference with usual activities and warrant possible intervention

Severe: Incapacitating (an inability to do usual activities) or significantly affects clinical status and warrants intervention

A serious adverse event was defined as an adverse event experienced by a subject that results in any of the following outcomes:

- Death
- A life-threatening adverse experience
- Hospitalization (unplanned hospital stay) or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect

The following stop criteria were used for the study: If a severe adverse event occurs, no further subjects would be given study medication or recruited into the study until it was determined if the adverse event was likely due to the study medication. If more than 2 severe adverse events occurred, the study would be stopped.

<u>Admission labs prior to drug administration:</u> Complete blood count, albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bilirubin, blood urea

nitrogen, carbon dioxide, chloride, creatinine, glucose, potassium, sodium, and total protein.

Electrocardiogram (EKG): The study physician monitored the EKG immediately prior to, during, and for 15 minutes post-infusion.

Blood Pressure & Pulse: A nurse checked and recorded blood pressure and pulse immediately prior to infusion, every 2 minutes during the intravenous infusion, then every 15 minutes for 1 hour after infusion, then every 8 hours until discharge.

Assessment of CNS Toxicity: A physician monitored the following signs of CNS toxicity prior to drug infusion and post-infusion:

Ataxia and nystagmus: 0=none, 1=mild, 2=severe

Injection Site Irritation: The nurse observed and recorded signs of irritation or inflammation at the injection site.

Other Side Effects/Tolerability: Side effects and tolerability of the intravenous and oral topiramate were also collected as a self-report solicited at each blood draw.

3.3.9 Sample size

The study enrolled 20 adult patients. The sample size was powered to detect a 20% difference in AUC between oral and intravenous administration (power=0.90, α=0.05). The decision to detect a 20% difference in AUC was intended to identify a clinically relevant change in exposure. The power calculation was performed using the estimated mean AUC of 76 mg*hr/L and standard deviation of 20 mg*hr/L.[81]

3.4 PHARMACOKINETIC ANALYSIS METHODS

3.4.1 Noncompartmental analysis

The concentration-time data was analyzed by non-compartmental methods to obtain area under the curve (AUC), clearance, half-life, volume of distribution, and bioavailability. The absolute bioavailability of oral topiramate versus intravenous topiramate was characterized in order to determine appropriate dosing in future studies as patients switch from intravenous to oral therapy or vice versa. To determine the absolute bioavailability, the area under the concentration-time curves for both the oral and intravenous topiramate were calculated. The AUC was determined by the log-linear trapezoidal rule with the tail area calculated from C_{last}/k. The terminal rate constant (k) was determined by linear regression of the terminal phase on log concentration versus time plots. Terminal half-life ($t_{1/2}$) was calculated as 0.693/k.

Concentration-time data from the intravenous and oral were analyzed using a non-compartmental pharmacokinetic approach with WinNonlin software (version 5.2; Pharsight Corporation, Mountain View, CA, USA). All data was weighed using $1/y^2$. The maximum concentration (Cmax) was the highest observed plasma concentration. Clearance was estimated by dose divided by $AUC_{0-\alpha}$. Volume of distribution was estimated by dose divided by $k*AUC_{0-\alpha}$. Steady-state oral area under the concentration time curves for the dosing interval ($AUCss_{0-tau}$) was calculated using steady-state settings in WinNonlin using log-linear trapezoidal rule.

Determination of k was done using WinNonlin using the default setting. During the analysis, WinNonlin repeats regressions using the last three points with non-zero concentrations, then the last four points, last five, etc. Concentrations prior to Cmax were not used. For each regression, an adjusted R^2 is computed. WinNonlin estimates k using the regression with the largest adjusted R^2 and if the adjusted R^2 did not improve, but was within 0.0001 of the largest adjusted R^2 value, the regression with the larger number of points was used. Each analysis was visually checked to make sure adequate and sufficient points were used to characterize the terminal rate constant.

Bioavailability (F) was determined by calculating the ratio of the dose normalized oral steady-state oral area under the concentration time curves for the dosing interval ($AUCss_{0-tau}$) to the dose normalized intravenous area under the concentration time curves

($AUC_{0-\alpha}$). Bioavailability was only computed for subjects with once daily dosing or if they were on equal oral topiramate doses taken either every 8 or 12 hour.

$$F = \frac{(AUC_{0\text{-tau}})_{oral} / Dose_{oral}}{(AUC_{0-\infty})_{IV} / Dose_{IV}}$$

The effects of sex, age, comedication, weight, creatinine clearance, and indication (epilepsy or migraines) on elimination half-life, clearance, volume of distribution, and bioavailability were examined using a univariate analysis of variance. A $p<0.01$ was considered significant.

3.4.2 Compartmental analysis

Compartmental analysis of the concentration time data was completed using 1 and 2 compartments models in WinNonlin 5.2. Data was analyzed using different weighting schemes ($1/y$, $1/y^2$, $1/\hat{y}$, $1/\hat{y}^2$). Models were compared using visual inspection, goodness of fit plots, weighted residuals, and Akaike's information criterion.

3.5 SAFETY ANALYSIS METHODS

Descriptive statistics were used to summarize the safety data from this study. The descriptive statistics included numbers of subjects, mean, median, standard deviation, minimum, and maximum for continuous data and frequencies and percentages for categorical data.

Safety assessments included adverse events, clinical laboratory tests (serum chemistry, hematology, and urinalysis), vital signs, and 12-lead electrocardiograms. The investigators assessed the relationship of the adverse event to the study drug as not related, unlikely, possibly, probably, or definitely related. All reported adverse events are included in the summary.

Mean laboratory test changes from baseline, if they occurred, were summarized using descriptive statistics. For vital signs, descriptive statistics were used to summarize the change from baseline. For EKG and physical examination findings, changes from baseline were summarized if they occurred.

3.6 RESULTS

Twenty patients with epilepsy or migraines completed the study. Thirteen of the patients were females and 7 were males. The average age was approximately 40 years (range 26-74 years). Six patients were on interacting comedications; one patient was on oxcarbazepine, 2 patients were on phenytoin, 3 patients were on carbamazepine, and 1 patient was on both carbamazepine and phenytoin. Sixty percent of patients were receiving topiramate for treatment of epilepsy and 40% were taking it for prevention of migraines. The total daily oral topiramate dose ranged from 50 mg to 600 mg. Table 4 shows the baseline characteristics of patients in the study.

Table 4: Patient demographics

Mean age (years) (range)	39.9 (26-74)
Sex (n)	
Female	13 (65%)
Male	7 (35%)
Race	
Caucasian (n)	20
Interacting Comedications (n)	
Phenytoin	3 (15%)
Carbamazepine	4 (20%)
Oxcarbazepine	1 (5%)
Medical Diagnosis (n)	
Epilepsy	12 (60%)
Migraines	8 (40%)
Mean daily topiramate dose (mg)	178 (50-600)
Mean weight (kg) (range)	92.3 (54.5-150.3)
Mean CrCL (ml/min) (range)	107.2 (66-158)

Figure 9 and Figure 10 display the mean concentration time profiles (96 hours and 12 hours, respectively) for the 25 mg intravenous topiramate dose infused over 10 minutes (error bars are one standard deviation from mean).

Figure 9: Mean concentration-time profile after 25 mg intravenous (0 to 96 hours)

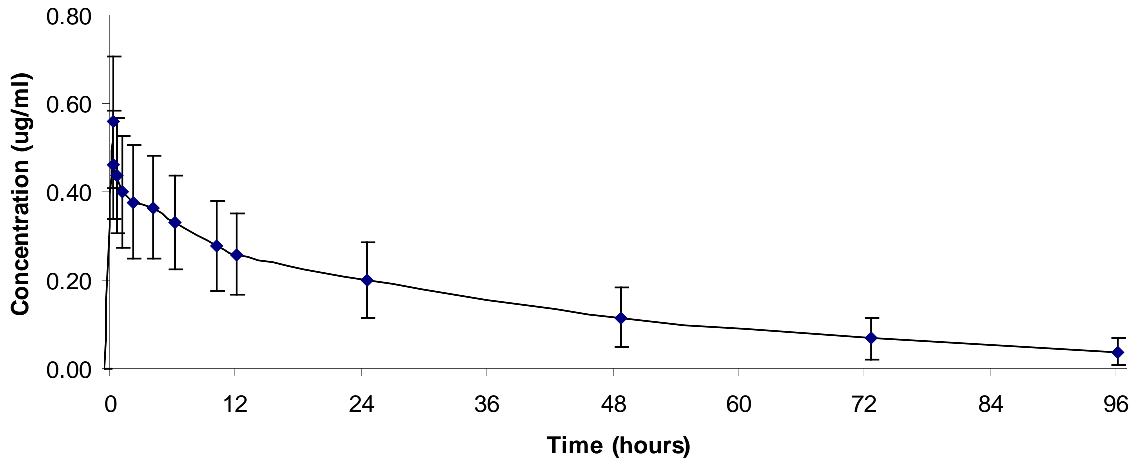


Figure 10: Mean concentration-time profile after 25 mg intravenous (0-12 hours)

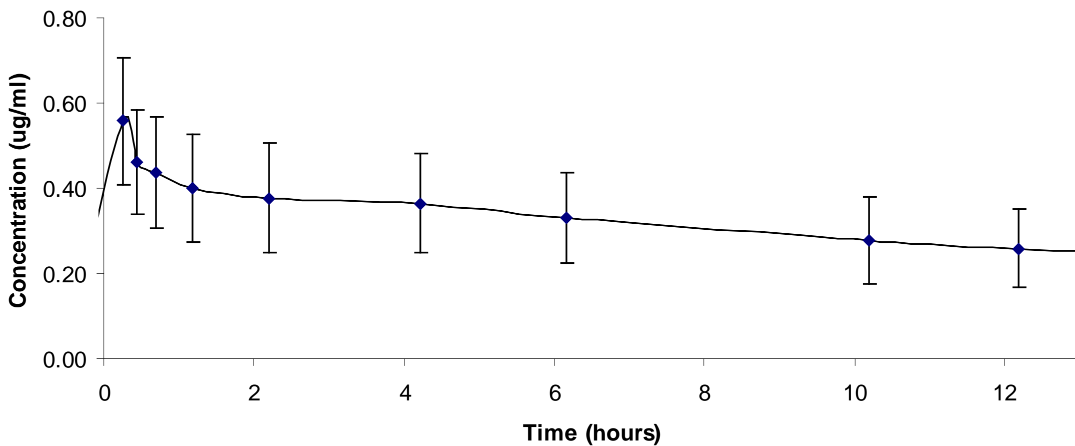
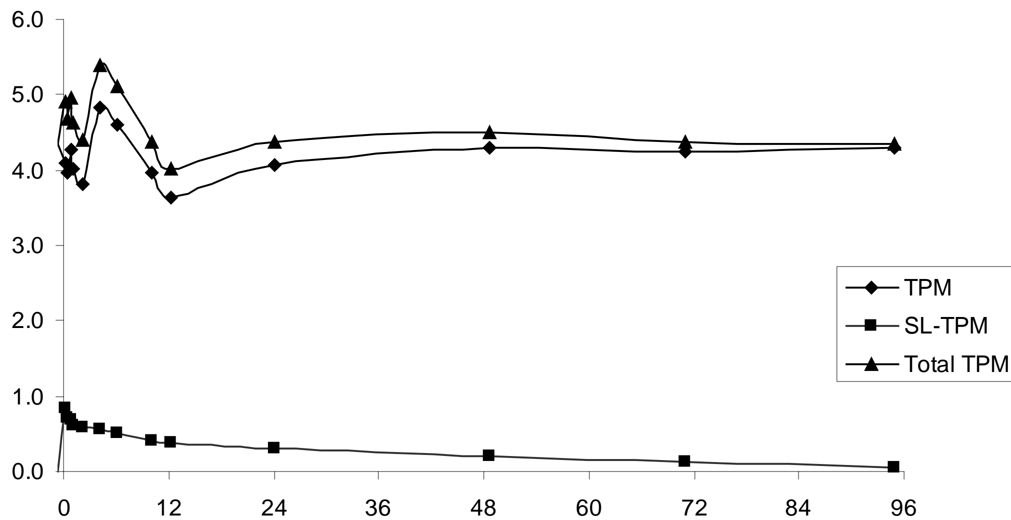


Figure 11 shows a representative patient from the study. This patient was taking 50 mg of oral topiramate twice daily. This graph displays the oral topiramate (TPM), stable-labeled intravenous topiramate (SL-TPM), and total topiramate (Total TPM). The single

25 mg dose of intravenous stable-labeled topiramate added less than 20% one time increase in daily topiramate exposure for most patients. This figure also clearly demonstrates the patient was at steady state, because the pre-dose, 24 hour, 48 hour, 72 hour, and 96 hour concentrations were all very similar (pre-dose= 4.4 ug/mL, 24 hour= 4.3 ug/mL, 48 hours= 4.5 ug/mL, 72 hour=4.4 ug/mL, and 96 hours= 4.3 ug/mL). The double peak in the concentration time profile is a result of the oral topiramate dose being given 1 hour after the intravenous topiramate administration.

Figure 11: Topiramate concentration-time profile of a typical patient after intravenous and oral administration



3.6.1 Noncompartmental analysis

Table 5 displays the individual pharmacokinetic parameters for patients in the study. The clearance of topiramate ranged 5 fold from 0.84 L/hr to 4.23 L/hr. The patients with the highest clearance were on at least one inducing antiepileptic drugs (carbamazepine,

oxcarbazpine, or phenytoin). The volume of distribution varied from 0.39 to 1.57 L/kg. The half-life ranged from 15.5 hours to 53.5 hours and was affected by inducing comedication use (see covariate testing section).

Table 5: Individual pharmacokinetic parameters

ID	CL (L/hr)	V_d (L/kg)	$t_{1/2}$ (hr)	$Cmax_{iv}$ (ug/ml)	$AUC_{0\text{-}\alpha\ IV}$ (hr*ug/ml)	$AUC_{0\text{-}tau\ oral}$ (hr*ug/ml)	F (%)	Medical Condition	Inducing Comed
1	1.04	0.65	24.9	0.83	24.0	51.2	101	Epilepsy	no
2	1.07	0.74	28.4	0.77	23.3	99.3	107	Migraine	no
3	1.14	0.73	36.4	0.57	21.9	25.2	103	Migraine	no
4	1.59	0.64	31.2	0.40	15.7	45.0	148	Migraine	no
5	1.81	0.80	16.6	0.87	13.8	*	*	Epilepsy	no
6	1.14	0.59	53.5	0.49	21.8	47.5	107	Migraines	no
7	1.19	0.63	33.1	0.66	21.1	*	*	Migraines	no
8	1.86	0.59	27.6	0.49	13.5	35.5	86	Migraine	no
9	0.97	0.39	20.6	0.67	25.9	*	*	Epilepsy	no
10	1.84	0.60	27.8	0.38	13.6	*	*	Epilepsy	no
11	1.32	0.77	38.3	0.52	19.0	*	*	Epilepsy	no
12	1.73	0.85	28.7	0.52	14.5	42.7	97	Migraines	no
13	4.23	1.05	13.7	0.44	5.9	26.9	106	Epilepsy	yes
14	2.65	1.00	29.5	0.39	9.4	11.0	102	Epilepsy	yes
15	3.05	1.12	21.0	0.48	8.2	19.7	116	Epilepsy	yes
16	3.92	0.64	14.9	0.39	6.4	31.2	118	Epilepsy	yes
17	2.47	1.07	31.3	0.48	10.1	50.7	60	Epilepsy	yes
18	3.35	1.28	22.2	0.60	7.47	15.0	100	Epilepsy	yes
19	3.53	0.92	15.5	0.53	7.1	43.9	77	Epilepsy	yes
20	0.84	0.75	37.2	0.74	29.7	121.4	102	Migraines	no

*Patients 5, 7, 9, 10, and 11 were excluded from bioavailability analysis

Table 6 displays the mean topiramate pharmacokinetic parameters obtained from non-compartmental analysis. The mean half-life was 27.6 hours (SD=9.7). The mean clearance was 2.03 L/hr (SD=1.07), which was affected by inducing comedication use.

The maximum concentration of the stable-labeled topiramate after 25 mg given intravenously was 0.56 ug /mL ± 0.15 ug /mL. This was similar to predicted concentrations (0.45 to 0.6 mcg/ml) calculated when designing the study.

Table 6: Mean topiramate pharmacokinetic parameters

Parameter	Mean +/- SD
CL (L/hr)	2.03 +/- 1.07
V_d (L/kg)	0.79 +/- 0.22
$t_{1/2}$ (hr)	27.6 +/- 9.7
$Cmax_{iv}$ (ug/mL)	0.56 +/- 0.15
$AUC_{0-\alpha\ IV}$ (hr*ug/ml)	16.0 +/- 7.0
$AUC_{0-24\ oral}$ (hr*ug/ml)	92.4 +/- 78.8
F (%)	110 +/- 16

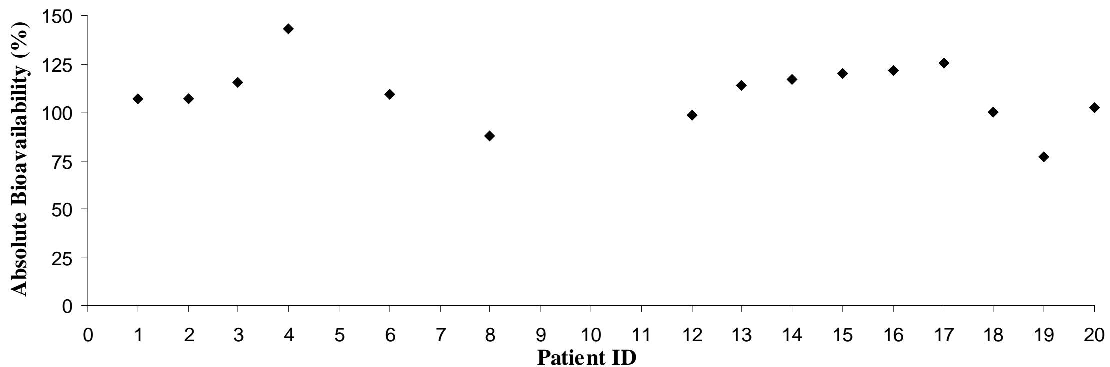
Table 7 displays the mean topiramate pharmacokinetic parameters based on inducing comedication use. Clearance, volume of distribution, and half-life were significantly different between patients taking inducing antiepileptic medications and those not taking inducers. The mean half-life was 21.2 hours and 31.1 hours, the mean volume of distribution was 1.01 L/kg and 0.67 L/kg and the mean clearance was 3.31 L/hr and 1.35 L/kg in inducers versus .non-inducers respectively.

Table 7: Mean topiramate pharmacokinetics based on comedication

Parameter	Inducers	Non-Inducers	P-value
CL (L/hr)	3.31 +/- 0.64	1.35 +/- 0.37	<0.001
Vd (L/kg)	1.01 +/- 0.20	0.67 +/- 0.12	<0.001
$t_{1/2}$ (hr)	21.2 +/- 7.1	31.1 +/- 9.2	0.023
$Cmax_{iv}$ (ug/ml)	0.47 +/- 0.08	0.61 +/- 0.16	0.052
$AUC_{0-\alpha\ IV}$ (hr*ug/ml)	7.8 +/- 1.6	19.8 +/- 5.3	<0.001

The mean absolute bioavailability for orally administered topiramate was was 110% +/- 16% with a range of 77% to 143% (Figure 12). Five patients were excluded from the analysis of bioavailability because they were not taking equal oral topiramate doses every 8 or 12 hour.

Figure 12: Absolute bioavailability by patient ID



Covariate testing

The effects of sex, age, inducing comedication, weight, creatinine clearance, and indication (epilepsy or migraines) on elimination half-life, clearance, volume of distribution, and bioavailability were examined by a univariate analysis of variance.

Age, creatinine clearance, and weight had no effect on elimination half-life, clearance, volume of distribution, and bioavailability. Indication had no effect on volume of distribution or bioavailability. Indication had a significant effect on clearance and half life ($p=0.01$ and $p=0.005$, respectively). Patients with epilepsy had a higher clearance and shorter half-life than patients with migraines (shown in equations below). Seven of 12 patients with epilepsy were also taking inducing antiepileptic drugs known to affect topiramate clearance. When inducing comedication use was included in the model, the effect of indication on clearance and half-life were no longer significant ($p=0.79$ and $p=0.67$). Therefore, indication is confounded with inducing comedication use.

Clearance (L/hr) = 1.32 + 1.19 (if epilepsy patient)

Half-life (hr)= 34.5 -11.5 (if epilepsy patient)

As expected, inducing comedication use did not influence bioavailability. Inducing medication use did alter clearance and volume of distribution ($p<0.0001$ and $p=0.0001$, respectively). The effect on half-life was significant at a $p=0.05$ but not a $p=0.01$ level ($p=0.02$) Patients taking inducers had on average a larger clearance, larger volume of distribution, and decreased half-life than those not taking inducing medications (see equations below).

