CLINICAL PHARMACOLOGY AND PHARMACOMETRIC ANALYSES OF CNS DRUGS USED IN THE ACUTE MANAGEMENT OF SEIZURES AND SPASTICITY

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Dedication

To my parents and family for their love and encouragement

&

To all my teachers for their unending support

Abstract

The overall goal of my thesis projects was to characterize the pharmacokinetics, bioavailability and tolerability of CNS drugs used in the acute management of seizure emergencies and spasticity. Two drugs were studied: diazepam and baclofen. Both of these drugs are already approved by the FDA and have been extensively used. For diazepam, two novel formulations were evaluated for intranasal delivery as rescue therapy in seizure emergencies. In the case of baclofen, commercially available intrathecal baclofen solution was administered intravenously to characterize its pharmacokinetics and safety in humans.

Diazepam rectal gel (Diastat®) is the only FDA-approved product indicated for acute repetitive seizures. Despite its proven efficacy, most older children and adults object to this route of administration. As a result, many patients do not realize the benefit of a therapy that can improve outcomes and decrease healthcare costs. Intranasal administration of benzodiazepines offers a potential alternative. The primary objective of this study was to compare the bioavailability and pharmacokinetics of two novel intranasal diazepam formulations versus intravenous administration in healthy volunteers. Twenty-four healthy volunteers were randomized into an open-label, three-way crossover study. Ten mg doses of two investigational intranasal diazepam formulations (solution, suspension) and a 5 mg intravenous dose of commercially available diazepam injectable, USP were given. A two-week washout period separated treatments. Plasma samples for diazepam analysis were collected pre-dose and at regular intervals up to 240 hours post-dose. diazepam concentration-time data were analyzed using a non-compartmental pharmacokinetics approach. Exposure following administration of diazepam intranasal solution (absolute

bioavailability – 97%) was greater than the intranasal suspension (absolute bioavailability-67%). Both investigational intranasal formulations were well tolerated. The results of this pilot study indicate that development of an intranasal diazepam formulation with high bioavailability, reasonable variability, and good tolerability is feasible. Further, a PK model was developed and simulation studies were performed to optimize future bioequivalence trials. Absorption characteristics of rectal and nasal diazepam formulations were compared using the deconvolution analysis.

An additional identified problem that this thesis work aims to address is management of baclofen withdrawal. The current recommended management strategies for baclofen withdrawal are inadequate and availability of intravenous baclofen would permit rapid attainment of drug concentrations in plasma as well as accurate and precise dose titration, thus allowing for prevention and expeditious treatment of withdrawal symptoms and reduced risk of adverse effects. The objective was to characterize baclofen pharmacokinetics and safety given orally and intravenously. Twelve healthy subjects were enrolled in a randomized, open-label, crossover study and received single doses of baclofen: 3 or 5 mg given IV and 5 or 10 mg taken orally with a 48-hr washout. Blood samples for baclofen analysis were collected pre-dose and at regular intervals up to 24 hours post-dose. Clinical response was assessed by sedation scores, ataxia and nystagmus. Mean absolute bioavailability of oral baclofen was 74%. Dose-adjusted areas under the curve (AUC) between the oral and IV arms were statistically different ($p=0.0024$), while AUC variability was similar (co-efficient of variation: 18-24%). Adverse effects were mild in severity and not related to either dose or route of administration. Three and 5 mg IV doses of baclofen were

well tolerated. Seventy-four percent oral bioavailability indicates that smaller doses of IV baclofen are needed to attain comparable total drug exposures.

Both intranasal diazepam and intravenous baclofen hold promise in management of seizure emergencies and baclofen withdrawal, respectively. Results from studies described in this thesis will inform the design of subsequent clinical studies that are needed for market approval.

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INTRODUCTION

1.1 Epilepsy

Epilepsy is the 4th most common chronic neurological disorder in United States after migraine, stroke and Alzheimer's disease [1] with episodic manifestations that are associated with abnormal electrical discharges in the brain [2]. Approximately 1-2% of the global population suffer from epilepsy with estimates from the World Health Organization data indicating a prevalence of 50 per 100,000 of the general population [3]. Nearly 3 million Americans (~1% of US population) have epilepsy, 10% of those affected are under the age of 15 (300,000 children) and 25% are above the age of 65 [1]. Typical seizures in ambulatory patients are self-limited and last less than 3 minutes [4]. More than half of patients with a first seizure will go on to have another. Epilepsy was defined conceptually by a task force of the International League Against Epilepsy (ILAE) in 2005 as a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures. This definition is usually practically applied as having two unprovoked seizures >24 h apart and has some limitations [5]. Therefore, the ILAE commissioned a second task force to develop a practical (operational) definition of epilepsy, designed for use by doctors and patients and the results of several years of deliberations on this issue have been published recently [6]. The second task force proposed that epilepsy be considered to be a disease of the brain defined by any of the following conditions: (1) at least two unprovoked (or reflex) seizures occurring >24 h apart; (2) one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years; (3) diagnosis of an epilepsy syndrome.

Approximately 30-40% of patients who have achieved seizure control with the use of antiepileptic drugs (AEDs) experience breakthrough seizures due to non-adherence to AEDs, infection/fever, severe stress, sleep deprivation, and metabolic or hormonal events/changes [2]. Moreover, these breakthrough seizures often result in seizure emergencies. Adequate and effective treatment in a timely fashion is critical to the successful management of patients encountering seizure emergencies.

1.1.1 Seizure Emergencies

Seizure emergencies, while not well-defined, include a wide spectrum of seizures including acute repetitive seizures, prolonged episodes lasting many minutes, and status epilepticus (SE) [7]. Acute seizures are the most common emergency related to epilepsy and account for about one million emergency department (ED) visits a year, or 1% of all ED visits in the US [8]. The worldwide annual incidence of acute seizures ranges from 70 to 100 in 100,000 [9]. The annual cost of prehospital and ED care alone has been estimated at $1 billion in the US [10]. Often identification of acute seizures occurs by observation of motor symptoms such as clonic jerking, known as convulsive seizures. However, it is to be noted that the majority of seizures in adults do not have prominent motor activity and are typically referred to as nonconvulsive seizures. Seizures which are prolonged (30 minutes) or recurrent without a return to baseline are referred to as SE (Figure 1).

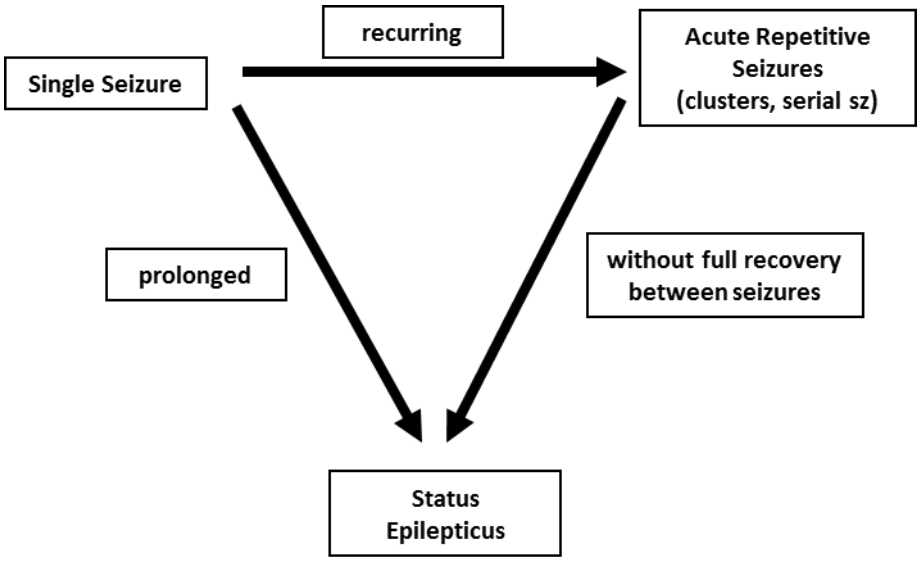

Figure 1: Evolution of Severe Seizures [11]

SE cause an estimated 42,000 deaths each year with a case-fatality rate ranging from 15% to 22% [12]. Mortality in acute symptomatic SE is up to 34% and is between 38-67% in the elderly [13] . SE can cause serious neuronal damage; it has been shown in an animal model that even if the systemic effects of SE are controlled, 30 minutes of SE may cause significant histologic damage [14] and thus treatment that is initiated early is much more likely to improve outcomes. Although early treatment leads to better outcomes [15], an examination of database of patients admitted in the greater Richmond, Virginia area between 1989– 1994 revealed that approximately 60% of patients receive treatment after a delay of more than 30 minutes [16]. Many neurologists use an operational definition of SE as any seizure lasting more than 5 minutes, or 2 or more seizures between which there is incomplete recovery of consciousness to enable timely and effective treatment [17].

1.1.1.1 Acute Repetitive Seizures (ARS)

Acute repetitive seizures are a predictable component of a patient's seizure disorder, historically distinct from other epileptic seizures in type, frequency, duration, or severity and with an onset easily recognized by the caregiver and physician [18]. Approximately 80% of seizures are spontaneous and unpredictable. Although rare, seizures may be provoked by specific precipitating events (~1%) [18]. The rest of the approximately 19% of seizures have a cyclical pattern recurring at more or less foreseeable intervals of days to weeks but with high variability from patient to patient. Although several terms such as serial, repetitive, recurrent, cluster or crescendo seizures have been used in literature to describe this cyclical pattern of seizures, none has ever constituted a standardized definition.

In the mid-1990s, the emergence of rectal diazepam product for treatment of these cyclical pattern seizures led the US Food and Drug Administration (FDA) to define for labeling purposes, the entity for which patients could be safely and effectively treated. The National Institute of Health Epilepsy Advisory Committee defined acute repetitive seizures as a characteristic episode of multiple seizures within 24 hours for adults (12 hours for children) despite optimal therapy and is recognizable by the patient's caregiver and distinguishable from other seizures. Upon FDA's recommendation, a nine-member panel of epilepsy experts in 1995 signed an affidavit describing acute repetitive seizures as a "…recognized chronobiological entity that can be defined as a form of seizures that

1) are severe
2) are a predictable component of the patient's seizure disorder

3) are historically distinct from the patient's other seizures in type, frequency, severity, or duration
4) have an onset that is easily recognized by the family and physician
5) demonstrate patient recovery between seizures
6) have a consistent component (such as an aura, prodrome, or characteristic single or multiple seizures) that is predictably and temporally linked to subsequent seizures, and
7) are a constellation of seizures variously referred to as recurrent serial, cluster, or crescendo seizures" (Peripheral and Central Nervous System Drugs Advisory Committee Meeting # 45; November 15, 1996).

1.1.2 Available Modes of Treatment and Unmet Need

Rescue therapy such as rectal diazepam is given as needed in an attempt to disrupt progression of a given seizure, and forestall what would otherwise be a more prolonged or more severe clinical event i.e. status epilepticus [7]. Treatment of seizure emergencies commonly involves intravenous administration of benzodiazepines (lorazepam or diazepam) and/or other antiepileptic drugs such as phenytoin, valproic acid, and levetiracetam by medical personnel [19]. The Neurocritical Care Society recommends use of intravenous (IV) lorazepam for treatment of status epilepticus when skilled health care personnel are available [20]. In a recent randomized trial in pediatric patients with convulsive status epilepticus, treatment with lorazepam did not result in improved efficacy or safety compared with diazepam [21]. Chamberlain et al [21] recommended that either diazepam, lorazepam, or midazolam could be chosen as a reasonable

first-line therapy. Although the IV route is the most effective option for quick cessation of seizures, therapy is often delayed when skilled medical personnel and transportation to medical facility is required for drug administration. A retrospective medical review of 30 patient records admitted to the ED revealed that the average total time from onset of seizures to treatment was 85 minutes [22] (Table 1). Studies have also shown that prolonged or repetitive seizures can produce morphological cortical brain damage [23] and the efficacy of routinely used benzodiazepines for treatment of such seizures decreases as the duration of seizures increases [24]. Due to this delay in therapy and resulting brain damage from untreated seizures, there is a great need for safe and effective rescue therapies which are quick and easy to administer as well as socially acceptable.

Table 1. Average Time for Events Taking Place in a Patient Experiencing Seizure Emergency

From	To	Average time to event (range)
Onset of seizures	Arrival of the emergency medical technician (EMT)	30 minutes (15-140 minutes)
Arrival of the EMT	Arrival of patient at the ER	20 minutes (10-40 minutes)
Arrival of patient at the ER	Initiation of a seizure treatment protocol	35 minutes (15-83 minutes)
Onset of seizures	Treatment of seizures	85 minutes

In 1997, rectal diazepam gel, Diastat® was approved by the FDA for the management of selected, refractory, patients with epilepsy, on stable regimens of antiepileptic drugs (AEDs), who require intermittent use of diazepam to control bouts of increased seizure activity. Two well-controlled clinical trials have shown that rectal diazepam is safe and effective in treating acute repetitive seizures. Despite the proven efficacy of diazepam rectal gel, many older children and adult patients object to this route of administration due to privacy, legal, and social concerns [25]. Because of these concerns, there

continues to be an unmet need for an alternate route of administration that is not only safe and effective, but also acceptable by patients, families and providers.

1.1.3 Benzodiazepines for Seizure Emergencies

For management of seizure emergencies, a drug should: have a wide therapeutic index; be sufficiently potent so as to permit use in small volumes; lend itself to methods of administration that are quick, easy, and safe; have a rapid onset of action (minutes) and an intermediate duration of action (hours) [26]. Benzodiazepines (BZDs) meet many of these criteria and are considered the drugs of choice for treating seizure emergencies as rescue therapy [26].

1.1.3.1 Mechanism of Action of Benzodiazepines

All BZDs have the same mechanism of action i.e., binding to the gamma amino butyric acid type A ($GABA_A$) receptors. Their binding to gamma subunit of $GABA_A$ receptors causes an allosteric (structural) modification of the receptor, which results in an increase in $GABA_A$ receptor activity. BZDs do not substitute for GABA, which bind at the alpha subunit, but increase the frequency of channel opening events which leads to an increase in chloride ion conductance resulting in hyperpolarization of the neuron. This causes an inhibitory effect on neurotransmission by diminishing the chance of occurrence of a successful action potential (Figure 2).

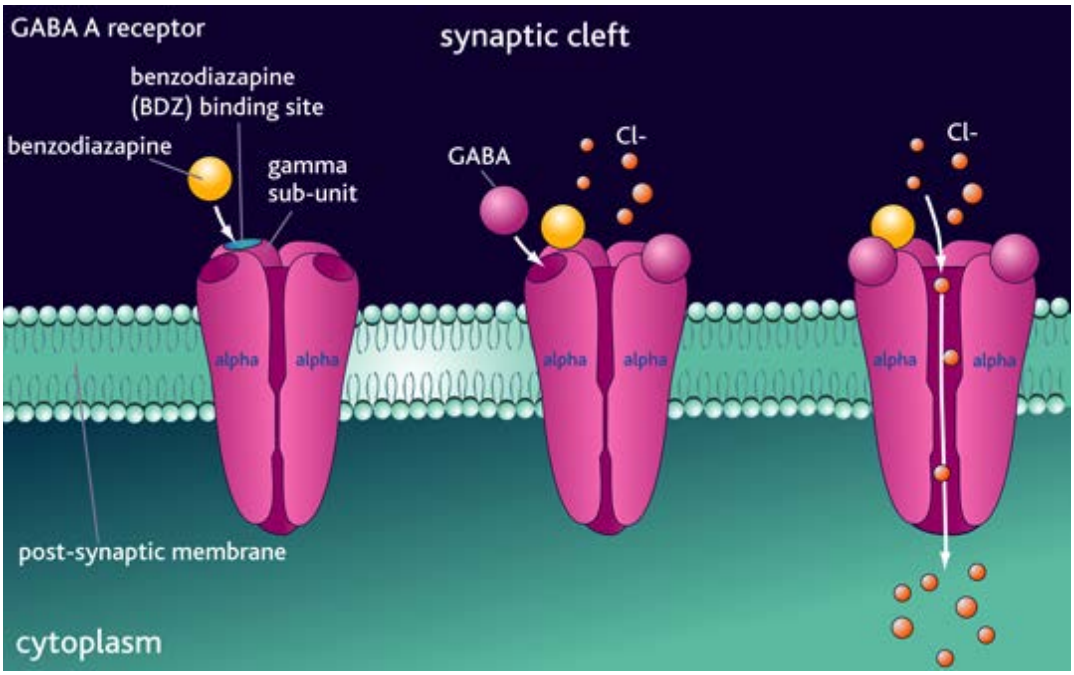

Figure 2: Mechanism of Action of Benzodiazepines [27]

This enhanced effect of the neuroinhibitory transmitter, GABA, at the $GABA_A$ receptor results in sedative, hypnotic, anxiolytic, anticonvulsant, muscle relaxant, and amnesic actions, which are useful in a variety of indications such as alcohol dependence, seizures, anxiety, panic, agitation, and insomnia. In general, benzodiazepines are well-tolerated and are safe and effective drugs in the short term for a wide range of conditions. Tolerance can develop to their effects and there is also a risk of dependence, and upon discontinuation a withdrawal syndrome may occur. These factors, combined with other possible secondary effects after prolonged use such as psychomotor, cognitive, or memory impairments, limit their long-term applicability. The effects of long-term use or misuse include the tendency to cause or worsen cognitive deficits, depression, and anxiety.

1.1.3.2 General Consideration Affecting Choice of Drug

While the mechanism of action is same for all BZDs, there are significant differences in physico-chemical properties and pharmacokinetic (PK) characteristics, which affect the choice of drug and route of administration. Below is a brief discussion of physico-chemical and PK differences of three benzodiazepines commonly used in the management of seizure emergencies and their impact on selection of drug and route of administration for treating seizure emergencies.

1.1.3.2.1. Lipid Solubility Considerations

An important physico-chemical property affecting the absorption and distribution of a drug is its lipid solubility. The greater a drug's lipid solubility, the faster is its absorption across membranes including rectal, muscular, buccal, nasal tissues as well as the blood-brain-barrier. Diazepam and midazolam are 4 and 6 times more lipid soluble than lorazepam (see Partition Ratio, Table 2). With respect to midazolam, the commercial injectable formulation is buffered to a pH of 3 that produces an ionized open ring compound with good water solubility. Following administration, as midazolam encounters physiologic pH, the open ring closes resulting in a highly lipid soluble structure. When given IV, diazepam, lorazepam, and midazolam enter the central nervous system (CNS) within a few minutes with a corresponding fast onset of effect. However, lorazepam redistributes out of the CNS into muscle and fat tissue more slowly resulting in a longer duration of effect. This property makes lorazepam the drug of choice to treat seizure emergencies when given IV.

When treating a seizure emergency by an extravascular route, the differences in lipid solubility and pharmacokinetics result in a different choice of benzodiazepines. Diazepam and midazolam when given extravascularly display significantly faster rates of absorption than lorazepam due to their high lipid solubility and re-distribution to muscle and fat tissues is not as pronounced. Consequently, diazepam and midazolam are preferred when non-IV routes are indicated, but their performance will vary by formulation, route of administration and type of seizure emergency.

Table 2. Physico-chemical Properties and Pharmacokinetic Parameters of Diazepam, Midazolam and Lorazepam

Drug	**Molecular Weight[28]**	**Partition Ratio[28]**	**Oral Bioavailability[29] (%)**	**Protein Binding[29] (% bound)**	**Elimination Half-life[29] (hours)** *
Diazepam	309	309	100	96-99	21-70
Lorazepam	321	73	99	93.2	7-26
Midazolam	362	34 at pH 3, 475 at pH 7.4	40-60	96	1-4

*Note: Half-lives of benzodiazepines are shorter in patients with concomitant enzyme inducing drug therapy

1.1.3.2.2. Absorptive Surface Area and Dose Volume Considerations

Each route has certain physiological characteristics that effect selection of drug and formulation. For example, all the routes have limited absorptive surface areas across which the drug must permeate to reach systemic circulation and a limited volume of fluid that can be instilled / injected into the tissue or cavity. According to Fick's Law of

diffusion (Equation (1)), the greater the absorptive surface area, the faster the drug absorption.

$$Rate\ of\ Diffusion = D \times A \times \Delta C/h \quad \text{.....Equation (1)}$$

Rectal and nasal routes have larger absorptive surface areas when compared to buccal, intramuscular and subcutaneous routes. The volume of fluid that could be instilled or injected is as follows: Rectal cavity (~ 10mL) > IM (2-5 mL) > Subcutaneous and buccal (2 mL) > Nasal (~200 µL per nostril). Routes which necessitate small volumes of fluid require potent drugs which are effective in small doses and/or can be formulated in very high drug concentrations.

Shortly after the introduction of Diastat® (diazepam rectal gel), commercial development of innovative formulations began involving several BZDs and one or more routes of administration, including buccal, intramuscular, nasal, and subcutaneous. Table 3 provides an overview of benzodiazepine rescue therapies which are either approved, used off-label, and/or under development. Currently, rectal diazepam (Diastat®) and buccal (BUC) midazolam (Epistatus® and Buccolam™, Europe only) are approved. Investigational products include intranasal (IN), intramuscular (IM) and subcutaneous (SQ) formulations.

Table 3. Benzodiazepine Seizure Rescue Therapies

Route	**Drug**	**Sponsor**	**Development Status***
Rectal	Diazepam	Valeant	FDA-approved product for out of hospital treatment of acute repetitive seizures (Diastat®)

IN	Diazepam	Neurelis Neuronex/Acorda	Phase I Phase II
IN	Midazolam	Upsher Smith	Phase III
IM	Diazepam	King/Pfizer	Phase III successfully completed.
IM	Midazolam	Meridian/Pfizer	Phase III completed
SQ	Diazepam	Xeris Pharmaceuticals	Pre-clinical phase
Buccal	Midazolam	Specialty Products ViroPharma	Marketed in selected European countries (Buccolam™ and Epistatus®)

*Source of information: clinicaltrials.gov, press releases, grant awards, marketing information

1.1.3.2. Route of Administration Considerations

As discussed previously, both diazepam and midazolam have physico-chemical and PK properties which favor non-IV routes. However, it is important to consider the significant differences in the rates of absorption when given by different extravascular routes. A review of diazepam and midazolam pharmacokinetics literature following rectal, IN, IM, SQ and buccal administration is given below to identify and compare key differences between both drugs across each route of administration.

1.1.4 Literature Review of Benzodiazepines Pharmacokinetics Following Rectal Administration

1.1.4.1 General Considerations for Rectal Administration

The rectal route is commonly used, particularly in young children, for the out-of-hospital treatment of seizure emergencies. Rectal administration is easily done by minimal training, partially avoids first pass metabolism and drug decomposition by stomach acids.

The most common obstacle to rectal administration is social resistance or embarrassment. Other negative aspects to consider in rectal drug administration include the risk of drug expulsion, variable absorption, degradation of drug molecules by microorganisms, and adsorption to fecal material.

1.1.4.2 Rectal Diazepam Pharmacokinetics in Healthy Volunteers

Various diazepam formulations have been administered rectally including solutions, suppositories, and a gel. Table 4 summarizes results of several pharmacokinetic studies of rectal diazepam in healthy volunteers using the commercially available rectal formulation (Diastat®). Results from pharmacokinetic studies conducted using diazepam solution and suppositories are not presented.

Table 4. Pharmacokinetics of Rectal Diazepam in Healthy Volunteers

Dose (mg)	Sample size (N)	Formulation	Cmax (ng/mL)	Tmax (hours)	Comparator/ Other comments	Ref.
10	12	Diastat gel	160±109[a]	0.75 (0.3-6.0)[b]	IN microemulsions	[30]
10	20	Diastat gel	220 (39)[c]	0.5 (0.17-1)[b]	IM autoinjector	[31]
10 10	24 24	Diastat gel (IR)[d] Diastat gel (RR)[e]	233 (42)[c] 209 (44)	1.4 (168)[c] 0.6 (76)	IM autoinjector	[32]
15	20	Diastat gel	447±91[a]	1.18±0.6[a]	IV infusion F = 90±10 %	[33]

[a] Mean ± SD
[b] Median (range)
[c] Mean (% CV)
[d] IR – 'Ideal' rectal (diazepam given after a cleansing enema the evening prior to dosing, overnight fast, and another enema in the morning prior to drug administration)
[e] RR – 'Real' rectal (diazepam given after a standard evening meal and bedtime snack on the day before dosing, and at 2 hours after a standard breakfast on the day of dosing)

An analysis of PK data in healthy volunteers from Table 4 confirms that there is high variability in both Cmax and Tmax when rectal diazepam gel is used. Dose-adjusted mean Cmax ranged from 16–30 ng/mL/mg (% Co-efficient of variability (CV) range for Cmax across studies: 20-60%). Similarly, the rate of absorption was variable with Tmax ranging from 18 minutes to 6 hours (Mean: ~1 hour, %CV range across studies: 50-168%). The mean absolute bioavailability of rectal diazepam gel in one study was shown to be 90% [33]. Across several studies of rectal diazepam gel, intermediate to high variability in exposure was reported (%CV for AUC0-inf: 30-45%).

1.1.4.3 Rectal Diazepam Pharmacokinetics in Patients

PK studies of diazepam rectal gel in patients were not found in my literature review. However, results of a rectal solution administered to patients (Table 5), show similar or shorter Tmax on average compared to that seen with rectal gel in healthy volunteers.

Table 5. Pharmacokinetics of Rectal Diazepam in Adult Patients

Dose (mg)	Sample size (N)	Formulation	Cmax (ng/mL)	Tmax (hours)	Comparator/ Other comments	Ref.
10	6	Rectal solution	309±68[a]	0.61±0.33[a]	Oral tabs & IV F=81±13	[34]
10	6	Rectal solution	Range (121-200)	Range (10-20 min)	IM and IV F=50±17%	[35]

[a] Mean ± SD

1.1.4.4 Rectal Midazolam Pharmacokinetics

In late 1980s and early 1990s, the results of pharmacokinetic studies involving rectal midazolam in infants, children, and adults following rectal midazolam administration were published [36-40]. Although average times to peak concentration were similar (~30

minutes) compared to rectal diazepam, mean rectal bioavailability of midazolam was very low in both children (18%) and adults (52%). This may be the reason why the commercial development of rectal midazolam has not been under taken.

