INTRANASAL AND RECTAL DIAZEPAM FOR RESCUE THERAPY: ASSESSMENT OF PHARMACOKINETICS AND TOLERABILITY

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ABSTRACT

The use of rectal diazepam has improved the management of acute repetitive seizures (ARS) outside a health care facility. Two placebo controlled trials have shown that rectal administration of diazepam is safe and effective for treatment of this condition. Diastat® is the only FDA approved treatment for ARS in the United States. Although some older children and adults are willing to use Diastat®, many patients in these age groups as well as physicians and caregivers object to the route of administration and instead use other therapies not approved for this purpose, receive no treatment, or use emergency medical services or acute care systems.

We developed and evaluated three nasal spray formulations of diazepam which can be easily administered with rapid absorption characteristics intended as an alternative to rectal administration. One formulation used a supersaturated glycofurol based co-solvent system while the remaining two (Nas-A & Nas-B) used microemulsion based co-solvent systems. These formulations were studied for their pharmacokinetics and tolerability in healthy adult volunteers. Data from these studies were then compared to the pharmacokinetics after rectal administration using both model-based analysis (NONMEM) and graphical methods.

The primary finding from this work was that, only the microemulsion-based formulations, particularly Nas-B could be used for further development as the glycofurol formulation was not well tolerated by subjects. The pharmacokinetic profiles after intranasal administration were associated with high variability. However, we are able to show that the dose-normalized partial area under the curve (AUC - an exposure parameter) after nasal administration, at times when the drug concentrations are most important, are 60-80 % of that when given via the rectal route. Given the ease and social acceptability of nasal administration compared to rectal, equivalent exposures can be easily attained by giving a second nasal dose, and we thus conclude that intranasal diazepam is a feasible and preferable alternative to rectal diazepam in the management of

ARS outside a hospital. This work also provides some recommendations for future studies in the development of an intranasal product.

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CHAPTER 1

INTRODUCTION

1.0 Epilepsy

Epilepsy is a chronic neurologic disorder with episodic manifestations (CDEM) that are associated with abnormal electrical discharges in the brain [1]. It is manifested in the form of recurrent, spontaneous seizure episodes or convulsions, the latter reflecting sudden, stereotyped episodes with accompanying changes in motor activity, sensation and behavior. Depending on the type of epilepsy, seizures may be accompanied with muscle spasms and a loss of consciousness. Seizures are thought to result from imbalance occurring in the discrete anatomical pathways in the brain between the major excitatory and inhibitory systems, glutamate and gama-amino-butryric acid (GABA) which leads to abnormal electrical discharges. Further, seizures may also induce alterations in neurons, glia and neuronal circuits that may affect membrane receptors and neurotransmitter uptake sites, both neurogenesis and apoptosis, astrocyte proliferation, and axonal sprouting. All these phenomena make an individual more susceptible to additional convulsive episodes and cognitive dysfunction.

Epilepsy is one of the most common neurological disorders in humans, affecting 1–2% of the global population. Estimates from the World Health Organization data indicate that approximately 50 million people worldwide, or a prevalence of 50 per 100,000 of the general population, suffer from epilepsy [2]. Treatment in the form of medications, vagal nerve stimulation, or surgery at defined resectable seizure foci are available options to treat epilepsy. Even with current medications approximately 30–40% of patients with epilepsy fail to achieve freedom from seizures. Also a large number of patients are known to be refractory to drug treatment which may involve one or combination or more than one drug. An individual who fails to achieve freedom from seizures with adequate doses of two anticonvulsants is known to be at high risk for failure on other anticonvulsant medications. Moreover, approximately 10–20% of patients have resistant seizures, despite the use of drug combinations and the potential problem of side effects for most drugs. For example, the two most commonly prescribed anticonvulsants, sodium

valproate (valproic acid; VPA) and carbamazepine (CBZ), even though highly effective in controlling seizures, produce fatigue, weight gain, and dizziness in addition to the latter having 'black box' warnings for hepatoxicity and teratogenicity [3].

There are numerous economic and social issues for individuals with epilepsy that result in a poor quality of life and if left uncontrolled, epilepsy results in significant morbidity, mortality, and financial burden to the healthcare system. As of 2004, health system costs due to epilepsy in the USA have been estimated at approximately $12.5 billion annually [4].

1.0.1 Disease State

Epilepsy is usually classified as idiopathic, symptomatic and cryptogenic. When no etiology other than a genetic predisposition has been identified epilepsy is referred to as idiopathic; symptomatic when epilepsy can be associated with trauma, e.g., poisoning, stroke, head injury or a brain lesion, Lennox–Gastaut syndrome, hippocampal sclerosis, and cerebral palsy; or cryptogenic where a symptomatic cause is suspected but unproven. Approximately 70% of epilepsies can be attributed to specific brain pathology [5].

1.0.2 Seizure Classification

The International Classification of Epileptic Seizures (ICES) established guidelines in 1981 that were refined by the Commission on Classification and Terminology of the International League Against Epilepsy (ILAE) in 1989 [6]. Although the classification systems developed concise definitions of the various types of epilepsy, they only described phenotypes and provided little information regarding the causality or severity of the condition or prognosis for the patient [3].

1.0.2.1 Partial seizures

Partial seizures are divided into: (1) simple, where only a part of the brain is involved and consciousness is not impaired, and (2) complex, which is differentiated from simple due to impairment of consciousness. Complex partial seizures are frequently preceded by a simple partial seizure or an aura.

1.0.2.2 Generalized seizures

Generalized seizures involve both sides of the brain and result in tonic and clonic movements (primary or secondary generalized) or another type of primary generalized epilepsy (e.g., absence or atonic seizure).

1.0.2.2.1 Tonic–clonic or major motor seizures

Tonic–clonic, grand mal or major motor seizures are characterized by a loss of consciousness, falling, muscle rigidity and jerking, and an electrical discharge that involves all or most of the brain.

1.0.2.2.2 Absence or petit mal seizures

An absence, formerly called petit mal seizures is a primary generalized epileptic episode that usually lasts less than 20 s and is characterized by a stare, sometimes associated with

blinking or brief automatic movements of the mouth or hands. These usually begin in childhood, are well controlled with drugs, and are seen in approximately 75% of children.

1.0.2.2.3 Atypical absence seizures

Atypical absence seizures are usually characterized by a staring spell characterized by partial impairment of consciousness that often occurs in children with Lennox–Gastaut syndrome, a rare disorder beginning in childhood that is characterized by mental retardation, multiple multifocal seizures that do not respond well to therapy and an electroencephalograph (EEG) that shows slow (less than 3 s^{-1}) spike-and-wave discharges.

1.0.2.2.4 Atonic or drop seizure

This type of seizure is characterized by a sudden loss of muscle tone that is usually not associated with loss of consciousness.

1.0.2.3 Febrile seizures

Febrile seizures are tonic–clonic convulsions that occur in children aged between 6 months and 5 years that are instigated by fever and are benign. Some children however can go on to develop hippocampal sclerosis, a primary cause of idiopathic temporal lobe epilepsy. Febrile seizures are typically treated with phenobarbital. This sometimes produces hyperactivity and behavioral and learning problems, leading some pediatric neurologists to believe that treatment of febrile seizures is worse than the seizure itself.

1.0.2.4 Status epilepticus

Status epilepticus was originally described as a series of repeated seizures or seizures lasting longer than 30 min that can occur in almost any seizure type [7]. More recent guidelines state that treatment should begin after 5 min of convulsive seizures [8]. Status epilepticus is associated with a high rate of mortality, 3% in children and 30% in adults, and is defined as a medical emergency, requiring rapid diagnosis and treatment. The probability of developing epilepsy after a first episode of status epilepticus is 41% in a 2-year period after the episode, which supports a relationship between the prolonged seizures of status epilepticus and eliptogenesis [3].

1.0.2.5 Seizure Emergencies

Seizure emergencies can occur in all types of patients, including patients with a diagnosis of epilepsy and those experiencing a single but prolonged seizure, as well as patients with newly diagnosed or longstanding epilepsy whose treatment regimens are being changed. Seizure emergencies may range from isolated, brief recurring seizures to status epilepticus. Recurrent unprovoked seizures and prolonged or acute repetitive seizures (ARS) are also known as cluster, crescendo, multiple recurrent, serial or sequential seizures [9]. Both acute repetitive seizures and prolonged seizures can evolve into status epilepticus. All seizure emergencies alter a patient's physical and cognitive functions, disrupt usual activities, have the potential for injury, and, in the case of status epilepticus, carry an increased risk of mortality. A detailed discussion on acute repetitive seizures will follow in the next section.

1.1 Seizure Emergencies

Emergencies include a wide spectrum of seizures including isolated, brief events and prolonged episodes lasting many minutes and status epilepticus. Figure 1.1-1 illustrates the range of seizure emergencies as determined by duration of seizure and potential for neuronal injury [9].

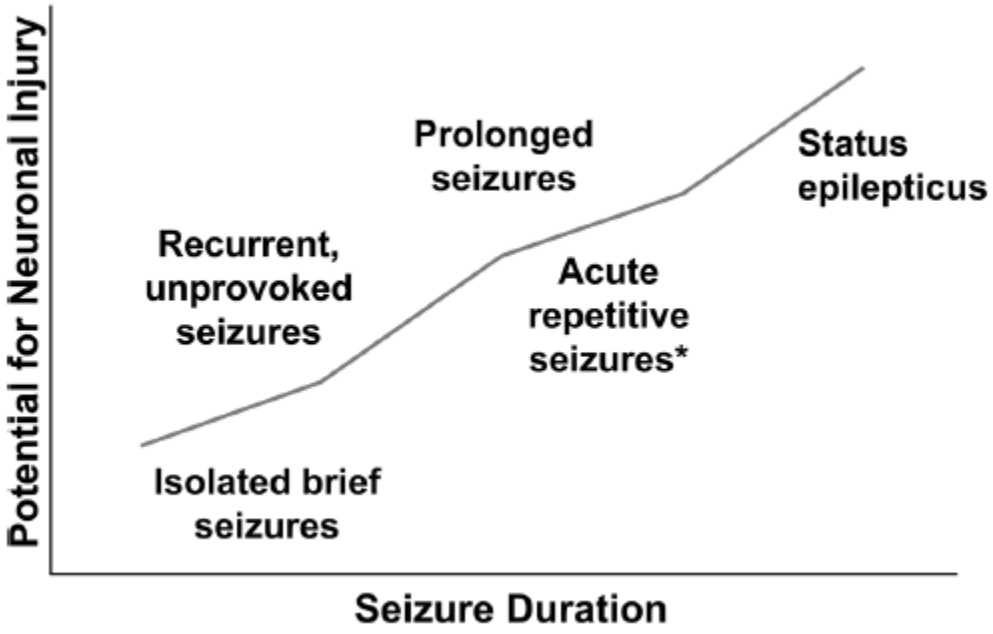


Figure 1.1-1: Spectrum of Seizures. * Also known as cluster, crescendo, multiple-recurrent, serial or sequential seizures.

A first isolated seizure may be self-limiting but most (>50%) are prolonged of more than 5 minutes duration [10]. It has been shown that of first seizures, 10 to 12 % are status epilepticus. Further, patients with prolonged seizures or status epilepticus are at a greater risk of experiencing another seizure of this type [11]. A prolonged seizure is usually defined as a seizure lasting from 5 to 10 minutes to 29 minutes of continuous or intermittent seizure activity without regaining consciousness. The definition for status

epilepticus has been evolving. A consensus conference held in 1993 changed the term to encompass an epileptic seizure lasting 30 minutes or more without recovery of consciousness between episodes [7]. However, Lowenstein and Alldredge have proposed a more operational definition for status epilepticus: “either continuous seizures lasting at least five minutes or two or more discrete seizures between which there is incomplete recovery of consciousness” [12]. Acute repetitive seizures are a predictable component of a patient’s seizure disorder, historically distinct from patients other epileptic seizures in type, frequency, duration, or severity, and with an onset easily recognized by the caregiver and physician.

1.1.1 Acute Repetitive Seizures (ARS) / Seizure Clusters

Table 1-1 shows examples of acute repetitive seizures (ARS). As mentioned earlier, these are a predictable component of a patient’s seizure disorder. The onset has a consistent predictable component (such as aura or prodrome, which may be convulsive or nonconvulsive symptom, or characteristic single or multiple seizures) that is predictably and temporally linked to subsequent seizures. Typically there is recovery between these seizures, which differentiates it from status epilepticus. The episodes of ARS may or may not progress to a prolonged seizure or to status epilepticus but may be predictable for each patient based on history. ARS may include any time of epileptic seizure and may occur at any age. ARS is also usually described by other terms such as cluster, serial, recurrent or crescendo seizures [10].

Table 1-1: Examples of ARS. Adapted from [10]

ARS definition	Criteria to treat
Three generalized tonic clonic seizures in 2 hours	After the fourth generalized tonic clonic seizure in 2 hours
Patient asleep and towards morning generalized tonic clonic seizures begin; never has just one seizure but will predictably have as many as 10 seizures 15-30 minutes apart	Treat after first sleep related seizure
In and out of complex partial seizures followed by 10 minutes of unresponsiveness, which will predictably continue for the rest of the day	Treat if patient is in and out of seizures for > 1 hour

1.1.1.1 Mechanism of ARS

The concept of clustering implies either impaired termination or increased excitation, possibly due to secondary alterations from an initial seizure that promote a second attack, or an excess of seizure-promoting factors [13]. It has been shown that seizures occurring within 8 hours of a prior seizure are significantly more likely to arise from a concordant focus than seizures more widely separated in time [14]. This has led to the speculation that an ictal focus may be more excitable, or less inhibited, following a first seizure, leading to a seizure cluster. A commonly used definition of a seizure cluster is three seizures per 24 hours, and is derived from the fact that seizures within 8 hours are not truly independent of each other [1]. Some studies have evaluated the concept of dependence between seizures, to identify whether a seizure may influence the possibility of a second seizure. Hopkins *et al*. [15] developed a Markov model in which the

transitional probability of a subject being in a particular seizure-susceptibility state depended on the state of the previous day which allowed the calculation of probability estimates for the expected incidence of subsequent seizure days.

1.1.1.2 Definitions of Seizure Clustering

Seizure clusters are defined clinically as, a closely grouped series of seizures, or statistically as, a deviation from randomness, which is typically addressed as a statistical concept.

Different clinical definitions based on empiric facts for seizure clusters have been proposed, including two to four seizures within 48 hours; 3 seizures in 24 hours; or two generalized tonic–clonic or three complex partial seizures in 4 hours. However, these definitions, especially in clinical and out-of-hospital settings may be inappropriate and usually rely heavily on the ability to identify these seizures and the record keeping of patients and caregivers [1]. In a large randomized controlled trial of treatment for acute repetitive seizures, the condition was defined as ‘‘multiple seizures occurring with a 24 period for adults or 12 hour period for children, with a pattern distinguishable from the usual seizure pattern’’[16].