Clearance (L/hr) =1.35 + 1.96 (if taking inducer)

Volume (L/kg)= 0.67 + 0.34 (if taking inducer)

Half-life (hr)= 31.1 -9.9 (if taking inducer)

Sex did not effect half-life or bioavailability. Sex was correlated with clearance and volume of distribution ($p=0.0005$ and $p=0.004$, respectively). Females on average had a lower clearance and volume of distribution compared to males (see equations below). Six of the 7 males in the study were taking an inducing antiepileptic medication, which is known to increase clearance. When inducing comedication use is included in the model, the effect of sex on clearance and volume of distribution was no longer significant ($p=0.97$ and $p=0.77$). Sex is confounded with inducing comedication use.

Clearance (L/hr) =3.04-1.54 (if female)

Volume (L/kg)= 0.97 - 0.28 (if female)

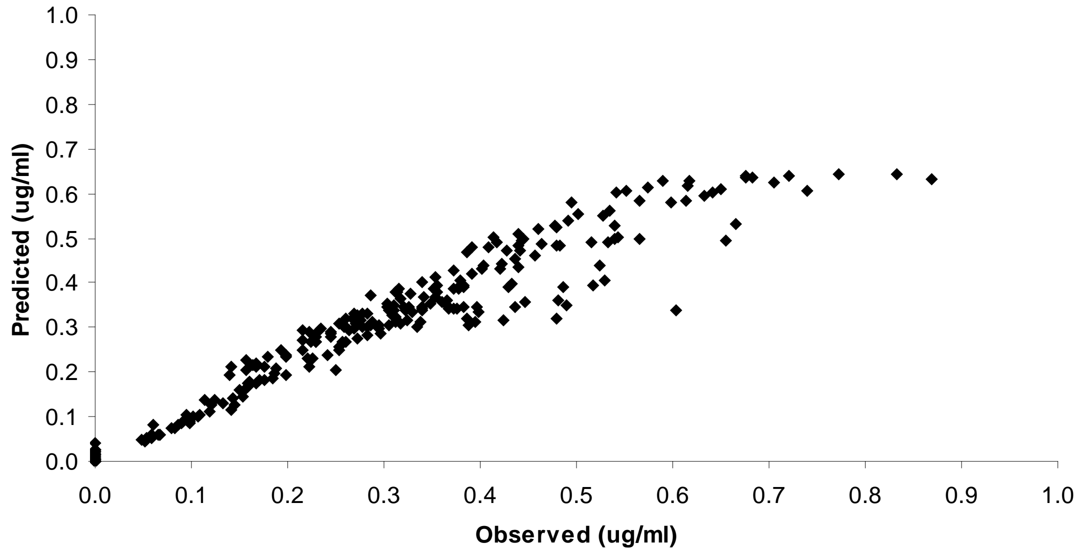
3.6.2 Compartmental analysis

Compartmental analysis of the intravenous concentration time data was completed using 1 and 2 compartments models in WinNonlin 5.2. After intravenous dosing, a 2 compartment model fit the plasma concentrations better in most subjects. This was determined by goodness of fit plots, weighted residuals, Akaike's Information Criterion, and visual inspection. Goodness of fit plots, including weighted residuals versus time and observed versus predicted values, improved with a 2 compartment model over a 1 compartment model (Figure 13 and Figure 14). With the 2 compartment model, the

weighted sum of squared residuals improved in all patients and the Akaike's Information Criterion score improved in half of the patients. Final data was modeled using $1/\hat{y}^2$.

The mean and individual 1 compartment model pharmacokinetic parameters are shown in Table 8 and Table 9. The mean pharmacokinetic parameters from the 1 compartment model compared closely to the results from the noncompartmental analysis. The mean clearance was 2.03 L/kg with the noncompartmental methods compared to 2.09 L/kg with the 1 compartment model. The mean half-life was 27.6 hours and 24.6 hours with the noncompartmental and 1 compartment methods, respectively. The mean volume of distribution was larger with the noncompartmental analysis (0.79 L/kg) compared to 1 compartment methods (0.675 L/kg).

Figure 13: Goodness of fit plots for one compartment model



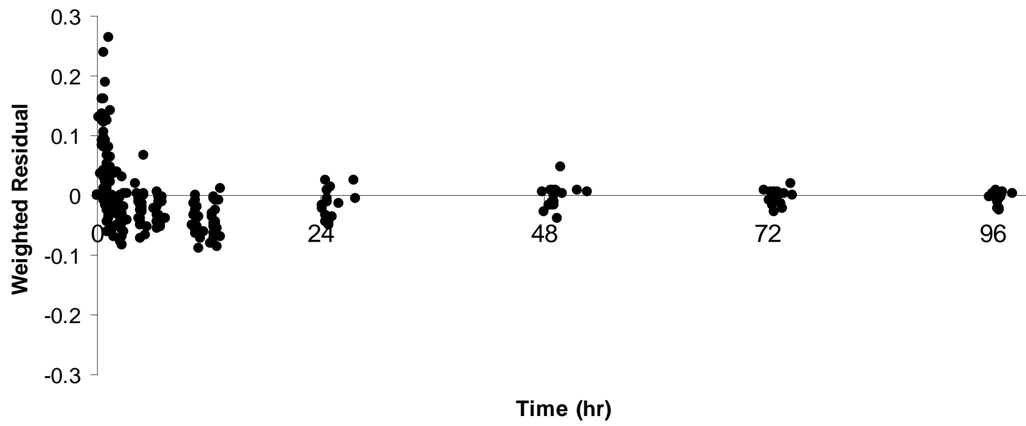
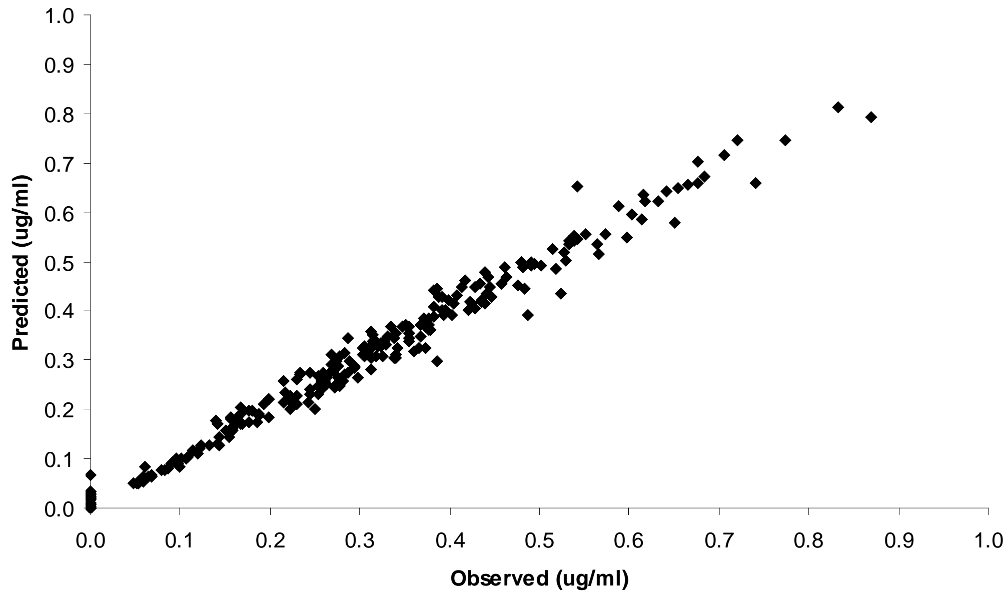


Figure 14: Goodness of fit plots for two compartment model



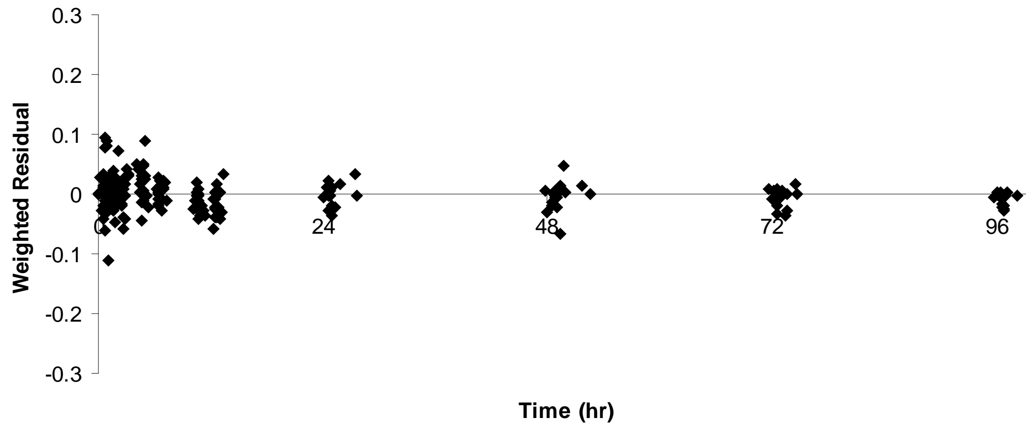


Table 8: Mean one compartment pharmacokinetic parameters

	Mean +/- SD
V_d (L/kg)	0.675 +/- 0.11
$t_{1/2}$ (hr)	24.6 +/- 9.3
CL (L/hr)	2.09 +/- 1.2
K_{10} (1/hr)	0.033 +/- 0.011

Table 9: Individual one compartment pharmacokinetic parameters

ID	Vd (L/kg)	$t_{1/2}$ (hr)	CL (L/hr)	K_{10} (1/hr)
1	0.672	27.8	0.96	0.025
2	0.655	26.1	1.03	0.027
3	0.600	31.3	1.10	0.022
4	0.647	34.4	1.46	0.020
5	0.722	15.3	1.78	0.045
6	0.476	42.6	1.16	0.016
7	0.560	31.3	1.12	0.022
8	0.512	24.5	1.80	0.028
9	0.637	35.5	0.92	0.020
10	0.586	27.4	1.82	0.025
11	0.671	33.9	1.30	0.020
12	0.669	22.2	1.77	0.031
13	0.907	10.9	4.56	0.063
14	0.725	20.8	2.72	0.033
15	0.838	16.0	3.01	0.043
16	0.602	13.3	4.13	0.052
17	0.748	16.3	3.30	0.043
18	0.875	17.3	2.94	0.040
19	0.716	10.6	3.99	0.065
20	0.683	34.7	0.82	0.020

The mean and individual 2 compartment model parameters are shown in Table 10 and Table 11. The mean pharmacokinetic parameters from the 2 compartment model compared closely to the results from the noncompartmental analysis. The mean clearance was 2.03 L/kg with the noncompartmental analysis compared to 2.11 L/kg with the 2 compartment model. The mean half-life was 27.6 hours using noncompartmental methods compared to a beta half-life of 27.4 hours with 2 compartment method.

Table 10: Mean two compartment pharmacokinetic parameters

	Mean +/- SD
V_1 (L)	40.95 +/- 13.8
V_2 (L)	28.12 +/- 18.1
CL (L/hr)	2.11 +/- 1.18
K_{10} (1/hr)	0.0538 +/- 0.027
K_{12} (1/hr)	1.606 +/- 2.7
K_{21} (1/hr)	1.43 +/- 0.725
Alpha (1/hr)	3.063 +/- 3.18
Beta (1/hr)	0.0293 +/- 0.013
Beta $t_{1/2}$ (hr)	27.4 +/- 9.8

Table 11: Individual two compartment pharmacokinetic parameters

ID	V_1 (L)	V_2 (L)	CL (L/hr)	K_{10} (1/hr)	K_{12} (1/hr)	K_{21} (1/hr)	Alpha (1/hr)	Beta (1/hr)	Beta $t_{1/2}$ (hr)
1	28.4	15.0	1.01	0.036	0.478	0.91	1.40	0.023	30.0
2	31.1	9.7	1.06	0.034	0.483	1.54	2.03	0.026	26.9
3	45.0	13.6	1.13	0.025	0.030	0.10	0.135	0.018	37.8
4	55.5	21.7	1.48	0.027	0.779	2.00	2.78	0.019	36.3
5	28.0	17.7	1.88	0.067	0.742	1.17	1.938	0.041	17.1
6	13.0	65.6	1.17	0.090	12.192	2.42	14.69	0.015	46.9
7	23.4	31.1	1.15	0.049	3.342	2.52	5.89	0.021	32.9
8	61.9	7.1	1.85	0.030	0.160	1.40	1.57	0.027	25.9
9	22.3	27.3	0.92	0.041	3.924	3.21	7.16	0.018	37.7
10	60.2	14.9	1.84	0.031	0.384	1.55	1.94	0.024	28.4
11	46.3	24.9	1.30	0.028	0.696	1.29	2.00	0.018	38.0
12	54.1	13.8	1.96	0.036	0.382	1.50	1.89	0.029	24.1
13	53.3	26.6	4.58	0.086	0.639	1.28	1.95	0.057	12.3
14	48.2	47.6	2.78	0.058	1.223	1.24	2.49	0.029	24.1
15	43.9	40.3	3.13	0.071	0.949	1.03	2.02	0.036	19.0
16	53.7	32.5	4.15	0.077	1.125	1.86	3.02	0.048	14.5
17	40.2	58.1	2.67	0.066	1.407	0.97	2.42	0.027	26.0
18	28.9	63.2	3.21	0.111	2.428	1.11	3.62	0.034	20.3
19	44.4	23.5	4.05	0.091	0.696	1.32	2.05	0.059	11.8
20	37.3	8.4	0.84	0.022	0.053	0.23	0.29	0.018	38.5

3.6.4 Safety results

No serious adverse events were reported by patients following intravenous administration of topiramate. No changes in heart rate, blood pressure, EKG, or infusion site reactions were observed (Table 13). Figure 15 displays the mean (solid line) and individual patients (dashed lines) blood pressure after intravenous topiramate dosing.

Seven patients (35%) experienced an adverse event during the study. Subject (TUMN03) experienced a vasovagal response during placement of the intravenous catheter prior to drug administration. The event was not related to the study drug. The patient fully recovered after placement of the intravenous line. One patient (TUMN07) had nausea and vomiting following the study drug. The event was considered mild and probably related to the study drug. The patient recovered fully without an intervention. Three patients (TUMN10, TUMN16, and TUMN17) experienced a seizure while on the research unit. Because these patients had uncontrolled epilepsy and the seizures were typical for their history, these events were considered not likely related to study drug. It is possible that being in the study (due to stress, getting up early for the study, or other factors) contributed to the seizures. Patient TUMN19 had increased seizure activity 3 days after the study drug was given. This event was not likely related to study drug. Patient TUMN11 experienced tingling and numbness in arms and legs. The patient occasionally in the past had this adverse event, which was presumed to be related to her oral topiramate therapy. The degree of her tingling and numbness increased after the intravenous topiramate dose was given. The event was possibly related to the study drug.

The patient followed up with her neurologist to adjust her oral topiramate dosage. One patient (TUMN17) experienced mild tingling around his lips during infusion. This was possibly related to the study drug and fully resolved by the end of the infusion. Table 12 summarizes the adverse events that occurred during the study.

Table 12: Adverse events after 25 mg intravenous topiramate

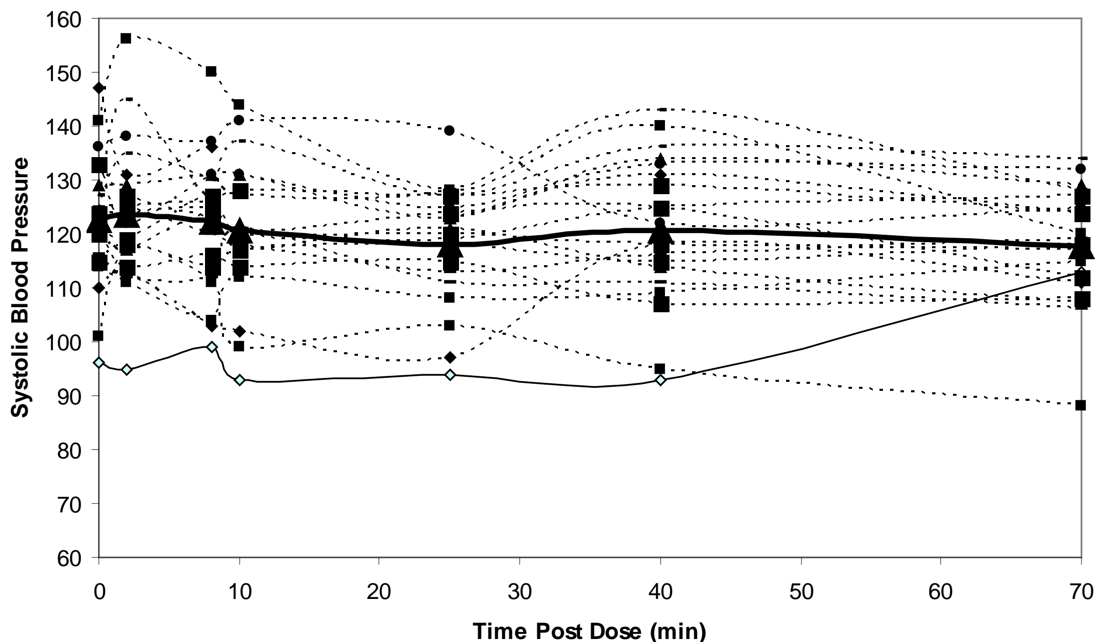
Adverse event	**Severity**	**Relation to study drug**	**Outcome**
Vasovagal response	Mild	Not related	Resolved
Nausea/vomiting	Mild	Probably	Resolved
Seizure	Mild	Unlikely	Resolved
Seizure	Mild	Unlikely	Resolved
Seizure	Mild	Unlikely	Resolved
Seizure	Mild	Unlikely	Resolved
Parasethia	Moderate	Possibly	Resolved
Tingling around lips	Mild	Possibly	Resolved

Table 13: Blood pressure and heart rate after intravenous topiramate

	INFUSION				POST-INFUSION		
	Pre	2 min	8 min	10 min	+15 min	+ 30 min	+1 hr
TUMN01	110/55	113/66	103/55	102/47	97/55	118/54	111/52
TUMN02	101/63	117/75	111/76	99/73	103/69	95/67	88/67
TUMN03	129/71	129/68	131/64	131/67	123/60	134/59	129/69
TUMN04	124/89	124/87	125/83	120/96	120/80	118/87	117/80
TUMN05	115/63	118/72	114/69	117/65	118/68	115/68	118/58
TUMN06	133/59	122/62	131/55	131/55	125/56	133/59	132/60
TUMN07	120/76	114/76	116/63	114/70	115/63	107/60	108/62
TUMN08	125/70	135/69	130/70	127/61	128/99	143/65	128/68
TUMN09	125/77	125/96	120/66	118/73	113/70	116/81	119/69
TUMN10	96/97	95/64	99/50	93/60	94/78	93/63	113/81
TUMN11	114/65	111/60	104/52	112/54	108/63	109/64	115/68
TUMN12	120/66	117/80	124/78	117/75	121/74	114/73	107/69
TUMN13	130/89	125/91	126/90	128/90	127/89	129/87	124/64
TUMN14	121/63	119/77	127/75	120/71	119/65	125/74	127/69
TUMN15	136/71	138/78	137/83	141/79	139/75	122/77	125/71
TUMN16	133/79	127/76	123/73	120/78	124/67	125/83	112/74
TUMN17	127/75	145/85	126/74	137/81	127/84	136/79	134/89
TUMN18	116/59	112/61	112/56	121/65	111/64	111/42	106/54
TUMN19	147/92	131/82	136/801	n/a	123/68	131/75	124/78
TUMN20	141/65	156/93	150/52	144/72	128/69	140/70	120/69

	INFUSION				POST-INFUSION		
	Pre	2 min	8 min	10 min	+15 min	+ 30 min	+1 hr
TUMN01	66	59	57	60	55	59	59
TUMN02	77	83	86	75	74	64	60
TUMN03	103	82	82	77	76	73	78
TUMN04	84	83	81	81	82	84	83
TUMN05	63	70	66	70	68	68	65
TUMN06	82	80	85	80	78	79	80
TUMN07	78	71	70	71	70	66	66
TUMN08	69	62	63	60	62	58	62
TUMN09	84	84	83	81	78	86	81
TUMN10	60	98	56	54	97	55	53
TUMN11	68	60	62	61	60	58	66
TUMN12	85	80	81	81	93	73	76
TUMN13	82	87	88	80	80	75	68
TUMN14	92	93	90	90	86	87	89
TUMN15	58	61	64	62	58	54	52
TUMN16	95	90	90	90	85	95	90
TUMN17	63	75	66	68	67	63	62
TUMN18	40	43	46	41	41	55	44
TUMN19	88	88	80	n/a	81	78	76
TUMN20	57	63	60	54	51	53	52

Figure 15: Mean and individual systolic blood pressure



3.7 DISCUSSION AND CONCLUSIONS

Neonatal seizures are a rare, but significant medical condition. This study was the first step to developing an intravenous topiramate formulation for treatment of seizures and neuroprotection in neonates. Prior to administering the investigational formulation in neonates, the safety and pharmacokinetics needed to be determined in adults. In this study, infusion of small doses (25 mg) over 10 minutes appeared to be safe. Based on an apparent volume of distribution of approximately 0.6 to 0.8 L/kg, this small dose was predicted to produce a maximum concentration of approximately 0.45 to 0.6 mg/L.[68] The study minimized adverse events by limiting the exposure of topiramate and administering

to patients who were already taking oral topiramate. No serious adverse events were reported by patients following intravenous topiramate. No changes in heart rate, blood pressure, EKG, or infusion site reactions were observed. Seven patients experienced minor adverse events: nausea and vomiting, tingling around the lips, paresthesia in the arms and legs, and one case of a vasovagal response with intravenous catheter placement. Four patients with intractable epilepsy experienced a typical seizure during the study period. Topiramate is known to cause nausea/vomiting and paresthesia. These effects were likely due to the increased concentration of topiramate. Because of the overall safety of the 25 mg intravenous dose, the next study was planned with larger and more clinically relevant doses (50 mg and 100 mg).