1.1.5 Literature Review of Benzodiazepines Pharmacokinetics Following Intranasal Administration

1.1.5.1 General Considerations for Intranasal Administration

Intranasal administration is simple, convenient, does not require patient cooperation, and offers potentially rapid and extensive absorption of highly potent, highly lipid soluble drugs, resulting in a fast onset of effect. Challenges include overcoming the necessity for very small dose volumes (< 200μL per nostril) and slow absorption due to limited absorptive area of the nasal cavity (200 cm^2) that limit the potential for less potent, less lipid soluble compounds. Additional caveats include the need for understanding the effects of seizures on nasal absorption of these drugs, minimizing irritation of nasal tissues by organic solvents used in the formulations, and limiting loss of drug due to anterior or posterior drainage from the nasal cavity. Several of the benzodiazepines have the requisite physico-chemical and pharmacokinetic properties to use in IN formulations for seizure emergencies (see Table 1) Within a few years after the introduction of diazepam rectal gel, there was an accelerated interest in developing a more acceptable approach/route to treat seizure emergencies. To that end, IN formulations of both diazepam and midazolam were investigated to demonstrate the feasibility of this route in an emergency setting and pilot studies were conducted in healthy subjects to investigate both pharmacokinetics and tolerability of innovative formulations administered intranasally.

1.1.5.2 Intranasal Diazepam Pharmacokinetics in Healthy Volunteers

Intranasal diazepam pharmacokinetics in healthy volunteers have been investigated and reported in several studies using various formulations of diazepam. Table 6 summarizes results of intranasal diazepam studies in healthy volunteers. The intranasal formulations used by Agarwal et al [41] and Ivaturi et al [30] (shaded below in Table 6), or a close analogue, are under development in the US.

Table 6. Pharmacokinetics of Intranasal Diazepam in Healthy Volunteers

Dose (mg)	**Sample size (N)**	**Formulation**	**Cmax (ng/mL)** Mean±SD	**Tmax (min) Median(range)**	**F (%)[a]**	**Ref.**
10 10	24 24	Solution Suspension	272±100 221±78	90 (48–240) 60 (36-120)	97 67	[41]
10 10 13.4	12 12 12	Nas-A and B Microemulsions	181±84 151±108 180±82	45 (15 – 90) 45 (15-180) 45 (15-240)	88* 70* 89*	[30]
10 5	8 8	60-40% V/V Glycofurol+H_2O	247±60 134±60	30 (10-120) 20 (15-180)	74±53 75±41	[42]
5	3	60-40% V/V Glycofurol+H_2O	179±9	28.8 ± 20.96[b]	-	[43]
7	8	PEG 300	179(126-232)[a]	42 (25-59)	42 (22-62)	[44]
4	8	PEG 300	99 (83-115)[a]	18 (11-25)	45 (32-58)	[44]
2	9	PEG 200 + 5% Glycofurol	39±17	18±11[b]	50±23	[45]

*Relative bioavailability compared to Diastat rectal gel
[a] Mean (95% CI)
[b] Mean±SD

An analysis of diazepam pharmacokinetics reveals that absorption following intranasal administration appears to be somewhat faster (Median Tmax: < 1 hour) than rectal route based on Tmax (see Table 4 and 6). However, average relative bioavailability of the IN

formulation studied by Ivaturi et al [30] was 70-90% relative to rectal formulation. It also has to be noted that there is intermediate to high variability in both Tmax and total drug exposure of diazepam given by the intranasal route (% CV range across studies: 30-50%).

1.1.5.3 Intranasal Diazepam Pharmacokinetics in Patients

A multi-center, open-label, PK study was conducted in 30 adult patients using 0.2mg/kg dose of diazepam nasal spray. This study demonstrated that pharmacokinetics were not affected by seizure type or status at the time of dosing [46]. Dose-normalized average Cmax and AUC for patients dosed during a tonic-clonic ictal state relative to all other patients were similar (Mean Cmax: 194 vs. 215 ng/mL, and mean AUC0-12: 1258 vs. 1212 h.mg/mL, respectively). However, Tmax was not reported.

1.1.5.4 Intranasal Midazolam Pharmacokinetics

Table 7 summarizes results of several reports describing pharmacokinetics of IN midazolam in healthy volunteers. The IN formulation used by Wermeling et al [47] or a close analogue is being developed in US and has orphan designation. Although not tested head-to-head, the time to reach Cmax for midazolam appears shorter than for diazepam (see Table 6 and 7). Its bioavailability overlaps with diazepam, but trends toward a lower, intermediate range of 60-85%. Thus, IN midazolam may have an advantage over diazepam due to its faster absorption. Its shorter elimination half-life may be beneficial in that patients may more quickly return to normal function due to rapid offset of effect. Alternatively, the faster rate of elimination may expose patients to a higher rate of seizure recurrence. Ongoing clinical trials may provide insights into this issue.

Table 7. Pharmacokinetics of Intranasal Midazolam in Healthy Volunteers

Dose (mg)	Sample Size (N)	Formulation	Cmax (ng/mL) Mean±SD	Tmax (Minutes) Mean±SD	F (%) Mean±SD	Ref.
2.5 5.0 7.5	25 25 25	USL261 USL261 USL261	58.8 73.5 92.7	Range for all doses: 11.5-16	73 SD?	[48]
5	17	PEG 400 + Prop. Glycol	83.9±29	10 (5-20)[a]	60±23	[47]
3 6	12 12	Beta CD + Chitosan	52 (78)* 97(49)	8.4 (5-31)[a] 7.6 (5-22)	81(41 – 102)[a] 76(52 – 102)	[49]
5	7	Propylene glycol	78±40	43±19	82±38	[50]
3 3	8 8	Beta CD (12%) + Chitosan (0.5%) Beta CD (12%)	80.6±15 68.9±20	7.2±1 13±4	76±12 85±8	[51]
5	6	Prop. Glycol +Water pH - 4	71±25	14±5	83±19	[52]
5	3	Aqueous solution pH = 3	62.8±14.51	21.6±7.63	-	[43]
12-20	8	Aqueous solution	147 (91-225)[b]	25 (10-48)[b]	50±13	[53]

CD: Cyclodextrin
* Geometric mean (CV)
[a] Median (range)
[b] Mean (range)

1.1.5.5 Intranasal Administration Summary

Intranasal administration is a potential alternative for treatment of seizure emergencies in patients who object to rectal administration. Pharmacokinetics in healthy volunteers show that absorption may be somewhat faster than rectal diazepam, but bioavailability is widely variable. Two diazepam formulations and one midazolam formulation are being commercially developed in the US. All three formulations can be administered in small dose volumes, with no evidence of second peaks in plasma concentration-time profiles

presumably due to delayed absorption from swallowing a portion of a dose. Absorption from midazolam formulations appear to be relatively rapid compared to diazepam formulations but display lower bioavailability. Decreased bioavailability issues can be overcome by administering a large enough dose, but high variability might become a problem. Variability in bioavailability was wide with both diazepam and midazolam formulations. Nasal irritation following drug administration was noted in most studies with one exception (Agarwal et al [41]).

1.1.6 Literature Review of Benzodiazepines Pharmacokinetics Following Intramuscular Administration

1.1.6.1 General Considerations for Intramuscular Administration

Intramuscular injections can be self-administered or can be given by a caregiver using auto-injector devices as they do not require IV access. Although simple and easy to self-administer, some limitations of this route are pain at the injection site, need to remove clothing before administration, need for long needles (0.8-1.5 inch), and muscle mass related issues in elderly. Interest in IM auto-injectors of benzodiazepines to treat seizure emergencies was sparked by the commercial success of Diastat. An IM auto-injector of diazepam has been available for several years and is included in the Chempak (diazepam 5mg/mL, auto-injector).

1.1.6.2 Intramuscular Diazepam Pharmacokinetics

Table 8 summarizes results of pharmacokinetics of diazepam given intramuscularly by an auto-injector. The rate of absorption is slow to intermediate with average Tmax around 1 to 1.5 hour across studies. Although these trials did not include an IV arm in any of these

studies, one could assume that the bioavailability would be 100% by intramuscular route. Inter-individual variability in Cmax and AUC was wide (%CV: 20-30% for Cmax and 30-60% for AUC). This product was recently granted orphan designation in May 2013. Abou-Khalil et al. reported the results of a randomized, placebo-controlled study of IM diazepam auto-injector for treatment of ARS in which the drug was significantly better at preventing seizure recurrence when compared to placebo [54].

Table 8. Pharmacokinetics of Intramuscular Diazepam in Healthy Volunteers

Dose (mg)	Sample Size (N)	Formulation/ Device	Cmax (ng/mL) Mean(CV)	Tmax (Minutes) Mean (CV)	Comparator	Ref.
10	24	Meredian Medical Tech. Auto-injector (AI)	303 (23)	52 (62)	Rectal Diastat	[32]
5 10 15	23 23 22	King Pharmaceuticals /Meredian AI	124 (31) 226 (25) 360 (28)	60 (15 -75)[a] 60 (15-90) 60 (15-120)	Rectal Diastat	[31]
10 20	24 24	Meredian AI	272 (33) 511(22)	94 (34) 90 (35)	None	[55]

[a] Median(range)

1.1.6.3 Intramuscular Midazolam Pharmacokinetics

Table 9 provides the pharmacokinetic results of intramuscular midazolam studies in healthy volunteers. Intramuscular administration of midazolam using an auto-injector results in rapid absorption compared to IM diazepam (Median Tmax for IM midazolam is about 30 minutes and that for IM diazepam is about 1 hour). Variability in Cmax and AUC was relatively low compared to diazepam (%CV: 13-35% for Cmax and 10-44% for AUC).

Midazolam has been shown to be most potent and rapidly acting in terminating soman-induced seizures as compared to IM diazepam and IM lorazepam [56]. A non-inferiority trial funded by the Department of Defense and the National Institute for Neurological Disorders and Stroke compared the efficacy and safety of intramuscular midazolam (5-10 mg) versus intravenous lorazepam (2-4 mg) for children and adults in status epilepticus treated by paramedics. Results of this study showed that intramuscular midazolam was superior to intravenous lorazepam in terminating seizures [57]. Both treatment groups were similar with respect to need for endotracheal intubation and recurrence of seizures. As the IM route does not require establishing an IV line, the median time to initiate treatment was shorter (1.2 minutes) in the midazolam group than in the IV lorazepam group (4.8 minutes), with a corresponding median time from initiating treatment to cessation of convulsions of 3.3 minutes and 1.6 minutes, respectively.

Table 9. Pharmacokinetics of Intramuscular Midazolam in Healthy Volunteers

Dose (mg)	**Sample Size (N)**	**Formulation /Device**	**Cmax (ng/mL) Mean(CV)**	**Tmax (Hours)** Median (range)	**Comparator**	**Ref.**
5 10 15 20 25 30	4 3 8 5 9 10	Meredian Medical Tech. Auto-injector (AI)	61.3(13.5) 111.2(27.1) 196.6(26) 207.2 (34.6) 266.9(15.9) 417.7 (25.8)	0.6 (0.5, 0.75) 0.5 (0.25, 0.5) 0.4 (0.25, 0.85) 0.5 (0.33, 1.00) 0.5 (0.33, 0.75) 0.5 (0.25, 1.5)	None	[58]
5	4	Conventional IM injection	100.5 (21)	0.3 (0.15, 0.4)	Conventional IM diazepam injection	[59]

1.1.6.4 Intramuscular administration Summary

Intramuscular administration of benzodiazepines offers a potential alternative to treatment of out-of-hospital seizure emergencies. Midazolam, given by the intramuscular route, is absorbed faster than diazepam and is the preferred benzodiazepine when given by this route. A diazepam auto-injector is being developed for marketing in US and has orphan designation for ARS. Midazolam auto-injector has orphan designation for soman-induced seizures and may be developed for marketing.

1.1.7 Subcutaneous Administration

There are no published pharmacokinetic studies of diazepam given subcutaneously to humans. Two pharmacokinetic studies of midazolam given subcutaneously were found in literature [60, 61]. Healthy subjects received single doses of 0.05-0.1mg/kg of subcutaneous and IV midazolam. In both studies, average Tmax was in the range of 25-30 minutes. Pecking et al. reported average Tmax (SD) and absolute bioavailability (SD) in their study to be 0.5 (0.18) hours and 96 (14)% respectively [60].

1.1.8 Buccal Administration

Midazolam has been studied by this route by several investigational groups. Two products (Epistatus™ and Buccolam®) are commercially available in the United Kingdom. Muchohi et al [62] investigated the pharmacokinetics of buccal midazolam in 8 children with severe malaria and convulsions. Median (range) Cmax of 186 (64-394) ng/mL were attained within a median (range) Tmax of 10 (5-40) minutes following administration. A study of buccal and intravenous midazolam pharmacokinetics in 8

healthy volunteers indicated that the buccal absorption is comparable to rectal diazepam with good bioavailability [63]. Scott *et al.* studied the buccal midazolam administration in 10 healthy volunteers who were administered 2mL of the intravenous preparation of midazolam (5mg/mL), flavored with peppermint. The subjects were asked to hold the solution in the mouth for 5 minutes and then spit it out. Changes in EEG were observed within 5 to 10 minutes after drug administration indicating rapid absorption and onset of effect [64].

1.1.9 Development of Intranasal Diazepam for Management of Acute Repetitive Seizures

Intranasal benzodiazepines appear to be particularly promising and several research groups have carried out studies to investigate the pharmacokinetics, bioavailability and tolerability [65-77]. Diazepam has been proven safe and effective and has been used for about 15 years for treatment of ARS. It is likely that clinicians would be more comfortable prescribing IN diazepam because of their clinical experience with diazepam in ARS patients. For IN diazepam, market approval of a commercial product is possible without conducting a safety and efficacy trial. A sponsor of IN diazepam could rely on efficacy performance of rectal diazepam and conduct only pharmacokinetics and safety studies for intranasal diazepam, but not necessarily a well-controlled, efficacy and safety trial. In contrast, clinicians lack such experience with midazolam in ARS patients and a midazolam product will need a well-controlled trial to show efficacy. From a drug development perspective, it would be more cost effective to conduct safety and PK studies for IN diazepam than to conduct a well-controlled efficacy trial for nasal midazolam.

1.2 Spasticity

Spasticity is defined as velocity-dependent resistance to movement associated with increased deep tendon reflexes and clonus. Spasticity is a well-known muscle tone disorder, characterized by tight or stiff muscles. In addition, reflexes may persist for too long and may be too strong (hyperactive reflexes). Spasticity occurs in disorders of the central nervous system (CNS) affecting the upper motor neuron (Figure 3) [78], most commonly arising after stroke, multiple sclerosis, spinal cord injury (SCI), some traumatic brain injuries, cerebral palsy and other CNS lesions. Causes of spasticity may include tumor, central nervous system infarct, neurodegenerative disorders, metabolic disorders, or hydrocephalus.

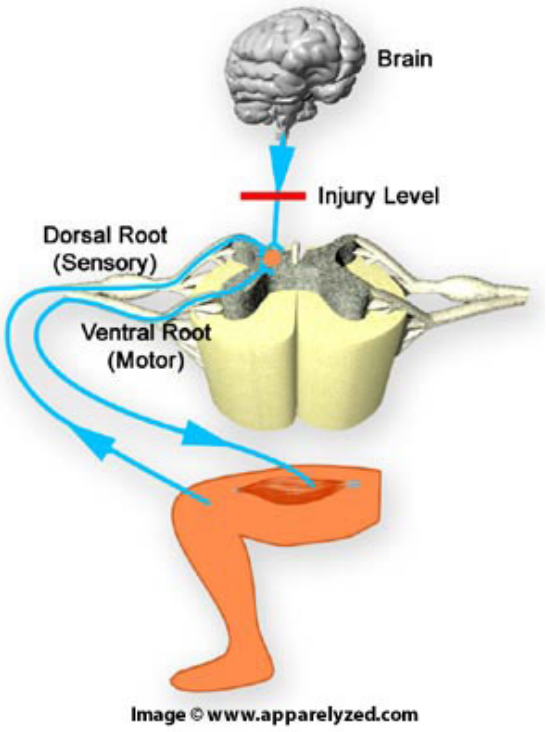

Figure 3: Upper Motor Neuron Injury Level Causing Spasticity

In general, spasticity develops when an imbalance occurs in the excitatory and inhibitory input to α motor neurons caused by damage to the spinal cord and/or brain [79]. The damage causes a change in the balance of signals between the nervous system and the muscles, leading to increased tonicity in the muscles. Spasticity affects approximately 70% of those with cerebral palsy (CP) and is the most common tonal abnormality associated with CP [80]. Dystonia is another common type of increased muscle tone associated with CP. Dystonia is defined as an involuntary alteration in the pattern of muscle activation during voluntary movement. Dystonia is characterized by varying tone often increased with intent or emotion and associated with assuming abnormal postures [81]. Hypertonicity in muscles interferes with functional abilities and contributes to the development of bony deformities and contractures. The pain associated with spasticity can be as mild as a feeling of tight muscles, or it can be severe enough to produce painful spasms of the extremities, usually the legs. Spasticity also can cause low back pain and result in feelings of pain or tightness in and around joints.

The presence of spasticity alone is not considered sufficient to warrant its treatment [82]. It is essential to set goals that are to be achieved through spasticity reduction. Treating spasticity will improve functional abilities and limit contracture formation [83]. Treatment for spasticity may include medications like baclofen, clonidine, tizanidine, dantrolene, gabapentin, diazepam, or clonazepam. Occupational and physical therapy programs, involving muscle stretching and range of motion exercises, and sometimes the use of braces, may help prevent tendon shortening. Rehabilitation also may help to reduce or stabilize the severity of symptoms and to improve functional performance.

Local injections of phenol or botulinum toxin may be used to relax specific muscles. Surgery may be recommended for tendon release, to cut the nerve-muscle pathway, or to implant a baclofen pump (intrathecal baclofen therapy). No single medication has been universally effective in the treatment of spasticity [84], which is not surprising given that spasticity is a result of multiple factors caused by several different mechanisms. Table 10 and Figure 4 provide the sites and mechanisms of action of anti-spasticity medications.

Table 10. Sites and Mechanisms of Action of Anti-spastic Drugs [78]

Drug	Site of Action	Mechanism of Action
Diazepam and Clonazepam	Spinal cord – presynaptic inhibition	Increase GABA affinity for $GABA_A$ receptors
Baclofen	Spinal cord – presynaptic inhibition	Binds to $GABA_B$ receptors
Clonidine and Tizanidine	Spinal cord	Hyperpolarize neurons, decrease excitatory amino acids, possible role of substance P
Dantrolene Sodium	Skeletal muscle	Inhibits release of calcium at sarcoplasmic reticulum
Gabapentin	Spinal cord	GABA

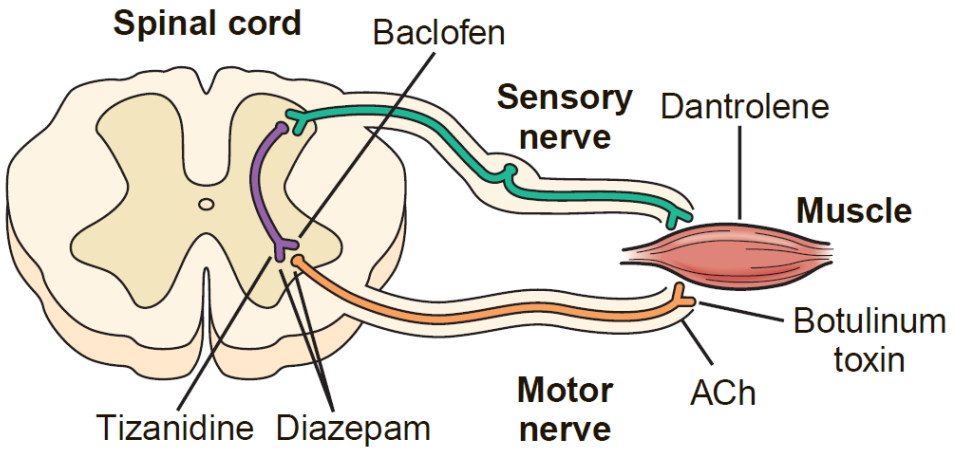

Figure 4: Sites of Action of Anti-Spasticity Drugs [85]

1.2.1 Baclofen

Baclofen is used in the treatment of spasticity. Its mechanism of action is to act at the level of spinal cord as an agonist at $GABA_B$ receptors. It suppresses the release of excitatory neurotransmitters and results in presynaptic inhibition, inhibition of mono- and poly-synaptic reflexes [86], reduces muscle-spindle activity and reduces gamma motor activity. It is available both as tablets and as an injectable solution, of which the latter is used in intrathecal pumps. The $GABA_B$ activity of racemic baclofen is known to be due to R-(-)-enantiomer (Figure 5) [87]. Table 11 shows the physicochemical properties of baclofen.

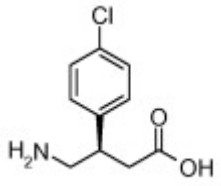

Figure 5: Chemical Structure of R-(-) Baclofen. Chemical name: 4-amino-3-(4-chlorophenyl) butanoic acid

Table 11. Physico-chemical Properties of Baclofen [88]

Molecular Weight	pKa	Water Solubility (mg/mL)	Octanol/Water Ratio [89]	Protein Binding (%) [90]
213.6	4	4.3	0.11	30

1.2.1.1. Oral Baclofen

Baclofen is rapidly absorbed when given orally and eliminated with a plasma half-life of 3-7 hours [91]. 85% of baclofen is excreted unchanged in urine and feces, while 15% is metabolized [92]. Adults are typically started on 5 mg three times a day orally and then titrated up 5 mg every 3 days until optimal effect is achieved. Some patients, to

experience adequate symptomatic relief, require dosages of baclofen that significantly exceed the conventional 80 mg daily maximum [93]. However, as the doses are increased, side effects such as somnolence, dizziness, and muscle weakness are observed [94]. Baclofen given orally often fails to produce sufficient relief. This could be possibly due to low bioavailability of drug at the site of action (spinal cord). Bioavailability at spinal cord can be markedly improved by local administration of baclofen at the site of action i.e., intrathecal drug delivery. Intrathecal administration of baclofen in the treatment of severe spasticity was proposed in 1984 by Richard Penn [95].

1.2.1.2. Intrathecal Baclofen

The introduction of intrathecal baclofen (ITB) served to address some of the shortcomings of oral baclofen. Patients on intrathecal baclofen need lower doses and typically start with 50-100 mcg/day and are titrated up 5-15% of the dose once every 24 hours until optimal effect is achieved. Intrathecal therapy has the potential to lower side effects by reducing drug exposure at supraspinal sites. Other advantages include minimal protein binding and enzymatic degradation in cerebrospinal fluid (CSF) compared to plasma, leading to longer half-life in CSF [96, 97]. ITB is pumped slowly into the cerebrospinal fluid (CSF) at the subarachnoid space by an implanted catheter, allowing for reduced, but still efficacious doses to be delivered at the site of action with much smaller systemic exposure. ITB is an option for patients with spasticity or dystonia unresolved by other pharmacological and non-pharmacological treatments. Furthermore, patients who do not respond sufficiently to oral baclofen may respond to ITB [98]. To understand factors affecting spinal drug delivery, presented below is a discussion of

anatomy of spinal cord, physiology of CSF, and movement of drug following epidural and intrathecal drug delivery.

1.2.2 Anatomy of Spinal Cord

The spinal cord is about 16-18 inches long and is basically a uniform structure throughout its length. The spinal cord is contained in the vertebral arch (back bone) which protects it from injury. It has an inner mass of gray matter and an outer covering of white matter. It carries messages that coordinate movement and sensation. The cord is an ovoid shaped column of nerve tissue that extends from the medulla at the underside of the brain down in the spinal column to the second lumbar vertebrae. The spinal cord is protected by the bones of the spinal arch and enclosed in the protective tissue of the three meninges (dura mater, arachnoid mater and pia mater) and CSF. The center of the cord is gray matter and shaped like an H (Figure 6). The white matter is arranged in tracts around the gray matter and consists of axons that transmit impulses to and from the brain or between levels of gray matter in the spinal cord.

The spinal cord has two basic functions. It can act as a nerve center and can work without the brain. The spinal cord carries sensory impulses to the brain and motor impulses from the brain. The spinal cord also controls reflexive activity such as stretch reflexes, bowel and bladder control, and withdrawal from a painful stimulus. The brain can sometimes modulate these reflexive activities. Thirty-one pairs of nerves exit from the spinal cord and innervate our body and limbs. The spinal cord also acts as a nerve center between the brain and the rest of our body.