Statistical definitions for clusters are dependent on the belief of whether seizures are random or nonrandom events. If seizures are considered random, then the occurrence of one seizure does not increase or decrease the likelihood of a subsequent one. However, evidence suggests that many seizures are not random and in fact may have some cyclical patterns including circadian and catamenial patterns; furthermore, factors such as medication noncompliance and sleep deprivation also exist. The Poisson distribution process has been applied to seizure patterns, to evaluate randomness of seizures [15]. The Poisson process which is a memoryless system where the probability of an event is not influenced by the times of the occurrences of any past event, describes a stochastic

(random) system in which the numbers of events occurring in disjointed (nonoverlapping) time intervals are independent random variables, and the number of events within each time variable occurs as a random variable with a Poisson distribution [17]. Deviations from a Poisson process in seizure patterns may reflect clustering, periodic patterns, or may reflect regularity [1].

1.1.1.3 Risk factors for ARS

The etiology of seizures may play a role in identifying risk factors; head trauma has been reported to be significantly associated with patient-reported history of seizure clustering, independent of epilepsy localization. Seizure control is another risk factor for clustering where patients with more intractable epilepsy appear to be at higher risk of experiencing seizure clustering [18]. It has been shown that in this group of patients with intractable epilepsy the occurrence of seizure clusters defined clinically may be a random phenomenon of high seizure frequency with a significant departure from a Poisson process in subjects with more seizures and higher seizure frequencies [19]. Despite the demonstration of hormonal influence on seizure occurrence, gender has not been demonstrated to be associated with seizure clustering while age appears to be an independent risk factor for clustering with longer duration of epilepsy increasing the clustering risk [13].

1.1.1.4 Risks and implications of ARS

There is accumulating evidence that prolonged or repetitive seizures can produce lasting morphological brain damage [20] and that efficacy of treatment including that of benzodiazepines decreases as the duration of seizure increases [10]. Seizure clusters have a significant impact on patient health and well being although they are not as life

threatening as status epilepticus [21]. They more than usually result in emergency room visits and, if left untreated, have been reported to evolve into status epilepticus [22]. Seizure clusters have a socioeconomic impact which includes missed school and work, as well as greater utilization of health care resources. The clinical implications of clustering are its effect on the presurgical evaluation of patients with refractory epilepsy. An important implication of cluster of seizures is in regard to outcome of drug trials. Seizure frequency reduction in drug trials implies seizure randomness. If seizures are not occurring randomly, the presumed responses to trial agents may not reflect true response; additionally, it may be appropriate to include consideration for changes in seizure patterns as well as frequency in drug trial outcome measures [1, 15].

1.1.1.5 Treatment for ARS

A product to treat ARS, especially outside a hospital setting should ideally be [10]:

i. Effective against a variety of seizure types

ii. Rapidly absorbed, with rapid onset of action and consistent interpatient variability

iii. Easily prepared and administered not only by the caregiver but the patient also during intervals when consciousness is not altered

iv. Have a sustained duration of action

v. Have few or minimal side effects

The choice of route and drug are based on many biopharmaceutical and practical considerations of formulation development and drug delivery. Intravenous drugs for acute short-term use available in the United States include lorazepam, diazepam, midazolam, phenobarbital and pentobarbital. Intravenous route is usually accessible in clinical settings or when trained personnel are available. For out-of-hospital emergencies, rectal diazepam gel is the only FDA approved product in the United States for the

treatment of ARS available in a convenient dispenser. Out-of-hospital management of seizure emergencies has also been called rescue therapy.

1.2 Unmet Medical Need

In 1997, the FDA approved a rectal diazepam gel, Diastat®, for use in the treatment of increased bouts of seizure activity in patients on a stable regimen of an antiepileptic drug(s). Diastat quickly became the drug of choice for treating seizure emergencies outside the hospital in young children. Although some older children and adults also use Diastat, many patients in these age groups as well as physicians object to this route of administration. Nonetheless, as many as 70-80,000 patients receive a Diastat prescription every year. The widespread objection to rectal therapy suggests that an alternative route to administer this rescue therapy is needed.

1.2.1 Current Therapy

The management of ARS outside a hospital setting in the absence of medical supervision has been improved by the availability of Diastat® (Valeant Pharmaceuticals International), a formulation of diazepam for rectal administration, which has shown to be safe and effective for treatment of this condition in two placebo controlled and open-label extension trials. Diastat is the only approved treatment for ARS in the United States.

Administration of rectal diazepam by caregivers has been largely successful in the treatment of ARS in terms of both safety and efficacy in clinical trials and in the community setting. Pellock and Shinnar identified 9 respiratory events and 3 deaths reviewing data reported to MedWatch and the FDA [23]. They reported that none of the 3 deaths were associated with respiratory complications, although one was unwitnessed. All the 9 subjects with respiratory events had returned to baseline. The authors concluded

that diazepam rectal gel had very low rate of morbidity and mortality, especially considering that over 2 million doses had been prescribed and over 1.5 million administered at the time of their review. The results of 2 randomized double blind controlled trials were published by Kapur [24] in which children with status epilepticus received 20 mg of rectal diazepam by caregivers. No serious respiratory events were noted in any of the 185 children in the study. Rectal diazepam effectively controlled recurring seizures and although some degree of prolonged somnolence was observed, the method of treatment appeared to be safe in the hands of medical caregivers. Brown *et al* reported on 149 cases in which 10 patients received 51 overdosages of rectal diazepam by caregivers [25]. Three patients received accidental overdoses and 7 patients received intentional overdoses due to inadequate efficacy of previously standard doses (without side effects). In all cases, no patient experienced respiratory complications even though doses were 188 to 256 % higher than the recommended dose. Even when caregivers administered large doses, these data indicate that the risk of respiratory adverse events may be low, particularly in patients who do not respond to the standard recommended dose.

1.2.2 Existing Unmet Need

Despite the availability of a very safe and effective treatment in Diastat which some older children and adults use, many patients in these age groups as well as physicians object to the route of administration and instead use other therapies not approved for this purpose, receive no treatment, or use emergency medical services or acute care systems.

1.3 Alternate Treatment Options for ARS

Considering the discussion earlier on the safety and efficacy of the diazepam given via the rectal route and in order to improve social acceptability by all age groups, we proposed the development of an intranasal delivery system of diazepam as an alternative to rectal diazepam for rescue therapy. Below is a brief discussion regarding the choice of diazepam (as compared to other benzodiazepines such as midazolam, lorazepam and Clonazepam), and the intranasal route of administration (as compared to intramuscular, buccal, rectal, etc.).

1.3.1 Factors Contributing to Choice of Drug and Route of Administration

Benzodiazepines (BZDs) are the drugs of choice for treatment of seizure emergencies, both in and outside the hospital. Treatment in emergency departments and hospitals usually, but not always, involves intravenous (IV) administration of either lorazepam or diazepam. The options for drug administration outside the hospital include rectal (PR), intranasal (IN), buccal (BUC), and intramuscular (IM) routes. The BZDs used to treat seizure emergencies have the same mechanism of action i.e. binding to the gamma-aminobutyric acid type A ($GABA_A$) receptors, although they have different affinities for the binding site which affects drug potency and, in part, dosing requirements. Thus, there is no competitive advantage of one BZD over another with regard to their pharmacology. In contrast, there are important physical-chemical differences among clonazepam, diazepam, lorazepam, and midazolam, which affect formulation requirements, the choice of route of administration, rate of absorption, and duration of effect. Among the four BZDs which are potential candidates for rescue therapy outside the hospital, midazolam has a competitive advantage in terms of administration via multiple routes and rate of absorption, whereas diazepam may have superior bioavailability and duration of effect.

Clonazepam and lorazepam appear to be inferior to the other BZDs in terms of utility in treating seizure emergencies by non-intravenous routes of administration.

1.3.1.1 Mechanism of Action of Benzodiazepines

All BZDs primarily act on the central nervous system where the main effects include sedation, hypnosis, decreased anxiety, anterograde amnesia, centrally mediated muscle relaxation and anti-convulsant activity. BZDs potentiate the neural inhibition that is mediated by gamma-aminobutyric acid (GABA) [26]. There are two types of GABA receptors, which are membrane-bound proteins, ionotropic and metabotropic. Ionotropic include the $GABA_A$ and $GABA_C$ receptors which are chloride channels mediating fast synaptic inhibition. These ion channel receptors are put together from five subunits forming an integral transmitter gated chloride ion channel. GABA is known to be less potent at the $GABA_A$ than the $GABA_C$ receptors. Unlike the $GABA_C$ receptors, the $GABA_A$ receptors are blocked by alkaloid bicuculline and are modulated by benzodiazepines, steroids and barbiturates [27]. The $GABA_A$ receptors are mainly responsible for inhibitory neurotransmission in the central nervous system. $GABA_B$ receptors belong to the second type of GABA receptors, the metabotropic type that are made up of single peptides. These receptors couple with G proteins to facilitate signal transduction mechanism. BZDs act by binding to a specific site that is distinct from that of GABA binding on the $GABA_A$ receptors. BZDs do not act at $GABA_B$ receptors.

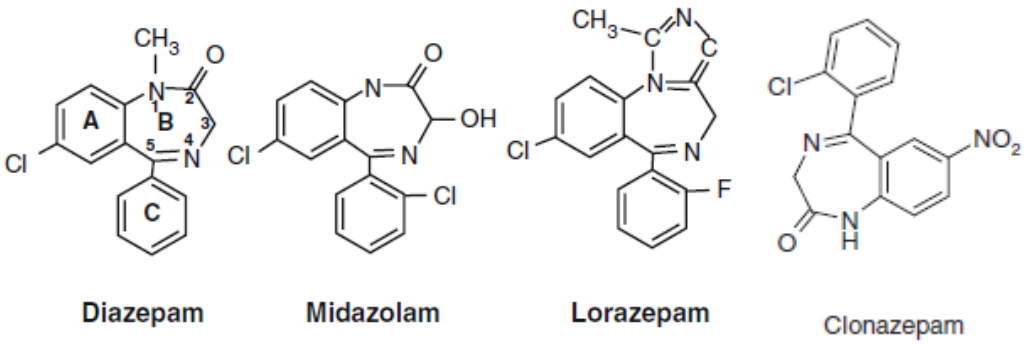


Figure 1.3-1: Structure of benzodiazepines. They are all composed of a benzene ring (*A*) fused to a seven-membered 1,4-diazepine ring (*B*).

The receptor binding properties and pharmacokinetics of benzodiazepines are defined by their chemical structure. Figure 1.3-1 shows the chemical structures of the four benzodiazepines in discussion here. The order of receptor affinity for these four benzodiazepines is clonazepam > lorazepam > midazolam >diazepam [26]. Benzodiazepines are classified as low, medium (e.g. clorazepate and diazepam) or high (e.g. clonazepam, lorazepam, midazolam) potency compounds. The binding affinities of for these compounds are provided in Table 1-2 along with other comparative properties [28]. Greenblatt *et al.* reported the in vitro binding affinities (nmol/L) of midazolam (0.44), clonazepam (0.41), lorazepam (1.64) and diazepam (9.57) using washed homogenates of cerebral cortices from adult male rats and based on displacement of [^{3}H] flunitrazepam [29]. Similar trend in the in vitro binding affinities were reported in another study using human cerebral cortex homogenates [30]. Data from in vivo studies showed that the receptor occupancy of clonazepam and lorazepam was a sigmoidal function of the brain concentrations. Miller reported that the brain concentrations of the drug producing 50 % receptor occupancy (IC_{50}) increased with the dose and reached its maximum at 1 and 4 mg/kg for clonazepam and lorazepam respectively [31]. The IC_{50} values were 22 and 133 ng/mL for clonazepam and lorazepam respectively. They also showed that the brain and plasma concentrations remained parallel for both drugs and hence speculated that receptor occupancy was related to plasma concentrations. The dose producing 50 % of the maximum effect (ED_{50}) was observed at levels of 30 and 60 %

binding for both drugs. These studies shows that clonazepam has relatively higher affinity than other benzodiazepines although the there is no advantage of one over the other in terms of pharmacology as the change in potency can be overcome by adjusting the dose, assuming no drug delivery limitations.

1.3.1.2 Physicochemical Properties of Benzodiazepines

The rate and extent of entry of drugs into the brain and other tissues is regulated by their physicochemical properties. Benzodiazepines are substituted 1,4-benzodiazepines (Figure 1.3-1). The term benzodiazepine is the chemical name for the heterocyclic ring which is a fusion between the benzene and diazepine ring systems. The benzodiazepines differ in the side groups attached to the central ring structure. The different side groups affect the binding of the molecule to the $GABA_A$ receptor which modulate the pharmacological properties. Benzodiazepines are low molecular weight compounds with different lipid solubilities Table 1-2. At physiological pH, clonazepam, lorazepam and diazepam are almost undissociated. Midazolam, on the other hand exhibits unique physicochemical properties. The diazepine ring in midazolam opens reversibly between the positions 4 and 5 at a pH of 4. This ring opening produces a highly water stable primary amine derivative, allowing aqueous solutions to be well tolerated when injected intravenously or intramuscularly. At a pH above 4 and at a physiological pH the ring closes, resulting in increased lipophilicity [32-34].

Benzodiazepines elicit their pharmacological action after crossing the blood-brain barrier. The crossing, either from the site of the drug delivery into the systemic circulation or across the blood brain barrier is primarily a diffusion process governed by the physicochemical properties according to Fick's law of diffusion:

$$Rate\,of\ Diffusion = D \times \Delta C \times \frac{A}{d}$$

where,

D is the diffusion constant of the drug. This parameter is related to the size and lipid solubility of the drug and the viscosity of the diffusion medium, the membrane. As lipid solubility increases or molecular size decreases then D increases and thus diffusion rate also increases.

ΔC is the drug concentration gradient across the membrane. Since V, the apparent volume of distribution, is at least four liters and often much higher the drug concentration in blood or plasma will be quite low compared with the concentration in the GI tract or the actual site of administration. It is this concentration gradient which allows the rapid complete absorption of many drug substances. Hence, the pharmacokinetics of the drug defines the concentration gradient.

A is the area of exchange. As the surface area increases the rate of diffusion also increase. This rate is further enhanced in the presence of villae and microvillae such as in the intestinal lining which will facilitate much faster absorption.

d is the membrane thickness. The smaller the membrane thickness, the quicker is the diffusion process. As one example, the membrane in the lung is quite thin thus inhalation absorption can be quite rapid.

Once into the systemic circulation, the benzodiazepines have to cross the blood brain and the blood cerebrospinal fluid barrier to elicit the pharmacological action. The speed of equilibration between plasma and the effect site (CNS) is described by the equilibration half-life which is measured by the elimination of the drug from the effect site. As midazolam, diazepam, clonazepam and lorazepam have almost equal molecular weights, the lipophilicity or partition ratio of these drugs define the equilibration half-life of the unbound drug [32].

All benzodiazepines show a rapid entry into the CNS and highly perfused tissues consistent with their short distribution half-lives [33], but relatively based on the partition coefficients shown in Table 1-2 it can be seen that midazolam and diazepam may have a

faster onset of action and a shorter equilibration half-life as compared to clonazepam and lorazepam.