This study is the first to investigate the absolute bioavailability of oral topiramate. In the past, determining the bioavailability of oral topiramate was not possible due to the lack of an intravenous formulation. There are no previous reports of oral topiramate bioavailability. The mean absolute bioavailability for orally administered topiramate in the study was 110% +/- 16%. Determination that the oral absorption is close to 100% indicates patients may take the same dose intravenously as they are taking orally. This will simply dosing as patients are changed from oral to intravenous topiramate and vice versa. CyDex, the manufacturer of the solvent used in our topiramate formulation, is interested in developing intravenous topiramate for replacement therapy in patients who are unable to take medication orally. Therefore, an accurate estimation of absolute bioavailability is needed to properly convert patients from one route of administration to another.

To accurately estimate bioavailability in this study, patients were assumed to be at steady-state. All patients were on a stable regimen of topiramate for at least 2 weeks prior to beginning the study. In reality, steady-state conditions are hard to meet. Patients may take their medication a few hours late or may miss a dose. Some of the patients in the study likely were not at steady-state. Their pre-dose morning topiramate concentrations (pre-dose, 24 hours, 48 hours, 72 hours, and 96 hours) varied up to 2 fold. This makes calculations of bioavailability difficult under the steady-state assumptions. The next crossover study was planned to further and more accurately characterize bioavailability and possible bioequivalence. Using a crossover design, where each subject received both oral and intravenous topiramate in a controlled setting, allows an accurate assessment of bioequivalence by reducing within-subject variability.

The mean half-life is the study was 27.6 hours (SD=9.7). The half-life ranged from 15.5 hours to 53.5 hours and was influenced by inducing comedication use. The mean half-life was 21.2 hours in patients on inducing comedication and 31.1 hours in those not on inducers. Previous studies report that when topiramate is administered alone, the mean half-life is approximately 20-30 hours.[69, 71, 78] The results from this study are similar to previous reports. The extended elimination half-life of the topiramate found in some patients may permit once or twice daily dosing.

The mean clearance was 2.03 L/hr (SD=1.07). The clearance was also significantly affected by inducing comedication use. Approximately 20% of topiramate is metabolized

when administered in the absence of enzyme inducers.[73] When topiramate is administered with an enzyme inducer, the metabolic clearance increases and up to 50% of the dose undergoes metabolism.[70, 75, 76] Therefore, there can be more than a doubling of metabolic clearance in the presence of inducers. The current study found a 5-fold range of clearances, from 0.84 L/hr to 4.23 L/hr. Similar to previous reports, patients on an inducing comedication had a significantly greater clearance than those not taking other inducing medications. The mean clearance was 3.31 L/hr in those on inducers compared to 1.35 L/kg in those not on inducers. This is important for the treatment of neonatal seizures as many babies are on multiple medications. Phenobarbital, which is currently the most commonly prescribed neonatal seizure treatment, is a known metabolic inducer. Babies given previous or concurrent phenobarbital therapy, may have a great clearance. and require higher or more frequent dosing.

The mean volume of distribution in this study was 0.79 L/kg (SD=0.22). In previous reports, the apparent volume of distribution was 0.6-0.8 L/kg, which is similar to the distribution of total body water.[70] Since an intravenous formulation was not available in the past, this is the first study reporting the absolute volume of distribution. The distribution volume of approximately 0.8 L/kg permits calculation of intravenous loading doses to quickly and accurately attain targeted topiramate concentrations. Future studies are needed to investigate the safety of using higher dosing for loading patients.
The effects of sex, age, inducing comedication, weight, creatinine clearance, and indication (epilepsy or migraines) on elimination half-life, clearance, volume of

distribution, and bioavailability were examined. In this study of adults patients, age, creatinine clearance, and weight had no effect on elimination half-life, clearance, volume of distribution, and bioavailability. Creatinine clearance is known to affect topiramate clearance.[77] Overall, the patients in the study had normal renal function (mean creatinine clearance= 107.2 ml/min). Only one patient (TUMN20) had a considerably lower creatinine clearance (66 ml/min). This patient's topiramate clearance was 0.86 L/hr, which is less than mean clearance for those not on inducers 1.35 L/hr. This patient's creatinine clearance was approximately 40% below normal, as was total topiramate clearance demonstrating possible proportionality. If more patients with poor renal function were enrolled in the study, it is possible a relationship between creatinine clearance and topiramate clearance would have been found. When taking into account inducing comedication use in the model, sex and indication did not have an affect on elimination half-life, clearance, volume of distribution, and bioavailability. Patients taking inducers had on average a larger clearance, larger volume of distribution, and shorter half-life than those not taking inducing medications. This is similar to previous literature reports.[70, 75, 76]

This study was conducted in patients already taking oral topiramate, as it was the safest population to begin investigating intravenous topiramate The goal of this study was to demonstrate, in adults with epilepsy or migraine, the safety of small doses of intravenous topiramate and to characterize topiramate pharmacokinetics. The results from this pilot study provide previously unreported information about topiramate. The safety of this

small, intravenous topiramate dose was needed prior to initiating the next phase of the project, a safety and bioequivalence study comparing larger doses of the investigational intravenous formulation with the commercially available oral tablet. This next study will provide information regarding safety and pharmacokinetics at more clinically relevant doses, which is needed prior to proceeding to children and neonates. Studying in naive volunteers is important because a majority of babies given the intravenous topiramate in the clinical setting will not have been taking topiramate previously. Results from this pilot study will inform the design of subsequent studies and eventually a controlled clinical trial intended to determine the efficacy and safety of intravenous topirmate for neuroprotection and seizure control in neonates.

3.8 FUNDING SOURCE

This study was funded by a grant from the FDA Office of Orphan Products Development.

CHAPTER 4

TWO-WAY CROSSOVER STUDY OF ORAL AND INTRAVENOUS TOPIRAMATE SAFETY AND PHARMACOKINETICS IN ADULT HEALTHY VOLUNTEERS

4.1 INTRODUCTION

The long-term goal of this research project is to develop a more effective, safer therapy for neonatal seizures. Prior to using an investigational intravenous topiramate formulation in children and neonates, the pharmacokinetics and safety of the formulation must be well characterized in adults. In first in human studies, the most important concern is usually safety. In many trials the subjects are healthy volunteers for where is not expectation of therapeutic benefit. Even though topiramate is already approved as an oral tablet, the investigational intravenous formulation also requires initial safety and pharmacokinetic studies. Although first in human studies are usually completed in healthy volunteers, the first in human study of intravenous topiramate was completed in adult patients already taking oral topiramate. They received only a small 25 mg intravenous topiramate dose in addition to their normal oral topiramate. This was done to minimize adverse events and gain initial pharmacokinetic and safety data.

The immediate aims of the next study was to demonstrate, in healthy volunteers, the safety and cognitive effects of an intravenous topiramate formulation clinically relevant doses and to characterize topiramate pharmacokinetics, particularly the relationship between intravenous and oral dosing. Because the main purpose of this Phase I study was not to determine efficacy, healthy volunteers were used. Healthy volunteers also allowed intravenous topiramate to be studied in naive subjects. This is clinically relevant, as a majority of babies receiving intravenous topiramate for neonatal seizures will not have had previous exposure to oral topiramate.

This was a pharmacokinetic and safety study of intravenous topiramate formulation in healthy adult volunteers. Two subjects received 50 mg of intravenous topiramate infused over 15 minutes and 50 mg of oral topiramate on 2 separate occasions following at least a 2 week washout period. The remaining 10 volunteers received 100 mg of intravenous topiramate infused over 15 minutes and 100 mg of oral topiramate on 2 separate occasions following at least a 2 week washout period. Topiramate concentrations were measured for 120 hours after dosing and safety was assessed throughout the 2 week study period.

Results from this pilot study will inform the design of subsequent studies, including controlled clinical trials intended to determine the efficacy and safety of intravenous topirmate for neuroprotection and seizure control in neonates.

4.2 SPECIFIC AIMS

The primary objectives of this study were to determine, in healthy adult volunteers, the intravenous dose needed to produce equivalent exposure as an oral dose and to assess the safety and cognition of the intravenous topiramate formulation at higher doses.

The Specific Aims of this project are to:

Aim #1: Characterize topiramate pharmacokinetics following oral and intravenous administration in adult healthy volunteers.

Hypothesis 1: The absorption of orally administered topiramate is close to 100%

Alternative Hypothesis 1: The absorption of orally administered topiramate is significantly less than 100%

Aim #2: Determine the safety profile of the intravenous topiramate formulation.

Hypothesis 2: Intravenous administration of intravenous topiramate formulation is safe.

Alternative Hypothesis 2: Intravenous administration of an investigational topiramate formulation results in infusion site, systemic, or neurological adverse effects.

4.3 STUDY METHODS

4.3.1 Study procedures

The study was approved by the Institutional Review Board at the University of Minnesota. Subjects were admitted into a clinical research facility (Prism Research) the day before the studies. Subjects underwent a brief physical and neurological examination. An EKG recording and routine blood tests were obtained. Routine blood tests including sodium, potassium, glucose, serum creatinine, chloride, urea nitrogen, hemoglobin, albumin, carbon dioxide, ALT, AST, alkaline phosphate, bilirubin, and total plasma protein. Subjects were confined to the facility for 24 hours following dosing for blood draws and safety assessments. They returned at 48, 72, 96, and 120 hours after dosing for additional blood draws and safety assessments including vital signs and reporting of adverse events. Subjects received a physical examination on day 6 of each dosing period. There was a minimum of a 2 week washout period between the two

treatments (oral and intravenous). Two weeks after the last dosing period, subjects returned for a final study visit. During the final study visit, subjects completed health questionnaires, underwent a physical, and laboratory tests were obtained.

4.3.2 Selection of subjects

Twelve (12) healthy volunteers 18 years of age and older were enrolled in the study. Subjects will be recruited by Prism Research, primarily from the Minneapolis/Saint Paul metropolitan area. Prospective subjects were identified from Prism databases, eligibility was reviewed, and those individuals who appear eligible were contacted. Patients agreeing to participate had the details of the study explained to them and were screened. Subjects signed the consent form at the screening visit. The original signed consent forms are kept on file.

Subjects were screened for inclusion and exclusion criteria. Other information collected at screening included date of birth, ethnicity, race, sex, medical and surgical history, height, weight, heart rate, blood pressure, respirations, temperature, EKG, physical exam, baseline laboratory tests, and medication use. The screening window was 21 days before the first dosing period.

4.3.3 Inclusion and exclusion criteria

To be eligible for enrollment, subjects must have met all of the following inclusion criteria:

- 18 to 65 years of age
- BMI range 18 – 32
- CrCl > 70 ml/min
- If female:
    - Subject was not of childbearing potential, defined as postmenopausal for at least 1 year or surgically sterile (bilateral tubal ligation, bilateral oophorectomy or hysterectomy), or if childbearing potential, used a method of birth control acceptable to the investigator during the study
    - Subjects of childbearing potential must have a negative serum pregnancy test

Subjects who have any of the following were not eligible for enrollment in this study:

- Pregnant
- Breast feeding
- A history of intolerance to intravenous administration of medication
- Taking any medication (over-the-counter medications can not be taken up to 7 days before the study)
- Known hypersensitivity to topiramate
- A significant history of cardiac, neurologic, psychiatric, oncologic, endocrinologic, metabolic, or hepatic disease

- Use of any investigational drug or device in the 30 days prior to screening

4.3.3 Design and dose rational

Subjects in the study were healthy volunteers. They were given single doses of oral and intravenous topiramate. The study design stipulated that 2 subjects receive a 50 mg intravenous topiramate dose administered over 15 minutes, followed two weeks later by a 50 mg oral dose. If no serious adverse events occurred after the intravenous dose, the remaining 10 subjects received 100 mg intravenously and 100 mg orally. The doses used were clinical relevant in that some patients are given this amount of topiramate as initial therapy in an acute care setting and they fall within the range of maintenance doses used to treat migraines and epilepsy. Single oral 100 mg doses of topiramate have been given to healthy volunteers in the past with few adverse events. Preliminary studies of intravenous topiramate at lower doses had been completed in twelve patients already on oral topiramate. There were no serious adverse events following a 25 mg intravenous dose given in addition to patients' usual oral dose. Thus, the doses selected for the study were clinically relevant and likely provided information on tolerability, but were unlikely to cause serious adverse affects.

Using a crossover design, where each subject received both oral and intravenous topiramate, allowed an accurate assessment of bioequivalence by reducing within-subject variability. The two treatments were be separated by at least a 2 week washout period

based. A 2 week washout period would be of adequate length (at least 5 half-lives) as long as topiramate has a half-life of less than 65 hours.

4.3.4 Concomitant medications

Use of concomitant medications was not allowed prior to or during the study. If a subject required a medication, continuation in the study was determined. The determination was based on the indication for the medication and the expected effect of the drug on the safety and pharmacokinetic evaluations. The dose and reasons for use of each concomitant medication was recorded in the subjects' case report form.

4.3.5 Pharmacokinetic sampling

Ten milliliter blood samples were collected in EDTA tubes for determination of plasma topiramate concentration at pre-dose, 5 ± 1, 15± 2 , 30 ± 3 minutes, and 1, 2, 4, 6, 10, 12, 24, 48, 72, 96, and 120 hours after administration of the intravenous and oral doses. The actual blood collection times for hours 1 through 12 were allowed to deviate by ± 10 minutes from the specified times and up to 4 hours from 24 to 120 hours. In all cases, the actual time of the blood draw were recorded. Missed samples or breakage of a sample were recorded.

4.3.6 Safety monitoring

A physician was present during and for 2 hours following both intravenous and oral drug administration as well as on call throughout the study. Safety assessments include adverse events, clinical laboratory tests (sodium, potassium, glucose, serum creatinine, chloride, urea nitrogen, hemoglobin, albumin, carbon dioxide, ALT, AST, alkaline phosphate, bilirubin, and total plasma protein), vital signs, and monitored 12-lead electrocardiograms, cognitive tests, and physical examinations. An adverse event was defined as any reaction, side effect, or other untoward event, regardless of relationship to study drug, that occurred anytime after the subject has signed the consent form and extended until 14 days after the last dose of study drug. This could be a clinically significant adverse change in clinical status, a treatment-emergent sign or symptom, a new illness, or a clinically relevant abnormal laboratory finding. All adverse events were recorded with the following minimum information: the specific event or condition, whether the event was a worsening of an existing medical condition, the dates of occurrence, severity, relationship to study medication, specific countermeasures (example: concomitant medications/procedures), and outcome. The investigators assessed the relationship of the adverse event to the study drug as either not related, unlikely, possibly, probably, or definitely related.

The intensity of an adverse event was be assessed by a physician using the following guidelines:

Mild: Awareness of signs, symptoms, or events that are otherwise easily tolerated

Moderate: Discomfort enough to cause interference with usual activities and warrant possible intervention

Severe: Incapacitating (an inability to do usual activities) or significantly affects clinical status and warrants intervention

A serious adverse event was defined as an adverse event experienced by a subject that results in any of the following outcomes:

- Death
- A life-threatening adverse experience
- Hospitalization (unplanned hospital stay) or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect

The following stop criteria were used for the study: If a severe adverse event occurred, no further subjects were given study medication or recruited into the study until it was determined if the adverse event was likely due to the study medication. If more than 2 severe adverse events occurred, the study would be stopped. If a subject had a severe adverse event in the first phase of the study, he or she would have been excluded from the alternate treatment.

Admission labs: complete blood count, albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bilirubin, blood urea nitrogen, carbon dioxide, chloride, creatinine, glucose, potassium, sodium, and total protein.

EKG: A study physician monitored the EKG immediately prior to and for 1 hour post dosing for the intravenous and oral drug. EKG strips were obtained prior to and immediately after the dosing and any time physician requested a strip.

Blood pressure & pulse: Blood pressure and pulse were monitored and recorded prior to dosing, every 5 minutes during the intravenous infusion, every 15 minutes for 1 hour after the infusion, and then every 8 hours until 24 hours after dosing. Blood pressure and pulse were also measured and recorded on each of the subsequent outpatient visits (48, 72, 96, and 120 hours after dosing). For oral topiramate administration, the blood pressure and pulse were monitored immediately prior to dosing, then every 15 minutes for 1 hour, then every 8 hours until 24 hours after dosing. Blood pressure and pulse were monitored and recorded on outpatient visits (48, 72, 96, and 120 hours).

Assessment of CNS toxicity: A physician monitored the following signs of central nervous system toxicity prior to drug administration and 30 minutes after drug administration:

Ataxia and nystagmus: 0=none, 1=mild, 2=severe

Injection site irritation: The injection site was monitored and signs of irritation or inflammation were recorded.

Cognitive testing: Cognitive side effects were assessed at baseline and after intravenous and oral topiramate administration.

Baseline 1: Upon their first admission, prior to receiving any study drug, each subject was given a neuropsychological battery.

Baseline 2: At the end of study visit (2 weeks after receiving their second study drug), a second neuropsychological baseline that consisted of the same tests used for Baseline 1. Baseline 1 and 2 scores were averaged in order to correct for any practice effects that might have occurred across the testing sessions. This average was used to compute the change score of each test administered under drug conditions. Each testing session (baseline and drug) was audiotaped for speech and language analysis.

Testing after topiramate: A neuropsychological battery was designed to take between 15-20 minutes to complete and comprised of tests below, were given at the following times after intravenous and oral topiramate administration: 15 minutes, 2.5 hours, and 6 hours.

Word-level Language/Verbal Tests:

- *Controlled Oral Word Association Test*: generate words beginning with a specific letter of the alphabet

Psychomotor Speed:

- *Symbol Digit Modalities Test*: graphomotor and psychomotor speed

Other Side Effects/Tolerability: Side effects and tolerability of the intravenous and oral topiramate were also collected as a self-report solicited at each blood draw.

4.4 PHARMACOKINETIC ANALYSIS METHODS

4.4.1 Noncompartmental analysis

The concentration-time data was analyzed by non-compartmental methods to obtain area under the curve (AUC), clearance, half-life, volume of distribution, and bioavailability. The absolute bioavailability of oral topiramate versus intravenous topiramate needed to be characterized in order to determine appropriate dosing in future studies as patients switch from intravenous to oral therapy. To determine the absolute bioavailability, the area under the concentration-time curve for both the oral and intravenous topiramate was calculated. The AUC was calculated by the log-linear trapezoidal rule with the tail area calculated from C_{last}/k. The terminal rate constant (k) was determined by linear

regression of the terminal phase on log concentration versus time plots. Terminal half-life ($t_{1/2}$) was calculated as 0.693/k.

Concentration-time data were analyzed using a non-compartmental pharmacokinetic approach with WinNonlin software (version 5.2; Pharsight Corporation, Mountain View, CA, USA). Data was weighed using $1/y^2$. Area-under-the-curve ($AUC_{0-\alpha}$) was calculated using a log-linear trapezoidal method. The maximum concentration (Cmax) was the highest observed plasma concentration. Clearance (CL or Cl/F) was estimated by dose divided by $AUC_{0-\alpha}$.

Determination of k was done using WinNonlin using the default setting. During the analysis, WinNonlin repeats regressions using the last three points with non-zero concentrations, then the last four points, last five, etc. Concentrations prior to Cmax were not used. For each regression, an adjusted R^2 was computed. WinNonlin estimates k using the regression with the largest adjusted R^2 and if the adjusted R^2 does not improve, but is within 0.0001 of the largest adjusted R^2 value, the regression with the larger number of points was used. Each subject was visually checked to make sure they were using adequate and sufficient points to characterize the terminal rate constant. If not, user defined points were used.

Bioavailability (F) was determined by calculating the ratio of the area under the concentration time curves ($AUC_{0-\alpha}$) for the oral and intravenous using the following equation:

$$F = (AUC_{0-\alpha})po / (AUC_{0-\alpha})iv$$

Bioequivalence was determined using WinNonlin version 5.2 using a nonreplicate crossover design. This is equivalent to the classical analysis method, but uses maximum likelihood instead of method of moments to estimate inter-subject variance. Using subject as a random effect this way, the correct standard errors were computed for sequence means and tests of sequence effects. In determining the bioequivalence of an intravenous and oral formulation, WinNonlin determines the least squares means and standard errors of the oral and intravenous formulations and the standard error of the difference of the oral and intravenous least squares means. The ratio (% reference) is the difference of the log transformed least square means. To obtain bioequivalence, the 90% confidence intervals for ratio (% reference) of the parameters of interest (LnCmax/D, $LnAUC_{0-\alpha}/D$, $LnAUC_{0-last}/D$) must fall between 80-125%. The parameters were dose normalized because 2 doses were used (50 mg and 100 mg). Both the classical confidence intervals and Westlake confidence intervals were calculated.