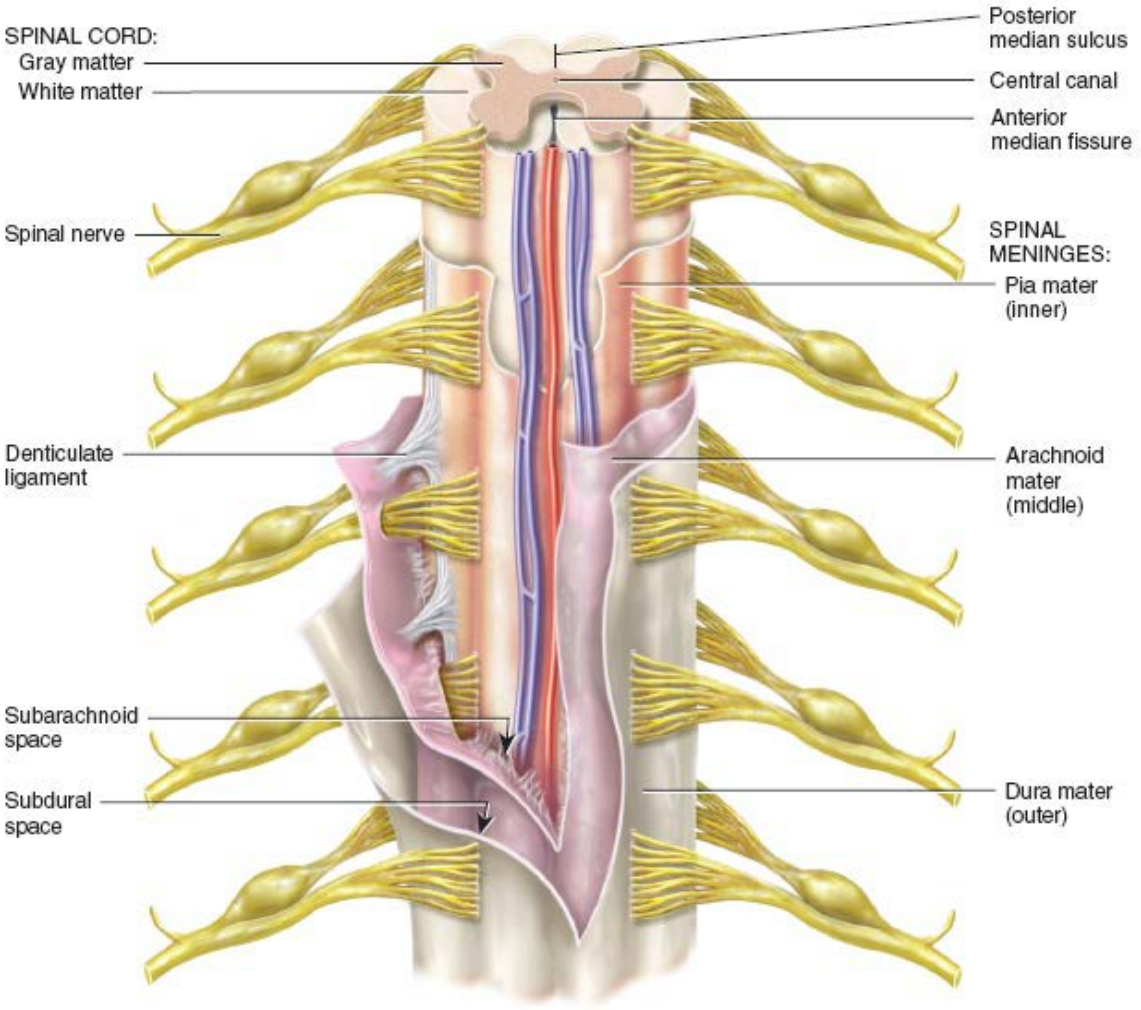

Figure 6: Anterior View and Transverse Section Through Spinal Cord [99]

1.2.3 Physiology of Cerebrospinal Fluid

1.2.3.1 CSF Formation

The rate of CSF formation is about 0.35-0.40 ml/min or 500-600 ml/day in humans. Approximately 0.25% of total adult CSF volume is replaced by freshly formed CSF each minute. The turnover time for total CSF volume is about 5-7 hours, yielding a turnover rate of about four times per day. Roughly 40-70% of CSF enters the macroscopic spaces via the choroid plexus, whereas 30-60% of CSF enters the macroscopic spaces from

extrachoroidal sites. Total CSF volume in man is 100-160 ml with ventricular volume being 16-27 ml.

Unlike the capillary endothelium (CE) of the blood-brain-barrier, the CE of the choroid plexus is fenestrated and does not possess tight junctions between cells (Figure 7). Blood entering choroid plexus capillaries is filtered across this endothelium and forms a protein-rich fluid within the choroid plexus stroma that is similar in composition to interstitial fluid in other tissues of the body. The choroid plexus stroma is separated from macroscopic CSF spaces by epithelial cells. These contain apical tight junctions that restrict passive solute exchange and constitute a "blood-CSF barrier" at the choroid plexus. Substances in the choroid plexus stromal fluid are transported across the relatively impermeable epithelial cells of choroid plexus by the combined processes of ultrafiltration and secretion. 60% of the extrachoroidal formation of CSF results from oxidation of glucose by the brain, and 40% results from ultrafiltration from cerebral capillaries. A very negligible amount of CSF forms in the spinal cord.

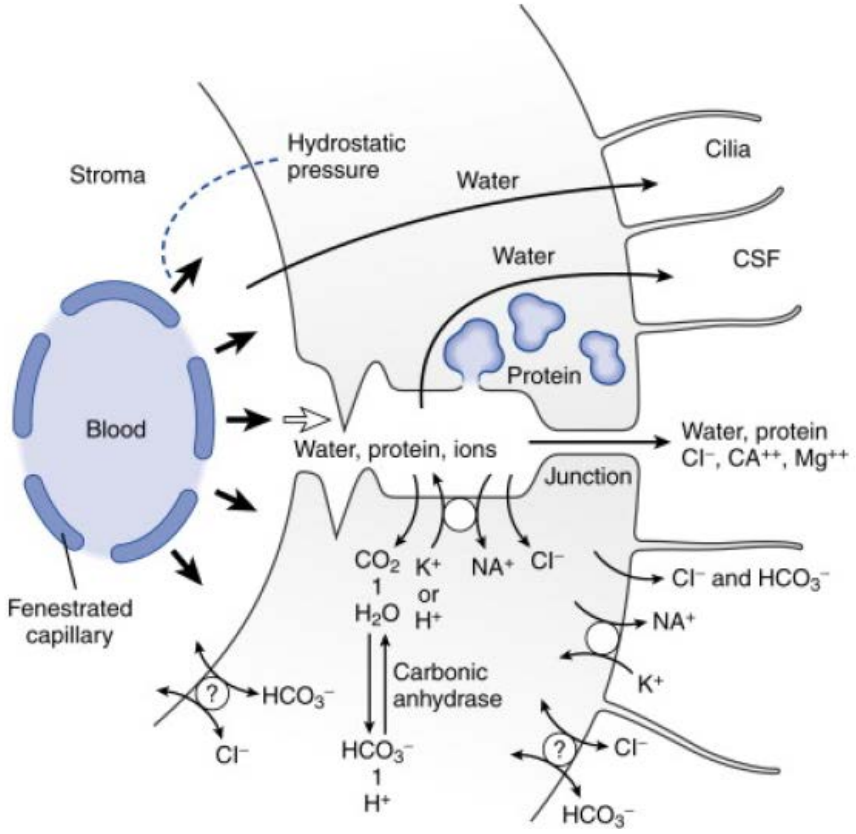

Figure 7: Processes Involved in CSF Formation at the Choroid Plexus [100]

1.2.3.2 CSF Circulation

Radioisotope studies indicate that labeled CSF flows from the ventricles to the basal cisterns within a few minutes, low cervical-high thoracic region at 10-20 minutes, thoracolumbar area at 30-40 minutes, lumbosacral cul de sac at 60-90 minutes, and basal cisterns at 2-2.5 hours [101] (round trip from brain to spinal cord to brain). About 20-33% of the labeled CSF reaches the intracranial cavity within 12h. CSF circulation concludes with reabsorption across arachnoid villi into the superior sagittal sinus and spinal dural sinusoids located on dorsal nerve roots. Circulation of CSF is not

substantially altered by posture or ambulation, although physical activity perturbs CSF concentration gradients by promoting CSF mixing.

1.2.4 Drug Kinetics Following Spinal Administration

Similar to any other route of administration, drugs injected into the epidural or intrathecal space follow Fick's law of diffusion and can be transported actively. The drug molecules will move down a concentration gradient by simple Brownian motion until they partition into the micro-environments (eg: epidural fat, collagen, CSF, white matter, myelin etc.,) in which they are thermodynamically most stable. The physicochemical properties of the drug play a crucial role in determining which micro-environment it will partition to, which in turn determines the bioavailability of a given drug at its intended target site. For example, a drug which is lipophilic will preferentially distribute and get sequestered into epidural fat and will not be highly available in the gray and white matter of spinal cord. Delivery of drugs into intrathecal or epidural space can achieve target site concentrations that are not readily attainable following conventional routes such as oral or intravenous delivery. Additional advantage from such delivery is to restrict drugs to their spinal site of action thereby avoiding drug related systemic side-effects or toxicities. It is important to understand the factors affecting drug distribution from the epidural and intrathecal space to the spinal cord while limiting redistribution to extra-spinal sites.

1.2.4.1 Systemic Distribution

Physico-chemical properties of the drug will govern the rate at which drug enters systemic circulation. It is interesting to note that rate of clearance from epidural space of etidocaine (a relatively hydrophobic, highly plasma protein bound (94%) drug) from the

epidural space is significantly slower than that of lidocaine (a relatively hydrophilic, less protein bound (64%) drug). So, this suggests that increasing lipid solubility allows drugs to be effectively sequestered into epidural fat. In combination, increased protein binding with high drug lipophilicity leads to longer drug residence time in epidural space.

1.2.4.2 Drug Distribution through Spinal Meninges to Spinal Cord

Drug distribution through the spinal meninges has been demonstrated to occur by simple diffusion *in vitro* [102-105]. Though possible, there is no evidence for active transport or facilitated diffusion of drug molecules through spinal meninges [106]. Conventional principles of drug diffusion suggest that increasing lipid solubility would result in greater drug permeability through the spinal meninges. Bernards and Hill [104] studied eight drug molecules and found no relationship between the drugs' permeability coefficients determined using the spinal meninges of monkeys and any measure of drug mass, molecular shape, or molecular size. There was, however, a biphasic relationship between the octanol: buffer distribution coefficient and the drugs' measured permeability coefficients. Drugs that were either very hydrophilic or very hydrophobic had permeability coefficients that were significantly less than drugs of intermediate hydrophobicity (Figure 8). One explanation for such “biphasic” relationship would be, as hydrophobicity increases presumably hydrophobic drugs may effectively get sequestered in the lipid bilayer of the arachnoid membrane. The same hydrophobic character which renders a drug to readily partition into the lipid bilayer of the cell membrane would lead to difficulty in partitioning out of the cell membrane to re-enter the aqueous intra- or inter-cellular space. Hydrophilic drugs would be expected to have quite the opposite

problem. They would have difficulty entering the lipid bilayer at the first place. Thus, drugs with intermediate hydrophobicity (log P of 2) will move through the arachnoid mater most rapidly. Although this explanation is not proved, it has been used successfully to increase the meningeal permeability of both hydrophilic and hydrophobic drugs. One way to improve the permeability of hydrophobic drugs is to increase their aqueous solubility. For example, combining sufentanil (lipophilic compound) with hydroxypropyl-b-cyclodextrin increases sufentanil's permeability across meninges by more than 2 fold *in vitro* [107]. Similarly, *in vitro* studies demonstrate that the meningeal permeability of hydrophilic drugs can be increased by addition of acyl-carnitines to the drug solution [105, 108]. Baclofen has a log P value of -0.96 and it preferentially partitions into hydrophilic environment. Using the model described above, baclofen given epidurally will not diffuse through arachnoid membrane. Addition of acyl-carnitines to baclofen might be helpful in increasing the permeability across spinal meninges.

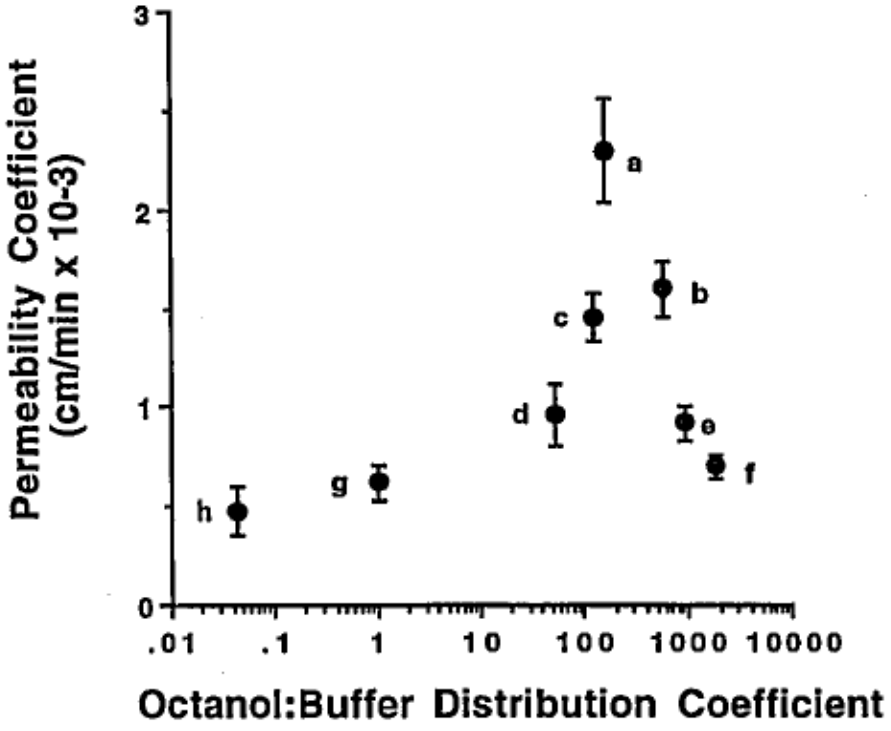

Figure 8: Plot of Octanol: buffer (pH = 7.4) Distribution Coefficient vs. the Experimentally Determined Permeability Coefficient;
(a = alfentanil; b=bupivacaine; c=lidocaine; d=haloperidol; e=fentanyl; f=sufentanil; g=morphine; h=tetracycline)

1.2.5 Drug Kinetics Following Intrathecal Administration

One would expect that the drug behavior in the CSF would be independent of whether it got there by diffusing across the spinal meninges from the epidural space or was injected directly into CSF. However, this is not the case. Direct injection into CSF via spinal needle or catheter produces different "initial conditions" compared to drugs which were initially delivered into epidural space. Intrathecal injection results in mixing of injectate with CSF, which does not occur with epidurally administered drugs. In addition, the patient position at the time of injection also affects the initial distribution of hyperbaric and hypobaric drugs.

One other factor which affects the initial distribution of intrathecal drugs is the baricity. Baricity is defined as the ratio of the density of the injectate compared to the density of CSF which averages 1.0003 g/mL at 37 degree centigrade. Hyperbaric solutions (density >1 g/mL) will move down in the CSF column in response to gravity to rest in the most dependent portion of the spinal column. In contrast, hypobaric solutions move up the CSF column and isobaric solutions (though difficult to make as we don't know the density of CSF of an individual exactly) don't move in response to gravity. Hyperbaric and hypobaric solutions only move until the solution is sufficiently diluted by CSF so that it becomes isobaric.

1.2.5.1 Drug Movement in Cerebrospinal Fluid

CSF flow within spinal canal significantly affects the distribution of drugs. CSF is produced within the brain and reaches the spinal cord by flowing caudal along the dorsal aspect of the spinal cord to the sacral cul de sac and then flows rostrally along the ventral surface of the cord to reach the basal cisterns. The total "round-trip" time in humans is 2-2.5 hours. In conjunction to flow of CSF, there is also a pulsatile to and fro motion imparted to the CSF by the normal pulsatile motion of the brain that occurs with heart beat.

1.2.5.2 Drug Clearance from Cerebrospinal Fluid

Drug in CSF is rapidly removed by uptake into spinal cord and also by diffusion across the meninges and into the epidural space. Drug clearance from the CSF can be studied by sampling CSF via a catheter at various time points following intrathecal injection.

However, this methodology has two shortcomings. One is that the method of sampling itself disturbs the CSF distribution of the drug by creating CSF turbulence and may thereby alter the drug's elimination kinetics. The second and even greater shortcoming is that it does not yield any information about the site to which the drug is cleared, which is critical information since one is rarely targeting the CSF *per se*.

Loss of drug to the epidural space is an underappreciated source of drug removal. *In vivo* studies in pigs show that 60% of the intrathecal dose of morphine was lost to the epidural space and the remaining was cleared into the spinal cord [109]. In the same study it was found that the epidural fat appears to act as a site for sequestration of intrathecally administered fentanyl and sufentanil. Loss of lipophilic drug to epidural space is greater than hydrophilic drugs and therefore, the bioavailability of lipid soluble drugs within the spinal cord receptors is much less than that of more hydrophilic drugs.

1.2.5.3 Drug Movement in Spinal Cord

Drugs administered in the epidural space or intrathecal space partition into the spinal cord by diffusion to reach the specific neuronal or receptor population that is being targeted. Spinal cord has two very different environments into which the drug molecules can diffuse (Figure 6). The outer portion of the spinal cord which is called white matter consists of myelinated axons running between the brain and spinal cord. The white matter surrounds the inner portion of the cord which is called gray matter.

Gray matter consists of unmyelinated neuronal cell bodies. Presence of myelin in the white matter is perhaps the most important difference between the gray and white matter with respect to drug diffusion in these two environments. Myelin is composed of 70%

lipid and 30% protein. Therefore, white matter largely represents a relatively hydrophobic environment compared to the gray matter and hydrophobic drugs would preferentially accumulate in it. In contrast, gray matter which consists of unmyelinated neuronal cell bodies will preferentially accumulate hydrophilic drugs. This preferential partitioning of hydrophilic and hydrophobic drugs in gray and white matter, respectively has not yet been adequately studied. So, it cannot be said with certainty if drugs will distribute within the spinal cord as predicted. However, drug movement in the brain has been extensively studied and can provide important clues for drug movement in spinal cord.

Herz and Teschemacher [110] injected radiolabeled morphine, hydromorphone and fentanyl into the lateral cerebral ventricle of rabbits and measured the depth of drug penetration at various times using autoradiography. They found that the hydrophilic drugs morphine and hydromorphone penetrated much deeper into the brain than the relatively hydrophobic drug fentanyl. It was also pointed out that fentanyl preferentially accumulated in the white matter and the other two drugs preferentially accumulated in the gray matter. Consequently, one would expect the bioavailability of lipid soluble drugs in the gray matter to be limited and therefore lead to reduced intensity and duration of drug effect if the site of action is in gray matter. For example, the relative analgesic potency of sufentanil compared to morphine in humans is approximately 20-40 times less following intrathecal administration compared to intravenous administration.

1.2.6 Transport of Baclofen from Systemic Circulation to Site of Action

Baclofen is currently available as oral tablets and an injection solution which is used in intrathecal pumps. When given orally it first gets absorbed into systemic circulation and then has to reach the site of action before it can elicit its therapeutic (eg: antispasticity) or side effects. The site of action for its antispastic effect is spinal cord and for some of the side effects such as sedation, dizziness and ataxia is brain. Therefore, one can conclude that baclofen can enter both into brain and spinal cord when given orally although the mechanism of transport to site of action is unknown.

From the above discussion, one can propose the following three mechanisms for baclofen transport from blood to its site of action in spinal cord.

1. **Passive diffusion** through spinal meninges into intrathecal space. The octanol-water distribution coefficient of baclofen is 0.11 (Log P = -0.96) [89]. From Figure 8, one can infer that a molecule with a log P value of 2 will diffuse rapidly through spinal meninges. Given the low lipophilicity of baclofen, passive diffusion across spinal meninges would be limited as the lipophilic arachnoid mater will act as a barrier to drug diffusion.

2. Although possible but not supported by any evidence, **active transport** of baclofen from blood across the spinal meninges and then into intrathecal space.

3. Transport of baclofen from **Blood to Choroid Plexus to CSF** and then percolation down into spinal canal. The transport of baclofen from blood to choroid plexus and eventually to CSF could be from passive diffusion and/or active transport at the tight junctions of epithelial cells of blood-CSF-barrier.

Baclofen concentrations determined by microdialysis after intravenous administration in rats were 27-fold higher in plasma than in interstitial fluid (ISF), whole brain tissue and CSF in [111]. This is regarded as surprising [89] because it was previously found to be a substrate of the neutral amino acid transporter [112], which should also be present in the BBB [113]. One hypothesis for limited distribution of baclofen to the CNS could be due to the affinity to an effective probenecid sensitive efflux system for organic anions [111].

1.2.7 Baclofen Withdrawal

Baclofen withdrawal occurs due to interruption in oral baclofen therapy as a result of illness such as gastroenteritis or ileus, non-compliance, or a scheduled surgery for which patients must temporarily stop taking oral medications. It could be also a result of interruption of intrathecal baclofen under circumstances of catheter displacement from the intrathecal space, pump infection or some other reasons of mechanical failure of pump. Baclofen withdrawal syndrome can be quite severe, resulting in a rebound increase in muscle tone and spasms, prolonged seizures, status epilepticus, hallucinations, dysesthesias, hyperthermia, and a neuromalignant syndrome-like picture potentially resulting in rhabdomyolysis and multisystem organ failure [114-120] . Some of these symptoms are due to withdrawal of baclofen from cerebral receptor sites. Therefore to treat baclofen withdrawal successfully, it is important to target both brain and spinal cord receptors.

1.2.8 Current Treatment Strategies and Limitations

The current recommended management of baclofen withdrawal is inadequate. In the case of interruption of ITB therapy, attempts to replace the medication with oral baclofen require large doses in an effort to control withdrawal symptoms [116, 121]. Moreover, in spite of

using large doses, symptoms are not controlled completely and adverse effects including sedation, nausea, vomiting and dizziness may occur. Diazepam is used by most clinicians for its anti-spasticity effect and to decrease the likelihood of acute seizures; however, could result in sedation and respiratory depression [116, 122].

1.2.9 Intravenous Baclofen for Management of Baclofen Withdrawal

Use of an intravenous (IV) baclofen formulation could prevent or minimize the complications in individuals in whom oral or intrathecal drug delivery is interrupted. Intravenous administration of baclofen would permit rapid attainment of drug concentrations as well as accurate and precise dose titration, thus allowing for more expeditious treatment of withdrawal symptoms and reduced risk of adverse effects.

1.2.10 Development of Intravenous Baclofen

Although oral and intrathecal baclofen have been used for decades, safety and PK of intravenous baclofen in humans is not well characterized. While there are several reports of IV baclofen administration in both animals and humans [123-126], details of the formulation used in these studies and pharmacokinetic results were not reported. In addition, very limited information was provided about the dose selection and adverse events in these studies. An FDA-approved intravenous formulation of baclofen is not currently available. A recent clinical study in patients used an extemporaneously compounded sterile formulation of baclofen (2.5mg/mL, pH 6.6) to reduce pain from muscle spasm and migraines (J Krusz, personal communication 2013).

These circumstances suggest that a FDA-approved commercial IV baclofen product would enhance management of patients with spasticity. The first step in the development of such a

product is demonstration of its safety in an animal model. Therefore, a pilot study in dogs was conducted to assess the bioavailability, short-term safety, and tolerance of intravenous baclofen in comparison to oral administration. IV baclofen at doses of 0.5 to 3 mg/kg was well tolerated in dogs [127]. The next phase in the development of an IV baclofen formulation is to conduct a series of investigations in humans. To that end, a pilot study was conducted to assess pharmacokinetics using non-compartmental analysis, and the tolerability of baclofen given intravenously to healthy volunteers.

CHAPTER 2

DISSERTATION RESEARCH OBJECTIVES

2.1 Development of Intranasal Diazepam for Management of Seizure Emergencies

To meet the need for an alternate therapy for seizure emergencies, we have investigated several intranasal (IN) formulations of diazepam. Studies conducted in our research group have demonstrated the feasibility of nasal administration of diazepam. Diazepam was absorbed rapidly following nasal administration and the pharmacokinetic (PK) profile of IN formulations compared favorably to that of the rectal diazepam gel. However, the tolerability was only moderate.

The results of earlier studies concluded that IN diazepam offers a viable alternative to rectal administration. However further enhancement of formulations was needed to both improve tolerability and the extent and consistency of absorption. To that end, two novel formulations of diazepam nasal spray were developed. A pilot study was conducted to characterize the PK and tolerability of these two novel nasal formulations of diazepam. Further, in addition to data from current work, archived rectal and intravenous diazepam PK data from five previously conducted studies were combined to build a population PK model and perform simulations to optimize future bioequivalence trials. The specific research objectives include:

1. Assessment of the PK using non-compartmental analysis, and tolerability of two different formulations of intranasally- administered diazepam.
2. Development of a PK model, using non-linear mixed effects modeling approach, to characterize diazepam absorption and disposition.

3. Perform a simulation study to test the hypothesis that an investigational IN product will produce exposure similar to that obtained following rectal administration.
4. Perform deconvolution analysis to compare the absorption characteristics of diazepam after IN and rectal administration

2.2 Development of Intravenous Baclofen for Management of Baclofen Withdrawal

To enhance management of patients with spasticity, our research group has proposed development of IV baclofen. A pilot study in dogs showed that 0.5- 3 mg/kg single doses of IV baclofen were well tolerated by dogs. The next phase in the development of an IV baclofen formulation would be to conduct a series of investigations in humans. To that end, a pilot study was conducted to assess PK and tolerability of baclofen given intravenously to healthy volunteers. The specific research objectives are:

1. Assessment of the PK, using non-compartmental analysis, of orally and intravenously administered baclofen, and
2. Assessment of tolerability of intravenously administered baclofen.