Table 1-2: Comparison of four benzodiazepines

BZD	Mol. Weight	pKa	Water Solubility (mg/L)	Octanol/ Water Ratio	Protein Binding %	Binding Affinities Ki (nM)
Clonazepam	317	1.5 10.5	100	257	86	0.51
Diazepam	309	3.4	50	309	97-99	9.57
Lorazepam	321	1.3 11.5	80	73	85	1.64
Midazolam	362	6.1	< 100 at neutral pH ↑ solubility in acidic pH	34 at pH 3, 475 at pH 7.4	96	0.44

1.3.1.3 Pharmacokinetic and Pharmacodynamics Characteristics of Benzodiazepines

The benzodiazepine pharmacokinetic and pharmacodynamic characteristics determine key factors such as the onset and duration of pharmacological activity (seizure cessation) and the plasma concentration effect relationship. The major difference in the benzodiazepines used to abort seizure emergencies are in their pharmacokinetic and physicochemical properties.

All benzodiazepines, when given via the intravenous route, enter the CNS at approximately the same rate due to its large absorptive area, thus negating any differences in the lipid solubility among the compounds [34].

With regards to duration of action, it is well documented that the pharmacological activity of benzodiazepines does not correlate well with the plasma concentration time profiles of these drugs, being either shorter or longer than their half-life [32, 34]. In the case of diazepam, seizure control is expected to occur at plasma concentrations greater than 200 ng/mL [35], although different studies widened this range between 200 to 600 ng/mL [36, 37]. In an animal model, after an intravenous bolus dose of diazepam, plasma concentrations fell below 200 ng/mL in less than 50 minutes [38]. In another study the duration of action of diazepam was assessed by the time elapsed before recurrence of seizures and it was reported that a substantial number of patients relapsed within 2 hours of intravenous administration of the diazepam [39]. When clonazepam was administered intravenously in a different study, response (seizure suppression) to most type of seizures lasted for up to 24 hours although no direct correlation was found between plasma clonazepam concentrations and efficacy. After intravenous administration of lorazepam seizure recurrence was prevented from about 2 hours to 72 hours, the latter being considerably longer than the half-life of the drug. Midazolam, on the other hand, has a shorter duration of action compared with clonazepam and lorazepam with seizures recurring in 3 out of 14 patients at 3-4 hours after an intramuscular dose of 0.2 mg. [32].

There are many studies describing the correlation between plasma concentration and pharmacodynamic effects for some of the benzodiazpines. Greenblatt *et al.* administered a single intravenous dose of diazepam (0.15 mg/kg), midazolam (0.1 mg/kg) or placebo as a 1 minute infusion to eleven healthy volunteers in a three-way cross over study. They collected electroencephalographic (EEG) spectrums and plasma concentrations over 24 hours and determined the total EEG amplitude occurring in the range of 13 to 30 Hz. They reported maximal EEG changes at the end of diazepam infusion and 5 to 10 minutes after midazolam infusion. Duration of action, as determined by the period of time over which the activity of EEG was 13-30 Hz, was about 5 hours for diazepam and only 2 hours for midazolam. 6 to 8 hours after administration, neither drug had significantly different values from baseline. Using an Emax (maximum effect) model to explain the concentration effect relationship, they reported almost similar values for Emax between diazepam and midazolam (19.4 % and 21.3 %), but the apparent EC_{50} values, the concentration producing 50 % of the effect, were 269 ng/mL and 35 ng/mL respectively [40]. Using similar EEG parameters for evaluating efficacy, Burher *et al.* observed a time lag between plasma concentrations and EEG effects after single dose infusions of diazepam or midazolam in three healthy volunteers [41]. Using an effect compartment model, they estimated the first order rate constant of equilibrium between plasma and effect compartment. They reported an equilibration half-life of 4.8 and 1.6 minutes for midazolam and diazepam, whereas the EC50 estimated for steady-state were on average 152 and 958 ng/mL for both drugs, respectively. A few other studies have described the relationship between the pharmacokinetics and effect, all of which are summarized in Table 1-3. These studies showed the onset of effect for midazolam was delayed compared with that of diazepam; however midazolam was 5-6 times more potent than diazepam.

Table 1-3: Pharmacodynamic parameters of benzodiazepines. Adapted from [32]

Drug	$t_{1/2,ke0}$ (min)	EC_{50} (ng/mL)	Effect Measures	Reference
Diazepam	NA	269	EEG (%β)	[40]
	1.6	958[a]	EEG (V/s)	[41]
	1.2	116	DSST	[42]
Midazolam	NA	32.5	EEG (%β)	[40]
	4.8	152[a]	EEG (V/s)	[41]
	3.2	18.1	DSST	[42]
Lorazepam	25.8	35.8	HE	[43]

a Estimated for steady state. DSST = Digit symbol substitution test; EEG = electroencephalogram; EC_{50} = drug concentration producing half maximum effect; HE = hand eye coordination; NA = not available; $t_{1/2,ke0}$ = plasma effect site equilibration half-life; V/s = volts/second; %β = percentage β-activity

1.3.1.4 Effect of Route of Administration on Pharmacokinetics and Drug Action

The benzodiazepines used for seizure emergencies are administered via different routes. Table 1-4 gives a brief overview of the available routes for the 4 benzodiazepines in discussion. The most common alternate routes of administration, other than the intravenous route for rescue therapy include rectal (PR), intramuscular (IM), intranasal (IN) and buccal (BUC). All four of these routes have certain physiological characteristics that effect selection of drug and formulation. First, each has a limited absorptive surface area consisting of epithelial tissue across which the drug must diffuse. Based on the Fick's Law of Diffusion (Section 1.3.1.2), the small absorptive surface area and the lipid-based membrane favors highly lipid soluble drugs, which have a relatively large partition

coefficient. A second consideration is the volume of fluid that can be instilled / injected into the tissue or cavity. The volume of the rectal cavity allows rectal instillation of up to 10 mL with little or no discomfort or alteration in absorption. In contrast, IM injections are typically limited to 5 mL, buccal administration 2-3 mL; while the maximum fluid volume that can be retained in the nasal cavity is approximately 200 μL per nostril. These factors dictate the use of drugs that are effective in very small doses i.e. micrograms to milligrams using dosing forms with very high drug concentrations.

Certain pharmaceutical and pharmacological principles influence the choice of a benzodiazepine and the route of administration. According to Fick's Law, the greater the lipid solubility the faster the diffusion across tissue membranes and greater the absorptive surface area the faster diffusion takes place. Absorptive surface areas of the cavities or tissues involved in drug administration are as follows: GI tract > Nasal Cavity = Rectal Cavity > Buccal Cavity > IM. Fick's Law also stipulates that the diffusion is optimized when a drug is in solution and is highly lipid soluble. As a general rule, the greater the lipid solubility is, the poorer the water solubility. All the benzodiazepines in development for rescue therapy are relatively insoluble in water (Table 1-2), although the aqueous solubility of midazolam is increased at least 500 fold to 5 mg/mL, at a pH of 3-4. Limited aqueous solubility requires use of organic solvents or other approaches to increase the concentration of drug to amounts required for IM, IN, or BUC administration. Organic solvents such as ethanol or propylene glycol can increase the concentration of benzodiazepines, but can cause local tissue injury. On the other hand, the greater the lipophilicity, the more rapidly a drug is absorbed across biological membranes, particularly membranes with small surface areas such as the rectal, nasal, and buccal cavities, and the tissue surrounding an intramuscular injection. Lipid solubility is typically expressed as the ratio of drug diffusion into octanol versus water. As shown in Table 1-2, the water solubility of clonazepam, diazepam, lorazepam, and midazolam are low and similar, whereas the lipid solubility among the 4 drugs varies 5 fold.

Rate of absorption is also influenced by the type of formulation. Only molecules in solution are available to diffuse across a membrane. Drugs that are only partially dissolved, such as suspensions, must first completely dissolve. Drugs in solid dosage forms such as tablets must first disintegrate and disperse, then dissolve before being absorbed. Thus, a solution of a drug given intranasally will be absorbed more rapidly than the same drug administered as a powder**;** Solutions > Suspension > Liquid-filled Capsules > Solids.

Table 1-4: Benzodiazepines used to treat seizure emergencies. Adapted from [34]

Route of Administration	**Drug**
Intravenous	Diazepam Midazolam Lorazepm
Oral (solution)	Diazepam Lorazepam
Nasal	Diazepam Midazolam Lorazepam
Rectal	Diazepam
Buccal/Sublingual	Clonazepam Diazepam Midazolam
Intramuscular	Midazolam

1.3.1.4.1 Intravenous Route

The universally recognized standard for treating the most severe forms of seizure emergencies is intravenous administration of a benzodiazepines, usually lorazepam or diazepam followed by phenytion or fosphenytoin [8]. This mode of therapy requires the presence of skilled medical personnel and equipment to administer drugs and provide cardio-respiratory support. After intravenous administration, most of the benzodiazepines exhibit almost similar half-lives of distribution ($t_{1/2\alpha}$). All benzodiazepines have large volumes of distribution suggesting extensive binding. It can be seen from Table 1-5, which shows a brief summary of the intravenous pharmacokinetic parameters, that $t_{1/2\alpha}$ is the shortest for diazepam and lorazepam as compared to slightly longer estimates for midazolam and clonazepam.

Table 1-5: Pharmacokinetic parameters of benzodiazepines following intravenous administration.

Drug	**$t_{1/2}\alpha$ (min)**	**Vd (L/Kg)**	**Clearance (L/h/Kg)**	**$t_{1/2}\beta$ (h)**
Diazepam	1.9-13.3	0.89 ± 0.18	0.0388 ± 0.015	32.9 ± 8.8
Clonazepam	30	2.4 (1.5-4.4)	0.057	29 (18-49)
Lorazepam	<11	1.14 ± 0.03	0.063 ± 0.009	14.3 ± 2.5
Midazolam	18.6 ± 14.4	0.80 ± 0.19	0.42 ± 0.17	2.4 ± 0.8

Values refer to adults receiving monotherapy and are from Rey at al. [32] unless otherwise specified**.**
$t_{1/2}\alpha$ = distribution half-life; **$t_{1/2}\beta$** = elimination half-life; **Vd** = volume of distribution

When diazepam, lorazepam and midazolam are given via intravenous injection, diazepam and midazolam have a faster onset of activity than lorazepam, roughly in proportion to

their lipid solubility (Table 1-2). In contrast, lorazepam displays a longer duration of activity because it more slowly re-distributes to muscle and adipose tissue elsewhere in the body. This, in part, along with its intermediate half-life makes lorazepam the drug of choice when treating seizure emergencies with intravenous therapy. These trends can be observed in Table 1-3. Lorazepam was significantly better than other four other treatments, phenytoin, diazepam + phenytoin, and Phenobarbital, in the treatment of overt status epilepticus when administered to patients with a verified diagnosis of generalized convulsive status epilepticus [8, 44]. In a different study evaluating the safety and efficacy of intravenous benzodiazepines administered by paramedics to treat status epilepticus, a 2-mg dose of lorazepam and a 5-mg dose of diazepam were compared with placebo in 205 adults. The results of this study were in agreement with the previous one, with diazepam and lorazepam significantly better than placebo and lorazepam having a slightly better response rate than diazepam (59 vs 43%) [45].

Summary for IV administration

Pros: Most effective option for treatment with quick onset of action. Lorazepam is the benzodiazepine of choice for this route.

Cons: Lorazepam and diazepam cause respiratory failure, arrhythmias and hypotension due to propylene glycol in the formulations; Administration requires skilled personnel or transport to medical facilities.

1.3.1.4.2 Intramuscular Route

Diazepam, lorazepam and midazolam can be administered intramuscularly. The combination of high water solubility of the commercial injectable midazolam combined with its high lipid solubility at physiologic pH (7.4) in the extracellular fluid at the injection site, results in very rapid absorption of intramuscular midazolam with resultant quick onset of antiseizure activity. Jawad *et al*. compared the effect of a 10 and 15 mg

intramuscular dose versus 10 and 20 mg intravenous doses given to 6 patients with epilepsy [46]. Time of onset and the extent of reduction in the number of spikes was similar between the 10 mg intramuscular midazolam and 20 mg intravenous diazepam doses, indicating that midazolam by the intramuscular route has a very rapid onset of action and a pharmacologic effect comparable to intravenous diazepam. Although spike suppression does not directly correlate with efficacy in aborting or preventing clinical seizures, this study suggested that a 10 mg intramuscular dose, which attains on average plasma midazolam concentrations of 100-200 ng/mL may be needed for a therapeutic effect. Midazolam bioavailability following an intramuscular injection ranges from 90-100% with a time to maximum drug concentration, t_{max} of 15-40 min.

These reports have lead to the recent initiation of a study funded by The Department of Defense and the National Institute for Neurological Disorders and Stroke. This status epilepticus study is comparing the efficacy and safety of a 10 mg dose of intramuscular midazolam administered by an autoinjector versus a 4 mg dose of intravenous lorazepam [47]. Results of the study should be available in 2011.

Earlier published studies on intramuscular diazepam describe the absorption process as poor, erratic, and irregular, with plasma levels lower than levels following oral administration. This is likely due to the fact that in the commercial injectable diazepam product the drug is solubilized with ethanol and propylene glycol. Following intramuscular injection, the organic solvents are rapidly absorbed leaving the diazepam in a more aqueous solution resulting in partial precipitation of drug and a slow rate of absorption. However, most of these studies had the intramuscular administration site as the buttock or, the site of administration was not specified [48-52]. The presence of more fatty tissue and poorer blood flow in the gluteus than in the deltoid and vastus lateralis muscles may be speculated as the cause of poor diazepam absorption after intramuscular injection in the buttocks [53]. Kortillia and Linnoila showed a more rapid and systemic absorption of intramuscular injection of diazepam in the thigh than obtained with oral diazepam [54]. Lehman and Wannarka recently reported the results of 2 studies of an intramuscular diazepam autoinjector sponsored by the US Army Medical Research and

Development Command in the early 90's [55]. The first study was a bioequivalence study comparing 10 mg diazepam dose administered using an autoinjector versus that administered via a conventional syringe. In the second study, dose proportionality of diazepam given via the autoinjector was assessed at doses of 10 and 20 mg administered as one or 2 injections. In both the studies, the site for the intramuscular injection was the midanterolateral thigh. The results of this study showed smooth consistent absorption of the dose with an average t_{max} of approximately 1.5 hours which is equivalent to the t_{max} after rectal administration. Moreover, contrary to earlier reports the results of this study show no lag time in absorption when administered via either device. The authors attribute this to choosing the right site for intramuscular administration. Mean diazepam C_{max} values of 272 ng/mL and 314 ng/mL were obtained from 1 diazepam 10-mg autoinjector in the bioequivalence and dose proportionality studies respectively. A mean C_{max} of 522 ng/mL was obtained with 2 simultaneously administered 10-mg diazepam autoinjectors (20 mg). Many factors influence the absorption of intramuscular diazepam; the location of the injection site, depth of injection, gender, muscle activity (such as exercise, which may increase the rate of absorption), and the amount of adipose tissue at the injection site [51, 56, 57]. The diazepam autoinjector was approved by the Food and Drug administration (FDA) in 1990 and was deployed in the 1991 Gulf War for the first time. Due to their durability and reliability and practical value in the treatment of patients with prolonged or repetitive seizures, these autoinjectors are currently stocked by the US military [55].