4.4.2 Compartmental analysis

Compartmental analysis of the concentration time data was completed using 1 and 2 compartments models in WinNonlin 5.2. Oral data was analyzed with and without a lag time. Data was analyzed using different weighting schemes ($1/y$, $1/y^2$, $1/\hat{y}$, $1/\hat{y}^2$). Models were compared using visual inspection, goodness of fit plots, weighted residuals, and Akaike's information criterion. Means of the oral and intravenous pharmacokinetic parameters were compared.

4.4.4 Cognitive testing

Controlled oral word association test (COWA) and the symbol digit modalities test were measured at baseline and after both oral and intravenous topiramate administration. Baseline 1 and 2 scores were averaged in order to correct for any practice effects that might have occurred across the testing sessions. This average was used to compute the change score (% of baseline) of each test administered at three time points (15 min, 2.5 hours, and 6 hours after dosing). All subject data was pooled and examined together. Individual subjects were not analyzed, because there were not enough time points to allow accurate determination of model parameters.

4.4.5 Statistical analysis

The mean and standard deviation were determined for all the pharmacokinetic parameters. Analysis of variance was used to compare the oral to the intravenous

treatments. A p-value<0.05 was considered statistically significant. Bioequivalence was determined using WinNonlin version 5.2. The 90% confidence interval bounds for the least-square mean ration (oral/IV) from log transformed data for Cmax/D, AUC_{last}/D, and $AUC_{0-\alpha}$ /D must fall between 80-125% for bioequivalence.

The effect of age, height, weight, and sex were examined on the pharmacokinetic parameters ($t_{1/2}$,Vd, CL, $AUC_{0-\alpha}$, $AUC_{0-\alpha}$/D) using a univariate analysis of variance. To account for multiple testing a p<0.01 was considered significant.

4.5 SAFETY ANALYSIS METHODS

Descriptive statistics were used to summarize the safety data from this study. The descriptive statistics included numbers of subjects, mean, median, standard deviation, minimum, and maximum for continuous data and frequencies and percentages for categorical data.

Safety assessments included adverse events, clinical laboratory tests (serum chemistry, hematology, and urinalysis), vital signs, 12-lead electrocardiograms, neuropsychological tests, and physical examinations. The investigators assessed the relationship of the adverse event to the study drug as not related, unlikely, possibly, probably, or definitely related. All reported adverse events are included in the summary.

Mean laboratory test changes from baseline, if they occurred, were summarized using descriptive statistics. For vital signs, descriptive statistics were used to summarize the change from baseline. For EKG and physical examination findings, changes from baseline were summarized if they occurred.

4.6 RESULTS

All twelve healthy volunteers, 6 female and 6 male, completed the study. The average age of subjects in the study was 35 years. All were given oral and intravenous topiramate separated by at least a 2 week washout period. All subjects in the study were in good health. None of the subjects were taking any medications prior to enrollment and for at least 2 weeks after the last dosing period. Table 14 shows the characteristics of subjects in the study.

Table 14: Subject demographics

Mean age (years)	35 (range 19-55)
Sex (n)	
Female	6 (50%)
Male	6 (50%)
Race	
Caucasian (n)	11 (91.7%)
Black	1 (8.3%)
Mean weight (kg)	78.2 (range 58.3-112.3)

Figure 16 through Figure 19 display the mean concentration time profiles (0-120 hours post dose and 0-12 hours post dose) after intravenous and oral topiramate, respectively.

These graphs illustrate that oral and intravenously administered topiramate produce similar concentrations in the plasma.

Figure 16: Mean concentration versus time profiles after intravenous topiramate (0-120 hours)

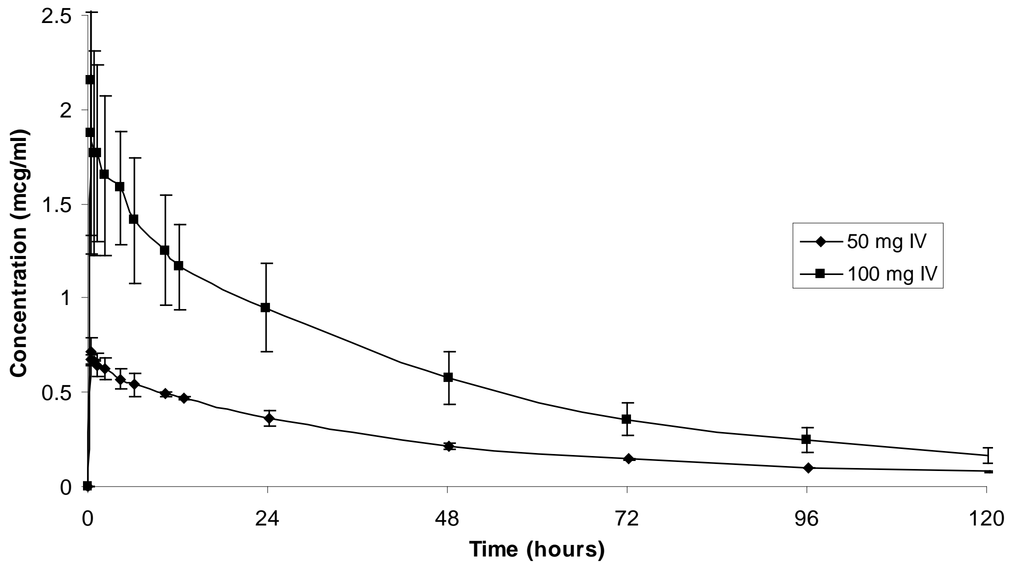


Figure 17: Mean concentration versus time profiles after intravenous topiramate (0-12 hours)

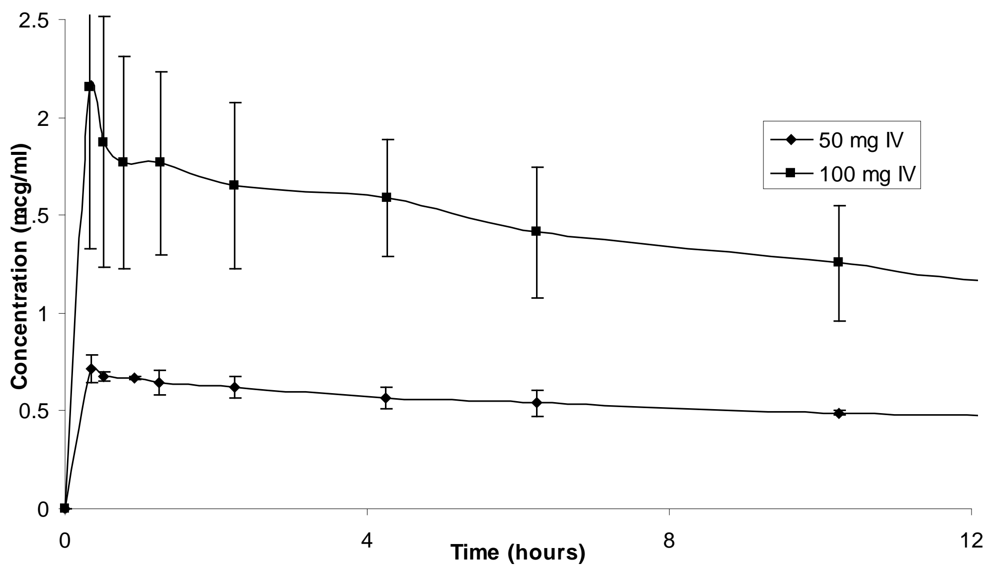


Figure 18: Mean concentration versus time profiles after oral topiramate (0-120 hours)

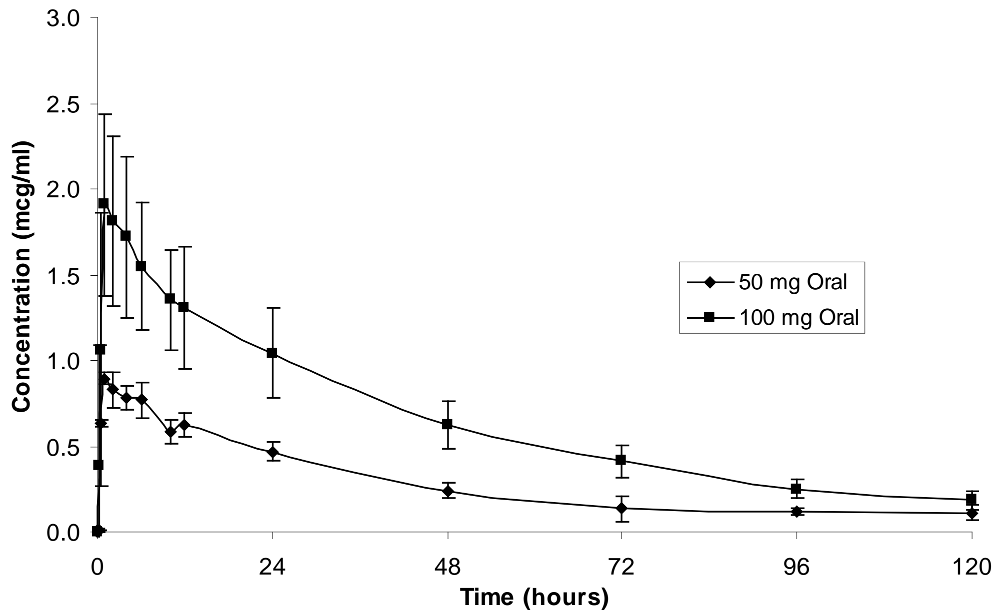
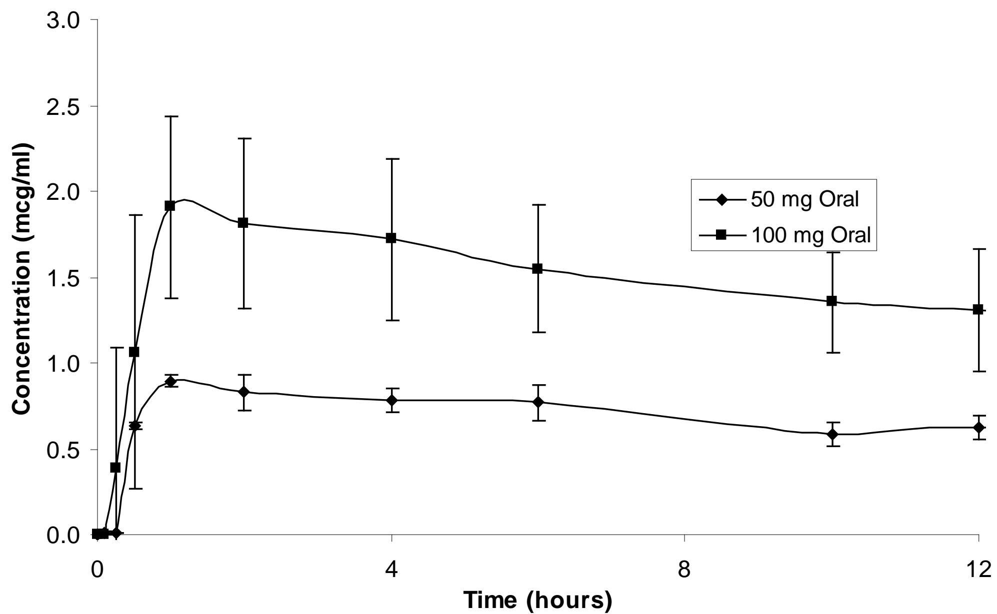


Figure 19: Mean concentration versus time profiles after oral topiramate (0-12 hours)



4.6.1 Noncompartmental analysis

There were no statistically significant differences between the pharmacokinetic parameters after oral and intravenous topiramate administration (Table 15). Table 16 and Table 17 show the individual pharmacokinetic parameters after intravenous and oral dosing.

Table 15: Mean pharmacokinetic parameters

Parameter	IV (mean+/- SD)	Oral (mean+/- SD)	P-value
CL (CL/F) (L/hr)	1.33 +/- 0.26	1.22 +/- 0.260	0.334
Vd (L/kg)	1.06 +/- 0.29	0.94 +/- 0.24	0.279
$t_{1/2}$ (hr)	42.3 +/- 6.2	41.18 +/- 7.5	0.693
Cmax (ug/ml)	1.99 +/- 0.89	1.801 +/- 0.64	0.542
Cmax/D (ug/ml/mg)	0.0212 +/- 0.007	0.0195 +/- 0.005	0.512
$AUC_{0-\alpha}$ (hr*ug/ml)	72.6 +/- 21.1	79.1 +/- 26.4	0.536
$AUC_{0-\alpha}$ /D (hr*ug/ml/mg)	0.78 +/- 0.17	0.85 +/- 0.19	0.337

Table 16: Individual pharmacokinetic parameters after intravenous topiramate

	Dose (mg)	$t_{1/2}$ (hr)	Cmax (ug/ml)	Cmax/D (ug/ml/mg)	$AUC_{0-\alpha}$ (hr*ug/ml)	$AUC_{0-\alpha}$ /D (hr*ug/ml/mg)	Vd (L/kg)	CL (L/hr)
T01	50	50.6	0.695	0.0139	32.32	0.647	1.60	1.55
T02	50	43.6	0.767	0.0153	33.57	0.672	1.55	1.49
T03	100	31.7	1.910	0.0191	65.28	0.653	0.95	1.53
T04	100	45.9	1.795	0.0179	83.92	0.839	0.79	1.19
T05	100	43.6	1.787	0.0179	64.15	0.642	1.23	1.56
T06	100	40.2	2.800	0.0280	68.37	0.684	1.17	1.46
T07	100	53.5	1.438	0.0144	58.61	0.586	1.17	1.71
T08	100	37.3	2.574	0.0257	105.31	1.053	0.88	0.95
T09	100	46.4	1.851	0.0185	87.37	0.874	0.91	1.15
T10	100	38.1	2.132	0.0213	73.51	0.735	0.94	1.36
T11	100	39.7	2.219	0.0222	95.31	0.953	0.69	1.05
T12	100	36.9	3.991	0.0399	103.97	1.040	0.86	0.96

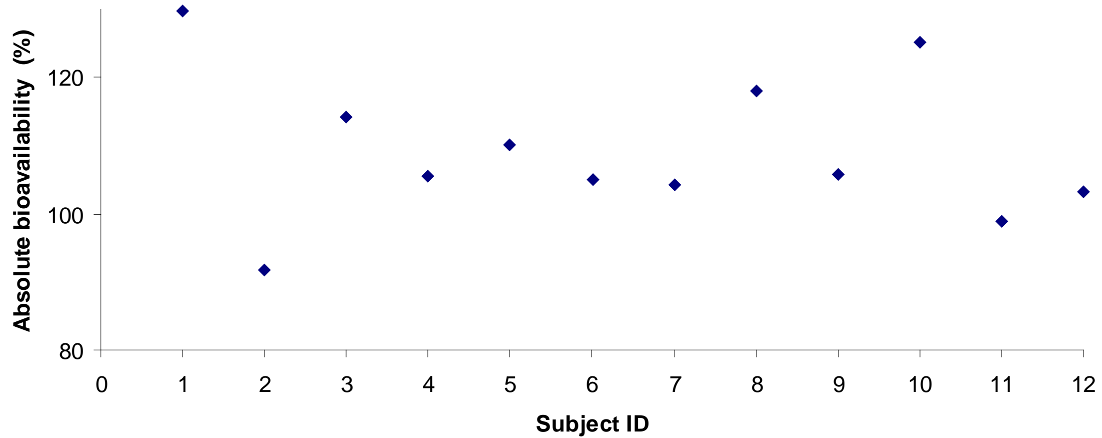
Table 17: Individual pharmacokinetic parameters after oral topiramate

ID	Dose (mg)	$t_{1/2}$ (hr)	Cmax (ug/ml)	Cmax/D (ug/ml/mg)	t_{max} (hr)	$AUC_{0-\alpha}$ /D (hr*ug/ml/mg)	Vd (L/kg)	CL/F (L/hr)
T01	50	42.2	0.919	0.0184	1	0.838	1.03	1.19
T02	50	39.2	0.869	0.0174	1	0.616	1.52	1.62
T03	100	35.8	2.233	0.0223	0.25	0.745	0.94	1.34
T04	100	53.6	1.479	0.0148	1	0.886	0.88	1.13
T05	100	46.3	1.632	0.0163	1	0.707	1.18	1.42
T06	100	31.4	1.842	0.0184	2	0.718	0.87	1.39
T07	100	32.0	1.251	0.0125	2	0.611	0.67	1.64
T08	100	39.9	2.654	0.0265	4	1.242	0.79	0.81
T09	100	39.6	1.739	0.0174	1	0.925	0.73	1.08
T10	100	55.5	2.059	0.0206	1	0.919	1.09	1.09
T11	100	40.7	1.969	0.0197	1	0.943	0.71	1.06
T12	100	37.8	2.971	0.0297	1	1.073	0.86	0.93

The mean half-lives for the intravenous and oral topiramate were 42.3 hours (SD=6.2) and 41.2 hours (SD=7.5), respectively. The volumes of distribution for the intravenous and oral were 1.06 L/kg (SD=0.26) and 0.94 L/kg (SD=0.24). The mean clearance for the intravenous and oral topiramate were 1.33 L/hr (SD=0.26) and 1.22 L/hr (SD=0.26). The mean time to maximum concentration after oral topiramate was 1.35 hr (SD=0.95).

The mean absolute bioavailability for orally administered topiramate was 109% +/- 10.8% with a range of 91.7% to 129.7% (Figure 20).

Figure 20: Absolute bioavailability by subject ID



The 90% confidence interval bounds for the least-square mean ration (oral/IV) from log transformed data is shown in Table 18. The Cmax/D, AUC_{last}/D, and $AUC_{0-\alpha}$ /D pass the bioequivalence 80-125% guidelines using the 90% confidence intervals. There were no significant sequence or period effects on Cmax/D, AUC_{last}/D, and $AUC_{0-\alpha}$ /D.

Table 18: Average bioequivalence

	Ratio (% Reference)	**Lower 90% CI**	**Upper 90% CI**	**Lower WL 90% CI**	**Upper WL 90% CI**
Ln(Cmax/D)	92.26	84.17	101.13	86.04	113.96
Ln(AUC_{last}/D)	109.24	103.20	115.62	85.96	114.04
Ln($AUC_{0-\alpha}$ /D)	108.44	102.85	114.34	87.13	112.87

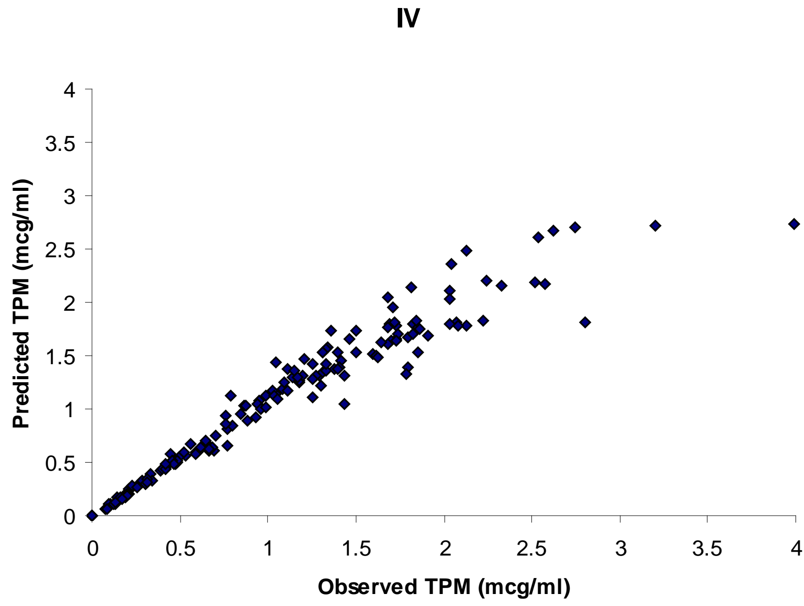
Covariate testing

The effect of age, height, weight, and sex were tested on the pharmacokinetic parameters ($t_{1/2}$, V_d, CL, $AUC_{0-\alpha}$, $AUC_{0-\alpha}/D$, F%). None of the covariates had a significant relationship with the pharmacokinetic parameters.

4.6.2 Compartmental analysis

Compartmental analysis of the concentration time data was completed using 1 and 2 compartments models in WinNonlin 5.2. In most subjects, after both intravenous and oral dosing, the data better fit a 2 compartment model. This was determined by goodness of fit plots, weighted residuals, Akaike's Information Criterion, and visual inspection. Goodness of fit plots, including weighted residuals versus time and observed versus predicted values, improved with a 2 compartment model over a 1 compartment model (Figure 21 and Figure 22). Adding a lag time to the oral model did not improve overall fit. Final data was modeled using $1/\hat{y}^2$.