2.3 My Role in Projects

Grant applications

Co-wrote and submitted two grant applications to Paralyzed Veterans of America Research Foundation for the following studies:

- Two-way crossover study of oral and intravenous baclofen in healthy adults
- Dose escalation study of intravenous baclofen in healthy adult volunteers

Investigational new drug applications

- Prepared and submitted an investigational new drug (IND) amendment for use of intranasal diazepam in healthy adult volunteers
- Prepared and submitted an investigational new drug (IND) application for use of intravenous baclofen in healthy adult volunteers
- Prepared and submitted an investigational new drug (IND) amendment for use of escalating doses of intravenous baclofen in healthy adult volunteers

Phase 1 study of two intranasal formulations of diazepam in healthy adult volunteers

- Assisted with designing the study and co-wrote the protocol
- Prepared, submitted and got approval for an IRB application
- Prepared and submitted an investigational new drug (IND) amendment for use of intranasal diazepam in healthy adult volunteers
- Served as study coordinator, supervised and managed the study
- Analyzed the pharmacokinetic data using non-compartmental analysis, non-linear mixed effects modeling, and deconvolution approach

- Developed an Orphan Drug Designation Application for Diazepam Nasal Spray Product

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

- Designed the study and co-wrote the protocol
- Prepared, submitted and got approval for an IRB application
- Prepared and submitted an investigational new drug (IND) application for use of intravenous baclofen in healthy adult volunteers
- Co-wrote and submitted a grant application to Paralyzed Veterans of America Research Foundation
- Served as study coordinator, supervised and managed the study
- Analyzed the blood plasma samples for baclofen concentrations using a validated liquid chromatography/mass spectrometry based assay.
- Analyzed the pharmacokinetic and safety data

Dose escalation study of intravenous baclofen in healthy adult volunteers

- Designed study and co-wrote the protocol
- Prepared, submitted and got approval for an IRB application
- Prepared and submitted an investigational new drug (IND) amendment for use of escalating doses of intravenous baclofen in healthy adult volunteers
- Co-wrote and submitted a grant application to Paralyzed Veterans of America Research Foundation

CHAPTER 3

DEVELOPMENT OF INTRANASAL DIAZEPAM FOR MANAGEMENT OF SEIZURE EMERGENCIES

3.1 A Pilot Study Assessing the Bioavailability and Pharmacokinetics of Diazepam after Intranasal and Intravenous Administration in Healthy Volunteers[1]

3.1.1 Introduction

Seizure emergencies are associated with high morbidity and mortality which can be reduced by prompt and appropriate pharmacological therapy. During a seizure, there is an increased release of excitatory neurotransmitters, usually glutamate and/or aspartate. Normally, an increase of the inhibitory neurotransmitter, gamma aminobutyric acid (GABA) will result in cessation of the seizure [128]. However, if GABA is not released promptly, excess excitation may lead to loss of neural control and convulsive seizures. In 1993, Epilepsy Foundation of America's Working Group on Status Epilepticus recommended that antiepileptic drug administration should be initiated whenever a seizure has lasted 10 minutes [129]. A recent review article suggested that most epileptic seizures last 1-4 minutes and seizures lasting greater than 5 minutes should be treated as status epilepticus [130]. Evidence suggests that the longer a seizure continues, the less likely it is to spontaneously stop [131] and can also progress to status epilepticus, which is associated with increased morbidity and mortality, suggesting a need for prompt therapy [132].

The standard treatment for seizure emergencies is intravenous administration of benzodiazepines, usually lorazepam or diazepam followed by phenytion or fosphenytoin [133]. Although intravenous route is the most effective option for quick cessation of

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seizures, therapy gets delayed as it requires skilled medical personnel and transportation to medical facility. Diazepam rectal gel (Diastat®) is the only formulation of diazepam indicated for the out-of-hospital management of selected, refractory patients who require intermittent use of diazepam to control bouts of increased seizure activity such as acute repetitive seizures. Introduction of rectal diazepam products in Europe and in the United States dramatically changed the management of seizure emergencies. With these formulations, caregivers were able to achieve good outcomes by initiation of early treatment after the onset of acute repetitive and prolonged seizures. As a result, emergency department admissions have declined with a decrease in health care costs and improved quality of life [134]. Nonetheless, social objections by older children and adults and legal concerns about rectal administration have limited its use. As a result, many patients do not realize the benefit of a therapy that can improve outcomes and decrease healthcare costs. Shortly after the introduction of rectal diazepam, a need for alternative route of administration was realized and interest emerged to investigate and develop different formulations of benzodiazepines (clonazepam, diazepam, lorazepam, and midazolam) using one or more routes of administration such as buccal, intramuscular and nasal.

Intranasal benzodiazepines appear to be particularly promising and several research groups carried out studies to investigate the pharmacokinetics, bioavailability and tolerability [65-73, 75-77]. To meet the need of an alternate therapy for seizure emergencies, our group has investigated several intranasal formulations of diazepam. Our earlier studies have demonstrated the feasibility of nasal administration of diazepam

[68]. Diazepam was absorbed rapidly following nasal administration and the pharmacokinetic profile of intranasal formulations compared favorably to that of the rectal diazepam gel. However, the tolerability was only moderate. The results of earlier studies concluded that intranasal diazepam offers a viable alternative to rectal administration, however further enhancement of formulations was needed to both improve tolerability and the extent and consistency of absorption. In the current study, two novel formulations of diazepam nasal spray have been evaluated and compared with intravenous administration. The primary objective of this study was to assess the bioavailability and pharmacokinetics (PK) of diazepam after intranasal administration of solution and suspension formulations in healthy volunteers under fasted conditions. The secondary objective of this study was to assess the safety and tolerability of these two diazepam nasal spray formulations after a single administration.

3.1.2 Methods

3.1.2.1 Subjects and Study Design

Subjects were healthy volunteers 18 to 45 years old with BMI between 19 and 30 kg/m^2, who provided informed consent and were compensated for participation. Subjects with known history of severe seasonal or non-seasonal allergies, having nasal polyps or any nasal passage abnormality that could interfere with nasal spray administration were excluded. Subjects who were pregnant or lactating, smoking or using tobacco products within the 6 months prior to the first dose of the study drug, allergic to diazepam, or have been on restrictive diet were also excluded. The study was approved by the Institutional Review Boards at the University of Minnesota and was conducted at PRISM Clinical

Research Unit (CRU) in St. Paul, MN. The principal investigator was present at the CRU during and following drug administration.

The study utilized a randomized, open-label, six sequence, three-way crossover design to compare the pharmacokinetics and bioavailability of a commercially available parental diazepam (DZP) administered intravenously (5 mg) with two novel intranasal DZP formulations (10 mg). Twenty four subjects received the two intranasal and one intravenous dose of DZP with a two-week washout period between doses. Prior to each of the three treatments, the subject's eligibility was reviewed. Subjects were instructed to abstain from prescription drugs and over the counter medications, 14 days and 7 days prior to the first dose of study, respectively. Treatment with any known enzyme altering drugs such as barbiturates, phenothiazines, cimetidine, carbamazepine, within 30 days prior to the first dose of study drug or during the study was also one of the exclusion criteria. Subjects were admitted to the study unit no later than 1900 hours of the evening prior to study drug administration. The next morning, following an overnight fast, subjects were randomized to receive 10 mg intranasal dose of DZP solution, or 10 mg intranasal dose of DZP suspension, or 5 mg intravenous dose of DZP. Subjects fasted for an additional 4 hours after the diazepam dose. Water consumption was restricted from 1 hour prior to dosing until 1 hour postdose.

3.1.2.2 Study Drugs

The intravenous formulation used in this study was the commercially available parenteral DZP (diazepam injectable, 5 mg/mL, USP). A single 5 mg diazepam injection was given intravenously, over one minute, as directed in the product package insert. The

intranasal DZP formulations were supplied by Neurelis, Inc. The intranasal diazepam dose of 10mg in 0.1mL was administered using the Pfeiffer/Aptar sprayer which is commercially available, with the subject lying in the semi-recumbent position.

3.1.2.3 Drug Assay

Blood samples (6mL) for the measurement of plasma concentrations of diazepam were collected in blood collection tubes containing K_2-EDTA at the following times relative to dosing: prior to dosing and at 2.5, 5, 10, 15, 20, 30, and 45 minutes and at 1, 1.5, 2, 4, 8, 12, 24, 36, 48, 72, 96, 144, 192, and 240 hours from the start of the intravenous infusion or after an intranasal dose. Plasma was separated by centrifugation and frozen at approximately -20°C, pending analysis by LC/MS/MS.

To prepare a sample for analysis, 20 µL of a 10ng/mL Diazepam-d_5 solution (internal standard) and 0.2 mL of water was added to a 50 µL of K_2-EDTA human plasma. The samples were vortexed, extracted using ABN SPE plates, eluted with methanol and taken to dryness under nitrogen at approximately 40°C. The dried residues were reconstituted in 200 µL of 1:1 methanol:water. After one minute of vortex mixing, 15 µL of the sample solution was injected onto the LC/MS/MS system. Standard curves over a range of 0.1 ng.mL to 100 ng/mL and quality control DZP samples containing 0.3 (low), 6 (medium) and 80 ng/mL (high) were prepared.

3.1.2.4 Pharmacokinetic Analysis

DZP concentration–time data were analyzed using a non-compartmental pharmacokinetic approach with Phoenix software (version 6.2; Pharsight Corporation, Mountain View, CA, USA). The terminal rate constant (λz) was determined from the slope of the terminal

log-linear portion of the plasma concentration-time curve, and the terminal half-life ($T_{1/2}$) was calculated as ln 2/(λz). Maximum plasma concentrations (C_{max}) and time to maximum concentration (T_{max}) were determined by direct observation of the data. The area under the concentration–time (AUC) curve to the last non-zero plasma concentration (C_{last}) that was above the lower limit of quantification (0.1 ng/mL) was calculated as AUC_{last}. The area under the concentration–time curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{last} + (C_{last}/\lambda z)$. Means and standard deviations for the parameters were also obtained using the descriptive statistics tool in Phoenix version 6.2.

3.1.2.5 Safety Evaluation

Safety was assessed by adverse event monitoring; clinical laboratory evaluations; changes in vital signs measurements, physical examinations, and ECG results. Nasal irritation was evaluated by a trained observer within two (2) hours before and post-dose following the one-hour PK sample and the 24-hour PK sample using the following nasal irritation scale:

0 - Normal appearing mucosa, no bleeding

1 - Inflamed mucosa, no bleeding

2 - Minor bleeding which stops within 1 minute

3 - Minor bleeding, taking 1-5 minutes to stop

4 - Substantial bleeding for 4-60 minutes, does not require medical intervention

5 - Ulcerated lesions, bleeding which requires medical intervention

A sedation scale was used to assess the degree of drowsiness of the subjects after administration of the intranasal and intravenous diazepam formulations. Sedation scores were reported by the subject (if awake) as well as by a trained observer using the same

rating scale, just prior to (baseline) and at 5, 15, 30, 60 minutes and at 2,3,4,6 & 8 hours post dose. The sedation scale used for rating drowsiness consisted of the following options:

0 - Alert, not drowsy; normal conversation
1 - Awake, talking; but somewhat drowsy
2 - Napping or sleeping, but easily awakened
3 - Sleeping, awakened only with loud voice or shaking
4 - Sleeping, very difficult to awaken; promptly returns to sleep
5 - Sleeping, cannot awaken

3.1.2.6 Statistical Analysis

Statistical analysis of PK parameters C_{max}, AUC_{last} and $AUC_{0-\infty}$ was done after log transformation. Repeated measures ANOVA followed by Tukey's multiple comparison test was used to test for statistical differences in AUC and $T_{1/2}$ among the intranasal and IV formulations. A paired t test was employed to determine if statistical differences existed in Cmax, the maximum observed concentration, between the two intranasal formulations. A Wilcoxon matched-pairs, signed-ranks test was used to test for a statistical difference in Tmax, the time to maximum plasma concentration, between the two intranasal formulations.

3.1.3 Results

All 24 subjects (19 male and 5 female) completed the study. The mean (±SD) weight of the 24 subjects was 78.1 (±11.0) kg. The age range of subjects was 21 to 45 years, with a mean of 32.6 years.

A summary of the pharmacokinetic parameters is presented in the Table 3.1-1. It is to be noted that there were 4 subjects each for the suspension and solution treatments for whom a valid estimate of λz could not be calculated and thus $AUC_{0-\infty}$ and T½ were not calculated either. This was either due to lack of a log-linear decay, a coefficient of determination (r^2) < 0.9000, or an extrapolated AUC that was >20% of $AUC_{0-\infty}$. The mean concentration time profiles for all the three arms are shown in the Figure 3.1-1. DZP concentrations rose rapidly and were maintained for several hours following administration of both intranasal formulations. The mean intranasal DZP suspension C_{max} and T_{max} were 221 ± 78.6 ng/mL and 1.25 ± 0.5 hours respectively. The mean intranasal DZP solution C_{max} and T_{max} were 272 ± 100 ng/mL and 1.51 ± 0.88 hours respectively. Median T_{max} values for the suspension and solution formulations were similar to the mean values: 1hr and 1.5hr, respectively. Total systemic exposure following administration of DZP intranasal solution (absolute bioavailability – 97%) was greater than that of the intranasal suspension (absolute bioavailability- 67%). The mean elimination $T_{1/2}$ was comparable for all three formulations indicating that there was no prolonged absorption of diazepam following intranasal administration (Figure 3.1-2).

Table 3.1-1. Mean ± SD of DZP pharmacokinetic parameters following intranasal (10 mg) and intravenous (5 mg) administration in 24 healthy volunteers.

Pharmacokinetic Parameter [a]	**10 mg Nasal Suspension Mean ± SD**	**10 mg Nasal Solution Mean ± SD**	**5mg Intravenous Mean ± SD**
C_{max} (ng/mL)	221 ± 78.6[d]	272 ± 100	-
T_{max}(hr) [b]	1.00 [0.6-2.0]	1.50 [0.8-4.0]	-
AUC_{last} (ng.h/mL)	5229±1463[e]	7340 ± 1882	3832 ± 1150
$AUC_{0-\infty}$ [c] (ng.h/mL)	5381 ± 1409[e]	7338 ± 2072	4104 ± 1318
Bioavailability	67 %	97 %	-
$T_{1/2}$ (hr) [c]	56.2 ± 23.0	49.2 ± 16.9	56.2 ± 21.0

a: Mean values are presented as arithmetic means
b: Median (Min, Max) reported for T_{max}
c: N=20 for Nasal suspension and Nasal Solution. There were 4 subjects each for the suspension and solution treatments for whom a valid estimate of λz could not be calculated and thus $AUC_{0-\infty}$ and T½ were not calculated either. This was either due to lack of a log-linear decay, a coefficient of determination (r^2) < 0.9000, or an extrapolated AUC that was >20% of $AUC_{0-\infty}$.
d: Two-tailed p value < 0.05 (Paired t-test)
e: Nasal Suspension vs. IV and Nasal Solution – p value < 0.05 (Tukey's multiple comparison test)

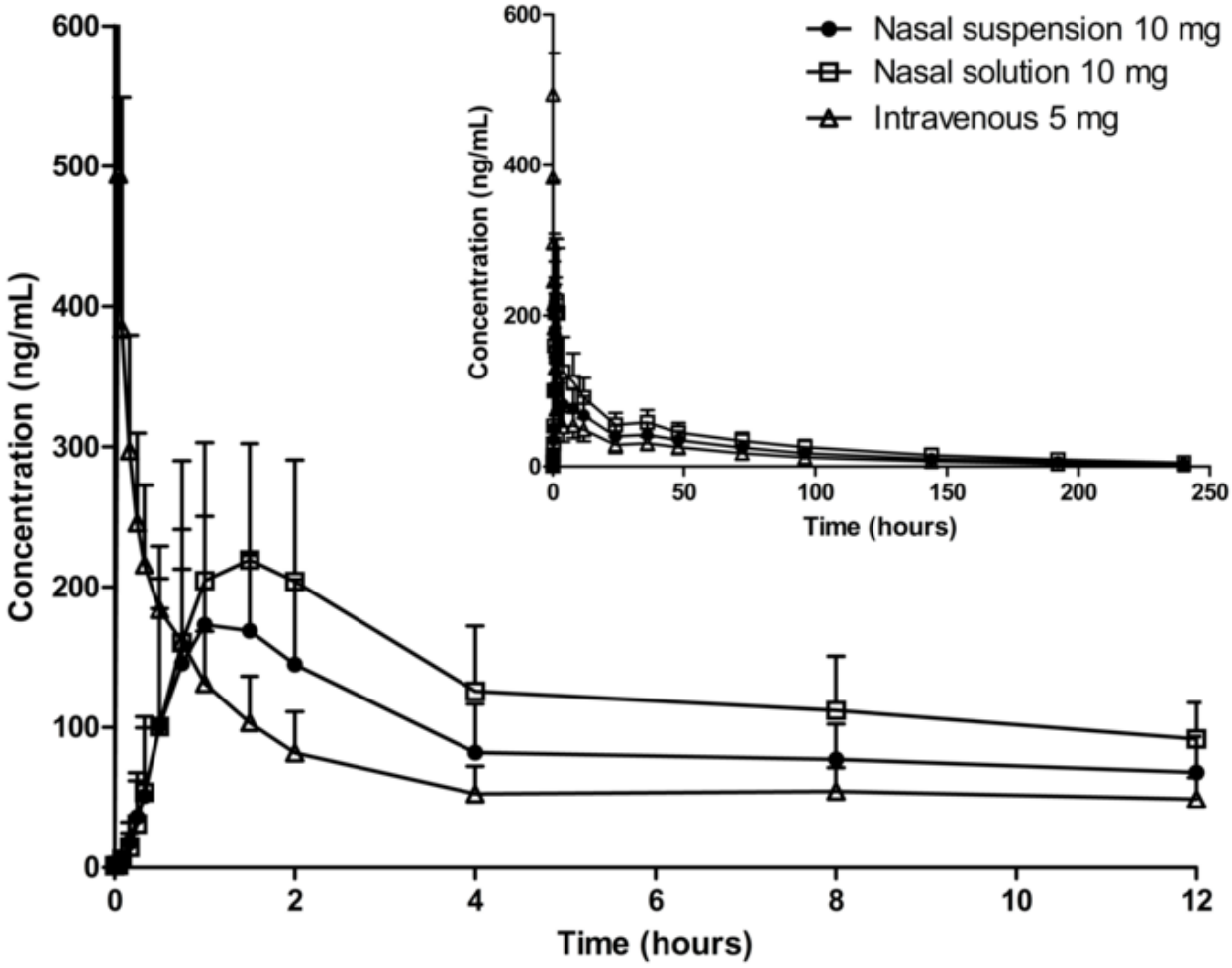

Figure 3.1-1: Mean (+ standard deviation) Plasma Concentration- time Profiles of Diazepam after Intravenous and Intranasal Administration in Twenty-Four Subjects (0—12 h). Inset shows the complete profile (0—240 h).

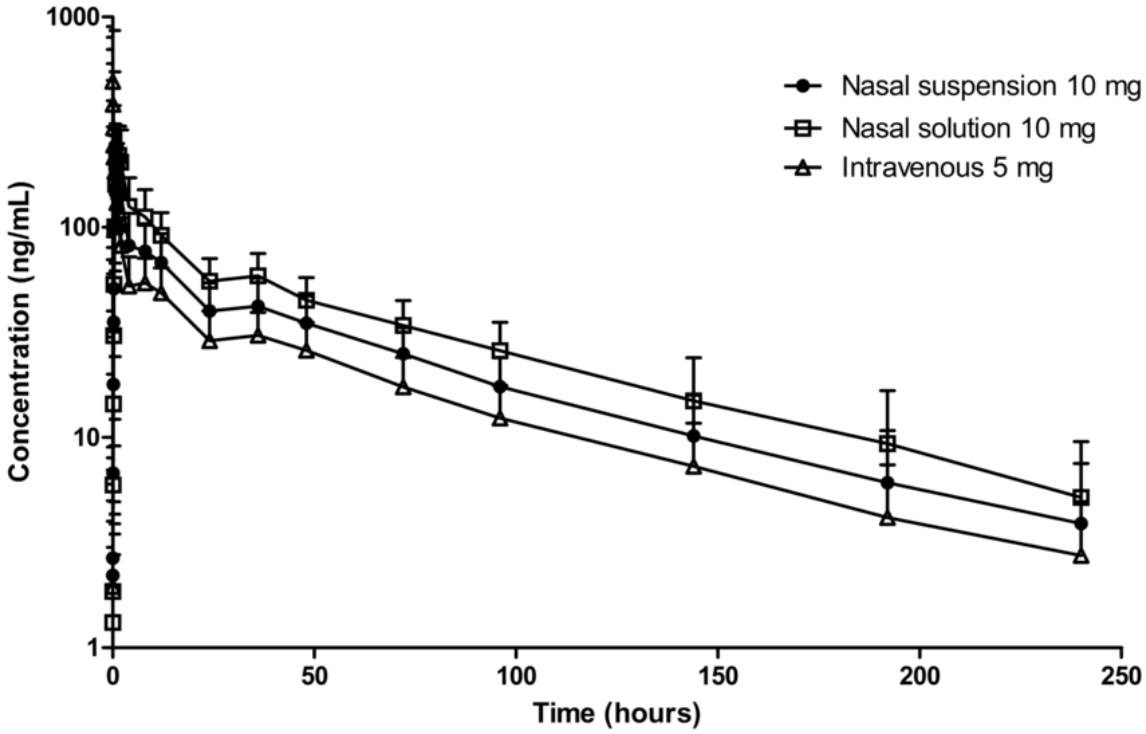

Figure 3.1- 2: Mean (+ standard deviation) Plasma Concentration-time Profiles of Diazepam after Intravenous and Intranasal Administration in Twenty-Four Subjects (Semi-Logarithmic Scale).

Both investigational intranasal formulations were well tolerated. Overall, 71% of subjects experienced ≥ 1 treatment-emergent adverse effects (AEs) during the study, with similar numbers of subjects experiencing AEs after administration of intranasal suspension (9 subjects), intranasal solution (8 subjects), and IV diazepam (10 subjects). The most commonly reported AEs were epistaxis and somnolence (6 subjects each). Mild, self-limited epistaxis (a few drops of blood in either nostril) was observed following administration of IV diazepam (3 subjects, 5 events), intranasal solution (3 subjects, 3 events) and intranasal suspension (1 subject, 1 event). Somnolence was more frequently associated with IV diazepam (4 subjects) than with either of the intranasal diazepam formulations (1 subject each). All treatment-emergent AEs were characterized

by the investigator as being mild or moderate in severity. There were no AE reports of nasal pain by any subject in any treatment period. Thirteen subjects experienced AEs that were considered by the investigator to be related to intranasal study drug formulations; most common AEs were headache, somnolence, epistaxis, and nasal discomfort. No AE met the criteria of a serious adverse event, and none resulted in a subject's withdrawal from the study.

3.1.4 Discussion

The absolute bioavailability of intranasal solution approached 100% and was higher than that reported in prior intranasal diazepam studies, while variability in exposure (AUC) for both the suspension and the solution was similar to the IV dose (Table 3.1-1). Our results compare favorably with studies involving rectal administration of diazepam. Milligan et al. administered a 20 mg rectal DZP solution to 10 epilepsy patients. The onset of reduction in EEG spike counts occurred approximately 15-20 min after drug administration and was associated with a plasma DZP concentration of approximately 200 ng/mL. The 60% reduction in spike counts and DZP concentrations above 200 ng/ml were sustained for the duration of the 3 hr observation period [135]. In our current study, for both the formulations, the mean C_{max} following a 10 mg dose was above the 200 ng/mL threshold associated with a reduction in spike counts. Following intranasal solution administration, 18 out of 24 subjects attained DZP concentrations above the 200 ng/mL threshold. By comparison, following the intranasal suspension administration 15 out of 24 subjects attained DZP concentrations greater than 200 ng/mL threshold.

The intranasal solution formulation had an absorption and elimination profile similar to that observed for the commercially available DZP rectal gel (Diastat®) as reported by our group. The absolute bioavailability of the nasal solution was approximately 10% greater than that reported for DZP rectal gel (Diastat®), but half-life, T_{max} and dose-adjusted C_{max} were comparable [136]. In that bioavailability study, we found a mean C_{max} of 447 ± 91.1 ng/ml following 15 mg dose of diazepam rectal gel administration. In the present study, the average C_{max} for the 10 mg dose of the intranasal solution formulation was approximately 270 ng/mL. Ivaturi et al. reported a linear increase in C_{max} with diazepam dose reflecting approximate dose proportionality [68]. Assuming a linear increase in C_{max} occurs with the intranasal solution of diazepam formulation used in the current study, increasing the dose to 15 mg from 10 mg should result in peak concentrations in the range of 400 ng/mL. The mean concentrations for the intranasal solution were maintained above100 ng/mL until approximately 8 hours after the drug administration. Assuming linear pharmacokinetics, doubling the dose from 10 mg to 20 mg will result in concentrations above 200 ng/mL for about 8 hours.

A critical factor when considering intranasal benzodiazepine therapy for seizure emergencies is the time to reach a targeted drug concentration. As compared to the rectal study using the commercially available diazepam rectal gel, the intranasal formulations in the present study exhibited a comparable rate of absorption. In our rectal gel study, we reported an initial peak concentration of 375 ng/mL at 45 minutes and a second peak of 447 ng/mL at 70 minutes. The intranasal suspension and solutions used in the current study attained average peak concentrations at 60 minutes and 90 minutes respectively.

One of our key findings was that the variability in exposure (AUC) for both the solution and suspension was similar to IV dose (coefficient of variation, 30%). The rectal gel study reported an absolute bioavailability of approximately 90%. Simulations are needed to determine the sample size required to show bioequivalence between the rectal gel and the intranasal solution. Our study was intended to serve as a pilot study to provide information on the suitability of one or both formulations for further development. The information gained from this study can be used to design future, adequately powered bioavailability and bioequivalence studies.

Prior studies with diazepam and midazolam reported dual peaks reflecting oral absorption in which the first peak representing nasal absorption occurs within 20-30 minutes and a second peak presumably due to enteral absorption occurs around 1-2 hours after drug administration. In some of the studies reporting dual peaks, subjects actually commented that they swallowed a portion of dose administered intranasally. Second diazepam peak concentrations were not observed in our current study results.