Both clonazepam and lorazepam are slowly absorbed following intramuscular administration, a reflection of their poor water solubility (Table 1-2) that requires they be formulated with organic solvents. In a study involving 12 healthy volunteers who were given a single dose of 2 mg clonazepam by intramuscular, oral, and intravenous routes, the absorption rate, as measured by t_{max} after intramuscular injection was significantly longer than that following oral administration: t_{max} = 3.1 vs. 1.7 hrs [58]. Similarly, Greenblatt *et al.* reported a mean t_{max} of 1.15 hrs following a 2 mg lorazepam dose given intramuscularly to healthy volunteers [59].

Summary for IM administration

Pros: No requirement of an IV line. Midazolam is the benzodiazepine with preferred qualities for intramuscular administration.

Cons: Absorption may be slow and erratic (diazepam and lorazepam) for the indication being treated.

1.3.1.4.3 Buccal Route

Buccal drug delivery is accomplished by placing either a solid or a liquid in the buccal pouch inside the oral cavity. If it's a solid dosage form, the formulation must rapidly disintegrate and the drug should dissolve if it is to be used as rescue therapy. For a liquid dosage form, the drug must be sufficiently concentrated to permit use of a few milliliters of solution. There are reports of buccal administration of clonazepam, using rapidly dissolving wafers [60], and lorazepam; but the greatest number of reports in the literature deal with buccal administration of midazolam liquids. 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 [61]. Scott *et al.* studied the buccal administration of midazolam in 10 healthy volunteers who were administered 2 mL 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 [62].

A number of studies have evaluated the safety and efficacy of buccal midazolam compared to rectal diazepam. In a large trial involving 330 children in Uganda (3 months to 12 years) presenting to the pediatric emergency department with a convulsion lasting greater than 5 minutes, Mpimbaza *et al.* evaluated and compared the treatment failure

rate (persistence of seizure beyond 10 minutes) between buccal midazolam and rectal diazepam, both administered at a dose of 0.5 mg/kg [63]. The medications were administered in a double dummy fashion with subjects receiving buccal midazolam and rectal placebo or rectal diazepam and buccal placebo in a randomized fashion. The results showed that significantly lower percentage of treatment failure in the buccal midazolam group compared to rectal diazepam (30.3% vs 43 % p=0.016). However, when the primary outcome was stratified by etiology of seizures (malaria-related or not malaria-related), no difference was found in the treatment failure between the buccal midazolam and rectal diazepam groups. Rectal diazepam group had a higher (17.5 %) recurrence of seizures within one hour (secondary outcome) compared to buccal midazolam (8%). However, respiratory depression, a measure of safety was uncommon in both groups. The authors concluded that both treatments are equally safe with buccal midazolam having a better efficacy in non-malarial related seizures. McIntyre *et al.* evaluated 219 seizure episodes in 177 children (≥ 6 months) presenting to a hospital setting with active seizures and no IV access [64]. These patients randomly received buccal midazolam or rectal diazepam. The primary outcome was defined as treatment success with cessation of seizures within 10 minutes without recurrence within one hour and without respiratory depression. Buccal midazolam performed better than rectal diazepam (33 vs 14 %) for the primary outcome and no difference was found between the two groups in terms of respiratory depression. Baysun *et al.* studied 43 children (2 months to 12 years) presenting to the pediatric emergency department with acute convulsion and compared the efficacy of buccal midazolam and rectal diazepam in cessation of seizure within 10 minutes. They did not find any significant difference between the two groups, 78 % and 85 % for buccal midazolam and rectal diazepam respectively [65]. In another study *Scott et al.* studied the same outcome of seizure cessation within 10 minutes while evaluating 79 seizure episodes of greater than 5 minutes in 28 young people at a residential center. They did not find a difference between the two treatments, buccal midazolam and rectal diazepam for the primary outcome [66].

Mpimbaza *et al.* and McIntnyre *et al.* found that significantly more children achieved seizure control within 10 minutes when treated with midazolam than with diazepam, while Baysun *et al.* and Scott *et al.* found no difference between treatments [63-66]. Seizure recurrence was significantly less common in children treated with midazolam than diazepam, in the two studies reporting on it [63, 64]. In all studies, respiratory depression was uncommon in either treatment group.

A commercial buccal midazolam product, Epistatus, is available in the United Kingdom for use as rescue therapy. It should be noted, however, that the efficacy, safety, duration of effect, and ease of buccal administration by nonmedical caregivers have not been evaluated in settings outside of hospitals.

Summary for Buccal Administration

Pros: Drugs administered by the buccal route reduce first-pass liver and gastrointestinal metabolism. It is much simpler and safer than intravenous or intramuscular injections, it is less embarrassing and inconvenient than intrarectal administration for patients, and it may permit the use of larger volumes than would be possible via intranasal administration. Midazolam is the drug of choice here with an available product in the market. There are however, buccal products of clonazepam and lorazepam used to treat seizures only as secondary indication or as an adjunct with other treatments.

Cons: If a patient's head is moving during a seizure, the dose administered in the buccal pouch may be swallowed or uniform distribution of the solution over the buccal pouch may not result which is essential for the maximum rate of absorption after buccal administration. There is a risk of pneumonitis secondary to aspiration of organic solvents such as propylene glycol and ethanol, which are present in diazepam and lorazepam liquid formulations. Moreover, placement of drug in the buccal pouch during a seizure may be hazardous for both the patient and the caregiver and runs counter to first aid guidelines for seizures [34].

1.3.1.4.4 Sublingual Route

Lorazepam and midazolam are the only benzodiazepines that have been studied for this route for the following indications: preanesthetic, anxiolytic, or as a sedative [62, 67-69]. The sublingual and buccal routes are often confused as the same route; however, the site of drug absorption differentiates the two. Sublingual absorption is rapid, avoids liver metabolism, and gastric degradation.

Greenblatt *et al.* compared the pharmacokinetics of sublingual lorazepam with intravenous, intramuscular and oral lorazepam [67]. Ten healthy volunteers randomly received 2 mg of lorazepam in the following five formulations: IV injection, intramuscular injection, oral tablet, sublingual administration of the oral tablet and sublingual administration of a specially formulated tablet. C_{max}, t_{max}, $t_{1/2}$, and relative bioavailability were not significantly different among the formulations. Peak concentrations were highest for the intramuscular route, followed by oral and sublingual; time to peak concentrations was most rapid for the intramuscular route, followed by sublingual and oral. Mean relative bioavailabilities were high for all routes: intramuscular (95.9%), oral (99.8%), sublingual of oral tablet (94.1%) and sublingual of special tablet (98.2%). This route of administration would only be helpful for replacement of a maintenance dose of oral lorazepam in patients with epilepsy.

There are no reported studies evaluating efficacy for the indication of epilepsy.

Summary for Sublingual Administration

Pros:

The drug can be given with little to no effort resulting in rapid absorption into the systemic circulation. There is minimal to no discomfort to the patient and may be beneficial for patients who are unable to take medications orally.

Cons:

This route may not be ideal for patients actively seizing. Clenching of the jaw during a seizure may inhibit drug administration by blocking access to the cavity underneath the tongue. Safety may also be compromised to both the patient and caregiver administering the drug by this route.

1.3.1.4.5 Intrarectal Route

The rectal route of administration can be used for both seizure emergencies and bridge therapy, depending on the rate and extent of absorption of a specific anti-epileptic drug. This is currently the most commonly used alternative route for the treatment of seizure emergencies. Rectal administration is easily achieved by untrained persons, partially avoids first-pass metabolism and prevents drug decomposition by stomach acids.

The most common obstacle for 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 micro-organisms, or adsorption to fecal material. The absorptive surface of the rectal cavity is 1/10,000 of the intestinal tract and rectally administered drugs experience a decreased transit time for absorption to take place.

Absorption from the rectum occurs almost exclusively via passive diffusion; therefore unionized, lipophilic small molecules with high solubility are most readily absorbed. Due to the shorter transit time and small fluid content in the rectum, drugs in solution are best available for absorption. A drug must also be soluble enough to be delivered in a volume small enough to be retained in the rectum (approximately 25 mL in adults).

Various formulations of diazepam have been administered rectally, including solutions, gel, and various suppositories. A diazepam gel is packaged and marketed in the United States for rectal administration.

Rectally administered diazepam solution has been shown to be approximately 80% bioavailable, with an average peak concentration (C_{max}) occurring 10-60 minutes post-dose [35, 70-76]. Plasma concentrations associated with clinical effectiveness ($\geq$ 200 ng/mL) were typically reached within five to ten minutes, similar to both IV and IM diazepam administration. Rectal diazepam gel (15 mg) was also found to be extensively and rapidly absorbed, with plasma concentrations exceeding 200 ng/mL 15 minutes after dose administration and a bioavailability of 90% (range 70–110%) [77].

Diazepam suppositories are more slowly absorbed, with therapeutically effective concentrations reached 20 to 120 minutes post-dose or later and concentrations peaking 45 minutes to 3 hours after administration, depending on the formulation [35, 50, 73-75, 78-83]. Bioavailability of diazepam suppositories is similar to solution, averaging 65 - 85% [73, 81]. Diazepam suppositories may be adequate to sustain plasma concentrations over long periods. Following administration of a single suppository (0.5 mg/kg) to infants, targeted concentrations were reached in 30 minutes and maintained for 8 hours, while giving a second suppository 8 hours after the first, maintained these concentrations over 24 hours [83]. In within-study comparisons, diazepam solution has been demonstrated to be more quickly absorbed, resulting in target concentration achievement and peak concentrations occurring earlier post-dose than suppositories [79].

Rectal diazepam has been shown to be effective in prevention of seizures and termination of prolonged or acute repetitive seizures. Following a seizure, patients in a double-blind study (n = 22) given a 20 mg diazepam suppository were seizure-free for 24-hours post-dose in 81% of cases, compared to 22% of cases following placebo [84]. In a separate study, continuous phenobarbital and intermittent rectal diazepam suppositories (in response to high temperature) were equally effective in prevention of febrile seizures [85].

Rectal diazepam has also been shown effective in treating prolonged seizures. A single dose of rectal diazepam solution effectively halted 71-80% of seizures within 5 minutes, while a second diazepam dose increased the success rate to 78-87% in 10 minutes [86,

87]. The elapsed seizure time prior to treatment was an important factor in effectiveness; seizures treated within 15 minutes were 81-96% successfully controlled, while delaying treatment for longer than 15 minutes reduced the success rate to 46-57% [86, 87]. Diazepam rectal gel was successful in termination of status on all 36 attempts in convulsive status epilepticus in an adult care facility [88]. Administration of rectal diazepam by paramedics prior to reaching the hospital also decreased the length of seizures and reduced the likelihood of recurrent seizures [89, 90].

Rectal diazepam solution (0.5 mg/kg) controlled 88% of acute convulsions (n = 65 episodes) within 15 minutes when administered at home [91]. Only 8% of seizures remained uncontrolled for over 30 min, with all treatment failures occurring in patients with a typical history of convulsing for 15 minutes or more. Diazepam was administered within 5 minutes of seizure onset in 85% of episodes.

In a study of home diazepam use and seizure duration, 76 subjects of all ages with severe epilepsy (or their caregivers) were instructed on the administration of rectal or oral diazepam (parenteral solution, 0.2-0.5 mg/kg) [21]. A second dose was allowed three hours after the first dose if the patient relapsed. 53% of diazepam users had seizures controlled within 10 minutes of administration, as compared to only 2% when diazepam was not administered. Furthermore, seizures lasting more than 30 minutes decreased from 76% to 4%. In a separate group of 12 adults, diazepam administered at home to people who experienced nonconvulsive status evolving into generalized tonic clonic (GTC) seizures. Despite daily antiepileptic therapy, 85% of the status episodes lasted longer than an hour and 76% evolved into convulsive seizures. When conventional therapy was replaced with intermittent diazepam, only 17% of the episodes lasted longer than an hour and 7% evolved into convulsive seizures.

In two placebo-controlled studies, the safety and efficacy of treating acute repetitive seizures at home with diazepam rectal gel, patients receiving active drug had fewer recurrent seizures during the 12 hours following the dose and required fewer ER visits

than those receiving placebo [16, 92]. In these studies, there were no significant occurrences of respiratory depression [93].

In a survey of home rectal diazepam users, 85% of those who administered a dose thought it was effective for most or all seizures [94]. A prospective study of home rectal diazepam gel use found diazepam administration successfully halted 84% of seizures episodes (> 5 min in length or 3 seizures within 2 hours) and parental stress scores dropped significantly from baseline [93]. An open-label trial found no loss of efficacy with repeated use [95]. In a review of data from 10 subjects who received an overdose in the home setting (> 180% of the protocol specified dose), the only side effect attributed to the overdoses was somnolence [25].

Overall, a variety of diazepam formulations have been administered rectally with high and relatively predictable bioavailability with few side effects. Rectal diazepam has been shown to be highly effective in halting seizures and preventing repetitive and febrile seizures in hospitals, ambulances, and at home. The use of home administered diazepam has been shown to decrease stress in caregivers and to be economically advantageous and reduces emergency room usage.

Pharmacokinetic data have been reported for infants, children, and adults following rectal midazolam administration. Peak plasma concentrations (92 – 156 ng/mL) occurred 31 (20-50) minutes post-rectal dose in adults following rectal administration of midazolam solution (0.3 mg/kg), with a bioavailability of 52 $\pm$ 8% [96].

A higher dose of rectal midazolam (0.6 mg/kg) administered to fifteen infants was well tolerated with maximum concentrations (147 $\pm$ 58 ng/mL) occurring an average of 32 (18-38) minutes post-dose [97]. Peak plasma concentrations occurred on an average of 10-30 minutes post-dose in children [98-100]. Following rectal administration of midazolam solution (0.3 mg/kg) to 16 children, peak plasma concentrations of midazolam were variable (99 $\pm$ 57 ng/ml) [98]. Rectal bioavailability of midazolam (0.15 mg/kg) in children was 18% [99]. An open-label, randomized trial (0.2 mg/kg midazolam) showed the maximum plasma concentrations following intravenous and

intranasal administration were significantly greater than that following rectal administration [100]. However, time to peak was similar for nasal (13 ± 6 min) and rectal (12 ± 6 min) administration.

To date, there are no reported double blind placebo controlled studies in which the efficacy of rectal administration of midazolam has been demonstrated. With low and widely variable bioavailability, rectally administered midazolam is not recommended.

Bioavailability of lorazepam was high and variable (86 ± 26%) following rectal administration of 2 mg lorazepam (parenteral solution diluted in saline) in a study of six adult males [101]. Peak plasma concentrations occurred 68 minutes post-dose. The rectal dose was well tolerated and resulted in less toxicity than an IV dose.

In non-placebo controlled trials, rectally administered lorazepam compared favorably with rectal diazepam. The efficacy of lorazepam (0.05-0.1 mg/kg IV or rectal) was compared with diazepam (0.3-0.4 mg/kg IV or rectal) for seizure arrest in the emergency room [102]. A second dose was allowed after 7-8 minutes if convulsions continued. Fifty-three children received diazepam (19 rectally) and 33 were given lorazepam (6 rectally). Patients responding to the first dose stopped convulsing in less than one minute. All of the rectal lorazepam patients and 63% of the rectal diazepam group were successfully controlled without additional AEDs. One patient receiving rectal diazepam and none of the rectal lorazepam patients exhibited respiratory depression and required ICU treatment.