Figure 21: Goodness of fit for one compartment model



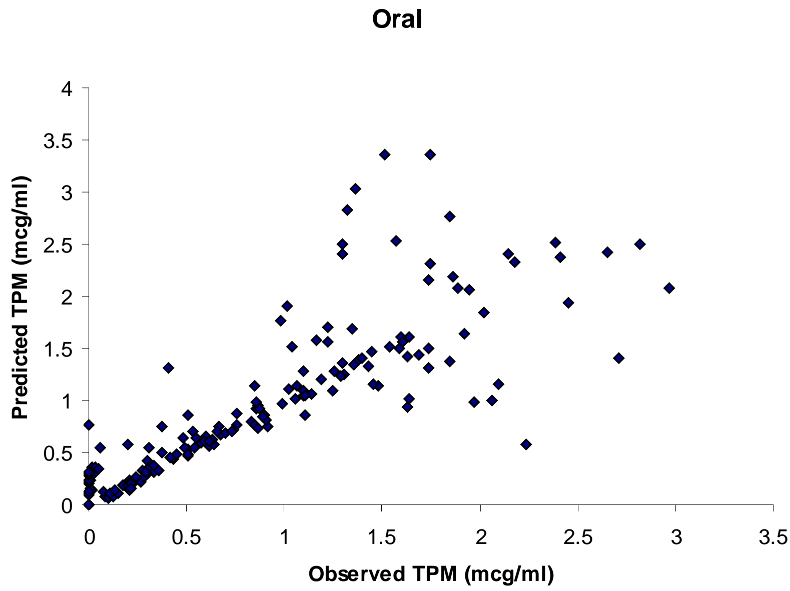
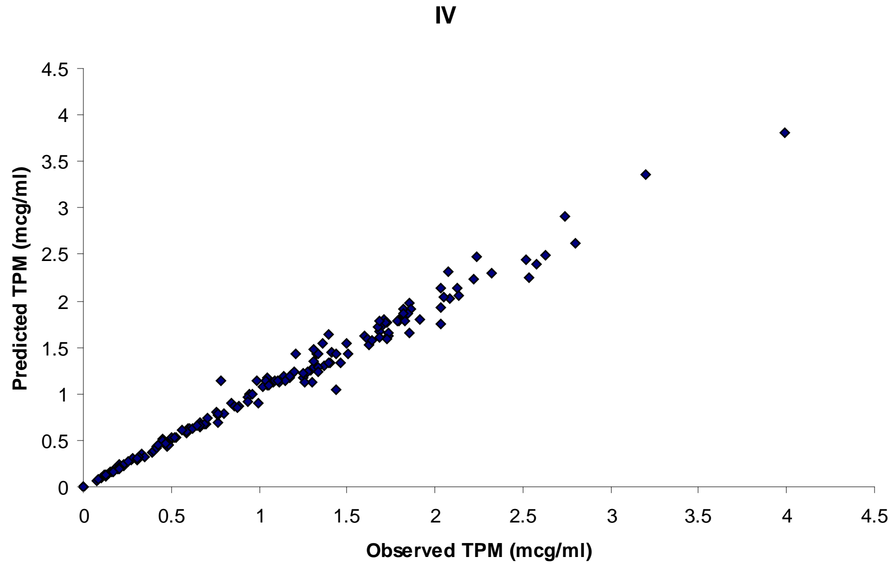
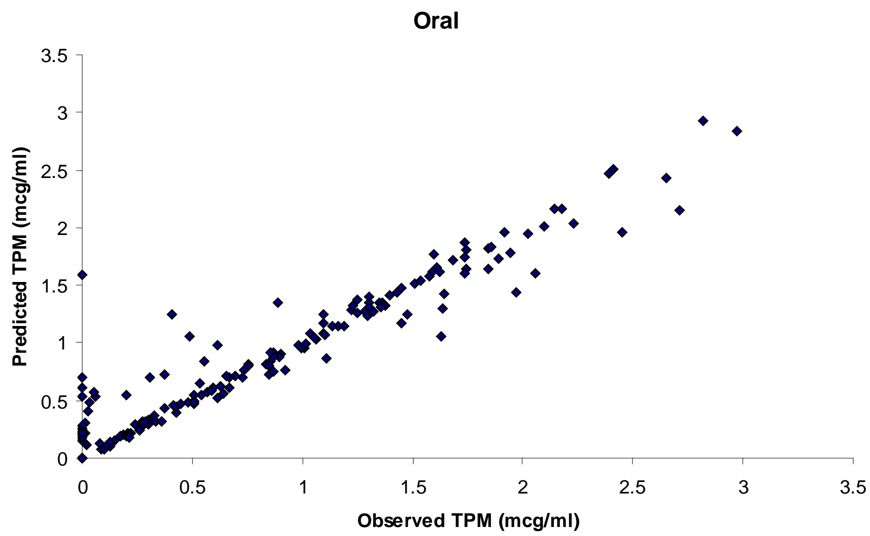


Figure 22: Goodness of fit for two compartment model





The average and individual one compartment model pharmacokinetic parameters are shown in Table 19 through Table 21. The pharmacokinetic parameters after oral and intravenous topiramate were similar. The clearance for intravenous and oral topiramate (1.3 L/hr and 1.1 L/hr) using 1 compartment methods was similar to the clearance using noncompartmental methods (1.33 L/hr and 1.22 L/hr). The volume of distribution using one compartment modeling (0.8 L/kg and 0.7 L/kg) is similar to estimates from the previous patient study. The half-lives for intravenous and oral topiramate (34.6 hours and 33.0 hours) using one compartment methods were shorter than the half-lives after noncompartmental analysis (42.3 hours and 41.1 hours).

Table 19: One compartment pharmacokinetic parameters

Parameter	**IV (mean +/- SD)**	**Oral (mean +/- SD)**
AUC (hr*ug/ml)	76.15 +/- 26.0	86.83 +/- 30.6
AUC/D (hr*ug/ml/mg)	0.818 +/- 0.18	0.93 +/- 0.222
Cmax (ug/ml)	0.0166 +/- 0.0046	0.019 +/- 0.0069
Cmax/D (ug/ml/mg)	1.56 +/- 0.603	1.76 +/- 0.81
K_{01} (1/hr)	n/a	1.56 +/- 0.853
K_{10} (1/hr)	0.0204 +/- 0.0028	0.022 +/- 0.0015
$t_{1/2}$ (hr)	34.64 +/- 4.72	33.03 +/- 7.5
CL (Cl/F) (L/hr)	1.275 +/- 0.268	1.135 +/- 0.278
Vd (L/kg)	0.826 +/- 0.202	0.70 +/- 0.19

Table 20: Individual one compartment pharmacokinetic parameters after intravenous topiramate

	T01	T02	T03	T04	T05	T06
Dose (mg)	50	50	100	100	100	100
AUC (hr*ug/ml)	32.87	35.12	68.29	86.34	65.76	77.23
AUC/D (hr*ug/ml/mg)	0.657	0.702	0.683	0.863	0.658	0.772
C_{max} (ug/ml)	0.609	0.650	1.685	1.394	1.327	1.820
C_{max}/D (ug/ml/mg)	0.012	0.013	0.017	0.014	0.013	0.018
K_{10} (1/hr)	0.019	0.019	0.025	0.016	0.020	0.024
$t_{1/2}$ (hr)	37.34	37.36	28.00	42.84	34.27	29.33
CL (L/hr)	1.521	1.424	1.464	1.158	1.521	1.295
V_d (L/kg)	1.159	1.272	0.798	0.720	0.941	0.755

	T07	T08	T09	T10	T11	T12
Dose (mg)	100	100	100	100	100	100
AUC (hr*ug/ml)	57.56	110.13	89.45	78.97	98.57	113.57
AUC/D (hr*ug/ml/mg)	0.576	1.101	0.895	0.790	0.986	1.136
C_{max} (ug/ml)	1.131	2.200	1.531	1.785	1.838	2.733
C_{max}/D (ug/ml/mg)	0.011	0.022	0.015	0.018	0.018	0.027
K_{10} (1/hr)	0.020	0.020	0.017	0.023	0.019	0.024
$t_{1/2}$ (hr)	35.18	34.61	40.42	30.58	37.09	28.71
CL (L/hr)	1.737	0.908	1.118	1.266	1.015	0.881
V_d (L/kg)	0.785	0.778	0.770	0.701	0.620	0.614

Table 21: Individual one compartment pharmacokinetic parameters after oral topiramate

	T01	T02	T03	T04	T05	T06
Dose (mg)	50	50	100	100	100	100
AUC (hr*ug/ml)	42.63	31.11	120.78	89.16	81.32	70.90
AUC/D (hr*ug/ml/mg)	0.853	0.622	1.208	0.892	0.813	0.709
C_{max} (ug/ml)	0.816	0.761	3.399	1.260	1.716	1.506
C_{max}/D (ug/ml/mg)	0.016	0.015	0.034	0.013	0.017	0.015
K_{01} (1/hr)	2.140	2.677	0.633	2.144	0.666	0.978
K_{10} (1/hr)	0.020	0.026	0.033	0.015	0.024	0.023
$t_{1/2}$ (hr)	34.65	27.08	20.93	47.40	29.02	29.79
CL/F (L/hr)	1.173	1.607	0.828	1.122	1.230	1.410
V_d (L/kg)	0.829	1.041	0.337	0.772	0.644	0.835

	T07	T08	T09	T10	T11	T12
Dose (mg)	100	100	100	100	100	100
AUC (hr*ug/ml)	64.26	126.69	92.18	117.89	91.88	113.14
AUC/D (hr*ug/ml/mg)	0.643	1.267	0.922	1.179	0.919	1.131
C_{max} (ug/ml)	1.095	2.444	1.610	2.559	1.444	2.559
C_{max}/D (ug/ml/mg)	0.011	0.024	0.016	0.026	0.014	0.026
K_{01} (1/hr)	2.879	1.410	2.373	0.393	1.021	1.463
K_{10} (1/hr)	0.018	0.021	0.018	0.026	0.017	0.024
$t_{1/2}$ (hr)	39.43	33.75	38.23	26.28	41.17	28.60
CL/F (L/hr)	1.556	0.789	1.085	0.848	1.088	0.884
V_d (L/kg)	0.788	0.659	0.707	0.404	0.738	0.614

The mean two compartment model parameters are shown in Table 22. The means for the intravenous data exclude two subjects (T02 and T07). These subjects' intravenous data did not fit a two compartment model. The volume of the second compartment in both of these subjects was driving unrealistic estimates of the beta half-life. These two subjects fit well to a one compartment model and their estimates of half-life, clearance, and volume of distribution were similar to the population estimates. Table 23 and Table 24 display the individual two compartment parameters after oral and intravenous dosing.

The terminal half-life, clearance, Cmax/D, AUC/D were similar between oral and intravenous topiramate dosing. The average clearance of intravenous and oral topiramate were 1.27 L/hr and 1.22 L/kg, respectively. This is similar to estimates found with the noncompartmental and one compartment methods. The mean terminal half life was 38.1 hours and 39.2 hours for the oral and intravenous topiramate.

Table 22: Two compartment pharmacokinetic parameters

Parameter	IV (mean+/- SD)	Oral (mean+/- SD)
V_1 (L)	41.04 +/- 16.9	47.15 +/- 14.14
V_2 (L)	26.5 +/- 13.3	18.26 +/- 6.29
CL (CL/F) (L/hr)	1.27 +/- 0.246	1.223 +/- 0.244
K_{01} (1/hr)	n/a	2.740 +/- 4.58
K_{10} (1/hr)	0.0347 +/- 0.0116	0.0272 +/- 0.006
K_{12} (1/hr)	1.664 +/- 2.27	0.101 +/- 0.075
K_{21} (1/hr)	1.219 +/- 1.38	0.220 +/- 0.110
Alpha (1/hr)	2.89 +/- 3.66	0.330 +/- 0.173
Beta (1/hr)	0.0185 +/- 0.0024	0.0182 +/- 0.003
Beta HL (hr)	38.1 +/- 4.92	39.2 +/- 6.83
A (mcg/ml)	1.361 +/- 0.917	1.48 +/- 1.85
B (mcg/ml)	1.413 +/- 0.512	1.39 +/- 0.557
C_{max}/D (ug/ml/mg)	0.0236 +/- 0.0078	0.0185 +/- 0.005
AUC/D (hr*ug/ml)	0.817 +/- 0.165	0.850 +/- 0.178

Table 23: Individual two compartment pharmacokinetic parameters after intravenous topiramate

	T01	T02	T03	T04	T05	T06
Dose (mg)	50	50	100	100	100	100
V_1 (L)	71.41	72.09	54.96	29.35	33.96	30.64
V_2 (L)	19.25	132.06	18.30	45.79	47.56	35.91
CL (L/hr)	1.57	1.00	1.47	1.18	1.57	1.45
K_{10} (1/hr)	0.022	0.014	0.027	0.040	0.046	0.047
K_{12} (1/hr)	0.092	0.013	0.013	4.817	3.327	1.073
K_{21} (1/hr)	0.343	0.007	0.040	3.087	2.376	0.916
Alpha (1/hr)	0.440	0.031	0.063	7.929	5.729	2.014
Beta (1/hr)	0.017	0.003	0.017	0.016	0.019	0.022
Beta HL (hr)	40.44	215.58	41.06	44.41	36.23	32.21
A (mcg/ml)	0.161	0.593	0.914	2.085	1.729	1.799
B (mcg/ml)	0.539	0.100	0.905	1.323	1.215	1.464
C_{max}/D (ug/ml/mg)	0.014	0.014	0.018	0.022	0.021	0.029
AUC/D (hr*ug/ml)	0.636	1.005	0.682	0.850	0.638	0.689

	T07	T08	T09	T10	T11	T12
Dose (mg)	100	100	100	100	100	100
V_1 (L)	86.86	39.59	59.73	47.77	21.42	21.56
V_2 (L)	1092.56	10.74	14.10	18.54	34.34	20.24
CL (L/hr)	0.21	0.95	1.15	1.37	1.03	0.96
K_{10} (1/hr)	0.002	0.024	0.019	0.029	0.048	0.044
K_{12} (1/hr)	0.021	0.071	0.024	0.054	6.138	1.035
K_{21} (1/hr)	0.002	0.260	0.104	0.138	3.828	1.102
Alpha (1/hr)	0.025	0.336	0.132	0.201	9.996	2.158
Beta (1/hr)	0.000	0.019	0.015	0.020	0.018	0.023
Beta HL (hr)	4299.94	37.46	45.83	35.04	37.73	30.60
A (mcg/ml)	1.081	0.604	0.409	0.724	2.886	2.295
B (mcg/ml)	0.070	1.922	1.265	1.369	1.783	2.344
C_{max}/D (ug/ml/mg)	0.011	0.025	0.017	0.021	0.028	0.041
AUC/D (hr*ug/ml)	4.790	1.057	0.867	0.728	0.973	1.046

Table 24: Individual two compartment pharmacokinetic parameters after oral topiramate

	T01	T02	T03	T04	T05	T06
Dose (mg)	50	50	100	100	100	100
V_1 (L)	38.83	56.83	47.75	57.72	66.86	41.42
V_2 (L)	24.17	16.07	9.92	26.48	19.36	18.95
CL/F (L/hr)	1.20	1.60	1.37	1.12	1.41	1.39
K_{10} (1/hr)	0.031	0.028	0.029	0.019	0.021	0.034
K_{12} (1/hr)	0.194	0.011	0.055	0.116	0.009	0.176
K_{21} (1/hr)	0.311	0.038	0.267	0.253	0.032	0.385
Alpha (1/hr)	0.517	0.058	0.327	0.376	0.048	0.572
Beta (1/hr)	0.019	0.018	0.023	0.013	0.014	0.023
Beta HL (hr)	37.14	38.12	29.61	52.79	49.69	30.66
A (mcg/ml)	0.991	0.464	0.427	0.781	0.743	2.224
B (mcg/ml)	0.768	0.433	1.678	1.157	0.790	1.633
C_{max}/D (ug/ml/mg)	0.018	0.016	0.020	0.014	0.014	0.017
AUC/D (hr*ug/ml)	0.830	0.626	0.729	0.889	0.708	0.719

	T07	T08	T09	T10	T11	T12
Dose (mg)	100	100	100	100	100	100
V_1 (L)	68.02	23.76	49.60	36.99	50.87	27.20
V_2 (L)	23.71	19.66	9.52	26.22	9.45	15.62
CL/F (L/hr)	1.56	0.83	1.08	1.13	1.05	0.93
K_{10} (1/hr)	0.023	0.035	0.022	0.030	0.021	0.034
K_{12} (1/hr)	0.072	0.236	0.064	0.134	0.024	0.121
K_{21} (1/hr)	0.206	0.286	0.333	0.189	0.129	0.210
Alpha (1/hr)	0.284	0.538	0.401	0.337	0.156	0.344
Beta (1/hr)	0.017	0.018	0.018	0.017	0.017	0.021
Beta HL (hr)	41.62	37.47	38.33	40.54	40.81	33.35
A (mcg/ml)	0.506	6.984	0.457	1.841	0.438	1.863
B (mcg/ml)	1.050	2.217	1.677	1.480	1.597	2.177
C_{max}/D (ug/ml/mg)	0.013	0.026	0.018	0.020	0.018	0.030
AUC/D (hr*ug/ml)	0.640	1.207	0.927	0.889	0.954	1.080

4.6.4 Cognition testing

Controlled Oral Word Association and Symbol Digit Modalities Tests were measured at baseline and after oral and intravenous topiramate administration. Controlled Oral Word Association Test showed a relationship for both the oral and intravenous concentration data. On average, as the concentrations of topiramate increased, the scores got worse (larger % change from baseline) (Figure 23). Test scores showed a similar relationship after oral and intravenous administration. The Symbol Digit Modalities Test did not show a clear concentration effect relationship (Figure 24). There was a weak relationship between concentrations and Symbol Digit Modalities Test scores after intravenous topiramate, but not oral. The relationship after intravenous dosing is being driven by a few data points at higher concentrations. A study with multiple doses that provides a larger range of concentrations would better explore these relationships. When determining the time of onset, the maximum changes from baseline for the Controlled Oral Word Association and Symbol Digit Modalities Tests occurred earlier after intravenous administration compared to oral administration (Figure 25 and Figure 26). After intravenous dosing, the maximum change in scores occurred 15 minutes post dosing, while the maximum change occurred 2.5 hours after dosing with oral administration.