Our earlier studies with a glycofurol based intranasal formulation of diazepam resulted in 75 % & 74 % bioavailability after administration of 5 and 10 mg DZP doses, respectively [68]. The formulations were absorbed rapidly but the tolerability was poor which made it unsuitable for further development. Subsequently, two other intranasal formulations compared the pharmacokinetics and tolerability with rectal diazepam gel. In that study, the pharmacokinetics profile of the two investigational diazepam formulations was comparable to that of rectal diazepam gel. No serious adverse effects were reported following administration of intranasal formulations; however, majority of the subjects

reported nasal discomfort or pain immediately after dose administration. In this current study, both diazepam nasal spray formulations were remarkably well-tolerated. There were no AE reports of nasal pain by any subject in any treatment period. It is noteworthy that intranasal administration of diazepam did not appear to cause epistaxis - more epistaxis events were reported following IV diazepam than after administration of either intranasal formulation. Nasal drug delivery can cause epistaxis and other nasal diazepam formulations may carry this risk, but the most plausible explanation for epistaxis in our study following IV administration is that individuals occasionally experience minor nose bleeds in harsh winters with dry interior environment.The nasal DZP solution shows promise as a socially acceptable, well-tolerated, easily administered formulation for use in seizure emergencies. Its pharmacokinetic profile is similar to rectally administered diazepam with high bioavailability, reasonable variability, and rapid attainment and maintenance of therapeutic drug concentrations. The absolute bioavailability of the intranasal solution was approximately 100% and was higher than that reported in prior intranasal diazepam studies. Variability in exposure (AUC) for both the suspension and solution was similar to the IV dose. The results of this pilot study indicate that development of an intranasal diazepam formulation with high bioavailability, reasonable variability, and good tolerability is feasible.

Acknowledgements

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diazepam concentrations were determined by LC/MS/MS at ICON Development Solutions LLC.

3.2 Application of Modeling and Simulation to Optimize a Bioequivalence Trial of Nasal and Rectal Diazepam

3.2.1 Introduction

Our research group has conducted several Phase-I clinical studies of diazepam comparing pharmacokinetics and tolerability of intravenous (IV), rectal and nasal (IN) formulations. The intranasal phase-I clinical data were generated from five different diazepam formulations. The glycofurol-based supersaturated formulation caused moderate irritability and was not tolerated well by subjects. Two other formulations which were microemulsion-based, showed low bioavailability. Results from a most recent pharmacokinetic study investigating two other IN formulations (NRL.1.A and NRL.1.B) have shown high bioavailability and good tolerability. Therefore, among the five IN formulations, NRL.1.A and NRL.1.B are being considered for further development.

Developmental Pathway for Intranasal Diazepam

There are two possible development paths for the potential approval of a nasal diazepam product. One pathway would be the traditional development route of having to prove safety and efficacy in at least one or more placebo controlled clinical trials after establishing the pharmacokinetics and toxicity over a range of doses. The other, possibly quicker pathway, would be to use either 505 (b) (2) or 505 (j) of the Hatch-Waxman act (see below).

Section 505 (b) (2): Drug products that may be submitted under section 505(b) (2) are not completely new products, yet they are not generics. These medications have both similarities and some differences from an innovator or brand drug. In the case of

intranasal diazepam the product has the same active ingredient as the previously approved rectal diazepam product, but now is formulated in a different delivery mechanism and a different route of administration.

The basis for the 505(b) (2) application is that there already is a certain amount of information that is known about the active ingredient. Under the rules in section 505(b) (2), the applicant can rely on information from studies it did not conduct and for which it does not have the raw data to base its conclusions (right of reference). The implication of this section for development of intranasal diazepam would be that the sponsor may rely on efficacy performance of rectal diazepam and conduct only pharmacokinetic and safety studies for intranasal diazepam. This would save both valuable time and resources for a sponsor.

Section 505 (j): Section 505 (j) refers to the generic drug approval process also commonly known as the Abbreviated New Drug Approval (ANDA) application. For an ANDA, the sponsor only needs to complete studies that demonstrate that the generic product is bioequivalent to the innovator product.

Thus, for intranasal diazepam, 505(b) (2) path may be chosen under which the sponsor may rely on efficacy performance of rectal diazepam and conduct only pharmacokinetic and safety studies for intranasal diazepam. Under this pathway, the sponsor will need to conduct a Phase I study to compare the pharmacokinetics of intranasal and rectal diazepam. Although not necessary for approval, it would be ideal if the sponsor could show that the intranasal product is bioequivalent to rectal diazepam. Typically

bioequivalence trials are done in 24-36 healthy volunteers. In the same study or a separate study, escalating doses of the intranasal formulation must be investigated to demonstrate linear pharmacokinetics and safety with increasing dose.

This project was aimed to develop a PK model, using data from current work and five previously conducted studies, which will be useful in designing a future bioequivalence study of rectal and intranasal diazepam.

Objectives

1. To characterize the pharmacokinetic parameters of different formulations of diazepam (IV, IN, and rectal) using a non-linear mixed effects modeling approach.
2. To test the hypothesis that the IN solution (NRL.1.B) will produce a similar exposure as that observed following rectal administration.

3.2.2 Methods

3.2.2.1 Study Design

All studies were conducted with the approval of the local Institutional Review Boards. Concentration-time profiles from 99 subjects in six Phase I studies of IV, IN and rectal diazepam comprised the final population PK database. Study design information, including subject populations, formulations, doses and PK sampling are given in Table 3.2-1. It is to be noted that extravascular pharmacokinetic data following single doses of two intranasal formulations (NRL.1A and NRL.1B) and the commercially available rectal diazepam gel (Diastat®) formulations were included in the database. All plasma samples with quantifiable concentrations of diazepam were used for the population

pharmacokinetic model development. Measurements below the limit of quantification and missing values were excluded from the analysis.

Table 3.2- 1. Description of studies included in the diazepam population pharmacokinetics database

Study	Design	Subjects	PK Sampling	Doses	Formulation
S01	Single blind, single dose phase 1	Females (n=3)	0-48 h	IV – 5mg	IV-Valium®
S02	Single blind, single dose, phase 1	Males (n=6) Females (n=2)	0-48 h	IV – 5mg	
S03	Open label, single dose, phase 1	Males (n=9) Females (n=3)	0-240 h	Rectal – 10 mg	Rectal - Diastat®
L01	Single blind, 2 way crossover, phase 1	Males (n=18)	0-240 h	IV – 7.5 mg Rectal – 15 mg	IV-Valium®
L02	Single blind, 3 way crossover, phase 4	Males (n=18) Females (n=16)	0-240 h	Rectal – 15 mg, in 3 periods	Rectal - Diastat®
S04	Single blind, 3 way crossover, phase 1	Males (n= 19) Females (n = 5)	0-240 h	IV – 5 mg IN – 10 mg	IV - Diazepam USP IN - NRL.1.A & NRL.1.B

3.2.2.2 Data Analysis

The population PK analysis was conducted via non-linear mixed-effects modeling in NONMEM (version - 7) software using PDxPOP version 5 for pre- and post-processing. The first-order conditional estimation method with eta-epsilon interaction (FOCE) was used for all model runs. Selection of models was guided by the likelihood ratio test (χ^2, $P<0.05$), diagnostic plots, physiological plausibility and precision of parameter estimates.

Final model parameter estimates were reported with a measure of estimation uncertainty based on the nonparametric bootstrap 95% confidence intervals.

Structural Model

Concentration-time profiles of IV, IN and rectal diazepam were simultaneously fit to various PK models. A sequence of compartmental models was tested and the results compared. An open two-compartment pharmacokinetic model with the first-order absorption and elimination was finally chosen for model fitting and was implemented with ADVAN4 and TRANS4 subroutines. The bioavailability (F) and first-order absorption rate constant (Ka) were estimated separately for each rectal and intranasal formulation.

Statistical Model

Between-subject variability (BSV) was estimated using an exponential error model, or log-normal parameter distribution (Equation 1) that expresses population variability as a coefficient of variation.

$$\beta i = \hat{\beta} * exp\ (\eta_{Pi}) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Equation\ 1$$

Where, βi is the estimated parameter value for individual i, $\hat{\beta}$ is the typical population value (geometric mean) of the parameter, and η_{Pi} is the individual-specific interindividual random effects for subject i and parameter β. These random effects are assumed to be normally distributed with a mean of zero and variance of ω^2; $\eta \sim N\ (0, \omega^2)$. Interindividual variability was included on clearance (CL), central volume of distribution (V2), intercompartmental clearance (Q), peripheral volume of distribution (V3),

absorption rate constants (Ka) and bioavailability (F) for rectal and two intranasal formulations. Residual variability was accounted for using a proportional error model (Equation 2).

$$Cij(obs) = Cij(pred) + Cij(pred)* \varepsilon ij \quad \text{Equation 2}$$

where, *Cij(obs)* and *Cij(pred)* are the observed and predicted diazepam values in the i^{th} individual at the j^{th} time point respectively, and εij are random effects which are assumed to be normally distributed with a mean of zero and variance of σ^2; $\varepsilon ij \sim N(0, \sigma^2)$.

Model-Based Simulation Studies

To test the hypothesis that one of the investigational intranasal product will produce a similar exposure as is obtained following rectal administration, model-based simulation studies were performed. The final PK model was used to simulate 100 trials with 24 subjects in a two-way crossover design comparing an IN dose of 8 mg from the NRL.1B formulation and a dose of 10 mg of the rectal product. Doses for simulation were decided based on the mean absolute bioavailability estimates obtained from the population PK model. Concentrations were simulated at 0, 2.5, 5, 10, 15, 20, 30, and 45 minutes and at 1, 1.5, 2, 4, 8, 12, 24, 36, 48, 72, 96, 144, 192, and 240 hours. Area under the curve (AUC0-inf) was derived using two methods. In the model-based approach, AUCs were calculated by dividing the dose by simulated individual clearances from 100 simulations. In the traditional NCA approach, AUCs were calculated using the trapezoidal area formula on each of the simulated concentration-time profiles within 100 simulations. The AUCs calculated from model-based approach, do not take into account the residual variability which represents model misspecification and assay variability.

For each of the simulated trials, the 90% CI of the geometric mean for the AUC ratio (IN/rectal) was computed in Phoenix (version 6.2), and the results were compared to the standard bioequivalence limit of 80–125%. Two products would be considered to result in similar systemic exposure if the 90% CIs of the geometric mean AUC ratios (IN/rectal) fell within the bioequivalence limits of 80–125%.

Model Evaluation

The final population PK model was evaluated using a predictive check and a nonparametric bootstrap. For the predictive check, 100 Monte Carlo simulation replicates of the original data set were generated using the final population PK model, and the distribution of the median concentration (Cmed) in the simulated data was compared with the distribution of the same characteristic in the observed data using exploratory graphics. For the nonparametric bootstrap procedure, 1000 replicate data sets were generated by random resampling from the original data set with replacement, using the individual as the sampling unit. Population parameters for each data set were subsequently estimated using NONMEM, and empirical 95% CIs were constructed by observing the 2.5^{th} and 97.5^{th} quantiles of the resulting parameter distributions for all bootstrap runs.

3.2.3 Results

Subject Demographics

Subject demographics are summarized in Table 3.2-2. The age of subjects ranged from 19 to 64 years, with a mean (SD) of 32.4 (9.6) years. The mean (SD) weight was 72.5 (14.0) kg.

Table 3.2- 2. Summary of subject demographics in the population pharmacokinetic database

Categorical Covariate		
	No. of Subjects	**Percent**
Sex Male Female	 70 29	 70.7 29.3
Continuous Covariates		
	Mean ± SD	**Range**
Weight (Kg)	72.5 ± 14	45-115
Age (Years)	32.4 ± 9.6	19-64

Population PK Model

There were 24 subjects who received both IV and IN diazepam, 46 received only rectal, 18 received both rectal and IV formulations, and 11 received only IV diazepam; these subjects contributed to a total of 53 IV profiles, 48 IN profiles and 132 rectal profiles. Diazepam pharmacokinetics were best described by an open two-compartment pharmacokinetic base model with first-order absorption and elimination. This model was implemented in NONMEM using ADVAN4 and TRANS4 subroutines.

Table 3.2-3 provides the parameter estimates of the structural and random variance parameters from the final model. The final model included a two compartment model with interindividual variability on clearance (CL), central volume of distribution (V2), intercompartmental clearance (Q), peripheral volume of distribution (V3), absorption rate constants (Ka) and bioavailability (F).

Table 3.2- 3. Population pharmacokinetic parameters for the typical individual after intravenous, rectal or nasal administration of diazepam

Parameter	NONMEM analysis		Bootstrap analysis	
	Estimate (% RSE)	95% C.I.	Median Estimate	95th percentile
Structural Model:				
CL(L/hr)	1.26 (3.94)	1.16 – 1.36	1.26	1.16 – 1.37
Vc (L)	17.9 (7.65)	15.2 – 20.6	17.9	15.8 – 20.5
Q (L/hr)	11.3 (7.4)	9.66 – 12.9	11.38	9.74 – 12.86
Vp (L)	58.4 (5.5)	52.1 – 64.7	58.72	53.5 – 68.8
Rectal Ka (1/hr)	1.29 (12.8)	0.967 – 1.61	1.29	1.01 – 1.58
Rectal F	0.798 (3.27)	0.747 – 0.849	0.80	0.74 – 0.86
IN (NRL.1.A) Ka (1/hr)	0.505 (12.0)	0.386 – 0.624	0.50	0.39 – 0.65
IN (NRL.1.A) F	0.706 (3.71)	0.655 – 0.757	0.70	0.65 – 0.75
IN (NRL.1.B) Ka (1/hr)	0.406 (13.8)	0.296 – 0.516	0.40	0.30 – 0.53
IN (NRL.1.B) F	0.991 (4.26)	0.908 – 1.07	0.99	0.91 – 1.06
Error Model:	**% CV (%RSE)**	**95% C.I.**		
IIV of CL	29.4(19.7)	23.06 – 34.6	28.98	23.95 – 34.35
IIV of Vc	33.9 (24.7)	24.35 – 41.35	33.72	26.04 – 43.64
IIV of Q	48.6 (28.7)	32.09 – 60.75	48.21	34.85 – 60.15
IIV of Vp	40.9 (17.0)	33.32 – 47.22	40.80	34.16 – 47.28
IIV of Rectal Ka	59.0 (32.2)	35.78 – 75.37	58.07	38.77 – 77.81
IIV of Rectal F	22.2 (29.3)	14.49 – 27.82	21.91	14.66 – 26.9
IIV of Ka (NRL.1.A)	53.5 (38.8)	26.15 – 70.99	52.19	31.38 – 74.42
IIV of F (NRL.1.A)	11.2 (56.3)	0 –16.28	10.95	3.18 – 15.95
IIV of Ka (NRL.1.B)	63.2 (24.5)	45.61 – 76.94	61.82	43.80 – 78.22
IIV of F (NRL.1.B)	16.2 (50.2)	2.07 – 22.85	15.97	3.15 – 22.53
RUV, Proportional	28.6 (9.08)	25.94 – 31.06	28.65	27.21 – 29.97

IIV – Interindividual variability
RUV – Residual unexplained variability

Diagnostic plots of the observed and population model predicted concentrations after intravenous, rectal and nasal administrations stratified by formulation are shown in Figure 3.2-1. Goodness-of-fit diagnostic plots for structural and error model are shown in Figure 3.2-2.

Estimates for the structural and random variance parameters demonstrated good precision with the exception of the inter-individual variability estimates of the absorption rate parameters (Ka). The bioavailability of the intranasal solution (NRL.1.B) approached 100% with a 95% confidence interval of 90% – 107%. In contrast, the bioavailability of the rectal product was estimated to be 80% with a 95% confidence interval of 75% to 85%.

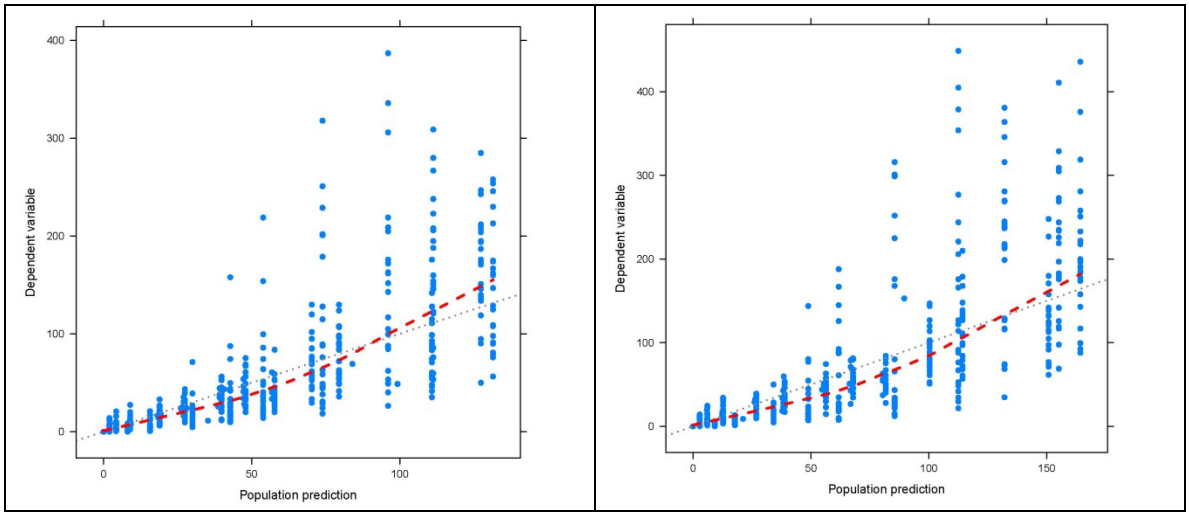

Figure 3.2- 1 (a): Model-based population predicted and individual predicted versus observed diazepam concentrations after intranasal administration of the two different formulations. Left Panel: Suspension Formulation. Right Panel: Solution Formulation

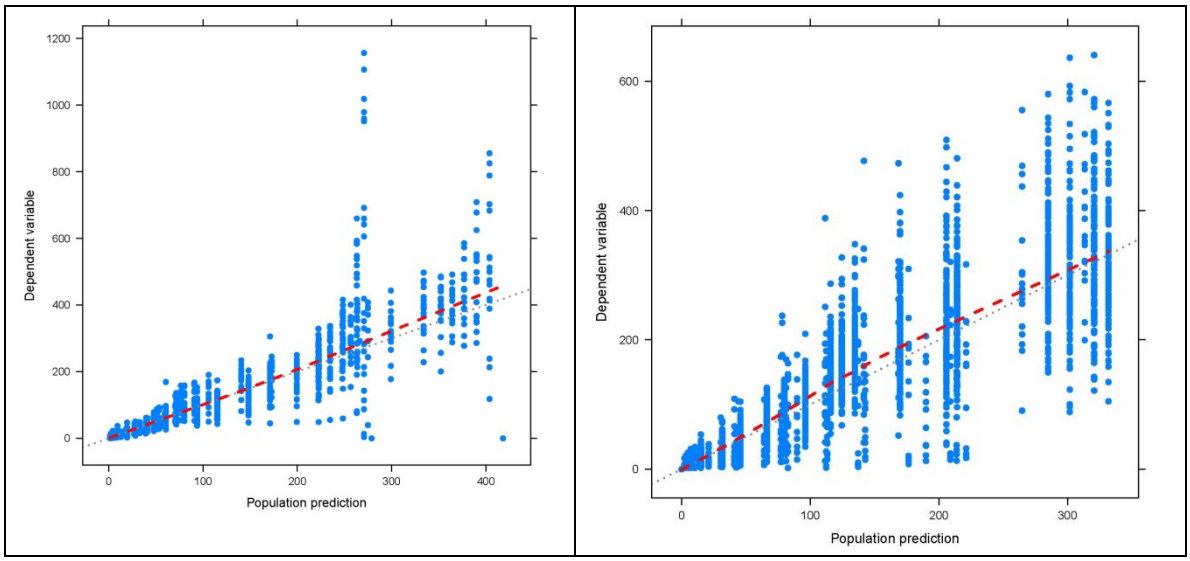

Figure 3.2-1 (b): Model-based population predicted and individual predicted versus observed diazepam concentrations after intravenous (Left) and rectal (Right) administration

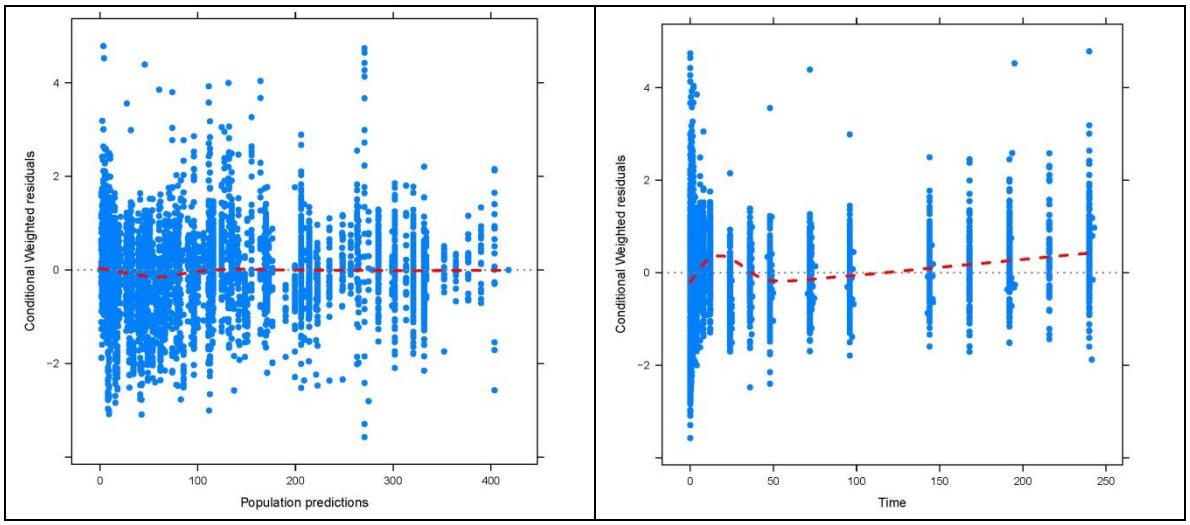

Figure 3.2- 2: **Conditional weighted residuals vs population predicted diazepam concentration (ng/mL and vs time (h). Values are indicated by solid blue dots with a smoothing spline trend line through the data. A dashed line at y=0 is included as a reference.**

Model Evaluation

The intranasal and rectal population PK model evaluation was carried out by predictive check and a nonparametric bootstrap procedure. Results reveal that the final model provided a reliable description of the data with good precision of structural model and most of the variance parameter estimates. The predictive check demonstrated that the simulated distributions of Cmed values were in agreement with observed values (Figure 3.2-3), except for rectal administration. For rectal administration, the 5^{th} percentile for observed concentrations was higher than the 90% CI of corresponding simulated concentrations for time points ≥ 150 hours. The lower limit of quantification (LLOQ) in all the rectal studies (Study S03, L01, and L02) was 2 ng/mL, whereas it was 0.1 ng/mL in the intranasal study (Study S04). As concentrations below LLOQ were excluded from analysis, the 5^{th} percentile for observed concentrations was higher than simulated concentrations due to exclusion of concentrations values below 2 ng/mL in rectal studies. The non-parametric bootstrap procedure resulted in 95% CIs for population PK parameter estimates, which are presented in the final model parameter table (Table 3.2-3). Overall, a typical structural model parameters and random variance terms were estimated with good precision, with the exception of absorption rate constant parameters.

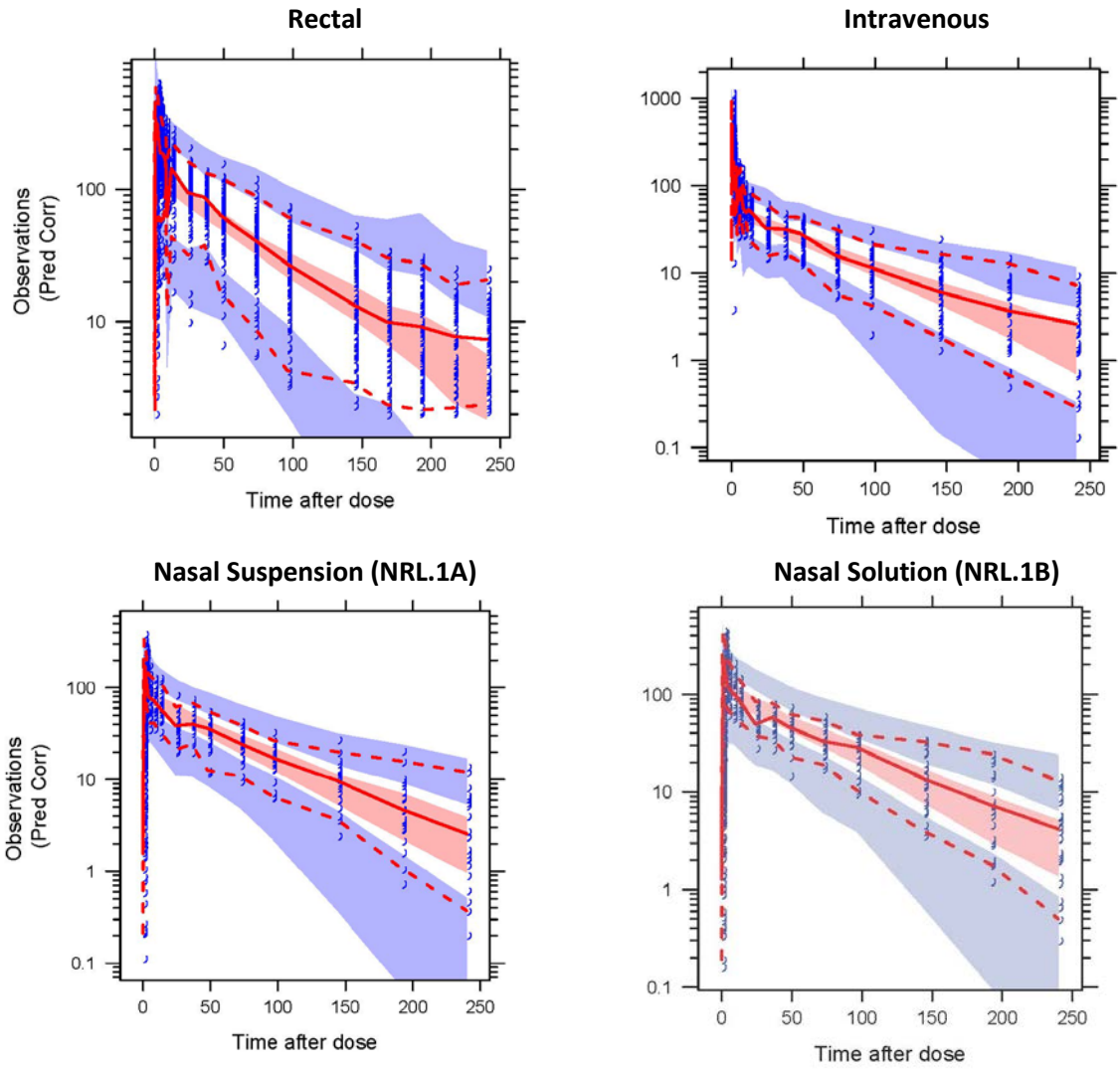

Figure 3.2- 3: Formulation and route stratified visual predictive check plots for diazepam concentrations over time. The solid line represents the median and the dotted line represents the 5th and 95th percentile for observed concentrations. The red band represents 90% CI of the median for model simulated concentrations. Blue bands denote 90% CI of the 5th and 95th percentile for model simulated concentrations.