Rectally administered lorazepam appears effective in halting seizures and shows high but variable bioavailability.

A study of 10 adults showed peak clonazepam concentrations 10-30 minutes following administration of a single rectal dose of diluted clonazepam suspension (0.02 mg/kg) [103]. Concentrations of clonazepam following IV administration (1 mg) exceeded those following rectal administration for the first 10 minutes, falling to similar concentrations for the remainder of the hour analyzed.

After 11 children were administered clonazepam rectally (0.05 – 0.1 mg/kg diluted parenteral solution), three subjects (one administered 0.05 mg/kg, two administered 0.1 mg/kg) failed to achieve measurable serum concentrations [104]. Peak concentrations occurred 10 – 120 minutes post-dose. All subjects were administered 0.1 mg/kg and showed detectable concentrations of 18 ng/mL (theorized to be an anticonvulsant level) within 20 minutes, while none of the subjects given 0.05 mg/kg reached this level.

Although measurable anticonvulsant concentrations may result after rectal administration of clonazepam, it may not be absorbed quickly enough to be used as a first line therapy in treating seizure emergencies. Although there are no clinical trials of efficacy of rectal clonazepam, it might be suitable for "bridge therapy" if oral administration is interrupted.

Summary for Rectal Administration

Pros: There is no requirement for placement of an IV line which would require skilled personnel and sterile equipment. It is a less invasive method and leads to reduced medical costs.

Cons: This route of administration is socially unacceptable with concerns of invasion of privacy/molestation. It can be difficult to administer via this route in a public setting.

Intranasal Administration

Intranasal (IN) drug therapy has been traditionally used for the treatment of local conditions such as allergic rhinitis or congestion. However, it has been increasingly used for systemic indications such as migraine, osteoporosis, and virus vaccination. Intranasal administration of midazolam is the most commonly cited benzodiazepine in the literature. Its use was first documented nearly twenty years ago as a pre-anesthetic in children

[105]. Nearly ten years later, clinicians theorized that intranasal midazolam may be an alternative treatment for acute seizures [106].

Diazepam, midazolam, lorazepam and clonazepam have been evaluated for pharmacokinetics of IN therapy. Lorazepam was intranasally administered to 11 healthy volunteers using 2 mg of the parenteral formulation. Lorazepam was absorbed with a mean percent bioavailability of 77.7 $\pm$ 11.1%. A double-peak concentration-time curve was observed, indicating possible secondary oral absorption. The time to peak concentration was variable, ranging from 0.25-2 hours [107]. For the treatment of seizure emergencies, IN lorazepam may not be an attractive option for this indication. Data does not yet provide evidence of rapid and reliable absorption. Amarin is using Elan's proprietary NanoCrystal® technology as the basis for an intranasal lorazepam formulation. NanoCrystals are small particles of drug substance less than 2000 nm in diameter that are prepared by special milling techniques; their stability against agglomeration is maintained by the use of surface adsorption agents. The final product is a dispersion of the drug substance that behaves like a solution but can be processed into a finished dosage form for any route of administration as either a solid or liquid [108].

Diazepam

There are four reports describing intranasal diazepam administration to healthy volunteers. Gizurarson *et al.* prepared an intranasal formulation containing 20 mg/mL diazepam dissolved in 5% glycofurol in polyethylene glycol 200 [109]. They compared a 2 mg IN dose of this formulation with the same dose given IV. Blood samples were collected for 5 hours following drug administration. The mean bioavailability was 50.4 ± 23.3% with a time to peak of 18 ± 11 minutes. All subjects complained of nasal discomfort immediately following drug administration, but the discomfort had resolved within 30 minutes. Lindhardt *et al.* evaluated an intranasal formulation of diazepam dissolved polyethylene glycol 300 in 7 healthy volunteers.[110] Using a crossover design, they compared 4 and 7 mg intranasal doses with a 5 mg intravenous dose and

collected blood samples for 60 minutes after drug administration. The intranasal formulation had a relative bioavailability of 45 and 42 %, a C_{max} of 99 and 179 ng/ml, and t_{max} of 18 and 42 mins for the 4 and 7 mg doses, respectively. Given that the half-life of diazepam ranges from 20 to 50 hrs, their bioavailability values are likely an underestimate of the actual extent of absorption. Slattery and Lau, using a 10 mg dose of diazepam dissolved in Cremophor EL and a longer period for collection for blood samples, reported a bioavailability of 78 % with a C_{max} of 175 ng/mL and a t_{max} of 1 hr in two subjects [111]. In recently reported studies, the intranasal absorption of diazepam from a supersaturated solution of glycofurol-water co-solvent system was explored [112, 113]. Cloyd *et al.* developed the supersaturated solution of diazepam that achieves a 40-mg/mL drug concentration in a 60/40% (v/v) cosolvent mixture of glycofurol and water. This formulation was designed to maximize diazepam concentration, thus allowing administration of small fluid volumes (≤200 μL). The addition of water in the solvent system improves the tolerability while increasing the driving force of permeation across the nasal mucosa [114]. In the first study, IN and IV midazolam and diazepam were studied in a cross over fashion in 4 healthy volunteers and the pharmacokinetics and tolerability were assessed. The C_{max} and t_{max} values for intranasal diazepam and midazolam were 179.2 ng/mL and 28.8 minutes vs 62.8 ng/mL and 21.6 minutes, respectively [113]. Immediately following intranasal administration, subjects reported tolerability scores of 6.75 and 6.0, and identical pain scores, 3.2, for diazepam and midazolam, respectively. In a follow up study, the supersaturated diazepam formulation was studied at 2 different doses, 5 mg and 10 mg in a three way crossover fashion where the third arm of the study was 5 mg IV diazepam. Each subject received two intranasal and one intravenous dose of diazepam and blood samples were collected up to 48 hours after dosing. The average bioavailability, *F*, was 75 and 74% for the 5 and 10 mg doses respectively. The mean C_{max}, t_{max} and $t_{1/2}$ were 134.3 ± 61.9 ng/mL, 55.6 ± 60.3 minutes and 49.1 ± 20.4 hours for the 5 mg dose and 247.0 ± 60.9 ng/mL, 39.3 ± 38.1 minutes and 57.0 ± 28.0 hours for the 10 mg dose. Using analog scales, subjects rated tolerability (0 = no change from normal;10 = maximum intolerability) prior to and 0, 5, 15, 60 minutes, and 8 hours after administration. The mean tolerability scores observed were 4.4

and 4.7 for the 5 mg and 10 mg doses. Both these scores dropped down to 3 and 2.5, 15 minutes post dose and to 1, 60 minutes post dose [112].

Midazolam

Midazolam is also administered intranasally and may be safe in the outpatient setting and effective in stopping seizure activity. Use of intranasal midazolam was first published in the late 1980s [105], and over 20 publications in the last 15 years describe the safety and efficacy of IN midazolam for control of seizures in a variety of patient populations including pediatrics and adults [106, 115-132]. The use of IN midazolam for treatment of seizures has also been advocated by several thoughtful review articles and editorials.

Several open-label studies using injectable midazolam (5 mg/mL) have described rapid onset of seizures cessation; however, bioavailability using the injectable solution intranasally is moderate and highly variable. Also, most investigators report nasal irritation, tearing, and a raw throat sensation immediately after administration, most likely due to a large volume (1 to 2 mL) and low pH (3 to 4) of the administered solution. Estimates of midazolam bioavailability in studies using this injectable formulation, ranged from 50-83 % [116, 117, 124, 129, 130, 133, 134]. Alternative intranasal midazolam formulations providing better tolerability as well as rapid and improved absorption have been studied [135-140]. The ingredients used in formulating these solutions include cosolvent combinations of polyethylene glycol, propylene glycol, and butylated hydroxytoluene [137, 139, 140] and cyclodextrin-based solutions [136, 138]. Gudmundsdottir *et al.* prepared a nonsterile aqueous solution, buffered to pH 4.2 and preserved with benzalkonium chloride, in which midazolam solubility was increased to 17 mg/mL by cyclodextrin complexation [136]. However, multiple sprays were necessary to deliver a 4- to 5-mg dose. The 28 mg/mL formulation used by Knoester *et al.* had water and propylene glycol as co-solvents, buffered to pH 4, with the addition of 1% benzyl alcohol as an antimicrobial preservative [137]. These investigators reported excellent pharmacokinetics, with a maximum concentration of 71 ng/mL achieved in 14

minutes on average, and an average bioavailability of 83%. Both products caused nasal irritation, suggesting midazolam itself may be the primary cause. A summary of the pharmacokinetic results for the studies of intranasal midazolam in children [141-148] and adults [113, 132, 135, 139, 140] is presented below.

The pharmacokinetics of intranasal midazolam doses of 0.06 to 0.25 mg/kg have been studied in adults, resulting in mean C_{max} values of 54 to 192 ng/mL with wide inter-individual variability at the different doses studied. Mean t_{max} occurred at approximately 15-20 minutes at all doses studied, and the half-life was short, 2 to 3 hours. Bioavailability was approximately 50 to 83 %, depending on the formulation used and the technique used to administer the IN doses. In children, higher IN doses of midazolam of 0.1 to 0.6 mg/kg have been studied, resulting in mean C_{max} values of 72 to 277 ng/mL, again demonstrating wide inter-individual variability. Peak concentrations generally occurred at 10 to 15 minutes after dosing, and the half-life was short, approximately 2 hours. Overall, pediatric patients appear to require and tolerate higher doses for sedation than adults.

Midazolam has a relatively fast elimination half-life especially in patients taking enzyme inducing drugs (0.5 to 2 hours). The rapid elimination of midazolam has the potential to reduce the duration of its effect. A highly concentrated intranasal midazolam formulation utilizing an organic solvent is undergoing a clinical trial (www.clinicaltrials.gov).

Some studies from above and a few additional also evaluated the midazolam's efficacy in aborting a seizure. The first paper describing nasal delivery of midazolam used 0.2 mg/kg to demonstrate the effect on aborting a seizure and on concurrent EEG activity in 19 children with long-standing epilepsy with difficult to control seizures [106]. The data suggest that 14 of the 19 patients were treated effectively, with a 60% reduction in spike counts/minute, and the appearance of beta-wave activity in 175 (80- 240) seconds on average for responders. Many additional papers describe relatively small numbers of pediatric patients (15-50) in open and controlled trials in which 0.2 mg/kg midazolam (up to 10 mg usually) was administered nasally [116, 117, 132-134]. All the papers are

consistent in describing rapid and successful abolition of seizures. Mean times to treatment success ranged from 50 seconds to 5-10 minutes. Nasal midazolam was considered the superior therapeutic alternative when compared to rectal or intravenous diazepam – primarily because there was less time used in drug administration preparation resulting in shorter overall times to being seizure-free. Limited publications in adult epilepsy patients demonstrate similar outcomes [118, 125]. A consistent outcome from these data is the rapidity of effect in aborting seizures that were not prolonged prior to drug treatment, with many patients having seizures eliminated in less than five minutes and for many under two minutes.

A recently patented intranasal clonazepam formulation uses a binary solvent mixture comprised of diethylene glycol monoethyl ether, triacetin, glycofurol, and propylene glycol in varying proportions [149]. A controlled trial using this formulation in patients with epileptic seizures is underway (www.clinicaltrials.gov). The only other intranasal clonazepam study was that by Schols-Hendriks *et al.* who reported a bioavailability of 40 % with peak concentrations appearing at 0.25-0.5 h after dose administration [150].

Summary for Intranasal Administration

Pros:

Administration of drugs by the nasal cavity can be useful for a variety of situations. The method is needle-less, easy to administer, and easily accessible for administration. It is non-invasive as compared to rectal, intravenous, or intramuscular administration; thus, an attractive feature for the pediatric population. Drugs can also be administered in an out-of-hospital setting, allowing patients or caregivers to administer the drug to young children in the event of limited or delayed access to medical personnel. Lastly, studies indicate that drug absorption can be quite rapid (within minutes), and can be reached earlier than oral or rectal therapy [98, 99, 142]. Intranasal administration may also

bypass hepatic first-pass metabolism to ensure high bioavailability of drug in some instances.

Cons:

The presence of allergic rhinitis and upper respiratory infection may inhibit absorption. Nasal preparations may cause mucosal irritation and alter ciliary activity. This route is not suitable for drugs with short half-lives which need to have sustained blood levels. Some drugs may be degraded by the enzymes present in the nasal mucosa. One of the major limitations is the volume of the drug that can be administered into the nasal cavity which is around 0.1-0.3 mL. In addition, a large portion of the drug may be swallowed leading to delayed absorption and first pass metabolism.

Table 1-6: Comparison of pharmacokinetic parameters of benzodiazepines after IM, IN, BUC or PR administration in healthy adults

BZD	**% Bioavailability**				**t_{max} (min)**				**$t_{1/2}\alpha$ (hrs)**	**$t_{1/2}\beta$ (hrs)**
	IM	IN	BUC	PR	IM	IN	BUC	PR		
CZP	93[58]	~40[b] [150]	~40[150]	NA	3.10[58]	0.25-0.5[150]	0.5-1.5[150]	10-30[103]	0.7-3.4	17-56
DZP	80-100[57]	70-80[112]	NA	90[77]	0.5-1.5[55, 57]	0.3-0.5[112]	NA	45-70[77]	0.3-0.5	24-60
LZP	100[107]	77.7[107]	NA	86[101]	3.0[107]	0.5[107]	NA	68[101]	0.1-0.5	17-56 hrs
MDZ	80-90[151]	60[140]	75-80[61]	52[96]	0.25-0.7[151]	0.15[140]	0.5[61]	31[96]	0.1-0.25	0.5-4 hrs

BZD = Benzodiazepine; CZP = Clonazepam; DZP = Diazepam; LZP = Lorazepam; MDZ = Midazolam; IM = Intramuscular; IN = Intranasal; BUC = Buccal; PR = Rectal; t_{max} = Time to maximum concentration; $t_{1/2}\alpha$ = Half-life of distribution; $t_{1/2}\beta$ = Elimination half-life

1.3.2 Summary for choice of Intranasal Diazepam

Among the four BZDs under development for rescue therapy outside the hospital, midazolam has a competitive advantage in terms of administration via multiple routes and a fast rate of absorption, whereas diazepam may have superior bioavailability and duration of effect. Clonazepam and lorazepam appear to be inferior to the other BZDs in terms of utility in treating seizure emergencies by non-intravenous routes of administration. Diazepam possesses properties that make it a particularly good candidate for intranasal administration. Its lipid solubility and potency are comparable to midazolam. Diazepam has a substantially longer elimination half-life which may provide a longer duration of action as compared to midazolam.

Table 1-6 provides a comparison of the four BZDs via different routes of administration. The literature review and available data suggest that IM midazolam has a superior profile to IM diazepam. Buccally administered midazolam is superior to other BZDs given by the same route. Both IM and BUC midazolam appear to have favorable pharmacokinetics and safety profiles that make them viable candidates for commercial products, although public concern about injecting medications may be a barrier to widespread use of IM midazolam. With respect to IN administration, midazolam appears to have a somewhat faster rate of absorption, but lower and more variable absorption, and a shorter elimination half-life as compared to IN diazepam. Further, the doses of diazepam needed to effectively and safely treat ARS and related conditions are well known, which is not the case with midazolam. Based on the Jawad [46] and Lahat [152] studies, midazolam doses of 10 mg may be needed. Based on all available data, diazepam and midazolam appear to be comparable and head-to-head clinical trials may be needed to determine if one is clinically superior to the other.