Figure 23: Controlled Oral Word Association Test changes with topiramate concentration

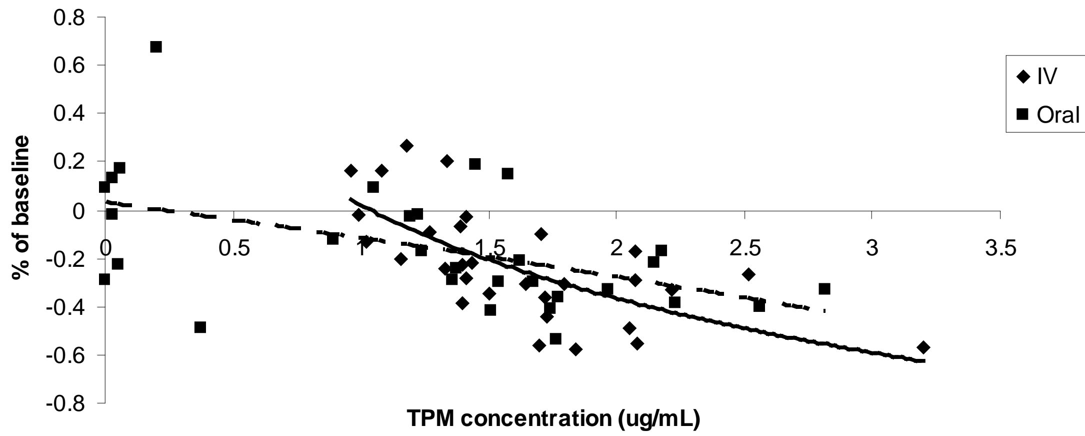


Figure 24: Symbol Digit Modalities Test changes with topiramate concentration

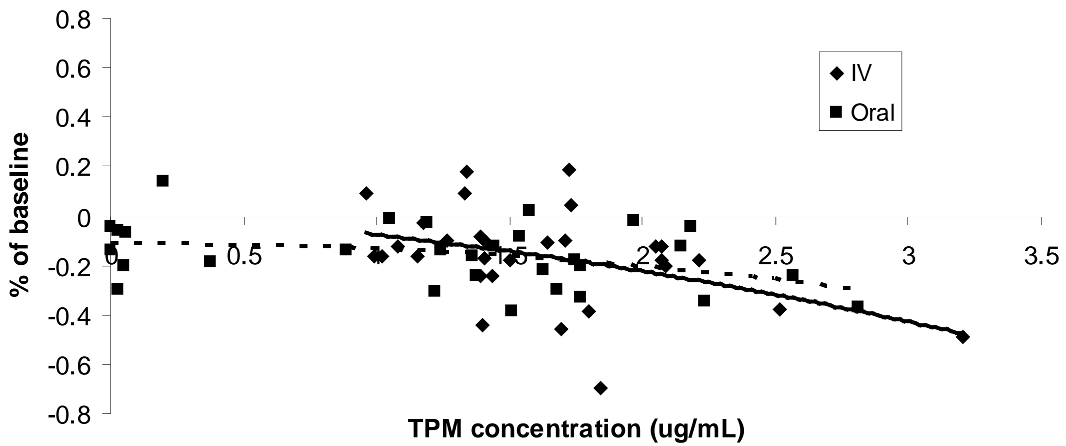


Figure 25: Controlled Oral Word Association Test change after topiramate

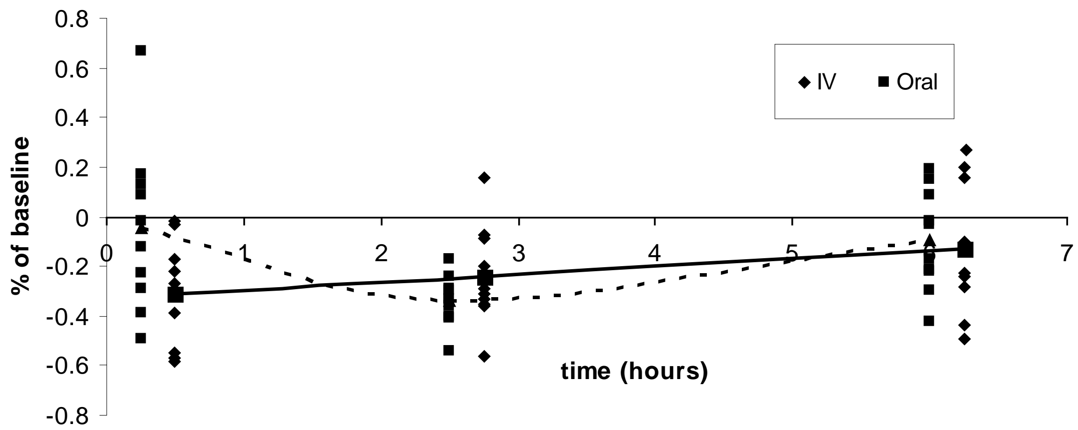
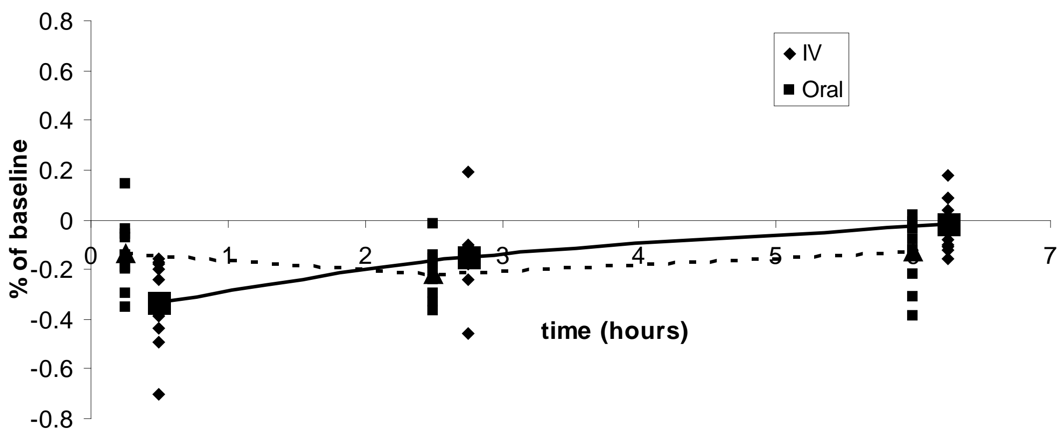


Figure 26: Symbol Digit Modalities Test change after topiramate



4.6.5 Safety results

No serious adverse events were reported by subjects following either intravenous or oral administration of topiramate. All adverse events were classified as either mild or moderate. No subjects discontinued the study. No changes in heart rate, blood pressure, EKG, or infusion site reactions were observed. Subjects reported no local discomfort due to administration of the intravenous formulation. All subjects could walk, talk, and follow commands after dosing.

When side effects were reported, they generally occurred between dosing and 2 hours following dosing. They usually resolved by 4 hours regardless of route of medication. Following intravenous administration, onset of cognitive adverse events and ataxia usually occurred during infusion or up to 15 minutes post-infusion demonstrating intravenous topiramate may have quick penetration into the brain.

Table 25: Adverse events reported

Oral

Adverse event	Severity	Relation to study drug	Duration	Outcome
Ataxia	Mild	Definitely	7 hours	Resolved
Headache	Mild	Possibly	16 hours	Resolved
Headache	Mild	Possibly	2 hours	Resolved
Cognitive impairment	Mild	Definitely	5 hours	Resolved
Ecchymosis at venipuncture site	Mild	Unlikely	n/a	Resolved

IV

Adverse event	Severity	Relation to study drug	Duration	Outcome
Abnormal taste	Mild	Possibly	15 min	Resolved
Hip arthralgia	Moderate	Unlikely	2 days	Resolved
Upper respiratory infection	Mild	Unlikely	n/a	Resolved
Headache	Mild	Definitely	1.5 hours	Resolved
Fatigue	Mild	Possibly	2 days	Resolved
Rash	Mild	Unlikely	2 days	Resolved
Lightheadedness	Mild	Definitely	2 hours	Resolved
Lightheadedness	Mild	Definitely	1 hour	Resolved
Nystagmus	Mild	Definitely	2 hours	Resolved
Ataxia	Mild	Definitely	2.5 hours	Resolved
Ataxia	Moderate	Definitely	1.5 hours	Resolved
Ataxia	Moderate	Definitely	4 hours	Resolved
Intoxication	Mild	Definitely	2 hours	Resolved
Cognitive impairment	Moderate	Definitely	4 hours	Resolved
Calm sensation	Mild	Definitely	2 hours	Resolved
Memory impairment	Mild	Definitely	n/a	Resolved
Dizziness	Mild	Definitely	1 hour	Resolved
IV infiltrate	Mild	Unlikely	n/a	Resolved
IV infiltrate	Mild	Unlikely	n/a	Resolved

Table 26: Blood pressure and pulse after intravenous topiramate

BLOOD PRESSURE

	INFUSION				POST-INFUSION			
	Pre-infusion	5 min	10 min	15 min	+15 min	+ 30 min	+8 hr	+ 24 hr
T01	126/82	120/79	124/88	124/83	120/84	120/84	108/73	97/66
T02	89/56	90/60	90/62	88/57	89/55	92/54	82/52	93/57
T03	110/70	113/68	125/67	117/71	108/72	121/65	109/71	116/70
T04	126/74	117/77	117/73	117/69	117/76	122/74	123/76	128/75
T05	116/68	109/66	106/60	109/69	117/62	115/61	104/63	117/75
T06	120/78	123/81	120/81	130/85	120/82	128/87	136/81	128/85
T07	118/73	124/87	134/86	120/79	131/83	130/88	120/73	124/82
T08	94/57	90/56	95/55	97/60	100/62	102/65	94/56	97/61
T09	125/88	131/78	122/76	126/81	127/81	127/84	123/76	121/76
T10	107/71	110/71	120/72	110/64	110/67	108/67	108/71	117/75
T11	121/83	116/83	120/81	122/85	126/87	125/85	116/78	123/87
T12	93/62	92/62	93/63	92/61	91/63	95/66	105/68	98/68

PULSE

	INFUSION				POST-INFUSION			
	Pre-infusion	5 min	10 min	15 min	+15 min	+ 30 min	+8 hr	+ 24 hr
T01	65	63	67	66	61	67	75	76
T02	45	60	58	n/a	44	51	47	46
T03	59	59	60	59	62	60	69	70
T04	67	58	62	57	64	57	71	73
T05	45	47	44	46	52	43	42	45
T06	57	55	55	61	65	66	87	79
T07	57	63	66	61	66	56	55	70
T08	72	73	72	73	74	68	68	66
T09	81	78	72	74	75	73	81	76
T10	62	52	57	63	63	67	58	67
T11	72	70	71	76	74	75	78	75
T12	64	59	61	67	79	66	62	67

Table 27: Blood pressure and pulse after oral topiramate

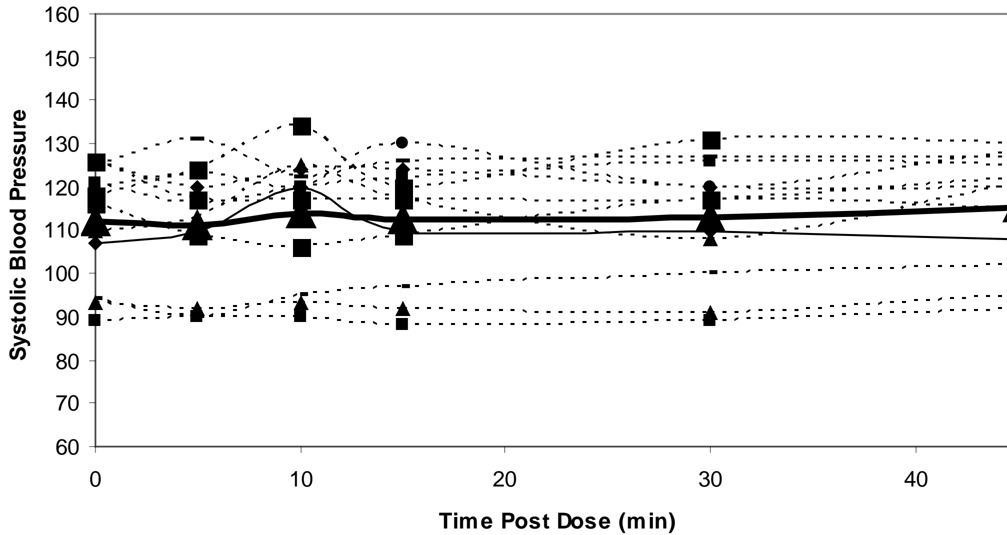
BLOOD PRESSURE

	Pre-infusion	+15 min	+ 30 min	+1 hr	+8 hr	+ 16 hr	+ 24 hr
T01	100/67	95/67	107/74	99/65	98/65	92/64	97/66
T02	87/60	82/60	82/60	82/62	91/56	79/50	88/51
T03	116/77	116/75	118/70	120/64	105/70	127/76	129/71
T04	128/76	126/82	135/78	126/75	122/73	128/85	122/81
T05	107/61	114/54	113/41	110/67	112/60	106/62	110/60
T06	128/85	133/85	136/84	127/84	125/83	124/72	130/80
T07	134/81	134/79	137/79	126/87	116/70	119/78	125/76
T08	96/58	96/65	100/64	91/60	92/60	92/56	98/62
T09	134/76	137/87	141/88	139/88	147/93	122/79	126/85
T10	105/68	105/70	108/68	108/68	108/69	missing	111/64
T11	119/78	114/78	111/77	115/81	125/82	110/71	133/89
T12	97/59	98/63	94/66	95/65	121/63	92/58	107/70

PULSE

	Pre-infusion	+15 min	+ 30 min	+1 hr	+8 hr	+ 16 hr	+ 24 hr
T01	64	65	69	62	82	65	76
T02	46	46	44	46	47	50	57
T03	51	60	62	56	63	60	60
T04	78	73	71	68	69	73	72
T05	51	51	47	50	45	44	45
T06	63	61	62	62	59	61	68
T07	58	58	51	56	57	56	54
T08	67	66	69	62	72	60	65
T09	82	80	82	79	80	77	81
T10	59	58	57	56	59	missing	87
T11	71	71	70	79	77	72	81
T12	54	64	55	57	64	57	69

Figure 27: Mean and individual systolic blood pressure after intravenous topiramate



4.7 DISCUSSION AND CONCLUSIONS

The first preliminary study of intravenous topiramate in humans was completed with a small dose in patients already were taking oral topiramate. There were no serious adverse events following a small dose given in addition to patients' usual oral dose. The second study was in normal subjects and was the first study investigating the bioequivalence and safety of intravenous topiramate in naive subjects at clinically relevant doses. The results from this study provide previously unreported information about topiramate.

Before this project, the absolute bioavailability of topiramate was unknown due to the lack of intravenous formulation. In the initial Phase I study in patients with migraines and epilepsy, calculation of bioavailability was difficult. To estimate bioavailability,

patients were assumed to be at steady-state. In reality, steady-state conditions are hard to meet. Patients may take their medication a few hours late or may miss a dose. Some of the patients were not at steady-state, as there morning trough concentrations varied up to 2-fold. A controlled study in healthy volunteers, more accurately investigates topiramate bioavailability and possible bioequivalence. The results show that oral topiramate is bioequivalent to intravenously administered topiramate. The absolute bioavailability of oral topiramate was 109 % +/- 10.8% (mean +/- standard deviation). These results are similar to the patient study where the absolute bioavailability was 110% +/- 16%. The plasma concentrations including peak concentrations attained by intravenous infusion were very similar to oral administration. The determination that the oral absorption is approximately 100% indicates if patients are switched from intravenous to oral, or vice versa, the same dose can be given. This will simplify dosing calculations for clinicians in the future.

There were no statistically significant differences between oral and intravenous pharmacokinetic parameters. The clearance, half-life, volume of distribution, and area under the concentration-time curve were not statistically different between oral and intravenous formulations. Topiramate clearances (mean 1.2-1.3 L/kg) after oral and intravenous in this study are similar to previous reports of oral clearance in subjects without inducing medications.[69, 71, 81] As expected, topiramate clearance in healthy volunteers was also similar to the clearance in those not taking inducing comedication in the patient study (1.35 +/- 0.37 L/hr).

Because of a lack of an intravenous topiramate, the absolute volume of distribution was previously unknown. Only the apparent volume of distribution could be calculated. The mean volume of distribution with the noncompartmental analysis (approximately 1.0 L/kg) is larger than the patient study results. The mean volume of distribution using compartmental modeling (0.7-0.8 L/kg) was similar to the results from the patient study. A distribution volume of approximately 0.7 to 1.0 L/kg with a low variability now provides a means to quickly attain desired drug concentrations using the intravenous formulation given as a loading dose. Future studies investigating the safety of using higher dosing for loading patients are needed.

The half-life of topiramate in this current study was longer than the half-life reported in the literature. The half-life of intravenous and oral topiramate was 42.3 +/- 6.2 hours and 41.2 +/- 7.5 hours, respectively. The half-life of topiramate in those not taking inducing comedication in our study with patients on maintenance therapy was 31.1 +/- 9.2 hours. This is likely due to the larger volume of distribution found in the healthy volunteer study. The extended elimination half-life of the intravenous and oral formulation indicates it may be given once daily or twice while maintaining targeted plasma concentrations.

Intravenous infusion of doses of 50 mg and 100 mg over 15 minutes appears to be safe. No serious adverse events were reported by subjects following intravenous or oral

administration of topiramate. Subjects reported no local discomfort due to administration of the intravenous formulation. If side effects were reported, they were generally mild and resolved by 4 hours regardless of route of medication. All of the adverse events that occurred were expected as topiramate is known to have cognitive and neurological adverse events that occur more frequently during the initiation of therapy. This was not a dose ranging study. Subjects in this study had very similar concentrations, therefore it was not possible to determine a relationship between topiramate concentration and severity of adverse event.

Topiramate has been associated with significant cognitive impairment in studies of healthy volunteers and patients with epilepsy.[124, 125] Topiramate is known to cause verbal function, memory, and attention impairment.[124] The Controlled Oral Word Association and Symbol Digit Modalities Tests were both found to be sensitive measurements for topiramate cognitive impairment in patients with epilepsy.[124] Controlled Oral Word Association Test measures verbal fluency and is used as an aid in determining neurocognitive ability and deficits.[126] This test has been used to measure verbal communication deficits after brain lesions, to monitor language development in children, and to assess cognitive adverse effects after medication use.[126] The Symbol Digit Modality Test also assesses neurocognitive function including attention, visual scanning, and motor speed.[127]

Both the Controlled Oral Word Association and Symbol Digit Modalities Tests scores decreased from baseline after both intravenous and oral topiramate administration. Controlled Oral Word Association Test displayed a more clear effect with topiramate concentration. On average, as the concentrations of topiramate increased the scores were lower. Although the Symbol Digit Modalities Test demonstrated impairment after topiramate dosing, there was not a clear concentration effect relationship. It is possible that topiramate concentration is not the best predictor of Symbol Digit Modalities Test scores. It is may be total exposure or specific concentration that better predicts changes to the Symbol Digit Modalities Test. A study with multiple doses that provides a larger range of concentrations would better explore these relationships.

The maximum changes from baseline for the Controlled Oral Word Association and Symbol Digit Modalities Tests occurred early after intravenous administration. The maximum impairment after oral administration occurred 2.5 hours after dosing. This corresponds to time to maximum concentration for oral topiramate. Onset of cognitive adverse events and ataxia occurred early post-infusion, demonstrating the intravenous infusion may have quick penetration into the brain. For the treatment of neonatal seizures, in which a fast onset of action is required, rapid penetration into the brain is beneficial. Reducing the duration and frequency of seizures in a short period of time is needed to prevent the long-term neurodevelopmental adverse outcomes that occur after neonatal seizures.

Cognitive effects of antiepileptic drugs are important factors to consider when managing patients. Impairment in cognition occurs more frequently in patients with epilepsy than in healthy subjects.[128] Cognitive decline in patients is complicated by numerous factors including seizure-related changes in neuron function, the underlying neuropathological basis of epilepsy, and exposure to multiple cognitive altering medications.[124, 129, 130] For the treatment of neonatal seizures, cognitive adverse events are not as important. This is a short lived condition and treatment is usually brief. The population is also critically ill babies in a hospital setting.

This study was conducted in healthy volunteers. Future research is needed to determine if intravenous topiramate administration can be used for more extended periods of time in adults and pediatric patients with epilepsy. Intravenous topiramate may be useful in situations where patients on topiramate are not able to take medications orally (undergoing surgery, vomiting, or malabsorption). Safety studies at higher doses used to load patients is required. Patient requiring higher loading doses will likely be in an acute care or hospital setting. Therefore, the neurological and cognitive adverse events, which are likely to occur with higher doses, may not be as clinically important. Also, if they are already taking oral topiramate and switched to intravenous therapy, adverse events will be minimal. Concentrations after oral and intravenous topiramate were very similar.

Prior to using an investigational intravenous topiramate formulation in children and neonates, the pharmacokinetics and safety of the formulation needed to be determined in

adults. The goal of this study was to demonstrate, in healthy volunteers, the safety of intravenous topiramate at higher doses and to characterize topiramate pharmacokinetics. Intravenous topiramate has been demonstrated to be safe in adult patients and healthy volunteers. Future studies in pediatric populations are now possible. Results from this pilot study will inform the design of subsequent studies, including controlled clinical trials intended to determine the efficacy and safety of intravenous topiramate for neonatal seizures.

4.8 FUNDING SOURCE

This study was funded by the New Therapies Grant from the Epilepsy Foundation.

CHAPTER 5

DESIGN OF A NEONATAL SEIZURE STUDY

5.1 CHALLENGES OF CONDUCTING RESEARCH IN NEONATES

5.1.1 Introduction

Over 40 years ago Harry Shirkey used the term "therapeutic orphans" to describe children receiving medications without pediatric studies demonstrating safety and efficacy. Although more drug studies are being done in children, neonates are still understudied. There are unique challenges in conducting research in neonates. Recent regulations to increase research and labeling for pediatrics has not benefited neonates as much as older pediatric populations. Many drugs used in neonates lack appropriate dosing, efficacy, and safety information about their use. Neonates are not "small infants" in the same way children are not "small adults."

There is an unmet need to conduct adequately controlled trials in neonates. Neonates have developmental differences such as renal and hepatic function and neurophysiology compared to children and adults. Therefore, weight or body-size scaling of dosing from older populations is not appropriate. Insufficient research leaves these babies at increased risk for unsafe or inadequate drug use. As presented in this chapter, neonatal and pediatric clinical researchers also face both ethical and practical challenges not usually encountered in the adult setting. Due to the lack of neonatal studies, newborns largely remain "therapeutic orphans."

5.1.2 Off-label use of drugs

Off–label drug use may expose neonates to ineffective therapies with unknown adverse events. Use of off-labeled drugs in neonates is far greater than any other childhood age group. In a neonatal intensive care unit study, 54.7% of prescribed drugs were used off-label, 9.9% were unlicensed drugs, and 90% of neonates receive at least 1 unlicensed or off-label drug.[131] Off-label drug use results at a higher risk of adverse drug reactions. A recent study found off-label drug use in pediatrics resulted in a 3.44 greater relative risk of adverse events compared to labeled medications.[132] Another study found 23-60% of off-label prescriptions were associated with an adverse event in pediatrics.[133]

5.1.3 Physiological difference

Newborns are a unique population. Diseases may be exclusive to this age group and physiological function and metabolic processes are substantially different from older children and adults. Neonatal growth and development is not a linear process, it can be dynamic and inconsistent. These factors complicate extrapolation from older populations.

Effect of neonatal physiology on pharmacokinetics

Although it has not been sufficiently studied, there is rapid, yet inconsistent, maturation of physiologic and pharmacologic processes during the first month of life that affect both pharmacokinetics and pharmacodynamics. This requires modification of treatments

based on age and development. Pharmacokinetic processes can rapidly change during the first few days to weeks of life.