Simulation Study Results

An 8 mg dose of IN diazepam (NRL.1.B. formulation) was selected to test the hypothesis that the investigational intranasal product will produce a similar exposure obtained following 10 mg rectal dose. Model-based calculated AUCs showed that in 88 out of 100 trials the 90% confidence intervals of the geometric mean intranasal/rectal (or test/reference) AUC ratios fell within the bioequivalence limits of 80–125%. Traditional NCA showed that in 77 out of 100 trials the 90% confidence intervals of the geometric mean intranasal/rectal (or test/reference) AUC ratios fell within the bioequivalence limits of 80–125%. None of the trials met bioequivalence criteria for Cmax.

Such discrepancy in the rates of success between the two approaches is expected. Model-based AUCs does not take into account the residual unexplained variability (RUV) whereas the traditional NCA approach does. The incorporation of the RUV (%CV 28.6%) in the traditional NCA approach resulted in wider CIs and therefore lower rates of success than the model-based analyses. Though the model-based approach showed high success rates, FDA only uses the traditional NCA approach to determine if two products are bioequivalent. Using the traditional approach, 23 out of 100 times, the 90% confidence intervals for the geometric mean nasal/rectal ratios of AUCs were out of the 80-125% limits (see Figure 3.2-4).

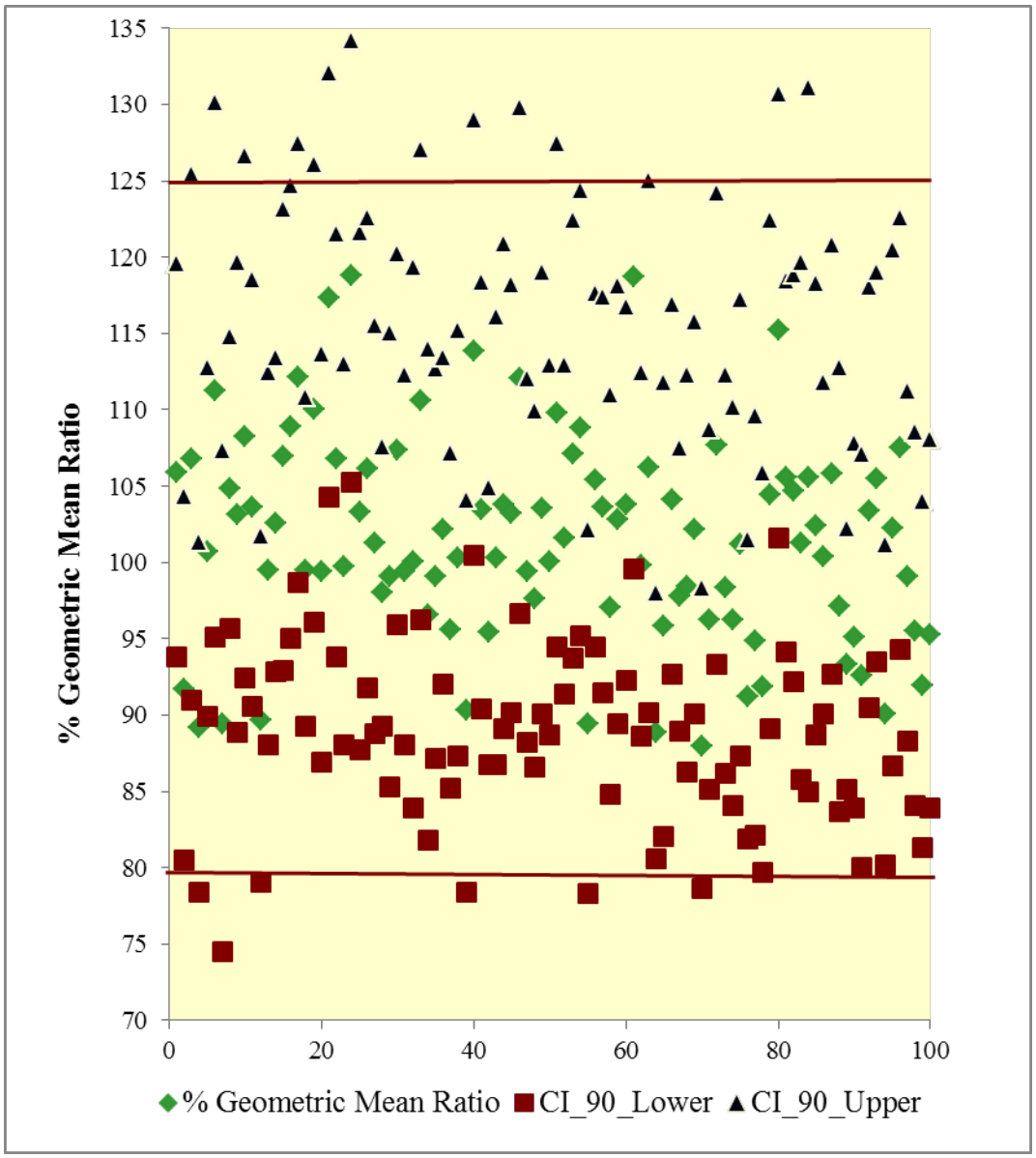

Figure 3.2- 4: Scatter plot of Geometric mean ratios (Nasal/Rectal) of AUCs and 90% CI for 100 simulations

3.2.4 Discussion

A two-compartment model with four parameters (CL, Q, Vc and Vp) best described the disposition of diazepam. Two additional parameters (Ka and F) were estimated per formulation to characterize the absorption phase. Model structural parameters and random variance parameters were estimated with good precision. Goodness-of-fit plots

revealed that the model described the observed data well. The parameter estimates for the fixed and random effects from the bootstrap procedure are comparable and within 5-10% of the estimates from NONMEM, indicating that the final model is robust [137].

To better characterize the absorption phase of data, we implemented more complex absorption models. These include transit compartmental model and Erlang frequency distribution, which describes asymmetric s-shaped absorption profiles. However, there was not a significant improvement seen with these complex models and therefore we chose to select the simpler first-order absorption model to characterize the absorption phase.

To demonstrate the utility of the developed PK model, we performed simulation studies to help designing a future bioequivalence trial. To prove bioequivalence, the sponsor has to show that the 90% confidence intervals of the geometric mean intranasal/rectal (or test/reference) of Cmax and AUC ratios fall within the bioequivalence limits of 80–125%. 77 out of 100 trials showed that total exposure from 8 mg of investigational intranasal product (NRL.1.B) and 10 mg of rectal product would pass bioequivalence limits. Although it would be ideal to prove that the intranasal product is bioequivalent to rectal diazepam, it is not necessary for approval. As the bioavailability of intranasal formulation (NRL.1B) is higher than rectal product, a 10 mg IN dose of diazepam produces greater exposure than a similar dose of rectal diazepam. However, given the bioavailability of oral diazepam approaches 100% and that the safety of a 10 mg dose of oral diazepam is well established, a three way Phase I bioavailability and safety study is proposed. If IN diazepam has greater bioavailability than rectal diazepam, but is

comparable to oral diazepam; then safety is established. A slightly greater exposure of diazepam following IN versus rectal will likely improve efficacy without affecting safety. In the same study or a different study, escalating doses of the intranasal formulation must be investigated to demonstrate linear pharmacokinetics and safety with increasing dose.

3.3 Characterization of Diazepam Absorption Profiles following Rectal and Intranasal Administration using Deconvolution Methods

3.3.1 Introduction

Both the intranasal formulations investigated in the pilot PK study exhibited comparable times to average peak concentration with those reported for the rectal formulation (60-90 minutes vs. 70 minutes, respectively); however, there were significant differences in drug concentrations achieved by these two formulations within the first hour. This was apparently due to differences in the rate of absorption in the first hour.

Milligan et al. rectally administered a 20 mg dose of diazepam solution or placebo to 10 adults with epilepsy and then observed spike wave activity and measured plasma concentrations. The onset of reduction in EEG spike counts occurred approximately 15-20 min after drug administration and was associated with a plasma DZP concentration of approximately 200 ng/mL [135]. This work demonstrated the exposure-response relationship for seizure control with diazepam level of 200 ng/mL, when given rectally. When developing the intranasal formulation, we would like to attain diazepam levels of > 200 ng/mL within minutes of dosing to quickly terminate seizures and avoid potential brain damage caused by seizures. Therefore, it is important to rigorously evaluate the times to attain target concentrations (> 200ng/ mL) of diazepam following rectal and nasal formulations.

Deconvolution can be used to evaluate *in vivo* drug release from a formulation such as oral tablets and the drug input rate following extravascular administration. Several deconvolution algorithms have been published in the literature [138-143]. Deconvolution

methods are based on the same assumptions as the AUC method (i.e. linearity and time invariance), but have certain advantages. Once an assumption regarding the concentration profile following an intravenous dose is made, the rate of drug input into the observation compartment and the cumulative amount absorbed can be calculated. Typically non-compartmental analysis techniques are used to calculate the absolute bioavailability of an extravascular formulation. It is calculated as the ratio of the dose-normalized areas under the plasma concentration-time curves from time zero to infinity ($AUC_{0\text{-}inf}$) following extravascular and IV administration. This method necessitates that plasma samples be collected for long time periods (up to 4-5 drug half-lives) to minimize percent extrapolation of area under the curve from last collection time point (AUC_{last}) to infinity. In contrast, when using deconvolution, plasma sampling is only needed until the drug absorption is completed, which may be only a few hours following drug administration [143, 144]. The deconvolution technique makes it possible to evaluate both rate of absorption and bioavailability, but does not require collection of plasma samples up to 4-5 drug half-lives. It offers the capability to examine complex absorption patterns that may be difficult to evaluate using conventional first-order absorption models.

3.3.2 Methods

The convolution principle may be expressed as follows:

$$C(t) = C(\delta) * f(t) \quad \text{Equation 1}$$

The response function, C(t), is obtained by the convolution of the unit impulse function, C(δ), with the input function, f(t). Mathematical convolution has been functionally expressed here by the asterisk.

Under conditions of linearity and time invariance, the transport of drug from a site i to a site j can be completely expressed by the three functions in eq. 1. C(t) refers to the concentration profile obtained when the drug is placed at site i and the concentrations measured at site j. C(δ) refers to the concentration profile at site j obtained after the drug is placed directly at site j, and f(t) represents the transfer function that governs the movement of mass from site i to j. The knowledge of any two of these three functions allows a determination of the third [145].

Deconvolution is the mathematical inverse of convolution. This refers to the situation where a knowledge of C(t) and C(δ) is used to obtain the input function, f(t). In the present work, C(t) is the concentration-time data following rectal and nasal administration, C(δ) is derived from the concentration-time data following intravenous administration and f(t) is the rate of absorption following nasal and rectal administration.

The deconvolution procedure included in Phoenix® WinNonlin® 6.2 was performed to obtain *in vivo* input rate or absorption rate profiles of diazepam from both rectal and two intranasal formulations. Data from study S04 and L01 were used (Table: 3.2-1). In study S04, 24 healthy volunteers received diazepam intravenously and nasally from a suspension and solution formulation in a three-way crossover design. Study L01 was a two-way crossover study in 18 healthy volunteers who received diazepam intravenously

and rectally. A tri-exponential expression (3-compartment open model) was fitted to the plasma concentrations obtained from intravenous administration to obtain individual parameter estimates. The individual parameter estimates were used to obtain unit impulse response function (C(δ)). Concentration-time data from nasal and rectal administrations were used as the response function (C(t)). Deconvolution was performed using these two functions to calculate input function, f(t). The deconvolution results are expressed as profiles of the input rate over time as well as cumulative fraction absorbed. The extent of absorption from both nasal formulations was compared to that of rectal administration from time zero to 15, 30, 45 minutes and 1, 1.5, 2, 3, 4,6,8,10,12 hours using a t-test to explore the pattern of statistical differences in amount absorbed at early time points in the concentration-time profile. In addition, the estimated extent of absorption within 2, 6 and 12 hours was compared for each formulation with the bioavailability calculated, based on the traditional NCA approach, using non-parametric ANOVA followed by a Dunn's multiple comparison test.

3.3.3 Results

The dose-normalized mean concentration time profiles for all three arms (nasal suspension, nasal solution and rectal gel) are shown in the Figure 3.3-1. Diazepam concentrations rise immediately following both nasal and rectal administration. However, the rate of absorption following rectal administration is faster than the two nasal formulations.

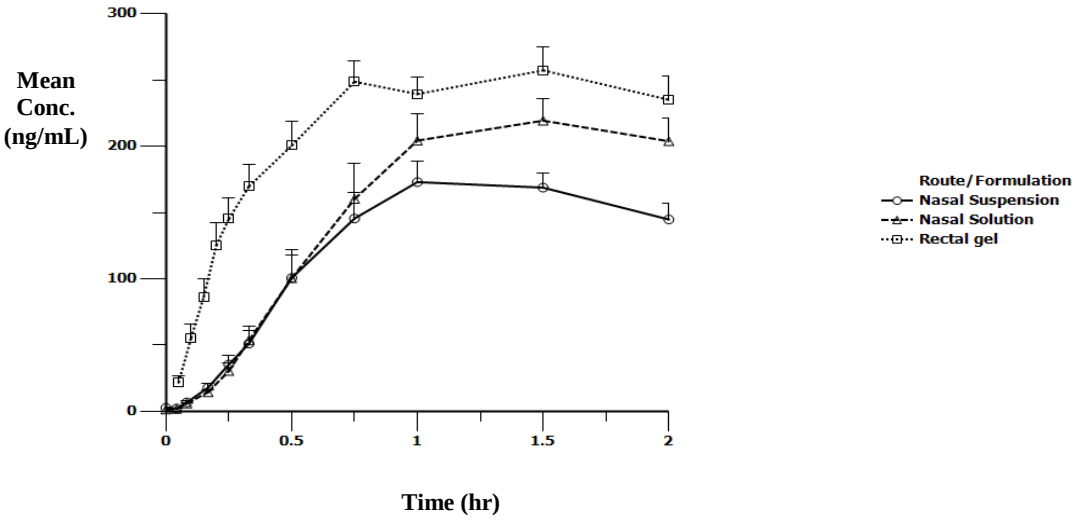

Figure 3.3- 1: Dose-normalized mean (+ standard deviation) plasma concentration-time profiles of diazepam after rectal and intranasal administration in healthy subjects (0—2 h).

Absorption rates of diazepam following intranasal and rectal administration were determined from the corresponding plasma profiles using deconvolution. Individual disposition parameters were obtained from the corresponding intravenous concentration-time profiles. Input rate or absorption rate profiles of diazepam from nasal and rectal administration as a function of time are shown in Figure 3.3-2(a-c).

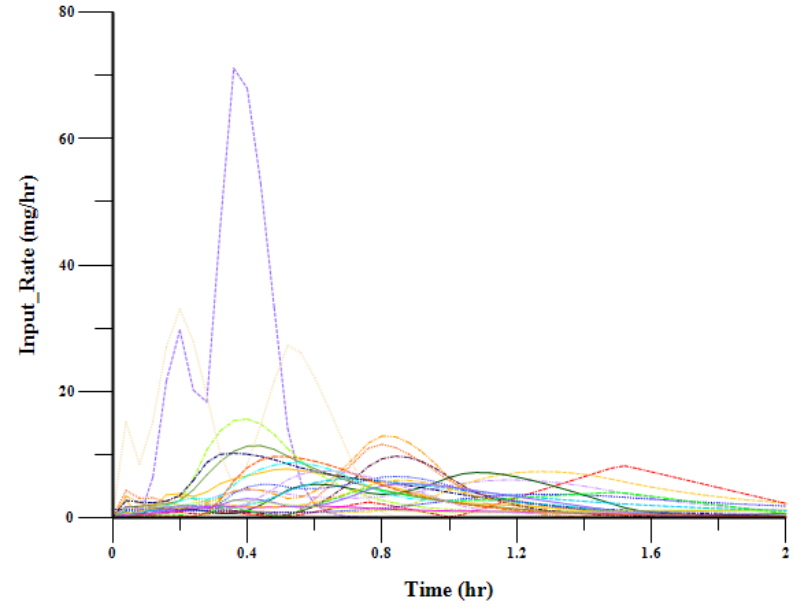

Figure 3.3-2.a: Input rate profiles of diazepam following administration of intranasal suspension in 24 healthy volunteers

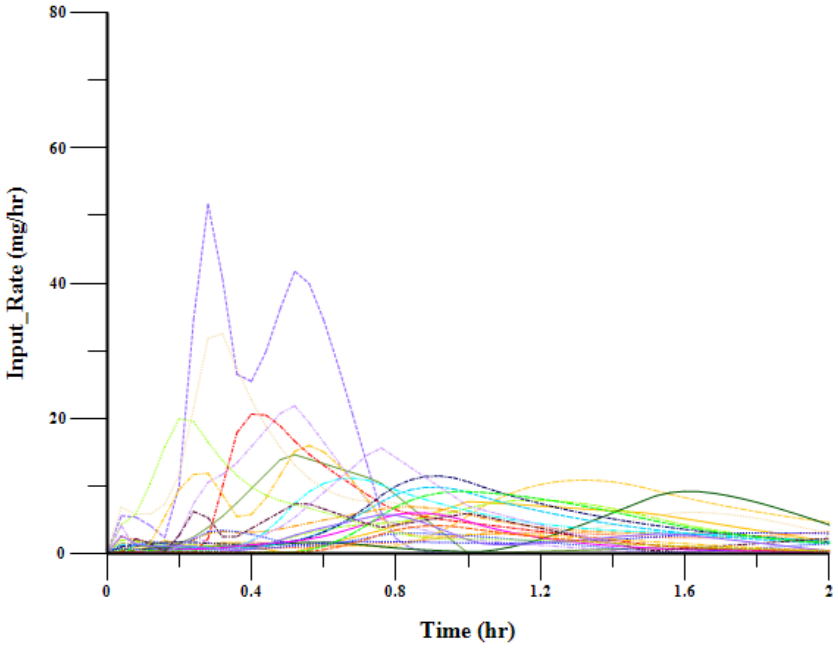

Figure 3.3-2.b: Input rate profiles of diazepam following administration of intranasal solution in 24 healthy volunteers

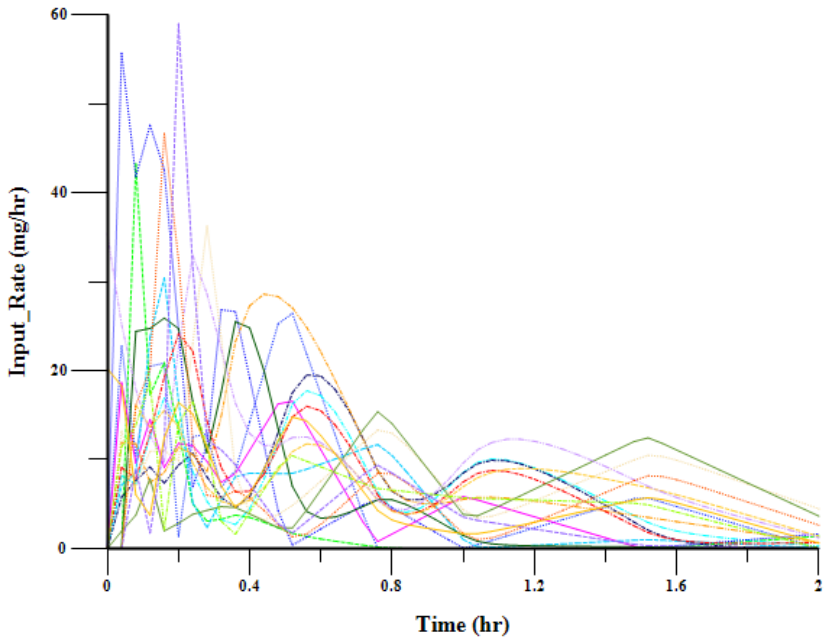

Figure 3.3-2.c: Input rate profiles of diazepam following rectal administration in 18 healthy volunteers

Table 3.3-1 and Figure 3.3-3 show average cumulative fraction absorbed at various time points following drug administration using nasal suspension, nasal solution and rectal gel. At a significance level of 0.05, extent of absorption following nasal suspension is different from rectal gel at all the time points. This result is not surprising given that both the rate and extent of absorption of nasal suspension is lower than rectal gel. In contrast, for nasal solution, the extent of absorption was different only at 15 and 30 minutes post-dose. At and after 45 minutes, the extent of absorption was similar for both the nasal solution and rectal gel. These differences in the early time periods could be explained by differences in the rates and extent of absorption.

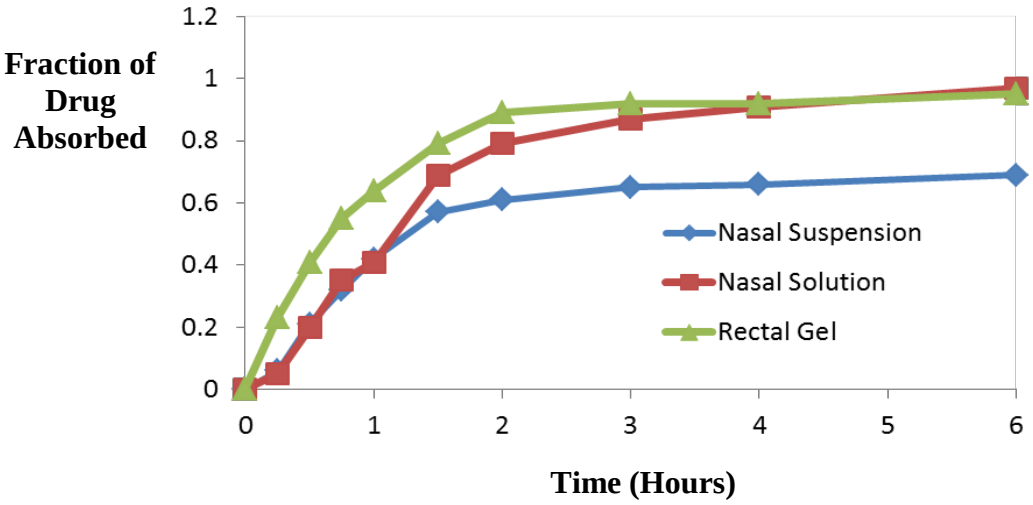

Figure 3.3- 3: Comparison of extent of absorption for diazepam at different time points

Table 3.3- 1. Comparison of extent of absorption for diazepam at different time points

Time (Hours)	Cumulative Fraction Absorbed (Mean ± SD)		
	Nasal Suspension	Nasal Solution	Rectal Gel
0.25	0.06 ± 0.09*	0.05 ± 0.06*	0.23 ± 0.11
0.5	0.21 ± 0.31*	0.20 ± 0.27*	0.41 ± 0.16
0.75	0.32 ± 0.36*	0.35 ± 0.41	0.55 ± 0.16
1.0	0.42 ± 0.34*	0.47 ± 0.41	0.64 ± 0.15
1.5	0.57 ± 0.30*	0.69 ± 0.37	0.79 ± 0.18
2.0	0.61 ± 0.29*	0.79 ± 0.37	0.89 ± 0.22
3.0	0.65 ± 0.29*	0.87 ± 0.35	0.92 ± 0.24
4.0	0.66 ± 0.29*	0.91 ± 0.33	0.92 ± 0.24
6.0	0.69 ± 0.28*	0.97 ± 0.31	0.95 ± 0.25
8.0	0.72 ± 0.28*	1.01 ± 0.32	0.96 ± 0.26
10.0	0.74 ± 0.28*	1.04 ± 0.33	0.96 ± 0.26

12.0	0.75 ± 0.28*	1.05 ± 0.33	0.96 ± 0.26

*t-test p-value comparing rectal gel <0.05

Eighteen out of 24 subjects attained diazepam concentrations above the 200 ng/mL threshold following intranasal solution. By comparison, following the intranasal suspension administration 15 out of 24 subjects attained diazepam concentrations greater than 200 ng/mL threshold. In contrast, dose-normalized concentrations following rectal administration revealed that all 18 subjects attained diazepam concentrations above 200 ng/mL.