The traditional development path for the potential approval of a nasal product for out-of-hospital seizure emergencies would be to prove the safety and efficacy in at least one or more placebo controlled clinical trials after establishing the pharmacokinetics and

toxicity over a range of doses. There is another possibly quicker path, which would be to use the generic drug approval application, 505 (j) in which case it would be sufficient to show bioequivalence between nasal and rectal diazepam. As rectal diazepam has orphan designation, a nasal diazepam product could be given this designation by using the clause of clinical superiority provided in 21 CFR 316.3(b)(3)(iii) for orphan drug products [153] which states the following:

"Clinically superior means that a drug is shown to provide a significant therapeutic advantage over and above that provided by an approved orphan drug (that is otherwise the same drug) in one or more of the following ways: (i) Greater effectiveness than an approved orphan drug (as assessed by effect on a clinically meaningful endpoint in adequate and well controlled clinical trials). Generally, this would represent the same kind of evidence needed to support a comparative effectiveness claim for two different drugs; in most cases, direct comparative clinical trials would be necessary; or (ii) Greater safety in a substantial portion of the target populations, for example, by the elimination of an ingredient or contaminant that is associated with relatively frequent adverse effects. In some cases, direct comparative clinical trials will be necessary; or (iii) ***In unusual cases, where neither greater safety nor greater effectiveness has been shown, a demonstration that the drug otherwise makes a major contribution to patient care.****"*

Thus, a nasal product may be developed which provides a major contribution to patient care in terms of providing better compliance and acceptability by all age groups.

In the event that one may be able to take the generic drug approval pathway, the development of nasal diazepam product would possibly require a bioequivalence study comparing with rectal diazepam followed by a range of safety studies. This is definitely more cost effective than having to conduct one or more placebo controlled trials as will definitely be the case for the development of a nasal midazolam product. Therefore, from the perspective of a cost effective development plan, pharmacologically viable drug and

most convenient and easy to use route of administration, an intranasal diazepam product clearly stands out as the candidate of choice for many reasons as discussed above.

CHAPTER 2

RESEARCH OBJECTIVES

The long term objective of this present work was to develop an intranasal formulation of diazepam as an alternate treatment option to the FDA approved rectal diazepam gel, Diastat®. Pilot studies were conducted to characterize the pharmacokinetics of intranasally administered diazepam using different formulation technologies. Also, for the purpose of a meaningful comparison, archived rectal diazepam pharmacokinetic data from two previously conducted studies were also collected. The specific research objectives include:

1. Assessment of the pharmacokinetics, using noncompartmental analysis, and tolerability of different formulations of intranasally administered diazepam solutions.

2. Development of an integrated population pharmacokinetic model for intranasal diazepam and identification of potential covariates that influence the absorption and disposition.

3. Development of an integrated population pharmacokinetic model for rectal diazepam gel, Diastat® and identification of potential predictors of absorption and disposition.

4. Comparison of intranasal and rectal diazepam pharmacokinetics using combined data from all studies.

CHAPTER 3

PHARMACOKINETIC CHARACTERIZATION OF INTRANASAL DIAZEPAM

3.0 Pharmacokinetics and Tolerability of Intranasal Diazepam and Midazolam in Healthy Adult Volunteers

3.0.1 Introduction

Individuals with uncontrolled epilepsy represent some of the greatest challenges in the management of this disorder [154, 155]. These patients are particularly prone to status epilepticus (SE) as well as prolonged or cluster seizures which are in themselves serious conditions that can evolve into SE [8]. Intravenously administered benzodiazepines (BZDs) are widely used for the treatment of seizure emergencies. When given within 30 minutes of seizure onset, intravenous (IV) BZDs are effective in more than 80% of patients [8, 33]. However, IV administration requires skilled personnel and transport to a medical facility which can delay initiation of therapy [156]. Treatment delay is associated with longer seizure duration, greater difficulty in terminating the seizure, prolonged hospitalization, higher mortality, and reduced quality of life [8, 90].

Administration of BZDs by other routes could permit earlier initiation of therapy outside of medical facilities. Rectal administration of diazepam (DZP) for the treatment of seizure emergencies is safe and effective, reduces medical costs, and improves quality of life, but many patients and their caretakers are reluctant to consider this mode of therapy especially when the patient is in a location which is socially embarrassing [16, 93, 94, 117].

The availability of a fast acting intranasal (IN) treatment that can be easily administered by the patient or a caregiver would greatly improve the management of seizure disorders. Essential characteristics for an intranasal drug delivery system in the treatment of seizure emergencies include: patients must be able to tolerate the formulation; administration volume of 0.5 mL or less; rapid, consistent absorption; and easy administration by non-medical caregivers and patients.

The purpose of this study was to evaluate the pharmacokinetics and tolerability of intranasally administered DZP and midazolam (MDZ) in healthy adult volunteers.

3.0.2 Methods

The study was approved by the Institutional Review Boards at the University of Minnesota and Hennepin County Medical Center. Four healthy, non-pregnant women of 20 to 24 years of age participated in the study. Subjects provided informed consent and were compensated for participation. Subjects were excluded if they were in poor health, unwilling or unable to receive intranasal or intravenous medications, pregnant, smokers, allergic to DZP or MDZ, or had narrow-angle glaucoma.

Subjects' treatment sequence was randomly assigned using a latin-square design. The study consisted of a four-way, randomized, single-blind, crossover design in which subjects received 5 mg doses of intranasal (IN) DZP, IN MDZ, IV DZP and IV MDZ. Subjects were admitted to the clinical research unit located at Hennepin County Medical Center and remained there for 8 hours on 4 separate occasions after a minimum 1-week washout period.

Commercial formulations were used for IV administration of DZP and MDZ. The IN DZP formulation consisted of an investigational supersaturated solution containing 40 mg/mL of DZP, glycofurol and water. The injectable MDZ formulation (5mg/mL) was also used for intranasal administration. The intranasal doses of 5 mg were administered using a 1.0 mL syringe such that 0.125 mL of the DZP solution and 1 mL of the MDZ solution were dripped slowly into either one of the nostrils Intranasal administration of normal saline (0.5 mL) given with a 1.0 mL dropper served as a control to compare tolerability of the drugs. Using a 10-point Global Tolerability Analog Scale, each subject rated overall tolerability of the intranasal (drug and normal saline) and intravenous doses (drug only) at 5 minutes prior to and 0, 5, 15, 60 minutes and 8 hours after drug

administration. A score of 10 was considered the least tolerable. This scale is analogous to Visual Analog Scales (VAS) and has been adapted from a previous study evaluating the tolerability of a nasal formulation [157]. Subjects also completed a pain and subjective discomfort questionnaire for the intranasal administrations. Using a 4 point analog scale with 4 representing extreme pain or discomfort, subjects rated specific pain characteristics: burning, stinging, and throbbing at -15 minutes, 0, 5, and 15 minutes.

Blood samples of 5 mL for pharmacokinetic analysis were collected, by means of a catheter inserted into a forearm vein, into glass tubes containing ethylenediamine tetraacetic acid as anticoagulant at -5, 0, 1, 5, 10, 20, 30, 60 minutes and 8 hours. For DZP, additional samples were obtained at 24 and 48 hours. Within 15 minutes of collection, the blood samples were spun in a centrifuge, and plasma was carefully separated. Plasma samples were stored at –80°C pending analysis.

3.0.2.1 Drug Assay

Plasma samples were analyzed for MDZ and DZP concentrations using an Agilent 1100 series HPLC system (Agilent Technologies, Palo Alto, CA, USA) with a C4 column. The mobile phase for the system consisted of 40% acetonitrile and 60% phosphate buffer (pH-6.0). The flow rate of the mobile phase was 0.5 mL ⁄min and the injection volume was 50 μL. Standard curves were prepared over the range of 5–500 ng ⁄ mL and quality control samples containing 15 (low), 50 (medium) and 250 ng ⁄ mL (high) of DZP and MDZ were prepared separately with blank human plasma. An aliquot of 0.2 mL of the plasma was added to a 12 x 75 mm glass tube. A sample of NaOH (200 μL) and the internal standard lorazepam (200 μL) were added and the solution was mixed well. A 2 mL volume of ether was poured in the tube as an extracting solvent and vortex mixed for 1 min and then centrifuged for 10 min at 769 g. A sample of the organic layer was collected and evaporated until dry with nitrogen at 34 ^{o}C, and then 200 μL of the HPLC mobile phase was added to dissolve the residue. After 30 s of vortex mixing, 50 μL of the

sample solution was injected into the HPLC system. The standards for DZP and MDZ were analyzed on separate days and the mean coefficients of variation were 5.6% and 5.0%, respectively. The mean coefficients of variation for the intraday variation of DZP and MDZ quality control samples were 8.6% and 7.5%, respectively.

3.0.2.2 Pharmacokinetic Analysis

Concentration-time data of DZP and MDZ were examined using non-compartmental pharmacokinetics analysis with WinNonLin software (version 5.2; 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 $\ln 2/(\lambda z)$. Maximum plasma concentrations (C_{max}) and time to maximum concentration (t_{max}) were determined by direct observation of the data. Means and standard deviations for the parameters were also obtained using the descriptive statistics tool in WinNonlin version 5.2.

3.0.3 Results

Four women, ages 20 to 24 years entered the study. One subject dropped out due to travel conflicts after completing the IN DZP arm and was excluded from all group analyses. The pharmacokinetic parameters for the three subjects are summarized in Table 3.0-1. The mean concentration-time profiles are shown in Figure 3.0-1 and the individual subject's concentration time profiles for both IN DZP and MDZ are shown in Figure 3.0-2. The average IN DZP C_{max} and t_{max} were 179.2 ± 8.8 ng/mL and 28.8 ± 20.9 minutes. The average IN MDZ C_{max} and t_{max} were 62.8 ± 14.5 ng/mL and 21.6 ± 7.6 minutes, respectively. The C_{max} and t_{max} of the subject who dropped out of the study were 109.48 ng/mL and 20 minutes, respectively.

Table 3.0-1: Mean (± S.D.) pharmacokinetic parameters of DZP and MDZ in healthy volunteers following IV and IN administration of 5mg dose.

PK Parameter	IV DZP	IN DZP	IV MDZ	IN MDZ
t_{max} (min)	-	28.8 ± 20.96	-	21.6 ± 7.63
C_{max} (ng/ml)	344.0 ± 92.81*	179.2 ± 8.85	165.2 ± 96.42*	62.8 ± 14.51
Half life (hrs)	59.1 ± 7.76	22.4 ± 3.45	0.9 ± 0.60	3.0 ± 0.74

** Concentration at 5 mins*

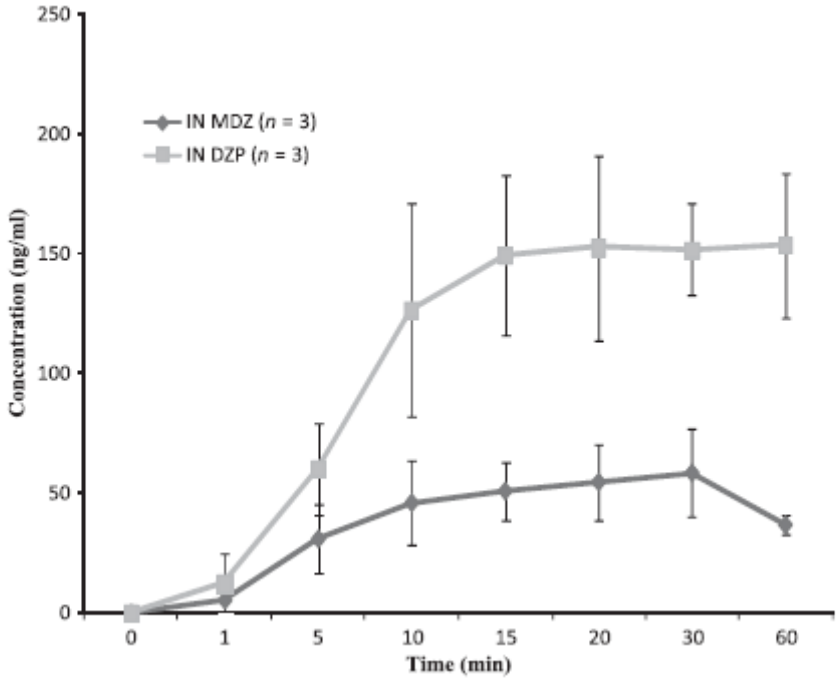


Figure 3.0-1: Comparison of mean intranasal DZP and MDZ concentration vs time profile.

Immediately following IN administration, subjects reported an average global tolerability score of 6.75 and 6.0 for DZP and MDZ respectively, which were statistically not different ($p>0.05$). Within 15 minutes, scores decreased to 3.3 and 1.5. respectively, which eventually returned to baseline (Figure 3.0-3).

Subjects rated both formulations as causing considerable pain with a maximum score of 3.2 immediately following nasal administration. Fifteen minutes later, the mean pain score for both drugs was 1.2. Posterior nasal drainage and watery eyes were reported by all subjects.

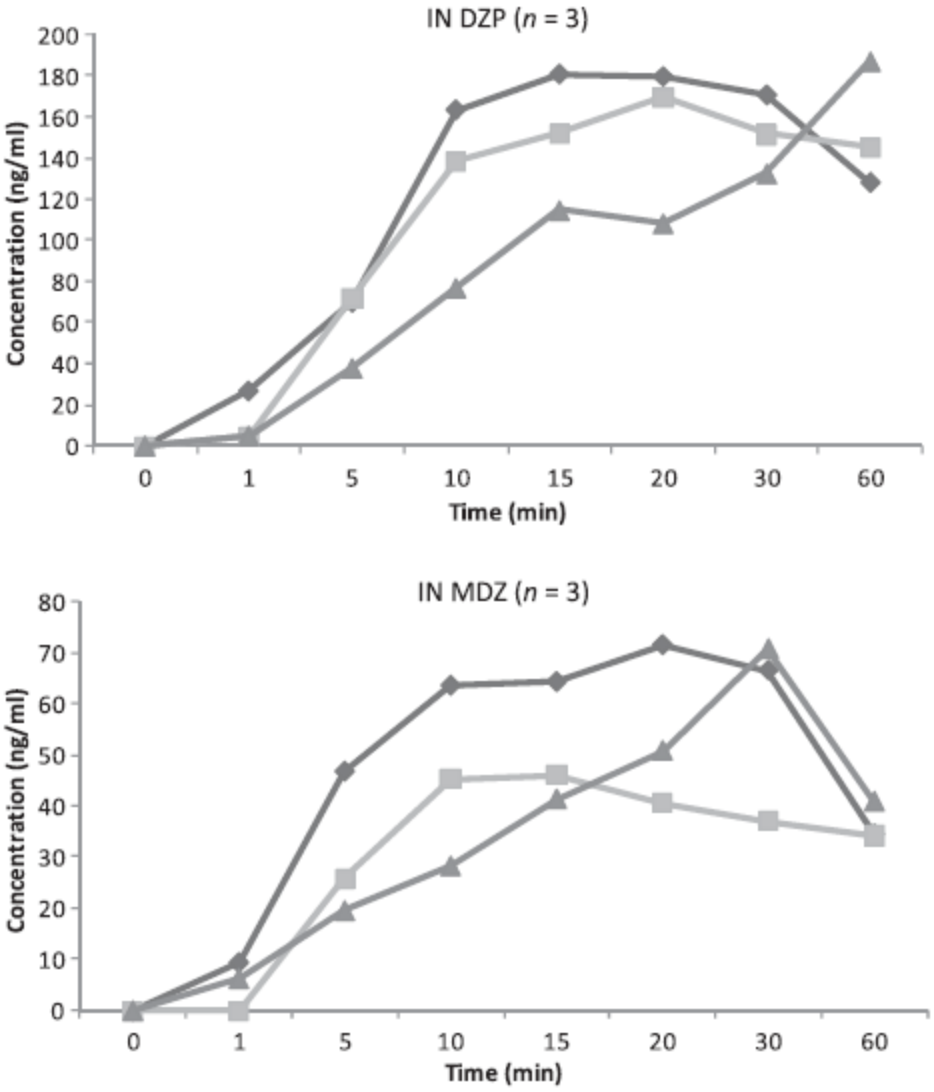


Figure 3.0-2: Concentration time profiles (0–60 min) of individual subjects (n = 3) for intranasal MDZ and DZP.