Immature physiological process and organ dysfunction can both lead to changes in absorption, distribution, metabolism, and excretion. Absorption can be altered as neonates have an increased gastric pH, delayed gastric emptying, and decreased gastric motility compared to children and adults.[134-138] Unfortunately, very few studies have investigated the effect of these changes on drug absorption in neonates. Overall, a majority of drugs are absorbed more slowly in neonates and the time to reach the maximum concentration increases.[139]

Depending on the characteristics of the drug, age-dependent development of body composition can change the distribution of drugs. Neonates have decreased body fat, small muscle mass, increased total body water, and decreased alpha-1-acid glycoprotein and albumin.[140, 141] Depending on lipid solubility and protein binding of a drug, the influence of age on distribution may vary. For example, in a study of neonates the average volume of distribution for phenobarbital was 0.97 +/- .15 L/kg compared to a typical adult volume of distribution of approximately 0.6 L/kg.[142, 143]

Developmental changes in metabolism occur in neonates due to decreased enzyme ontogeny and reduced blood flow to liver.[144, 145] Each phase I and II drug metabolizing enzyme has a distinct pattern of maturation. For example, CYP2E1 protein and activity

levels increase rapidly within the first 24 hours of birth.[146] Activity, protein, and mRNA levels rise steadily and reach adult levels by about one year of age.[146] The CYP3A subfamily, which the most abundant CYP enzyme in adult livers, is also developmentally regulated. CYP3A is important as it metabolizes a large number of drug substrates and endogenous compounds. Fetal livers express CYP3A7, which peaks at approximately one week postpartum and declines during the first year of life.[147] The fetal liver demonstrates little CYP3A4 activity and increases postnatally to reach 30 to 40% of adult levels by one month of age and adult levels by one year.[148] There is little correlation between CYP3A4 mRNA and protein levels during the first month of life. CYP3A4 mRNA are relatively low and increase rapidly following birth to plateau after one week of age.[147] The ontogeny of only a few UGT enzymes have been examined.[149] As an example, the fetal liver expresses only low levels of UGT1A1 protein and increases to adult levels at approximately 3 to 6 months of age.[150]

Developmental alterations in renal function can change the pharmacokinetics of a drug that undergoes extensive renal excretion. Both renal filtration and secretion are reduced at birth. The glomerular filtration rate at birth is 2-4 mLs per minute per 1.73 m^2 in full-term babies (as low as 0.6-0.8 in pre-term neonates) and reaches adult levels at approximately one year of life.[151]

The difficulty in assessing renal and hepatic function is that they do not always mature in a predictable way. Overall, more research needs to be done, especially in the critically ill neonate, to better understand and predict these processes.

Effect of neonatal physiology on pharmacodynamics

Disease processes in neonates may differ from those in adults. Both the underlying mechanism of the disorder and the clinical presentation may be altered. Some diseases have no close analogies to adults (example: Kawasaki's disease). Very limited research has been done demonstrating the effect of development on the interaction between drugs and receptors. See section 1.1.5 Pathophysiology that describes maturation of CNS receptors and the impact on potential mechanisms of action for neonatal seizure treatments.

Neonates are at increased risk of adverse events due to immature drug detoxification mechanisms, multiple drug exposures, multiple organ dysfunction, and anemia. Adverse events and outcomes in the neonate are difficult to assess compared to children and adults (lightheadedness, headache, nausea). Newborns also experience adverse events not seen in adults (examples: Reye’s syndrome after aspirin, gray baby syndrome after chloramphenicol, and hyperactivity after phenobarbital). Age appropriate pharmacodynamic endpoints are needed.

5.1.4 Lack of baseline data

For many conditions there is limited data available on their incidence or prevalence in newborns. Diagnosis may also be more difficult and predictors may be poorly defined. For some diseases, information is not available on outcome rates and treatment effects. These make designing a study and determining an adequate sample size for a study difficult, especially since many of the diseases that affect neonates are rare disorders.

5.1.5 Small sample size

For many neonatal diseases, the population of affected babies is usually small. Therefore, it is difficult to enroll an adequate number of patients to obtain statistical power. Designing a study to enroll sufficient subjects in a reasonable time frame may require a multi-center or international study. Again, baseline outcome rates and variability data needed to calculate sample size may be limited.

5.1.6 Choice of control group

Most pediatric drug studies have not been randomized controlled trials. Standards of care can undermine the feasibility of conducting necessary controlled clinical trials even though scant evidence exists in support of these treatments. For example, clinicians may have ethical concerns about withholding a drug even though it has not been proven effective or safe. Again, this makes it difficult to implement a randomized placebo controlled study.

Control groups should pose minimal risk, not exceeding that of standard care. The Declaration of Helsinki paragraph 29 states “the benefits, risk, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods.”[152] It also states a placebo or no treatment control should only be permitted when there is “no *proven* prophylactic, diagnostic, or therapeutic method.”[152] Difficulties arise when standards of care have not been adequately studied or "proven."

5.1.7 Measuring outcomes

Outcome measures developed for adults may not be appropriate in newborns or it is difficult or impossible to obtain desired outcomes**.** Mortality is objective but in many neonatal or childhood illnesses death is uncommon**.** Further, mortality may not be a sensitive measure and may require large sample sizes**.** Long-term outcomes usually are important for many neonatal or pediatric diseases. Developmental outcomes and later health status are also difficult and expensive to measure. Follow-up for studies may need to be decades or longer. Unlike older populations, clinical end points for neonatal conditions cannot be based on verbal questioning or patient cooperation with measurement. See section 5.2.2 Study features for neonatal seizure studies outcomes.

5.1.8 Obtaining consent

Older children should participate in the decision to participate in research to the extent their capacity allows. Obviously, this is not possible with neonates. Obtaining consent in pediatric studies requires more time as compared to adult studies. Approaching parents in times of stress raises the question whether consent is truly voluntary. A study investigating the timing of consent in neonatal studies found the proportion of parents who agreed to enter early (71%) was significantly higher than when asked for later consent (43%).[153] From questionnaires given to parents after neonatal intensive care studies, 90% of parents felt they had made an informed decision, 79% knew they had the right to refuse treatment, 76% knew what alternatives were available, but only 69% knew they could withdraw at any time.[154]

Alternative methods to traditional consenting processes include waiver of informed consent in emergency situations, consent before birth, consent early and re-consent later, use of an independent group of experts, and the Zelen design.[154] In studies using the Zelen design, patients are randomized to either the treatment or control group before giving informed consent. Only patients receiving standard care provide informed consent. Disadvantages of this design is that there is a lack of treatment blinding, which may produce bias. Also, statistical analysis should be performed with intention-to-treat. If patients refuse their original treatment, this will lead to a reduction in study power and the study will require a larger sample size.

5.1.9. Analytical challenges

Another important consideration when designing a study in neonates is analytical challenges. Newborns are at increased risk of anemia from multiple blood draws. A general guideline used by many Institutional Review Boards is a maximum of 3–5% of blood volume over a one to three month period for research purposes.[155] Studies using microsampling techniques, sensitive assays, sparse sampling, simulation studies, and population pharmacokinetic models are increasingly being used to overcome these challenges.[155]

5.1.10 Formulations

Another difficulty in studying neonates is pediatric formulations are not always available. Oral solutions or intravenous formulation are usually needed. More concentrated preparations may be needed to limit volume. Excipients need to be proven safe in the pediatric and neonatal populations even if safety is known in adults (e.g. diethylene glycol in the sulfanilamide disaster).[156] Many pediatric formulations are also extemporaneously compounded, which may lack the quality control of commercial formulations.

5.2 NEONATAL SEIZURE STUDY DESIGN

5.2.1 Introduction

Most neonatologists and pediatric neurologists agree a placebo-controlled or delayed treatment design for neonatal seizures is not possible.[157-159] When managing a serious problem such as neonatal seizures, it is extremely difficult to implement a placebo-controlled trial once a standard of practice exists, even if efficacy and safety of the standard therapy has not been determined.

Nevertheless, studies are feasible using novel and creative methods. Possible designs for evaluating the treatment of neonatal seizures include the following: add-on, active-controlled, placebo-controlled studies for prevention of seizures, or placebo-controlled studies of the treatment of subclinical electrographic seizures. Each of these designs has its own advantages and disadvantages. The optimal design for all study types relies on essential features: preliminary data, patient selection, accurate diagnosis, treatment protocols, and selection of meaningful outcome measures. If properly performed and analyzed, these studies will provide invaluable information for the treatment of a rare and devastating disorder.

5.2.2 Study features

Preliminary studies

A phase I pharmacokinetics and safety study of intravenous topiramate must be done in neonates before a controlled clinical trial can be undertaken. Currently, there is only one small study investigating oral topiramate pharmacokinetics in neonates.[85] Pharmacokinetic processes change rapidly during the first week to month of life. Therefore, topiramate pharmacokinetics should be characterized with respect to age and development. Many newborns with seizures also have significant multi-organ dysfunction that affect hepatic and renal processes, which in turn influence the elimination of topiramate. Many newborns with hypoxic-ischemic encephalopathy are now treated with therapeutic hypothermia that can also affect the pharmacokinetics and safety of intravenous topiramate. Hence, the impact of reduced body temperature on topiramate disposition also needs to be determined.

Once the pharmacokinetics of topiramate in the population is established, studies can be designed using dosing regimens intended to attain concentrations thought to be neuroprotective and control seizures. The neuroprotective target concentrations are based on experimental data demonstrating efficacy in neonatal animal models. However, it is possible effective and safe concentrations (or exposure) in human neonates will differ from those determined in animals. Consequently, preliminary dose escalation studies designed to determine safety and preliminary efficacy would help inform the design of controlled clinical studies. This would help determine initial safety and determine the

maximum tolerated dose. The babies enrolled early in the study would be given smaller doses. If safe, higher doses would be used in the next subset of patients enrolled in the study.

Study population

A target population would be term newborns with a gestational age at birth of 38-42 weeks and postnatal age <7 days with seizures caused by hypoxic ischemic encephalopathy. Choosing a homogenous population of newborns with a single etiology of seizures will simplify the pharmacokinetic, safety, and efficacy analysis. Hypoxic-ischemic encephalopathy is the most common cause of neonatal seizures. Newborns with hypoxic-ischemic encephalopathy have poor outcomes and might benefit from improved seizure control and neuroprotection.

Although these newborns have the potential for great benefit, this population is a challenging group to study. These babies are critically ill with significant multiorgan dysfunction and taking numerous other medications (example: vasopressors and antibiotics). Choosing term babies based on gestational age will allow a more homogeneous population as these neonates will be at similar stages of development. This is especially important when determining dose-exposure relationships and pharmacokinetics.

Hypoxic-ischemic encephalopathy after birth asphyxia is most likely to occur soon after birth, but it can occur unexpectedly and requires rapid initiation of intensive care. Most newborns are transferred to a neonatal intensive care unit. Seizures caused by hypoxic-ischemic encephalopathy typically escalate in frequency and duration, but normally subside after a few days.[9, 10] Rapid diagnosis and treatment is essential.

It needs to be acknowledged the results of studies in babies with hypoxic-ischemic induced seizures may not apply to other neonatal seizure populations. It is possible seizures might respond differently in other conditions such as in premature infants and seizures associated with developmental abnormalities, metabolic disorders, infections, or stroke.

Diagnosis of hypoxic-ischemic encephalopathy

Accurate diagnosis of perinatal asphyxia and hypoxic-ischemic encephalopathy is necessary but can be difficult Perinatal asphyxia is diagnosed based on neonatal distress with a hypoxic-ischemic insult. Diagnosis is defined clinically from the following: fetal heart rate abnormalities, cord blood $pH<7.1$, biophysical profile score $<7/10$, Apgar score <6 at 5 minutes, need for positive pressure ventilation or chest compressions, and encephalopathy.

Diagnosis of neonatal seizures

Continuous and prolonged EEG monitoring is required to accurately diagnose and monitor seizures in a study, especially because of the high incidence of electroclinical

dissociation.[160] A neonatal seizure study should require EEG-proven seizure activity rather than clinical seizures alone for inclusion. Recently, there has been increased interest in using 1 or 2 channel EEG monitors to detect seizures[161, 162] however full EEG still remains the gold standard for quantifying and detecting seizures.[11] Additional studies would be required to determine the feasibility of using reduced channel EEGs in future trials. When babies undergo therapeutic hypothermia with a cool cap, there is limited access for an EEG. Whole body hypothermia allows full access to the scalp for EEG during cooling.

Control group

A control group is needed to determine the safety and efficacy of intravenous topiramate. Hypoxic-ischemic encephalopathy causes a high incidence of medical complications. Adverse event rates and efficacy can only be determined by comparing rates in the treatment versus standard (or control) groups. Depending on the study design, either placebo or standard of care (phenobarbital) can be used for the control group.

Blinding and randomization

The most objective way to determine topiramate safety and efficacy is to blind investigators, clinicians, and family to treatment assignment. Subjects should be randomized to treatment or standard/control in order to distribute known and unknown confounding variables between groups. Assignment should be stratified by treatment with hypothermia because this may affect the rate of adverse events and efficacy.

Treatment should also be stratified based on clinical site, since this most likely will be a multicenter study. This will take into account any treatment or quality of care differences that occur based on site.

Outcomes

Customary clinical practice is to visually monitor high-risk neonates for the emergence of clinical seizures and perform EEG examinations when suspicious clinical activity appears.[10] In a clinical study, continuous video-EEG monitoring allows for the most accurate examination of the traditional end point of treatment, cessation of clinical seizures. For the treatment of neonatal seizures complete seizure cessation is the most critical end point. Percent seizure reduction (example: > 50 % reduction in seizures) would be a secondary endpoint.

Again, evidence from research in newborn animal models supports the view neonatal seizures themselves cause adverse neurodevelopmental outcomes.[163, 164 165, 166] Seizures, in addition to hypoxic-ischemic encephalopathy, can cause additive or supra-additive injury. However, demonstrating this in human newborns and designing clinical studies to separate the effects of the initial injury from the effect of the seizures themselves is difficult.

With respect to meaningful long-term outcomes, improvement of neurodevelopmental function is the optimal goal. Ideally, follow-up should extend to school age children. It

is important to understand how babies subsequently do in school and later in life. The long-term follow-up should test verbal function, motor function, and IQ and other neurodevelopmental outcomes to at least 7 years of age. Magnetic resonance spectroscopy is being used to assess injury within weeks to months after seizures.

Topiramate dose and duration

The projected dose, dose regimen, length of treatment and duration of intravenous topiramate for future Phase II-III studies would be based on current knowledge of topiramate pharmacokinetics/pharmacodynamics and the outcomes of Phase I studies in human neonates. Given present information, the projected topiramate regimen would consist of a 20 mg/kg loading dose followed by 7.5 mg/kg/day as a maintenance dose. Due to the extended half-life of topiramate, daily dose of topiramate could be given once and spilt to twice daily dosing. The initial preliminary pharmacokinetic studies in neonates will assist in determining the most effective dosing interval. Doses would be adjusted to attain concentrations in the range of 20-25 ug/mL.

If treatment failure occurs with topiramate or phenobarbital, a second agent (such as phenytoin or benzodiazepines) will be used. Treatment is considered a failure if the neonate has an episode of electrical seizures lasting longer than 2.5 minutes or a total of 2.5 minutes of seizure activity during any 5-minute period. Duration of treatment would be for 7 days. If seizures continue or reoccur, drug therapy would be continued.

Stop criteria

If a severe adverse event occurs, no further subjects would be given study medication or recruited into the study until it is determined if the adverse event was likely due to the study medication. If more a severe adverse events occurred in more than 2 patients, the study would be stopped.

Safety assessment

The heart rate and rhythm, mean arterial pressure, and respiratory status should be monitored continuously during treatment. Daily monitoring of liver and renal function tests, coagulation tests, blood cell count, and blood-gas measurements should be included in all studies.

An adverse event is defined as any reaction, side effect, or other untoward event, regardless of relationship to study drug, that occurs anytime after the study begins. This could be a clinically significant adverse change in clinical status, a treatment-emergent sign or symptom, a new illness, or a clinically relevant abnormal laboratory finding. All adverse events should be recorded with the following minimum information: the specific event or condition, whether the event was a worsening of an existing medical condition, the dates of occurrence, severity, relationship to study medication, specific countermeasures (example: concomitant medications/procedures), and outcome. The investigators should assess the relationship of the adverse to the study drug as: not

related, unlikely, possibly, probably, or definitely related. The intensity of an adverse event should be assessed by a study physician as mild, moderate, or severe.

A serious adverse event is defined as an adverse event experienced by a subject that results in any of the following outcomes:

- Death
- A life-threatening adverse experience
- Hospitalization (unplanned hospital stay) or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect

Cost

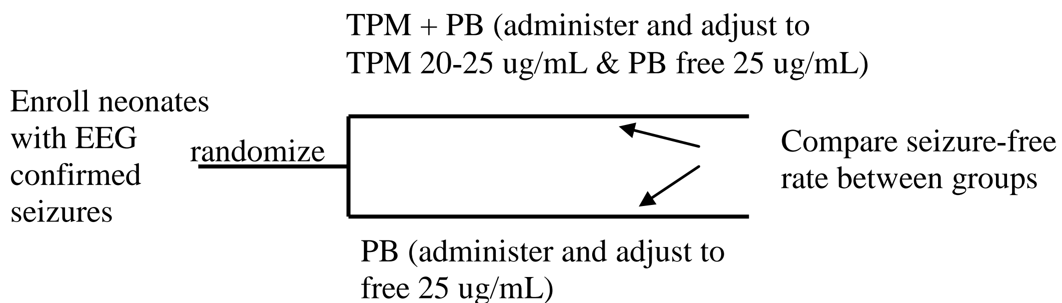
Development and investigation of intravenous topiramate for the treatment of neonatal seizures is a multimillion dollar project. Treatment of neonatal seizures is a rare condition, and there is a lack of financial incentives for a pharmaceutical company to bring a product to market. Future clinical investigation of intravenous topiramate safety and efficacy in neonates will mostly likely be funded by the National Institute of Neurological Disorders and Stroke and/or the FDA Orphan Products Grant Program.

5.2.3 Study designs for neonatal seizure trial

5.2.3.1 Add-on study

This study design would enroll term neonates with a gestational age at birth of 38-42 weeks and postnatal age <7 days with seizures caused by hypoxic-ischemic encephalopathy. Seizures would be confirmed by EEG prior to randomization. Babies would be randomized to receive either phenobarbital (standard of care) or phenobarbital plus topiramate (Figure 28). Doses would be adjusted to attain concentrations in the range of 20-25 ug/mL. Phenobarbital would be given at a loading dose and adjusted to a free level of 25 ug/mL. Duration of treatment would be for 7 days. If seizures continue or reoccur topiramate therapy would be continued.

Figure 28: Add on study schematic



The sample sizes were calculated using a comparison of two independent binomial proportions using the likelihood ratio statistic with a Chi-square approximation, a two-sided significance level of 0.05, and a power=0.8. The sample sizes were calculated assuming 43% of babies are seizure-free in the phenobarbital (control) group.[35] The sample size was calculated based on different treatment effects (Table 28). A sample size

of 42 per treatment group achieves a power of at least 0.8 to detect a proportion difference of 0.3 when the reference proportion is 0.43 (43%). A proportion difference of 0.3 represents a clinically significant difference between the two treatment groups. This study is in critically ill neonates and the drop out rate should be low. Patients are only being treated for 7 days while in the hospital setting and there will be little loss to follow-up. If significant drop outs do occur, the sample size should be adjusted. Death will be considered a treatment failure. All comparisons should be done using an intention-to-treat analysis.

Table 28: Sample size for add-on study

Proportion difference	N (per group)	N (total)
0.1	392	784
0.15	174	348
0.2	98	196
0.3	42	84
0.4	22	44
0.5	13	26

*significance level of 0.05, and a power=0.8

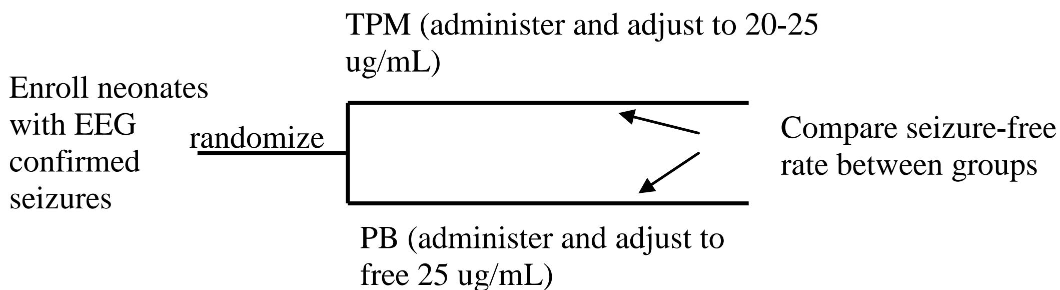
The advantage of this study design is it is ethically justifiable. Phenobarbital is the current standard of care and study design does not withhold treatment. A disadvantage of this design is the actual efficacy of topiramate is not determined. This study investigates topiramate as an adjunctive treatment. Evidence exists that phenobarbital causes long-term developmental adverse events when given in infancy. Data from animal studies shows phenobarbital is harmful to developing neurons.[41, 42] Therefore, the long-term goal

is to find a new treatment option that is safer and more effective than phenobarbital, not to find an adjunctive treatment to use in combination. This design does allow for a subsequent study of topiramate versus phenobarbital if the first study shows a difference with topiramate plus phenobarbital.