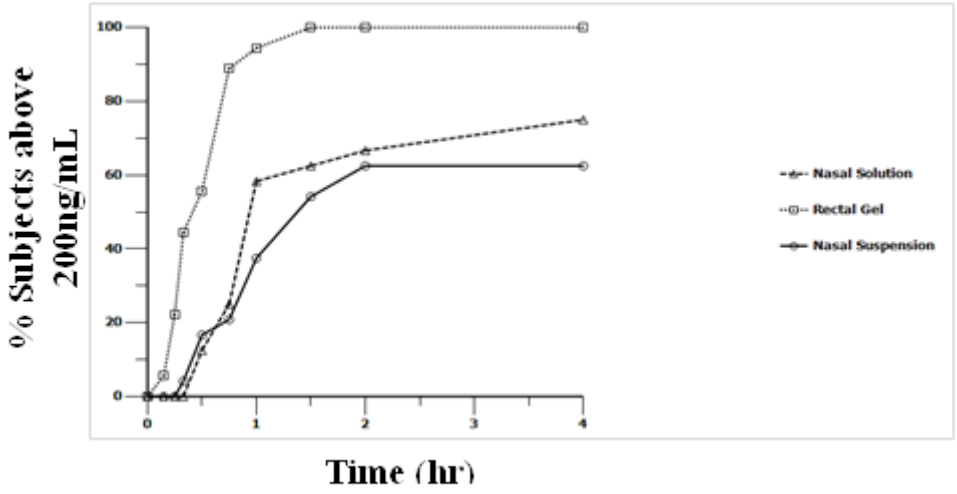

Figure 3.3- 4: Plot showing percentage of healthy subjects attaining concentrations greater than 200 ng/mL over time following rectal and nasal administration

The time to attain threshold concentrations for nasal formulations was considerably longer than rectal gel (Figure 3.3-4) in most subjects. There was significant difference between rectal and nasal formulations in the proportion of subjects attaining the threshold concentrations of 200 ng/mL in the first 30 and 60 minutes (Fisher's exact test p value < 0.05). Fewer subjects, < 20% and < 60%, achieved concentrations above 200 ng/mL by

30 minutes and 1 hour time points, respectively, following nasal solution compared to rectal group (~55% and 90%). It could be argued that this delay in attaining threshold concentrations could be overcome by increasing the dose but this approach would need to be demonstrated in a future PK study.

The bioavailability estimated by the traditional NCA approach was compared to the cumulative fraction absorbed (at 2, 6 and 12 hours) obtained from deconvolution results. The results are shown in Table 3.3-2 and Figure 3.3-5. It can be inferred from Table 3.3-2 that the extent of absorption at 2 and 6 hours (column II and III) was smaller than thebioavailability calculated from NCA for all the three formulations. However, there was no difference in the extent of absorption at 12 hours (column IV) and bioavailability calculated from NCA (column I).

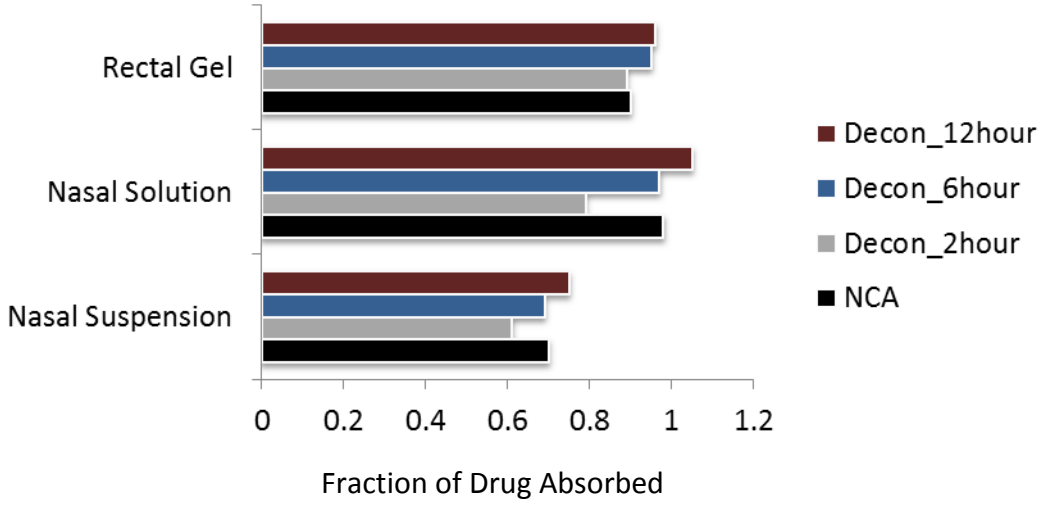

Figure 3.3- 5: **Comparison of extent of absorption for diazepam using NCA and Deconvolution at 2, 6 and 12 hours**

Table 3.3- 2. Comparison of extent of absorption for diazepam using NCA and Deconvolution. Column I shows the bioavailability calculated by NCA and Column II, III and IV show the extent of absorption at 2, 6, and 12 hours obtained by deconvolution

Route / Formulation	Bioavailability (NCA Approach) (I) Mean±SD	Cumulative Fraction Absorbed at 2 hours (II) Mean±SD	Cumulative Fraction Absorbed at 6 hours (III) Mean±SD	Cumulative Fraction Absorbed at 12 hours (IV) Mean±SD
Nasal Suspension	0.70 ± 0.12	0.61 ± 0.29*	0.69 ± 0.28*	0.75 ± 0.28
Nasal Solution	0.98 ± 0.17	0.79 ± 0.37*	0.97 ± 0.31*	1.05 ± 0.33
Rectal Gel	0.9 ± 0.09	0.89 ± 0.22*	0.95 ± 0.25*	0.96 ± 0.26

* Dunn's multiple comparison test comparing column (I): p-value <0.05

3.3.4 Discussion

The results from deconvolution analysis were comparable to those of non-compartmental approach (NCA). The mean absolute bioavailability calculated using NCA for all the three formulations (rectal, IN solution, and IN suspension) was similar to cumulative fraction of drug absorbed calculated by deconvolution. Both NCA and deconvolution techniques do not require any absorption model assumptions; however, input rates or absorption rates can be obtained from deconvolution but not from NCA. In contrast, the population PK model developed and presented in chapter 3.2 assumed a first-order absorption model. The first-order absorption assumption works well in most of the cases but does not allow for secondary peaks resulting from delayed absorption or complex absorption mechanisms. The first-order absorption model assumes that drug absorption is governed by a first-order rate constant which only allows for a monotonic function. On

the other hand, deconvolution makes it possible to explore presence of secondary peaks and any complex absorption patterns which are otherwise not possible by conventional first-order absorption assumption.

Graphical analysis of input rates following both nasal formulations and rectal gel revealed that there was considerable variation in input rates between individuals. Following rectal administration, there was greater variability compared to nasal administrations. A second peak between 30 to 90 minutes after dose administration was seen in the input rate profiles of most individuals receiving rectal gel. Secondary peaks were observed in only two subjects after intranasal administration. Secondary peaks in the input rate profiles suggest delayed absorption but they could also be due to sensitivity of deconvolution to errors in the input response data. Prior studies with intranasal benzodiazepines reported double peaks in concentration-time profiles in which the first peak representing nasal absorption occurs within 20-30 minutes and a second peak presumably due to enteral absorption occurs around 1-2 hours after drug administration. In some studies, subjects actually commented that they swallowed a portion of administered intranasally dose [68]. While second diazepam peak concentrations were not observed in our current study of the two intranasal formulations, this does not necessarily rule out the possibility for enteral absorption.

3.3.5 Conclusions

Deconvolution results indicate that the absorption rates and patterns are different when the drug is given rectally vs. the intranasal route. Absorption of diazepam was more rapid in the rectal group when compared to the nasal group subjects. Target concentrations of

> 200 ng/mL were achieved in greater number of subjects in rectal group at the early collection times compared to intranasal group. Secondary peaks were evident in the input rate profiles of the rectal group but not the intranasal groups. Although the bioavailability of intranasal solution approached 100%, the absorption in the first hour following drug administration is slower than rectal administration as noted previously. In the current study, we used a dose of 10mg which was administered in one nostril by using a 100µL spray. To achieve a target concentration of 200 ng/mL within an hour or even less time, one might have to increase the dose to 20 mg. This can be easily achieved by administering a 100µL spray consisting 10 mg diazepam in both nostrils, but the impact of this change in drug administration would need to be investigated.

CHAPTER 4

DEVELOPMENT OF INTRAVENOUS BACLOFEN FOR MANAGEMENT OF BACLOFEN WITHDRAWAL

4.1. A Pilot Study Assessing Pharmacokinetics and Tolerability of Oral and Intravenous Baclofen in Healthy Adult Volunteers

4.1.1 Introduction

Spasticity is a frequent and prominent symptom of upper motor neuron injury in individuals with cerebral palsy, multiple sclerosis, acquired spinal cord injury, brain injury and neurodegenerative disorders [115, 146, 147]. In general, spasticity develops when an imbalance occurs in the excitatory and inhibitory input to α motor neurons; this imbalance is caused by damage to the central nervous system. Baclofen is a drug used to treat spasticity. It is structurally similar to GABA (gamma aminobutyric acid) and acts as a $GABA_B$ agonist at the level of the spinal cord [148]. Baclofen is available as oral and intrathecal (ITB) formulations. The ITB formulation is used in conjunction with an implanted programmable pump to provide a constant infusion of the drug. Individuals treated with oral or ITB may experience a withdrawal syndrome if it is abruptly discontinued. Interruption in ITB therapy may be the result of problems with the programmable pump or catheter. Interruption in oral baclofen therapy may be the result of illness resulting in inability to take oral medications, non-compliance, or a scheduled surgery for which patients must temporarily stop taking oral medications. This withdrawal syndrome can be quite severe, resulting in a rebound increase in muscle tone and spasms, status epilepticus, hallucinations, and a neuromalignant syndrome-like picture potentially resulting in rhabdomyolysis and multisystem organ failure [114-120] .

The current recommended management of baclofen withdrawal is inadequate. In the case of interruption of ITB therapy, attempts to replace the medication with oral baclofen require large doses in an effort to control withdrawal symptoms [116, 121]. Moreover, in

spite of using large doses, symptoms are not controlled completely and adverse effects including sedation, nausea, vomiting and dizziness may occur. Diazepam is used by most clinicians for its anti-spasticity effect and to decrease the likelihood of acute seizures; however, could result in sedation and respiratory depresssion [116, 122]. In conditions such as ileus or gastroenteritis, baclofen is not absorbed when given orally and can result in baclofen withdrawal. Use of an intravenous (IV) baclofen formulation could prevent or minimize the complications in individuals in whom oral or intrathecal drug delivery is interrupted. Intravenous administration of baclofen would permit rapid attainment of drug concentrations as well as accurate and precise dose titration, thus allowing for more expeditious treatment of withdrawal symptoms and reduced risk of adverse effects.

Although oral and intrathecal baclofen have been used for decades, safety and PK studies of intravenous baclofen in humans have not been published. While there are several reports of IV baclofen administration in both animals and humans [123-126], details of the formulation used in these studies and pharmacokinetic results were not reported. In addition, very limited information was provided about the dose selection and adverse events in these studies. Since an FDA-approved intravenous formulation of baclofen is not currently available, a recent clinical study in patients used an extemporaneously compounded sterile formulation of baclofen (2.5mg/mL, pH 6.6) to reduce pain from muscle spasm and migraines (J Krusz, personal communication 2013). In the current clinical investigation, a commercially available intrathecal formulation of baclofen (Lioresal Intrathecal®, 2mg/mL) was used.

These circumstances suggest that a FDA-approved commercial IV baclofen product would enhance management of patients with spasticity. The first step in the development of such a product is demonstration of its safety in an animal model. Therefore, a pilot study in dogs was conducted to assess the bioavailability, short-term safety, and tolerance of intravenous baclofen in comparison to oral administration. IV baclofen at doses of 0.5 to 3 mg/kg was well tolerated in dogs [127]. The next phase in the development of an IV baclofen formulation would be to conduct a series of investigations in humans. In the current study, safety, tolerability and pharmacokinetics of an intravenous formulation of baclofen has been evaluated and compared with oral administration. The primary objective of this study was to characterize baclofen pharmacokinetics after oral and intravenous administration in healthy volunteers under fasted conditions in order to determine dosing of IV baclofen in subsequent studies. The other objective of this study was to assess the safety and tolerability of a single dose of baclofen given intravenously.

4.1.2 Methods

4.1.2.1 Participants

Twelve healthy adult volunteers gave informed consent and were compensated for participation. Participants who were pregnant or lactating, had a history of sensitivity to IV drug administration, or who had a history of significant diseases were excluded from the study. All female volunteers involved in the study were post-menopausal for 1 year, surgically incapable of bearing children, or were practicing at least one approved method of contraception. The volunteers were medication free for 48 hours before, during, and 24 hours after the administration of the study drug. The study was approved by the

Institutional Review Board of the University of Minnesota and was conducted at PRISM Clinical Research Unit (CRU) in St. Paul, MN. The principal investigator was present at the CRU during drug administration.

4.1.2.2 Study Drugs and Design

The study utilized a randomized, open-label, two-way crossover design to compare the pharmacokinetics and bioavailability of the oral tablet with an intravenous formulation in twelve healthy participants. The oral formulation used in this study was a commercially available 10 mg tablet (10 mg baclofen,Qualitest Pharmaceuticals). For administering the 5 mg oral dose in the initial three patients, the tablets were cut in half. A single intravenous dose of either 3 or 5 mg was administered over 15 minutes, using the commercially available 2 mg/ml intrathecal baclofen formulation (Lioresal Intrathecal®, Novartis Pharmaceuticals) that is used in baclofen pumps. Initially, three participants received single 3 mg intravenous and 5 mg oral doses on separate study days with at least a 48 hour wash out period between doses. After assessing the safety and clinical tolerance of IV baclofen for the three initial participants, an additional nine participants received a 5 mg intravenous dose and a 10 mg oral dose of baclofen after a washout period of at least 2 days. Blood samples (6mL) for the measurement of plasma concentrations of baclofen were collected in blood collection tubes containing K_2-EDTA at the following times: prior to dosing and at 5, 15, 30 minutes, and 1, 2, 4, 6, 8, 10, 12, and 24 hours after drug administration. Plasma was separated by centrifugation and stored frozen until analysis

4.1.2.3 Drug Assay

Study plasma samples were prepared by adding 50 µL of a 500ng/mL levetiracetam solution (internal standard) to 250 µL of K_2-EDTA human plasma. Baclofen and internal standard were extracted from plasma by precipitating protein with methanol and dried under nitrogen at approximately 40°C. The dried residues were reconstituted in 300 µL of mobile phase consisting of 20 mM ammonium acetate: methanol (75:25) solution. After one minute of vortex mixing, reconstituted sample solution was filtered and injected onto the HPLC-MS system. Standard curve samples over a range of 20-400 ng/mL baclofen and quality control samples containing 30 (low), 80 (medium) and 240 ng/mL (high) baclofen were prepared and analyzed in triplicate along with the study samples. The assay was linear over the range 20–400 ng/mL with a lower limit of quantification of 20 ng/mL.

4.1.2.4 Data Analysis

Baclofen concentration–time data were analyzed using a non-compartmental pharmacokinetic approach with Phoenix software (version 6.2; Pharsight Corporation, Mountain View, CA, USA). The terminal rate constant (λz) was determined from the slope of the terminal log-linear portion of the plasma concentration-time curve, and the terminal half-life ($t_{1/2}$) was calculated as ln 2/(λz). Maximum plasma concentrations (C_{max}) and time to maximum concentration (T_{max}) were determined by direct observation of the data. The area under the concentration–time curve (AUC) to the last non-zero plasma concentration (C_{last}) that was above half the lower limit of quantification (10ng/mL) was calculated by the trapezoidal rule and reported as AUC_{last}. The area under

the concentration–time curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{last} + (C_{last}/\lambda z)$. Mean and standard deviation values for the parameters were also obtained using the descriptive statistics tool in Phoenix version 6.2. A paired t-test was used to determine if statistical differences existed in log normalized, dose-adjusted AUC between oral and IV arms.

4.1.2.5 Safety Evaluation

Safety was assessed by ECG results, blood pressure and pulse monitoring, assessment of CNS toxicity, injection site irritation, side effects and tolerability, as well as physical exams at 12 and 24 hours after the drug administration. Ataxia assessment by observation of gait was done prior to drug administration, every 30 minutes for the first 4 hours, and at 12 and 24 hours after the drug administration. At the same time as ataxia assessments, nystagmus and sedation were also assessed. Nystagmus was assessed both at neutral and lateral gaze. Sedation was assessed using the Stanford Sleepiness Scale (SSS) given below [149] :

1 - Feeling active, vital, alert, or wide awake

2 - Functioning at high levels, but not at peak; able to concentrate

3 - Awake, but relaxed; responsive but not fully alert

4 - Somewhat foggy, let down

5 - Foggy; losing interest in remaining awake; slowed down

6 - Sleepy, woozy, fighting sleep; prefer to lie down

7 - No longer fighting sleep, sleep onset soon; having dream-like thoughts

4.1.3 Results

All 12 subjects (9 male and 3 female) completed the study. The mean (±SD) weight of the 12 patients was 79.6 (±13.5) kg. The age range of subjects was 26-56 years, with a mean age of 39.3 years.

A summary of the pharmacokinetic parameters is presented in Table 4.1-1. A valid estimate of λz could not be calculated in subjects receiving the 3 mg IV dose, and thus $AUC_{0-\infty}$ and $t_{1/2}$ were not reported. This was either due to a coefficient of determination (r^2) < 0.9000, or an extrapolated AUC that was >30% of $AUC_{0-\infty}$. The mean concentration-time profiles for both (5 mg IV and 10 mg oral) arms are shown in the Figure 4.1-1. When the subjects received the intravenous formulation, the observed maximum baclofen concentration occurred at the 5 minute time point, whereas, the median T_{max} for oral administration was 1 hour. The mean (SD) C_{max} values for the oral (10 mg) and IV (5 mg) doses were 176 (15) ng/mL and 313 (75) ng/mL, respectively. The mean $t_{1/2}$ was similar for both the oral and IV arms (4 and 4.52 hours, respectively). The mean absolute bioavailability of the oral baclofen tablets (Table 4.1-1) was 74 %. There was a significant difference in log-normalized, dose-adjusted AUCs ($p = 0.0024$) between oral and IV dosing with similar variability (coefficient of variation: 18-24%).

Table 4.1-1. Mean ± SD of baclofen pharmacokinetic parameters following oral (10 mg) and intravenous (5 mg) administration

Pharmacokinetic Parameter	5 mg IV Mean [a] ± SD	10 mg Oral Mean ± SD
C_{max} (ng/mL)	310 ± 74	174 ± 16
T_{max}(hr)[b]	-	1.0 [0.5-2.0]
AUC_{last} (ng.h/mL)	593 ± 111	878 ± 199
$AUC_{0-\infty}$ (ng.h/mL)	707 ± 166	1023 ± 232
$AUC_{0-\infty}$/Dose[c] (ng.h/mL/mg)	141 ± 33	102 ± 23
Bioavailability (%)	-	74± 15
$T_{1/2}$ (hr)	4.52 ± 1.6	4.03 ± 0.73

a: Mean values are presented as arithmetic means
b: Median (Min, Max) reported for T_{max}
c: Two-tailed p value < 0.05 (Paired t-test performed on dose-normalized AUC)

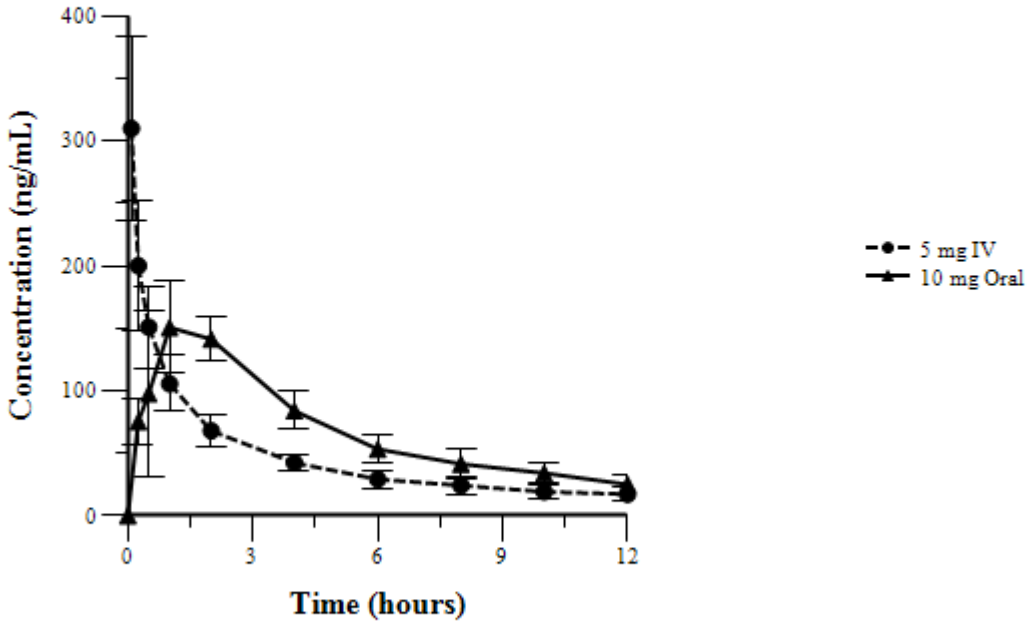

Figure 4.1- 1: Mean (±standard deviation) plasma concentration- time profiles of baclofen after intravenous and oral administration (0—12 h)

The investigational intravenous formulation was well tolerated. Most common adverse events (AEs) were somnolence, mild ataxia and mild nystagmus. One subject had mild end point nystagmus at baseline and following oral and IV baclofen. Two other subjects had mild nystagmus, one after IV and one after oral baclofen. Mild somnolence (SSS score ≤ 4) was observed with both the IV (7 subjects) and oral (3 subjects) doses. Mild ataxia was noted following administration of IV baclofen (4 subjects) and oral baclofen

(7 subjects). All treatment-emergent AEs were characterized by the investigator as being mild in severity, and all subjects returned to their baseline values within six hours of drug administration. All subjects were able to normally communicate and walk without assistance throughout the entire period of the observation. No AE met the criteria to be serious, and none resulted in a subject's withdrawal from the study.

4.1.4 Discussion

To our knowledge this is the first-in-human study investigating intravenous baclofen safety and pharmacokinetics in comparison with oral baclofen. Adverse effects were mild in severity and were not related to route of administration. All subjects in both oral and IV groups were awake; however "fogginess" was reported in both groups. Non-drug related factors possibly confounded sedation scores. For instance, one subject worked the night shift prior to a study day and was sleep deprived, which could have resulted in a higher sedation score. All subjects could walk without assistance, although mild ataxia was observed in both groups. However, very low doses were used in this study; tolerability at higher clinical doses remains to be established.

Absolute oral baclofen bioavailability is 74%, indicating that approximately 25% of a 10 mg dose is either not absorbed or undergoes first-pass metabolism prior to drug reaching systemic circulation. It also implies that a smaller IV baclofen dose will be needed when substituted for oral doses. For example, assuming linear kinetics, the total systemic exposure (AUC) after an intravenous dose of 15 mg would be equivalent to the total exposure achieved after 20 mg of oral baclofen dose. However, the assumption of linear pharmacokinetics needs to be evaluated in a dose-escalation study before one can

confidently use the 25% reduction to determine the IV dose that results in comparable total drug exposure as an oral dose. One of the key findings of this study was that the between subject variability in exposure (AUC) was similar in both IV and oral arms (CV: 18-24%). Variability estimates gained from this study will be helpful in determining the sample size required for future dose-escalation trials.

The results of this study indicate that the Cmax observed following IV administration would be about four times higher than observed with a similar oral dose. Generally when medications are given IV, a higher Cmax could be a safety and tolerability concern. However, in our studies with an animal model, there was no toxicity seen at 0.5 mg/kg IV doses (~10-12.5mg) of baclofen in dogs despite Cmax values in the range of 1000 - 1500 ng/mL. The dose escalation study in the dogs demonstrated that higher single doses of 2 and 3mg/kg (~38-75 mg total dose) eventually resulted in toxicity; but the onset was delayed by two to three hours after peak concentrations, which ranged from 4000-7000 ng/mL [127], further emphasizing the need for a dose escalation study in humans. The disconnect between peak plasma concentrations and drug effects was also demonstrated in a mouse model [150]. Moreover, delayed toxicity implies that clinicians might need to wait a sufficiently long time period (~2-3 hours) to see the maximum clinical effect of an IV baclofen dose in humans as well.

The results of this study differ somewhat from previously published pharmacokinetic studies of oral baclofen given to healthy subjects [91, 92, 151]. Kowalski et al. reported an average peak concentration of 540 ng/mL in eighteen healthy volunteers who received a 25 mg dose. [91]. Assuming linear kinetics, a 10 mg oral dose would result in

a peak concentration of approximately 215 ng/mL. However, in the present investigation, the average peak concentration was 176 ng/mL following a 10 mg oral dose. Average Tmax and half-life reported by Kowalski et al was 2.79 hours and 6.54 hours respectively, both of which are longer than those herein reported (1 and ~4 hrs, respectively). Differences in peak concentrations could be due to differences in the dissolution or disintegration of tablets, saturable absorption mechanisms at higher doses, assay methodology, or blood sampling times.

Intravenous baclofen could be useful in several clinical scenarios including prevention or treatment of withdrawal from oral or intrathecal baclofen. One such scenario would be to use as a bridge therapy when oral baclofen is discontinued for any reason. Another scenario would use IV baclofen when ITB therapy is interrupted due to pump failure or infection leading to removal of pump. IV baclofen could also be used to treat baclofen withdrawal. Finding the correct IV doses would be difficult when substituting ITB doses, as knowledge of bioavailability is not directly relevant. Moreover, it has been shown that there is no correlation between the intrathecal dosage infused and the corresponding CSF baclofen concentration, which further complicates the calculation of therapeutic IV doses in managing withdrawal resulting from ITB discontinuation [152]. Designing and conducting a study to determine equivalency of IV to ITB dosing would be challenging due to both ethical and technical concerns. For these reasons, studies in appropriate animal models may be informative but are lacking.

4.1.5 Conclusion

The PK data gained from this study will guide the design of future trials directed at the development of IV baclofen for bridge therapy. Dose escalation studies are needed to assess the safety of higher clinically relevant doses as well as determining the linearity of exposure at higher oral doses to aid in correct calculation of replacement IV doses. Once the safety and pharmacokinetics of higher IV doses is demonstrated, dose substitution studies in patients on chronic oral baclofen therapy will be necessary to assess multi-dose safety and pharmacokinetics. Lastly, clinicians should be aware of the potential cost of IV baclofen therapy. Nevertheless, in view of the lack of satisfactory alternatives, severity of the withdrawal signs and symptoms as well as the possible complications, the costs could be justified and would probably be part of inpatient hospital billing.