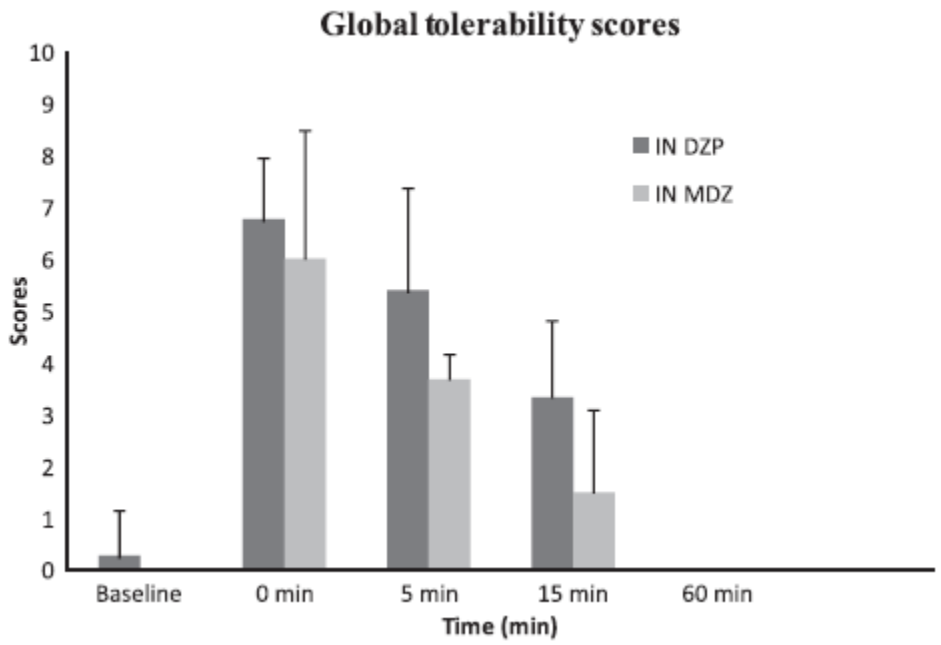


Figure 3.0-3: Comparison of mean global tolerability scores after intranasal administration (n = 3).

3.0.4 Discussion

Using PubMed with key terms "intranasal midazolam and diazepam", we found no published reports directly comparing IN DZP and IN MDZ. Various MDZ formulations given IN have been investigated with most studies using the commercially available injectable MDZ solution [158, 159]. These studies with doses between 10-20 mg (2-4 mL's) reported C_{max} and t_{max} values in the range of 147-192 ng/mL and 14-25 minutes, respectively. The absorptive area of the nose limits the volume administered to approximately 20 μL – 200 μL per nostril although smaller volumes are preferable [160]. When the commercially available injectable MDZ solution is given intranasally, volumes exceeding 0.20 mL are required in order to administer a clinically relevant dose [158]. This could affect both bioavailability and C_{max}. Highly-concentrated investigational nasal MDZ formulations, including a water and propylene glycol admixture (pH 4) [137], and a solution containing 14 % (w/v) sulfobutylether β-cyclodextrin (pH 4.3) [138] have also

been studied in humans. Although these formulations permit administration of smaller volumes (200-300 µL), there was no distinguishable difference in the values of C_{max} and t_{max}.

Three previous studies have investigated IN DZP in humans. Gizurarson *et al.* compared an intranasal 2 mg dose of a 20 mg/mL DZP solution dissolved in 5% glycofurol in polyethylene glycol 200 with the same dose given IV [109]. Blood samples were collected for 5 hours following drug administration. The mean bioavailability was 50.4 ± 23.3% with a time to peak concentration of 18 ± 11 minutes. All subjects complained of nasal discomfort immediately following drug administration, but the discomfort resolved within 30 minutes. Lindhardt *et al.* evaluated an intranasal formulation of DZP in polyethylene glycol 300 in 7 healthy volunteers. Using a cross-over design, they compared 4 mg and 7 mg intranasal doses with a 5 mg intravenous dose and collected blood samples for 60 minutes after drug administration. The intranasal formulation had a relative bioavailability of 45 and 42 %, C_{max} of 99 and 179 ng/mL and t_{max} of 18 and 42 mins for the 4 and 7 mg doses, respectively [110]. Given that the half-life of diazepam ranges from 24 to 48 hrs, their bioavailability values are likely an underestimate of the actual extent of absorption. Slattery and Lau, using a 10 mg dose of DZP dissolved in Cremophor EL, reported a bioavailability of 78 % with a C_{max} of 175 ng/mL and a t_{max} of 1 hr [111]. A recent study by Ivaturi *et al*. [112] determined the pharmacokinetics and dose proportionality of 5 mg and 10 mg doses of an intranasally administered diazepam formulation compared with intravenous administration in eight healthy volunteers using a cross over design. The formulation used was a 40 mg/mL supersaturated solution of diazepam in glycofurol-water cosolvent mixture. Each subject received two intranasal and one intravenous dose of diazepam and blood samples were collected up to 48 hours after dosing. The mean C_{max}, t_{max} and $t_{1/2}$ were 134.3 ± 61.9 ng/mL, 55.6 ± 60.3 minutes and 49.1 ± 20.4 hours for the 5 mg dose and 247.0 ± 60.9 ng/mL, 39.3 ± 38.1 minutes and 57.0 ± 28.0 hours for the 10 mg dose. Using analog scales, subjects rated tolerability (0 = no change from normal;10 = maximum intolerability) prior to and 0, 5, 15, 60 minutes, and 8 hours after administration. The mean tolerability scores observed were 4.4

and 4.7 for the 5 mg and 10 mg doses. Both these scores dropped down to 3 and 2.5, 15 minutes post dose and to 1, 60 minutes post dose.

The pharmacokinetic parameters for IV DZP and IV MDZ shown in Table 3.0-1 were comparable to those reported in the literature [161]. The relationship between DZP pharmacokinetics and pharmacodynamics is complex. Following rapid IV administration, relatively high plasma DZP concentrations occur prior to distribution to various body compartments including the CNS. This makes correlation of DZP levels with seizure control difficult. In contrast, the absorption of DZP following rectal or nasal administration, although relatively rapid, does permit equilibration of DZP concentrations between plasma and the CNS. Milligan *et al.* rectally administered a 20 mg dose of DZP solution or placebo to 10 adults with epilepsy and then observed spike wave activity and measured plasma concentrations. Rectal DZP significantly reduced EEG spike frequencies within 20 minutes at a mean serum diazepam level of 210 ng/mL. The mean C_{max} of DZP was 413 ng/mL and the mean t_{max} was 32 minutes [35]. Based on these results, subsequent controlled clinical trials using similar doses, and presumably similar plasma DZP concentrations, have demonstrated that rectal DZP is effective in treating acute repetitive seizures [16].

Although we administered 5 mg DZP intranasally in this study, doubling the dose to 10 mg by giving 5 mg DZP into each nostril should result in concentrations > 200 ng/mL that are attained within 5-10 minutes.

It is unclear if prolonged serum DZP concentrations are needed to achieve and maintain seizure control. The longer elimination half-life of DZP compared with MDZ as shown in the results conveys a theoretical advantage in preventing subsequent seizure recurrence. In controlled investigations DZP is effective in treatment of seizure emergencies [16, 162]. Such studies have yet to be conducted with MDZ.

All subjects reported moderate discomfort with both formulations. This is a major limitation of both the injectable MDZ solution and the investigational DZP formulation.

Measures to improve comfort level or tolerability are needed for greater patient acceptance. Nonetheless, some patients and caretakers would prefer the transient discomfort of the present intranasal formulations to rectal administration of medication in public settings. Similar views have been expressed in a comparative study of IN MDZ and rectal DZP [117]. Intranasal DZP may be useful in the treatment of seizure emergencies. However, this was a small study of healthy volunteers which precludes generalization to clinical use and further research is needed to improve tolerability of the formulation and to characterize the appropriate dose.

3.1 Bioavailability and Tolerability of Intranasal Diazepam in Healthy Adult Volunteers

3.1.1 Introduction

Frequently recurring seizures, prolonged seizures or status epilepticus are recognized as seizure emergencies [9]. Rapid treatment of such emergencies improves outcomes and minimizes associated morbidity [45]. The standard approach to treating seizure emergencies is intravenous administration of anti-seizure medications in an emergency center. There is now substantial evidence that the use of an out-of-hospital medication to terminate repeated or prolonged seizures reduces visits to the emergency department, lowers medical costs, and improves quality of life [16, 93, 94, 163].

Benzodiazepines are widely used in the treatment of seizure emergencies. When there is intravenous access, lorazepam is considered the drug of choice for the treatment of prolonged seizures and status epilepticus [8]. However, in the management of seizure emergencies outside a hospital setting, rectal administration of diazepam has been employed with good success. Although buccal and intranasal administration of benzodiazepines are used in clinical practice, only rectal diazepam has been shown to be safe and effective in terminating acute repetitive seizures in blinded, placebo-controlled studies [16, 92]. Nonetheless, older children and adults often refuse therapy because of social objections to this route of administration [106, 129, 164]. Hence, many patients are effectively without benefit of an approved therapy that can be administered outside the hospital.

Availability of a fast acting intranasal treatment that can be easily administered by the patient or a caregiver would greatly improve the management of out-of-hospital seizure emergencies. Essential characteristics for an intranasal drug delivery system include a well-tolerated formulation; administration volume of ≤ 0.3 mL (approximately 100-150 μL/spray/nostril); rapid, consistent absorption; and easy administration by non-medical caregivers and patients. Nasal drug delivery is well accepted as a mode of therapy for

treatment of seizure emergencies [117]. Benzodiazepines, especially midazolam, given intranasally have been studied in several open-label trials. These studies provide evidence that they can be easily administered, are reasonably safe, and exhibit a clinical effect comparable to rectal diazepam [117, 124, 125].

Relative to other benzodiazepines, diazepam (DZP) has certain physicochemical and pharmacological characteristics such as high lipid solubility and a long elimination half-life that support its use in intranasal therapy [34]. Our group has developed an investigational intranasal formulation of DZP. In this formulation, DZP dissolved in glycofurol is rapidly mixed with water, which is a poor solvent of DZP but is fully miscible with glycofurol. With proper care, the result is a supersaturated DZP solution that is thermodynamically unstable but kinetically stable for several tens of minutes. In the supersaturated state, DZP has a high activity, and is expected to be rapidly absorbed across the nasal musosal membrane [114].

The objective of the present study was to carry out a randomized, single-blind, three-way crossover study, to determine the bioavailability and pharmacokinetics of an investigational formulation of DZP administered intranasally at 5 and 10 mgs as compared to a 5 mg intravenous dose. Safety and tolerability of this investigational intranasal formulation were also evaluated.

3.1.2 Methods

3.1.2.1 Subjects and Study Design

Subjects were healthy volunteers 18 years or older who provided informed consent and were compensated for participation. Subjects were excluded if they were in poor health, unwilling or unable to receive intranasal or intravenous medications, pregnant, smokers, allergic to DZP, or had narrow-angle glaucoma. The study was approved by the Institutional Review Boards at the University of Minnesota and Hennepin County

Medical Center and was conducted at DaVita Clinical Research Unit (CRU) in Minneapolis.

The study utilized a randomized, single-blind, three-way crossover design to compare the pharmacokinetics and tolerability of a commercially available parental DZP administered intravenously (5 mg) and two intranasal DZP doses (5 mg and 10 mg). This investigation was intended to serve as a pilot study to characterize the tolerability and pharmacokinetics of a saturated glycofurol formulation. We chose a sample size which would give us sufficient data to do exploratory analysis and understand the performance of the formulation. The sample size was based on the ability to detect a 30% difference in AUC between nasal and intravenous administration, assuming one drop-out (Power = 90%, $\alpha = .05$). Eight 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 or over-the-counter medications beginning 24 hours prior to each admission through each 48 hour blood draw. They were also instructed to not consume alcoholic beverages 24 hours before and after drug administration study days. Subjects were admitted to the CRU where they would remain for 10 hours.

On the morning of the first day of the study, subjects were randomized to receive a 5 mg intranasal, or 10 mg intranasal, or 5 mg intravenous dose of DZP. Subjects were blinded to the dose of the intranasal treatment, as both doses involved administration into two nostrils. For the 5 mg dose, a control solvent was administered in the second nostril whereas for the 10 mg dose, subjects received 5 mg of the formulation in each nostril to maintain the blind and study conditions. Each subject had an indwelling catheter placed in her/his arm. All doses were administered while the subjects were in the supine position.

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). The intravenous drug was acquired by the CRU. The injectable DZP was stored as per the approved labeling in a secure location.

The intranasal DZP formulation was a freshly prepared supersaturated solution containing 40 mg/mL DZP in a 60 %-40 % (v/v) co-solvent mixture of glycofurol and water [114]. The intranasal dose was administered as 5 mg dose (0.125 mL) using a 1-mL syringe, with the subject lying in the supine position such that the 5 mg dose was instilled in one nostril, with a cosolvent blank of equal volume instilled in the other nostril. The 10 mg dose was divided into two equal volumes and instilled into each nostril.

3.1.2.3 Drug Assay

Blood samples (5 mL) were collected prior to dosing and at the following time points following instillation: 0, 1, 5, 10, 15, 20, 30, 45 min, and 1, 1.5, 2, 3, 4, 6, 8, 10, 24 and 48 h. Plasma was immediately separated, transferred to polyethylene tubes, and frozen pending analysis by HPLC.

To prepare a sample for HPLC, the sample was thawed, and an aliquot was transferred to a 12 x 75 mm glass tube. Following addition of NaOH (200 µL, 0.05 N) and the internal standard lorazepam (200 µL, 1 mg/mL), the solution was mixed well. DZP and lorezepam were extracted by pouring 2 mL ether into the tube, vortex mixing for 1 min, and centrifugation at 2000 rpm for 10 min and then by freezing the bottom portion of the tubes. A 200 µL sample of the organic layer was collected and evaporated until dry with nitrogen at 34 °C.