5.2.3.2 Active control study

This study design would enroll term neonates with a gestational age at birth of 38-42 weeks and postnatal age <7 days with seizures caused by hypoxic-ischemic encephalopathy. Seizures would be confirmed by EEG prior to randomization. Babies would then be randomized to receive either phenobarbital (standard of care) or topiramate (Figure 29). The projected topiramate dosage regimen would consist of a 20 mg/kg load followed by 7.5 mg/kg/day as a maintenance dose. Doses would be adjusted to attain concentrations in the range of 20-25 ug/mL. Phenobarbital would be given at a loading dose 35 to 45 mg and adjusted to a free level of 25 ug/mL. Duration of treatment would be for 7 days. If seizures continue or reoccur topiramate therapy would be continued.

Figure 29: Active control study schematic



The sample sizes were calculated using a comparison of two independent binomial proportions using the likelihood ratio statistic with a Chi-square approximation, a two-sided significance level of 0.05, and a power=0.8. The sample size was calculated assuming 43% of babies are seizure-free in the phenobarbital (control) group.[35] The sample size was calculated based on different treatment effects (Table 29). These calculations are the same as the add-on study, but the proportion difference to expect from each study design will differ.

Table 29: Sample size for active control study

Proportion Difference	N (per group)	N (total)
0.1	392	784
0.15	174	348
0.2	98	196
0.3	42	84
0.4	22	44
0.5	13	26

*significance level of 0.05, and a power=0.8

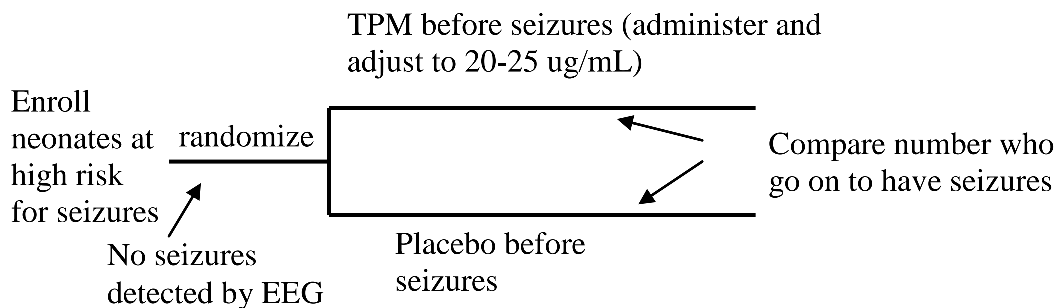
Advantages of this study design are that it compares the safety and efficacy of topiramate to standard treatment (phenobarbital). Babies are not withheld treatment in this design. The disadvantage is the actual efficacy of topiramate is not determined because this is no placebo group to compare. Most clinicians and parents would not withhold treatment to a seizing newborn. A problem could arise if topiramate was found to be inferior (in safety or efficacy) to phenobarbital. If this were to occur, it would be unknown whether

topiramate is better than no treatment, because there is no data available comparing phenobarbital to placebo.

5.2.3.3 Prevention of seizures

This study design would enroll term neonates with a gestational age at birth of 38-42 weeks with diagnosed hypoxic-ischemic encephalopathy who have not experienced seizures at enrollment. These newborns are at a high risk of seizures in the near future. Babies would then be randomized to receive either topiramate or placebo (Figure 30). The projected topiramate dosage regimen would consist of a 20 mg/kg load followed by 7.5 mg/kg/day as a maintenance dose. Doses would be adjusted to attain concentrations in the range of 20-25 ug/mL. Duration of treatment would be for 7 days. If seizures occur, escape criteria would be applied to transition the baby to standard of care.

Figure 30: Prevention of seizure study schematic



The sample sizes were calculated using a comparison of two independent binomial proportions using the likelihood ratio statistic with a Chi-square approximation, a two-

sided significance level of 0.05, and a power=0.8. The sample size was calculated assuming 50% of babies with hypoxic-ischemic encephalopathy have seizures.[167] The sample size was calculated based on different treatment (Table 28). A sample size of 39 per treatment group achieves a power of at least 0.8 to detect a proportion difference of 0.3 when the reference proportion is 50%. A proportion difference of 0.3 represents a clinically significantly difference between the treatment groups.

Table 30: Sample size for prevention of seizure study

Proportion Difference	N (per group)	N (total)
0.1	388	776
0.15	170	340
0.2	94	188
0.3	39	78
0.4	20	40

*significance level of 0.05, and a power=0.8

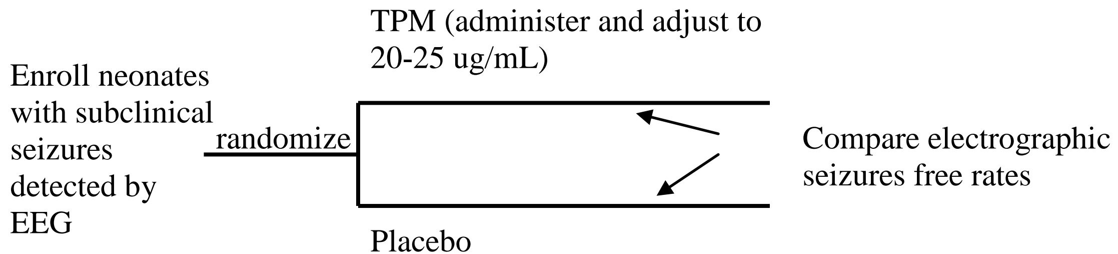
Advantages of this study design are that it is ethically justifiable and it is placebo-controlled. This study does not withhold standard treatment, since newborns are not usually given medication to prevent seizures. Since a placebo can be used, this design would allow determination of the actual benefit of topiramate. The disadvantage is it investigates prevention rather than suppression of seizures. It is possible that prevention of seizures in neonates is physiologically and mechanistically different than suppression of already occurring seizures. Hence, the safety and efficacy of topiramate for prevention

of seizures may differ from treatment of seizures. In addition, this study design exposes patients to a potentially harmful drug who may otherwise not receive it.

5.2.3.4 Treatment of subclinical seizure

This study design would enroll term neonates with a gestational age at birth of 38-42 weeks and postnatal age <7 days. Newborns with diagnosed hypoxic-ischemic encephalopathy would be monitored with an EEG. Only those with electrographic (subclinical) seizures would be included in this study (Figure 31). The projected topiramate dosage regimen would consist of a 20 mg/kg load followed by 7.5 mg/kg/day as a maintenance dose. Doses would be adjusted to attain concentrations in the range of 20-25 ug/mL. Duration of treatment would be for 7 days. If patients go on to have clinical seizures there would be an escape criteria to receive standard of care.

Figure 31: Treatment of subclinical seizure study schematic



The sample sizes were calculated using a comparison of two independent binomial proportions using the likelihood ratio statistic with a Chi-square approximation, a two-sided significance level of 0.05, and a power=0.8. The proportion of babies that stop

seizuring without treatment (placebo group) during a treatment period is unknown. Therefore, the sample size was calculated using different reference proportions (control group rates). This was done because the baseline data is unavailable. The sample size was then calculated based on different treatment effects (Table 31).

Table 31: Sample size for treatment of subclinical seizure study

Reference Proportion	Proportion Difference	N (per group)	N (total)
0.6	0.1	357	714
0.6	0.15	153	306
0.6	0.2	82	164
0.6	0.3	32	64
0.5	0.1	388	776
0.5	0.15	170	340
0.5	0.2	94	188
0.5	0.3	39	78
0.5	0.4	20	40
0.4	0.1	388	776
0.4	0.15	174	348
0.4	0.2	98	196
0.4	0.3	43	86
0.4	0.4	23	46
0.4	0.5	14	28
0.3	0.1	357	714
0.3	0.15	163	326
0.3	0.2	94	188
0.3	0.3	43	86
0.3	0.4	24	48
0.3	0.5	15	30
0.2	0.1	293	586
0.2	0.15	138	276
0.2	0.2	82	164
0.2	0.3	39	78
0.2	0.4	23	46
0.2	0.5	15	30

*significance level of 0.05, and a power=0.8

Advantages of this study design are it is ethically justifiable and it is placebo-controlled. This study does not withhold treatment, since there is no standard of treating subclinical seizures. If not enrolled in this study, it would not even be known that these newborns were experiencing electrographic seizures. Usually EEGs are only done after clinical seizure activity. Since a placebo can be used, this design would allow determination of the actual benefit of topiramate. The disadvantage of this design is it investigates electrographic rather than clinical seizures. It is possible that electrographic seizures in neonates are physiologically and mechanistically different than clinical seizures. The safety and efficacy of topiramate for electrographic seizures may differ from clinical seizures. This study design also exposes patients to a potentially harmful drug that would otherwise not receive it. It is currently unknown whether treatment of electrographic seizures improves long-term neurodevelopmental outcomes.

5.3 CONCLUSIONS AND DISCUSSION

Advances in the care of neonate with seizures over the past few decades has resulted in improved survival. Unfortunately, there have been minimal improvements in morbidity. There are unique challenges in conducting research in neonates. Newborns are a unique population. Diseases may be exclusive to this age group and physiological function and metabolic processes are substantially different from older populations. Therefore weight or body sized scaling from older populations is not appropriate. The past "trial and error" approach to dosing neonates and infants can have devastating consequences including

increased risk of adverse events. Insufficient research currently leaves these babies at increased risk for unsafe or inadequate drug use.

Neonatal clinical researchers also face practical challenges not usually encountered in the adult setting. For many neonatal conditions sparse data may be available on incidence or prevalence. Diagnosis may also be more difficult and predictors may be poorly defined. For many neonatal diseases, the population of affected babies is usually small. Outcome measures used in older population may not be appropriate in newborns. Developmental outcomes and later health status are also difficult and expensive to measure. Obtaining consent in pediatric studies requires more time and consideration as compared to adult studies. Neonatal studies must be designed with limited blood sampling, which can be accomplished by utilizing sensitive assays with small volumes, sparse sampling strategies, and population pharmacokinetic approaches. These factors make designing a study and determining an adequate sample size difficult, especially since many of the diseases that affect neonates are rare disorders

Even though there are unique challenges to conducting research in neonates, studies are feasible using novel and creative methods. Possible study designs for the treatment of neonatal seizures include add-on studies, active-controlled studies, placebo-controlled studies of the prevention of seizures, or placebo-controlled studies of the treatment of subclinical electrographic seizures. Each study design has advantages and disadvantages. An optimal study design for all of these studies relies on fundamental features:

preliminary data, patient selection, accurate diagnosis, treatment protocols, and selection of meaningful outcome measures.

Considering all designs, I recommend an active controlled study of intravenous topiramate versus phenobarbital. This design would compare the safety and efficacy of topiramate to the current standard treatment. This design best investigates the long-term goal of finding a new treatment option that is safer and more effective than phenobarbital. Investigating adjunctive treatment, treatment of electrographic seizures, and prevention would provide useful data, but these designs are not the most clinically applicable. The main disadvantage of this design is the actual efficacy or safety of topiramate is not determined, since there is no placebo group to compare. If topiramate safety or efficacy is not found to be superior to phenobarbital, it would be unknown whether topiramate is better than no treatment. If efficacy or safety is not superior to phenobarbital, then future development of intravenous topiramate for neonatal seizures needs to reconsidered. Phenobarbital is effective in less than 50% of cases and it has well documented long-term adverse events. The goal is to find a new treatment option that is more effective and safer than the current treatment options.

Research in neonates needs to become a priority of regulators, pharmaceutical industry, and academicians. Until then, neonates will continue to be at risk for adverse effects and ineffective treatment. Future research is needed to demonstrate the efficacy and safety of

drugs used in newborns. Unless this occurs, caring for neonates will continue to be similar to conducting thousands of studies with a population of one (N=1).

CHAPTER 6

CONCLUSIONS

The studies in this thesis include a study in newborn laboratory animals, a phase I study in patients with epilepsy and migraines taking topiramate, and a healthy volunteer dose ranging and safety study. Hypoxic-ischemic brain injury in newborns is a serious medical problem with a high mortality rate, grave neurological sequelae including impaired cognition and neonatal seizures, and significant treatment-related adverse effects that can cause further brain injury. A safer, more effective treatment for neonatal seizures combined with the potential for neuroprotection would be a considerable advancement in the care of newborns with hypoxic-ischemic brain injury. Intravenous topiramate has the potential to control seizures and provide neuroprotection in newborn babies.

Neonatal seizures are defined as seizures occurring during the first 30 days of life. Evidence exists that they can cause long-term developmental and cognitive dysfunction. They are estimated to occur in less than 1% of live births. Hypoxic-ischemic encephalopathy caused by birth asphyxia is the most common cause of neonatal seizures. Other less common causes include inborn errors of metabolism, intracranial hemorrhaging, infections, and metabolic irregularities. Regardless of etiology, neonatal seizures are a rare medical condition. The number of neonates with this condition at any particular time is far less than 200,000, the definition of a rare disorder. Classification as a rare medical disorder qualifies potential therapies to be designated as orphan drugs and, thus benefiting from incentives provided in the Orphan Drug Act including tax credits,

market exclusivity, exemption of prescription drug user fees, formal protocol assistance, and Food and Drug Administration (FDA) funding through a grant program.

Studies show neonates with seizures due to hypoxic-ischemic encephalopathy and those with increased seizure burden have poor outcomes. Seizures and brain injury arising from hypoxic-ischemia often result in life-long morbidity including cognitive impairment. These are the newborns most likely to benefit from improved seizure control and neuroprotection.

The current drugs of choice for the treatment of neonatal seizures, phenobarbital and phenytoin, have never been subjected to adequately controlled clinical trials. In uncontrolled studies, fewer than 50% of babies responded to therapy. Current therapies for neonatal seizures also continue to raise concerns regarding adverse effects on the developing and immature brain. Therefore, new treatment options are greatly needed. Thus far, no therapies have been specifically developed for the treatment of neonatal seizures.

Topiramate is an antiepileptic drug used to treat epilepsy in adults and children. Recent research has shown topiramate is neuroprotective in newborn laboratory animals in models of status epilepticus and cerebral ischemia. The proven safety and effectiveness of topiramate for seizures in older children and adults together with substantial laboratory

evidence of efficacy suggests topiramate may be beneficial for the treatment neonatal seizures resulting from hypoxic-ischemic encephalopathy and provide neuroprotection.

Development of an intravenous formulation is essential for improving the treatment of neonatal seizures. An intravenous formulation allows use of loading doses to quickly attain concentrations known to be neuroprotective and stop seizures. Stopping seizures quickly is vital to preventing further brain injury. Also, many babies with neonatal seizures are critically ill and have multiorgan dysfunction. As a result, their gastrointestinal motility is severely reduced. Without an intravenous formulation, treatment with oral topiramate requires placement of a gastrointestinal tube. An oral solution of topiramate is not commercially available and must be extemporaneously compounded. As a result of these problems, consistent absorption cannot be assured. Therefore, an intravenous formulation of topiramate would greatly facilitate appropriate treatment of seizures in neonates. However, prior to using an investigational intravenous topiramate in newborns, its pharmacokinetics and safety must first be demonstrated in adults and most likely in older children. Further, pre-clinical studies in newborn laboratory animals investigating optimal drug concentration would be helpful in guiding the design of studies in human neonates.

The studies in this thesis include a study in newborn laboratory animals, a phase I study in patients with epilepsy and migraines taking topiramate, and a healthy volunteer dose ranging and safety study. The goal of the animal study was to determine plasma

topiramate concentrations in rat pups given doses previously shown to result in neuroprotective effects. Postnatal day 7 rats were given 30 mg/kg or 50 mg/kg of intraperitoneal topiramate. Plasma samples were obtained at predetermined times up to 8 hours post-dose. Following an intraperitoneal injection, topiramate exhibited rapid absorption attaining maximum concentrations slightly higher than the steady-state concentrations reported when the drug is used orally to treat epilepsy. The neuroprotective doses produced maximum concentrations slightly above this range, while non-neuroprotective doses produced peak concentrations approximately twice as high as the therapeutic range for epilepsy. As this study involved a small number of animals, future neuroprotection studies of topiramate in laboratory animals should utilize dosing regimens that attain concentrations in this range to verify such levels are optimal. The results of the rat pup study indicate peak topiramate concentrations in the range of 20-40 μg/mL are associated with doses shown to be neuroprotective in rat pup models of hypoxic-ischemia. The concentrations determined in the rat put study are similar to those used in standard treatment for epilepsy increasing the likelihood topiramate will be safe in newborns. Although several putative neuroprotectants have demonstrated benefits in animal studies, none have shown significant benefits in humans. Thus, it is possible the neuroprotection demonstrated in animal models may not translate to human babies. Nonetheless, these results provide target concentrations and exposures that will inform the design of dosing strategies in future topiramate neuroprotection studies.

Results for the two human studies provide previously unreported information about topiramate. In adults, the bioavailability of oral topiramate is almost 100%, thus plasma concentrations attained by intravenous infusion were similar to oral administration. The determination that the oral absorption is essentially complete indicates patients switched from intravenous to oral, or vice versa, can be given the same dose. The steady-state elimination half-life of topiramate indicates it may possibly be given once or twice daily in some patients with minimal peak-trough fluctuations in plasma concentrations. A distribution volume of approximately 0.7-0.8 L/kg with a small variability now provides a means to quickly attain desired drug concentrations using the intravenous formulation given as a loading dose. Studies investigating the safety of using higher doses for loading patients are needed. Given present information, the projected topiramate dosage regimen for future studies would consist of a 20 mg/kg load followed by 7.5 mg/kg/day as a maintenance dose. Doses in babies would be adjusted to attain concentrations in the range of 20-25 ug/mL.

Intravenous infusions of doses of 25 to 100 mg over 10 to 15 minutes appear to be safe. No serious adverse events or local administration-related discomfort were reported by subjects following intravenous doses of topiramate. The side effects that were reported were generally mild and resolved by 4 hours regardless of route of medication. Following intravenous administration onset of cognitive adverse events and ataxia occurred early, usually within 15 minutes from the end of the infusion, demonstrating intravenous topiramate likely exhibits rapid penetration into the brain. For the treatment

of neonatal seizures, in which a fast onset of action is required, rapid penetration into the brain is beneficial. For the treatment of neonatal seizures, cognitive adverse events are not as clinically important.

The current treatment of neonatal seizures is hindered by the absence of even a single randomized, placebo-controlled efficacy study of any antiepileptic drug. Although it is standard of care to treat newborns with phenobarbital or phenytoin, their actual efficacy has never been established. Evidence exists these medications can have serious short term adverse effects, interact with other medications, cause long-term developmental delays in humans, and are harmful to the developing neonatal animal brain. For these reasons, most neonatologists and pediatric neurologists agree a placebo-controlled or delayed treatment design for neonatal seizures is not possible. Possible study designs for the treatment of neonatal seizures include an add-on study, an active-controlled study, a placebo-controlled study of the prevention of seizures, and a placebo-controlled study of the treatment of subclinical electrographic seizures. Each of these studies has its own advantages and disadvantages. The future study designs proposed in this thesis would provide evidence of efficacy and safety of topiramate for the treatment of neonatal seizures. Choosing seizures caused by hypoxic-ischemic encephalopathy for study of new therapies provides a homogenous population of newborns with a single etiology of seizures. This will simplify the design and analysis of pharmacokinetic, safety, and efficacy trials. The recognition and quantification of seizures would use EEG, the gold standard of seizure detection. Even with challenges of conducting research in neonates,

studies are feasible using novel and creative methods. These studies would provide invaluable information for the treatment of a rare and devastating disease.

Further research is needed to determine if intravenous topiramate can be used for more extended periods of time and in larger doses in adults and pediatric patients with epilepsy. The studies included in this thesis provide pharmacokinetic and safety data needed to begin studies in younger patients. A Phase I pharmacokinetics and safety of intravenous topiramate must be done in neonates before a controlled clinical trial can begin. These studies would be designed to determine the pharmacokinetics and initial safety of topiramate in this population. Once the topiramate pharmacokinetics in neonates is established, studies can be designed with dosing regimens to attain target concentrations (20-25 ug/mL) thought to be neuroprotective and to control seizures.

Lastly, the development of intravenous topiramate for neonatal seizures will require juvenile animal toxicology studies. Neither topiramate or Captisol® has been adequately investigated in formal neonatal animal toxicology studies. Prior to filing an IND for studies in neonates, toxicity studies are needed in age appropriate animal models.

The research presented in this thesis supports the eventual goal of developing a more effective, safer treatment for neonatal seizures. The results from the research included in this thesis will hopefully lead to a more effective and safer treatment for this devastating condition.

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