Acknowledgements

We would like to thank Usha Mishra, MS for her work on the development of the baclofen assay and measurement of baclofen concentrations.

Funding

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CHAPTER 5

CLINICAL DEVELOPMENT PLAN AND FUTURE DIRECTIONS

5.1 Clinical Development Plan for Intranasal Diazepam for Seizure Emergencies

There are two possible development paths for the potential approval of a nasal diazepam product. One pathway would be to prove safety and efficacy in one or more well-controlled clinical trials. The other, possibly quicker, pathway would be to show that the nasal product is safe and bioequivalent to the commercially available rectal product.

The results of the pilot study conducted in healthy volunteers indicate that neither of the two intranasal formulations would be bioequivalent to rectal diazepam. The rate of absorption from intranasal formulations was considerably slower than rectal diazepam. In addition, there were considerable differences in bioavailability among the three formulations. Although the simulation study showed that in 75% of the trials, the 90% confidence intervals of the geometric mean of AUC ratios (IN solution/rectal) fell within the bioequivalence limits of 80-125%, the nasal solution failed the bioequivalence test for Cmax. In the absence of pharmacokinetic data demonstrating comparable exposures following rectal and intranasal administration, a well-controlled clinical trial to prove safety and efficacy in patients would be needed. This chapter outlines the key study design elements of an efficacy trial that will be required for the approval of intranasal diazepam product and the associated challenges.

5.1.1 Design of a Safety and Efficacy Trial for Development of Intranasal Diazepam

Choice of control group

Recently, Abou-Khalil et al reported the results of a randomized, placebo-controlled study of IM diazepam auto-injector for treatment of ARS in which the drug was significantly better

than placebo at preventing seizure recurrence [54]. The sample size required for a placebo-controlled trial will be considerably smaller than that required if a non-inferiority trial is conducted using an active-control group. However, slow patient enrollment is a concern. In addition, a number of measures have to be taken to assure the safety of patients who are randomized to placebo group. In the context of ARS, most of the older children and adult patients decline rectal therapy and would agree to enroll in a placebo-controlled trial despite knowing that they might get randomized to placebo group. Pharmaceutical companies prefer placebo-controlled trials because they are cost-effective and less time consuming because of the smaller sample requirement. However, a clinician might prefer a non-inferiority trial because it provides data for comparative efficacy and safety of investigational drug to that of standard of care. Following below is the study design for a non-inferiority trial comparing IN diazepam to rectal diazepam. In order to appreciate the merits and demerits of active vs. placebo control group, an attempt is made to contrast the differences of a non-inferiority trial to that of placebo-controlled trial.

Primary aim

The primary objective of the study will be to show that the proportion of patients who were seizure free in the intranasal diazepam group was not inferior to that in the rectal diazepam group by more than a prespecified margin of 10% (the non-inferiority margin).

Secondary aim

The secondary objective of this study will be to assess the safety and tolerability of the diazepam nasal spray .

Study design

The efficacy trial would be a multicenter, double-blind, double-dummy, parallel, randomized, non-inferiority trial comparing the efficacy of intranasal diazepam and rectal diazepam. Diazepam doses for both intranasal and rectal routes would be determined as per the Diastat label.

Procedures

The Institutional Review Board at each study center will approve the protocol and consent procedures and the trial would be conducted under an Investigational New Drug (IND) application with the FDA. Diastat AcuDial syringes with diazepam and placebo will be purchased. Intranasal diazepam spray devices with active ingredient and placebo will be obtained from the sponsor. A Data and Safety Monitoring Committee (DSMC) will monitor the study for safety.

Study population

Inclusion criteria

1. Males and females of age 12-45 years with a documented history of ARS.
2. To ensure timely enrollment, patients need to have at least 4 episodes of ARS during the preceding year and at least one episode in the preceding three months.
3. Patients should be on stable antiepileptic drug dosage regimens for at least 4 weeks before enrollment.
4. A cranial CT scan or MRI without evidence of a treatable cause for the seizures.
5. Females of childbearing potential should have a negative pre-enrollment pregnancy test and on-going contraception.

6. All patients or their parent or legal guardian should provide assent/written consent for study participation.

Exclusion criteria

1. Phenobarbital concentrations in plasma >30mg/L.
2. Current treatment with drugs other than anticonvulsants.
3. Long term use of benzodiazepines or allergy to diazepam.
4. Use of CNS depressants or drugs interacting with diazepam.
5. Patients who had received another investigational medication or device within 30 days of study entry.
6. Patients with clinically significant abnormal baseline laboratory values or an unstable medical abnormality.
7. Patients with known history of severe seasonal or non-seasonal allergies, having nasal polyps or any nasal passage abnormality that could interfere with nasal spray administration.

Seizure types

For study purposes, the epileptic seizure type within the episode of ARS could be primary generalized, complex partial with or without becoming secondarily generalized, or simple partial with a motor component as defined in the International Classification of Seizures. (These seizure types are believed to be recognized more accurately by the caregiver for counting purposes) [153].

Assessments

Study nurses will train caregivers to identify episodes of ARS, give medication, and record in a study booklet respiration, skin, color, seizures, adverse events, and global assessment of treatment outcome. During episodes of ARS, study nurses will maintain telephone contact with the caregivers to review procedures, monitor patients, and intervene as necessary if patients need additional treatment. The observation period will begin 15 minutes after the first dose and will continue up to 12 hours. The patients and care givers will be scheduled to return to the clinic 72 hours after treatment to review the recorded data.

Sample size and power calculation

Published studies of similar patient populations show that 60% of subjects were seizure free when treated with rectal diazepam [153]. Sample size was determined to have 90% power to show the non-inferiority of intranasal diazepam with a margin of 10% using a one-sided test with the probability of a type I error of 0.025. The placebo-controlled trial conducted by Abou-Khalil et al calculated a sample size of 86 patients per group for 90% power [54]. In contrast, for the non-inferiority trial, sample size required for randomization is 722 patients per treatment group, assuming 70% of the patients randomized will be treated. Some patients, though enrolled, may not actually be treated due to several reasons. These include withdrawal of consent before treatment, and some may become ineligible post-enrollment. Practical issues such as absence of a caregiver, need of other concomitant medications, lost to follow-up, improper dosing, and incomplete data recording are some other factors to inflate the sample size. Assuming average enrollment rate of 6 patients per clinic per year with 50 participating centers,

patient enrollment will take place for 5 years for the non-inferiority trial. In contrast, the placebo-controlled trial, which would require enrollment of about 300 patients, can be completed in less than 2 years.

In addition to the 5 years of enrollment, factoring in the time for study start-up and end-of-study procedures, it will take 7 years to complete the non-inferiority trial. Though a non-inferiority trial provides comparative efficacy data, it will take a long time and require a large study. Therefore, a placebo-controlled trial would be feasible as it is less time consuming and cost effective.

Statistical analysis

All patients who receive study medication will be included in the analysis. Demographic and baseline variables such as sex, age, age group (6-12 and >12-17 years), race, weight, seizure etiology, ARS frequency, and seizure type (complex partial vs. generalized) will be analyzed for comparability between rectal and intranasal diazepam treated groups. Categorical variables will be analyzed using chi-square test. If any of the expected subgroups are less than 5 then Fisher's Exact Test will be used. Baseline values of age and weight are analyzed with a two-sample t test. ARS frequency at baseline will be analyzed with a Wilcoxon rank-sum test because literature review indicates that such data are not normally distributed.

The primary objective of the study is to show that the proportion of patients who were seizure free during the observation period of 12 hours in the intranasal diazepam group was not inferior to that in the rectal diazepam group by more than a prespecified amount

(the non-inferiority margin). The null hypothesis of inferiority will be tested with one-sided z statistic [3]. The secondary efficacy variable (seizure count) for the two treatment groups (Diastat versus Intranasal diazepam) will be compared using Wilcoxon's rank sum test at 0.05 significance level.

Primary efficacy analysis/Significance testing

If the one-sided 97.5% CI (or equivalently the two-sided 95% CI) of the absolute difference of two treatments ($\Delta = P_{IN} - P_{rectal}$) excludes -0.1 (-10%), then we show that intranasal diazepam is non-inferior.

Challenges to physician and patient cooperation

Designing and conducting a study to demonstrate safety and efficacy would be challenging. Clinical staff training, time to explain the study to patients, follow-up burden etc. are some likely challenges to physician participation. To overcome this challenge, the study design should be simple, data collection forms should be user friendly and not cumbersome. Patient's willingness to participate in research is one of the most common challenges in clinical research. Another challenge is the availability/willingness of caregiver to record the events, which is essential for proper study execution. Subjects who are already stable on rectal product might be interested in trying an intranasal product, but the need to fill out data collection forms might be a hindering factor for patient enrollment.

An Alternate Apporach to Study Design: Bayesian adaptive methods

One can employ Bayesian adaptive methods to address the large sample size requirements of a non-inferiority trial. Bayesian methods allow use of historical data and interim trial results to modify study design adaptively to reduce both time and expense. Bayesian adaptive designs allow for unlimited looks at accumulating data because of its different framework for testing, which will allow stopping a trial sooner. From a frequentist view, one cannot take a look at accumulating data unless the trial is completed or an interim analysis is planned. Bayesian methods are different in that you can take multiple looks and make decisions early if a particular arm is performing better than what was expected during the sample size calculations. If we have more than one investigational treatment in a trial, Bayesian methods will allow you to drop an unproductive or unpromising treatment, without completing the whole trial as initially planned. Bayesian adaptive methods will also allow the assignment of more patients to a more promising arm. For example, if a particular intranasal formulation is performing better than another formulation, adaptivity will allow more patients to get randomized to that particular formulation. In summary, frequentist statistical inference for hypothesis testing is design-based; if you change the design of the experiment, you change the reference set of outcomes, and thus the p-value. Therefore, you cannot change the study design when employing frequentist methods. However, Bayesian approach allows more flexibility as inference is model-based and does not use p-values. The non-inferiority study described here uses frequentist approach and an interim analysis is not planned given that it will inflate the already large sample size further and a Bayesian approach would be preferable in this case for the reasons outlined above.

5.2 Development of Intravenous Baclofen for Prevention of Baclofen Withdrawal

We are encouraged by the experience gained with the series of investigations with regard to developing an IV formulation of baclofen. Bolus doses and continuous infusions of IV baclofen were tolerated by dogs without instability of vital signs at doses much higher than those typically used in humans with oral baclofen. The results of current research in humans described in this thesis showed that 3 and 5 mg of baclofen doses given IV are well-tolerated by healthy adults. Future studies in humans are being planned to investigate the pharmacokinetics and safety of escalating single doses of intravenous baclofen, as well as the optimal approach to substituting intravenous baclofen administration when oral or intrathecal baclofen therapy is interrupted. Figure 5.2-1 lists a number of studies which we envision to be required for the commercial development and marketing approval of IV baclofen. The first two studies in Figure 5.2-1 are completed and Study III is in planning stage.

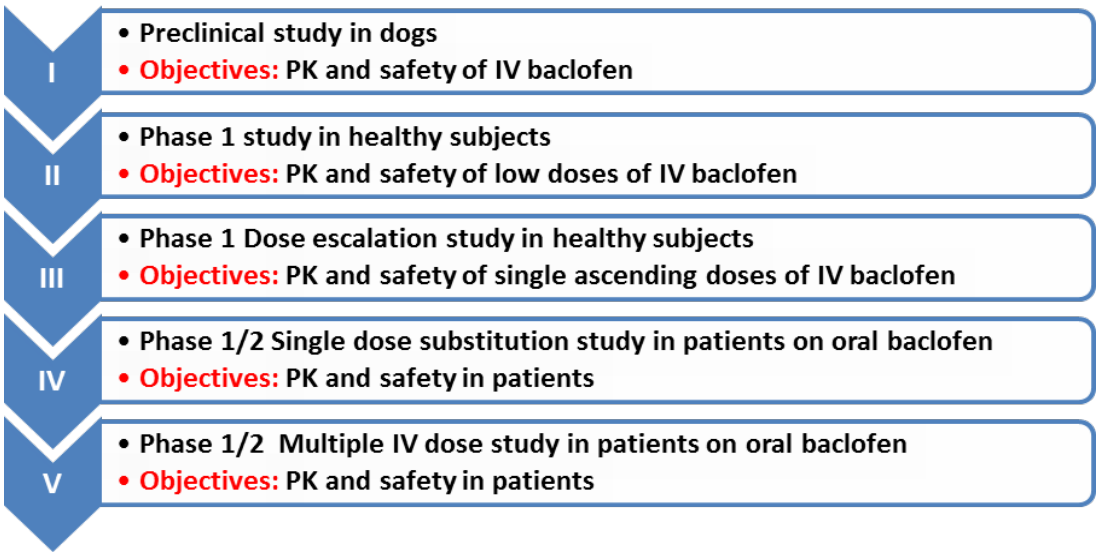

Figure 5.2- 1: Clinical Studies Required for Commercial Development of IV Baclofen

5.2.1 Designing Phase 1 Dose-escalation Study of Intravenous Baclofen in Healthy Subjects (Study III)

Objectives

1) Determine the safety profile of commonly used doses of baclofen when given orally and intravenously.
2) Characterize baclofen pharmacokinetics of escalating doses after oral and intravenous administration in healthy adults.
3) Determine oral bioavailability and assess dose-proportionality of single doses of 10, 15 and 20 mg of oral and 7.5, 11.5, 15 mg of intravenous baclofen, respectively.

Study Design

This study will utilize a randomized, open-label, three phase, two-way crossover design to compare the pharmacokinetics and bioavailability of the oral tablet with an intravenous formulation in thirty six healthy participants.

Dosing Scheme

Phase one: Twelve subjects will be randomized to receive 10 mg doses of oral and 7.5 mg of IV baclofen in a crossover fashion; 3 of these subjects will receive the 7.5 mg IV dose initially to evaluate safety and tolerance. If these 3 subjects tolerate the IV dose, the remaining 9 subjects at this dosing level will be randomized to receive either oral or IV dose first.

Phase two: If 7.5 mg intravenous dose of baclofen is tolerated by all subjects, then 12 different subjects will receive 15 mg baclofen orally and 11.5 mg baclofen intravenously;

3 of these subjects will receive the 11.5 mg IV dose initially to evaluate safety and tolerance. If these 3 subjects tolerate the IV dose, the remaining 9 subjects at this dosing level will be randomized to receive either oral or IV dose first.

Phase three: If 11.5 mg intravenous dose is tolerated by all subjects, then 12 different subjects will receive 20 mg baclofen orally and 15 mg baclofen intravenously; 3 of these subjects will receive the 15 mg IV dose initially to evaluate safety and tolerance. If these 3 subjects tolerate the IV dose, the remaining 9 subjects at this dosing level will be randomized to receive either oral or IV dose first.

Dose Justification

In this dose-escalation study, single oral doses of 10, 15 and 20 mg baclofen, which are commonly used clinically will be studied. Since the first-in-human study results demonstrated that the oral bioavailability of baclofen was 74%, equivalent IV doses (75% of oral doses) of 7.5, 11.5 and 15 mg will be studied.

PK Sample Collection

Blood samples (6mL) for the measurement of plasma concentrations of baclofen will be collected in blood collection tubes containing K_2-EDTA at the following times: prior to dosing and at 5, 15, 30 minutes, and 1, 2, 4, 6, 8, 10, 12, and 24 hours after drug administration. Plasma will be separated by centrifugation and stored frozen until analysis

Safety Assessments

Safety will be assessed by ECG results, blood pressure and pulse monitoring, assessment of CNS toxicity, injection site irritation, side effects and tolerability, as well as physical

exams at 12 and 24 hours after the drug administration. Ataxia assessment by observation of gait will be done prior to drug administration, every 30 minutes for the first 4 hours, and at 12 and 24 hours after the drug administration. At the same time as ataxia assessments, nystagmus and sedation will be assessed. Nystagmus will be assessed both at neutral and lateral gaze. Sedation will be assessed using the modified Stanford Sleepiness Scale (SSS) given below:

1 - Feeling active, vital, alert, or wide awake

2 - Functioning at high levels, but not at peak; able to concentrate

3 - Awake, but relaxed; responsive but not fully alert

4 - Somewhat foggy, let down

5 - Foggy; losing interest in remaining awake; slowed down

6 - Sleepy, woozy, fighting sleep; prefer to lie down

7 - No longer fighting sleep, sleep onset soon; having dream-like thoughts

8- Asleep, unable to awaken

Study Stopping Rules

If any subject develops severe ataxia (unable to walk without assistance) or is observed to have a sleepiness scale of 8, no further dose escalation will occur. In addition if an SAE occurs in any participant we will ask the Medical Monitor to determine whether the adverse event was due to study medication. If it is deemed due to study medication no further subjects will be given IV baclofen.

Data Analysis

Descriptive statistics will be used to summarize the data from this study. In addition to standard pharmacokinetic analysis to calculate absolute oral bioavailability, statistical analysis of dose-normalized PK variables Cmax, AUClast and AUC0-∞ will be done after log transformation. Dose proportionality will be assessed by ANOVA followed by Tukey's multiple comparison tests to detect statistical differences in AUC and Cmax among the oral and IV doses.

5.2.2 Dose Substitution Studies of IV Baclofen in Patients under Steady-State Conditions (Study IV and V)

Once the safety of escalating doses is established in healthy volunteers, the next step is to conduct dose substitution studies in patients chronically receiving baclofen. Based on the absolute oral bioavailability estimate obtained from studies in healthy volunteers, oral doses of baclofen in patients can be substituted with an equivalent IV dose. However, one needs to note that these patients are under steady-state conditions and characterizing the absolute bioavailability can be done using an IV formulation of stable-labeled isotope of baclofen. In this study a portion of the patient's usual oral dose will be replaced and co-administered with an intravenous stable isotope of baclofen. This approach permits measurement of the absolute bioavailability, clearance, distribution volume, and elimination half-life parameters that cannot usually be determined from only oral dosing studies. For drugs such as baclofen where discontinuation of therapy is not possible, stable-labeled (nonradioactive) isotopes followed by mass spectrometry analysis can be used to characterize pharmacokinetics in patients under steady-state conditions.

For faster approval of IV baclofen and in absence of data suggesting that spasticity alters bioavailability of oral baclofen, one can argue that a single dose substitution study (study

IV) is not required in patients. One can make an assumption that oral bioavailability in healthy adults and patients would be similar. This would then allow to directly conduct a safety and PK study following multiple doses of IV and oral baclofen.

5.2.3 Application of Modeling and Simulation to Optimize IV Dosing Regimen

When oral baclofen therapy is interrupted, similar total exposures can be attained by initiating IV therapy using equivalent doses based on the absolute bioavailability estimates obtained from the PK studies outlined above. However, the dosing regimen may have to be modified. IV doses given using the same dosing regimen as the oral therapy will result in higher peak and lower trough concentrations than those seen from oral administration. It will be necessary to modify the dosing regimen by decreasing the dose and increasing the dosing frequency to achieve similar peak and trough concentrations. A PK model developed with the data obtained from the studies outlined above could be used to simulate concentrations from several dosing regimens. These simulations could provide helpful information in optimizing IV baclofen dosing regimen to maintain target drug concentrations within desired therapeutic window. The same PK model can also be used to optimize other dosing regimens such as intravenous infusions of varying dose rates if precise concentrations are to be maintained.

On the other hand, calculating IV doses for preventing or treating baclofen withdrawal resulting from interruption of intrathecal therapy is not straightforward. Daily intrathecal baclofen doses are typically very low and a study analyzing 43 patients (28 males, 15 females; range 3-44years) reported a range of 70 to 1395 mcg/day (median 575 mcg/day)[152]. When given intrathecally, the drug is delivered near the site of action; there

is minimal enzymatic degradation; restricted distribution to supraspinal tissues explaining the reason for smaller ITB doses required to treat spasticity. One paradigm to calculate required IV doses would be to dose patients with an aim to achieve same cerebrospinal fluid (CSF) baclofen levels as those achieved after intrathecal baclofen delivery. However, in clinical practice CSF levels are rarely available. In the absence of CSF levels, the soleus H-reflex can be used to guide the IV dosing of baclofen. The soleus H-reflex measure provides an objective, reproducible, and sensitive index of changes in spinal excitability and future clinical trials of baclofen should explore its potential use.

CHAPTER 6

CONCLUSIONS

CONCLUSIONS

The overall goal of this project was to characterize the pharmacokinetics, bioavailability and tolerability of CNS drugs used in the acute treatment of seizure emergencies and spasticity. Two drugs were studied: diazepam and baclofen. Both of these drugs are already approved by the FDA and have been extensively used. In the case of baclofen, commercially available intrathecal baclofen solution was administered intravenously to characterize its pharmacokinetics and safety in humans.

For diazepam, the pharmacokinetics and tolerability of two novel formulations were evaluated for intranasal delivery as rescue therapy in seizure emergencies. While rectal diazepam (Diastat Acudial™) is safe and effective in treating ARS, its use is limited because of patient/family/teacher objections to the route of administration, particularly when the drug needs to be administered at work, school, classroom, or public locations. Furthermore, systemic exposure following rectal administration is widely variable given the potential for bowel movements or other factors that impact absorption of drugs. The availability of an intranasal diazepam product that is easily and safely administered and rapidly and completely absorbed would add significant clinical benefit to patients who experience ARS. The results of earlier studies by our research group concluded that intranasal diazepam offers a viable alternative to rectal administration, however further enhancement of formulations was needed both to improve tolerability and the extent and consistency of absorption. In the current work, two novel formulations of diazepam nasal spray were evaluated and compared with intravenous diazepam administration in a phase I pilot PK study. Of the two formulations that were tested, namely, nasal suspension and nasal

solution, the absolute bioavailability of the latter approached ~100% and this formulation was well tolerated with no reports of nasal pain or irritation. Therefore, this formulation has the potential to serve as an alternative mode of therapy to rectal diazepam and is preferred candidate for further development. Additional clinical trials including dose-escalation, and safety and efficacy studies in patients are required before market approval.

For baclofen withdrawal, the current recommended management strategies are inadequate and availability of intravenous baclofen would permit rapid attainment of drug concentrations in plasma as well as accurate and precise dose titration, thus allowing for prevention and expeditious treatment of withdrawal symptoms and reduced risk of adverse effects. In the current work described in this thesis, a commercially available intrathecal formulation of baclofen was administered intravenously to evaluate and compare with oral baclofen tablets in a phase I pilot PK study. The absolute bioavailability of the oral tablets was found to be 74 % and the small doses of baclofen given IV were well tolerated. The PK data gained from this study will guide the design of future trials directed at the development of IV baclofen for bridge therapy. Dose escalation studies are needed to assess the safety of higher clinically relevant doses as well as for determining the linearity of exposure at higher oral doses to aid in correct calculation of replacement IV doses. Once the safety and pharmacokinetics of higher IV doses is demonstrated, dose substitution studies in patients on chronic oral baclofen therapy will be necessary to assess multi-dose safety and pharmacokinetics.

In conclusion, the research described in this thesis suggests that both intranasal diazepam and intravenous baclofen hold promise in management of seizure emergencies and baclofen withdrawal, respectively.

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APPENDIX

NONMEM Code for PK Model

```
$PROB RUN# 201

$INPUT ID TIME AMT DV EVID CMT OID=DROP TRT FORM STDY PERI DLVL
AGE SEX RACE WT TAD

$DATA IVPRINDZP.CSV IGNORE=@

$SUBROUTINES ADVAN4 TRANS4

$PK

TVCL=THETA(1)
CL=TVCL*EXP(ETA(1))
TVV2=THETA(2)
V2=TVV2*EXP(ETA(2))
TVQ=THETA(3)
Q=TVQ*EXP(ETA(3))
TVV3=THETA(4)
V3=TVV3*EXP(ETA(4))

KAPR=THETA(5)*EXP(ETA(5))
F1PR=THETA(6)+ETA(6)

KASUSP=THETA(7)*EXP(ETA(7))
F1SUSP=THETA(8)+ETA(8)

KASOL=THETA(9)*EXP(ETA(9))
F1SOL=THETA(10)+ETA(10)

DUM6=0
IF(FORM.EQ.6) DUM6=1
DUM7=0
IF(FORM.EQ.7) DUM7=1
DUM8=0
IF(FORM.EQ.8) DUM8=1
KA=DUM6*KAPR+DUM7*KASUSP+DUM8*KASOL
F1=DUM6*F1PR+DUM7*F1SUSP+DUM8*F1SOL

SC=V2/1000
```

```
$ERROR

Y = F + F*ERR(1)
IPRED=F
IRES=DV-IPRED
W=IPRED
IWRES=IRES/W

$EST METHOD=1 INTERACTION PRINT=5 MAX=9999 SIG=3 MSFO=40001.MSF
NOABORT

$THETA

(0, 1);CL
(0, 20);V2
(0, 10);Q
(0, 60);V3
(0, 1);KAPR
(0,.8,1);FPR
(0, 1);KAINSUSP
(0,.8,1);FINSUSP
(0, 1);KAINSOL
(0,.8,1);FINSOL

$OMEGA

0.1 ;  CL
0.1 ;    V2
0.1 ;      Q
0.1 ;      V3
0.2 ; KAPR
0.005 ;FPR
0.2 ; KAINSUSP
0.005 ;FINSUSP
0.2 ; KAINSOL
0.005 ;FINSOL

$SIGMA
0.01

$TABLE ID TIME IPRED CWRES TAD IWRES ONEHEADER NOPRINT
FILE=sdtab001
```

```
$TABLE ID ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 ETA8 ETA9 ETA10 CL V2
Q V3 KA F1 NOAPPEND NOPRINT FILE=patab001


$TABLE ID AGE WT FIRSTONLY NOAPPEND NOPRINT FILE=cotab001

$TABLE ID SEX RACE FORM FIRSTONLY NOAPPEND NOPRINT FILE=catab001
```