HPLC analysis was carried out by dissolving the residue in 200 µL of the HPLC mobile phase, consisting of 40 % acetonitrile and 60 % phosphate buffer (pH-6.0). After 30 seconds of vortex mixing, 50 µL of the sample solution was injected into an Agilent 1100 series HPLC system with a C4 column. Flow rate of the mobile phase was 0.5 mL/min and the injection volume was 50 µL.

Standard curves were prepared over a range of 5 ng/mL to 500 ng/mL and quality control DZP samples containing 15 (low), 50 (medium) and 250 ng/mL (high) were prepared separately with blank human plasma. Standards for DZP were analyzed on separate days and the mean coefficients of variation were 5.6 % and 5.0 %. The mean coefficients of variation for the intraday variation of the DZP quality control samples were 8.6 %.

3.1.2.4 Pharmacokinetic Analysis

DZP concentration-time data were analyzed using a non-compartmental pharmacokinetic approach with WinNonLin software (version 5.2; 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 $\ln 2/(\lambda z)$. 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 to the last non-zero plasma concentration (C_{last}) that was above the lower limit of quantification was calculated as AUC_{last}. The area under the concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{last} + (C_{last}/\lambda z)$. To assess initial exposure after intranasal administration, area under the concentration-time curve was determined at various time points in the first few hours (AUC_{0-t} h).

Means and standard deviations for the parameters were also obtained using the descriptive statistics tool in WinNonlin version 5.2.

3.1.2.5 Safety and Tolerability Assessment

Safety was assessed based on the frequency of adverse events and/or serious adverse events, changes in vital signs, and changes in the physical examination findings for the entire duration of the study**.** Vital signs were measured once during screening, one time on each of the 3 dosing days and the last day or the study close out day.

Using a 10-point Global Tolerability Analog Scale, each subject rated overall tolerability of the intranasal and intravenous doses at 5 minutes prior to drug administration and 0, 5, 15, 60 minutes and 10 hours after drug administration. A score of 10 was considered the extremely intolerable. This scale is analogous to Visual Analog Scales (VAS) and has been adapted from a previous study evaluating the tolerability of a nasal formulation [157]. Subjects also completed a pain and subjective discomfort questionnaire for the intranasal administrations. Using a 4-point analog scale with 4 representing extreme pain or discomfort, subjects rated specific pain characteristics: burning, stinging, and throbbing at -15 minutes, 0, 5, 15 and 600 minutes. A composite pain score was calculated as the average of scores for the 3 pain characteristics.

3.1.2.6 Statistical Analysis

For each variable, the data were analyzed using an analysis of variance (ANOVA) appropriate for data from a crossover design. Among the effects included in this ANOVA are sequence, subjects within sequence, treatment, and period. A test of the sequence

effect using the subject within sequence effect as an error term was performed to investigate the presence of carryover. Significance was determined at the $\alpha < 0.05$ level. Dose proportionality was determined by using the confidence interval criterion.

3.1.3 Results

3.1.3.1 Pharmacokinetics

Eight subjects (6 male and 2 female; mean age, 28.3 years) were enrolled and completed the study. As shown in Figure 3.1-1, DZP concentrations rise rapidly and are maintained for several hours following intranasal administration. There was a linear increase in C_{max} and exposure (AUC_{0-t}) with DZP dose reflecting approximate dose proportionality (Table 3.1-1). Both t_{max} (median range, 20-30 minutes) and $t_{1/2}$ (mean range, 49.1 - 57.0 h) were comparable following the intranasal doses. Table 3.1-2 shows the ratio, expressed as a percent, of drug exposure after nasal administration relative to intravenous dose at different time points. Cross-over, sequence or period effects were not found to be significant in this study.

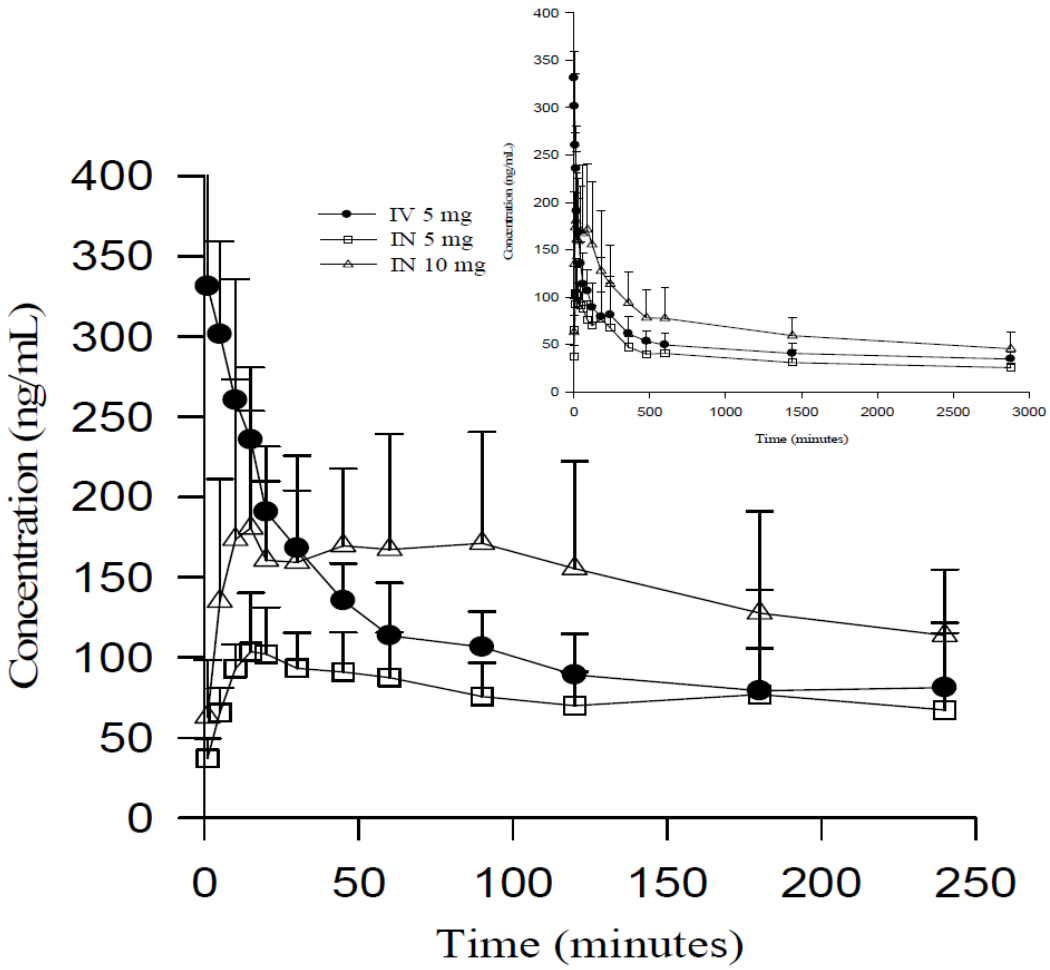


Figure 3.1-1: Mean plasma DZP-concentration time profiles after intravenous and intranasal administration in eight subjects (0—4 h). Inset shows the complete profile (0—48 h).

Table 3.1-1: Mean ± SD of DZP pharmacokinetic parameters following IV and IN administration.

Pharmacokinetic Parameter	5 mg IV Mean ± SD	5 mg IN Mean ± SD	10 mg IN Mean ± SD	Dose Corrected Ratio[a]	90 % Confidence Interval[a]
C_{max}, ng/mL	-	134.3 ± 61.9	247.6 ± 60.9	0.92	0.74,1.36
$AUC_{0\text{-}1h}$, ng.h/mL	187.3 ± 23.4	88.6 ± 16.0	157.6 ± 41.2	0.88	0.67, 1.45
$AUC_{0\text{-}last}$, ng.h/mL	2255.4 ± 628.9	1710.8 ± 540.5	3361.8 ± 916.1	0.98	0.88, 1.16
$AUC_{0\text{-}\infty}$, ng.h/mL	4484.0 ± 1275.4	3295.0 ± 1134.9	7739.6 ± 3645.2	1.17	0.73, 2.07
t_{max}, min	-	20 (15-180)	30 (10-120)	-	-
$t_{1/2}$, h	48.3 ± 17.8	49.1 ± 20.45	57.0 ± 28.0	-	-
F ($AUC_{0\text{-}\infty IN}$)/($AUC_{0\text{-}\infty IV}$)	75 ± 41 %	74 ± 53 %	-	-	-

[a] Ratios of dose normalized parameters, calculated by arithmetic means, 90% confidence intervals about the difference of the log-transformed parameters.

Table 3.1-2: Mean AUC_{IN}/AUC_{IV} ratios (%) at different time points after intranasal administration of 5 mg and 10 mg DZP to eight healthy volunteers.

AUC_{IN}/AUC_{IV} (%) Time (hours)				
IN dose	**1 h**	**4 h**	**48 h**	**0-∞**
5 mg	47	67	70	75
10 mg	42	65	68	74
p-value[a]	0.318	0.473	0.414	0.403

[a] *p-values from the comparison between the two doses.*

3.1.3.2 Safety and Tolerability

No unanticipated adverse events were reported by subjects following nasal administration of DZP. All subjects, however, reported swallowing a portion of the nasal dose. There were no observed clinically significant abnormalities in vital signs or ECG measurements and no clinically relevant changes in laboratory parameters during the study.

Immediately after intranasal administration, subjects reported an average global tolerability score of 4.4 and 4.7 for the 5 mg and 10 mg DZP doses, respectively. Within 15 minutes, scores decreased to 3.0 and 2.5 respectively, and returned to baseline by 10 hours (Figure 3.1-2). Differences in scores between the two doses were not statistically significant, suggesting absence of drug effect on tolerability and that the discomfort was primarily due to the vehicle.

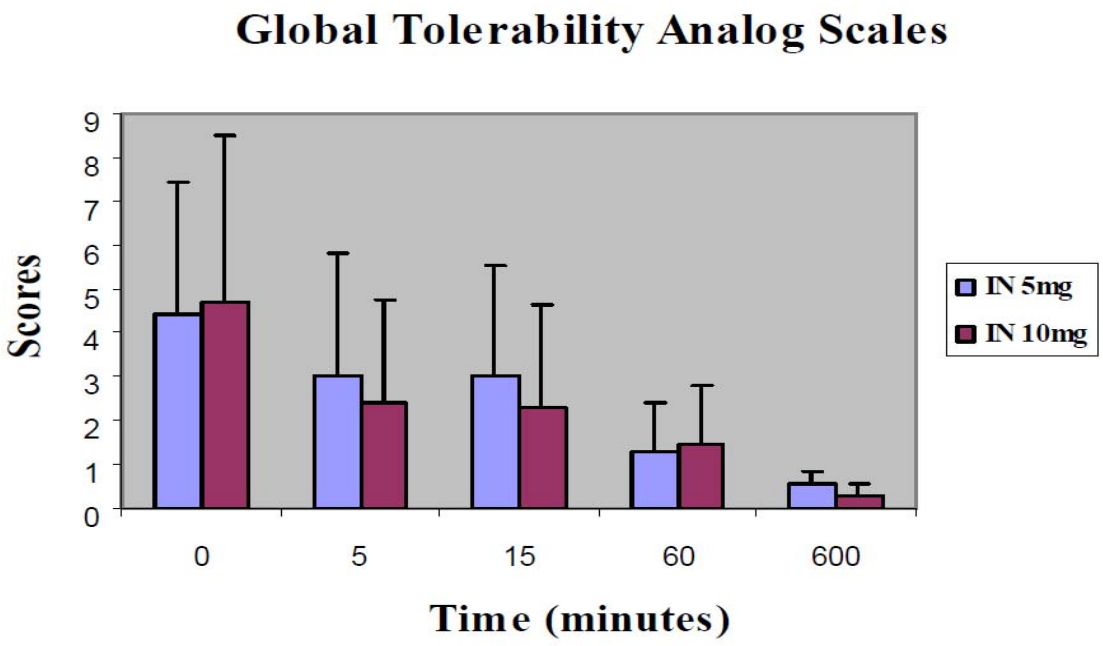


Figure 3.1-2: Comparison of Mean Global Tolerability Scores after IN administration (n = 8). Baseline scores were zero (0) for all subjects.

Subjects rated the formulation at both doses as causing considerable pain scores of 2 and 2.3 out of 4 immediately following nasal administration. Fifteen minutes later, the composite pain score for both doses were 1.8 and 1.3, and the scores returned to baseline by 10 hours. Posterior nasal drainage and watery eyes were reported by all subjects.

3.1.4 Discussion

The aim of this study was to evaluate the pharmacokinetics and tolerability of an investigational, supersaturated formulation of DZP administered intranasally at two doses. The results demonstrate that the formulation is rapidly absorbed with good, but incomplete bioavailability. Once C_{max} is attained, concentrations plateau for several hours before declining. However, the formulation caused considerable discomfort immediately following administration and was poorly tolerated. Two subjects reported tolerability scores of 8 up to 15 minutes, indicating significant discomfort.

Given limited resources, we chose to maximize the number of subjects and limit blood sample collection to 48 hrs, or approximately one DZP half-life. The estimated DZP half-life is consistent with previous reports in healthy volunteers, which provides external validation of the pharmacokinetic parameters determined in our study [77]. Nevertheless, the estimates for C_{max}, AUC, bioavailability and dose proportionality are less precise than would have been the case with a longer sampling period and more subjects. The 90% confidence intervals for difference in the log transformed parameters of C_{max} (0.74, 1.36) and $AUC_{0-\infty}$ (0.73, 2.07) for the 5 mg and 10 mg doses fell outside the accepted range for dose proportionality (0.80-1.25), while AUC_{0-last} (0.88, 1.16) was within the range (Table 3.1-1). The average bioavailability, F, was 75 and 74 % for the 5 and 10 mg doses respectively. Although nasal administration provided rapid drug levels in the body, these levels appeared to be sustained for a long time after dosing. The maintenance of these levels is reflected by exposure in terms of partial bioavailabilities at various time points

as shown in Table 3.1-2. The results for exposure in the first 60 minutes were comparable with the results demonstrated previously by Lindhardt *et al.* [110].

The pharmacokinetic parameters obtained following IN DZP in this study are consistent with those reported in previous investigations in healthy volunteers, although the sampling period in these studies was substantially shorter. Gizurarson *et al.* compared an intranasal 2 mg dose of a 20 mg/mL DZP solution dissolved in 5% glycofurol in polyethylene glycol 200 with the same dose given IV [109]. Blood samples were collected for 5 hours following drug administration. The mean bioavailability was 50.4 ± 23.3% with a t_{max} of 18 ± 11 minutes. Gizurarson reported that all subjects complained of nasal discomfort immediately following drug administration which resolved within 30 minutes. Lindhardt *et al.* evaluated an intranasal DZP formulation in polyethylene glycol 300 in 7 healthy volunteers. Using a cross-over design, they compared 4 mg and 7 mg intranasal doses with a 5 mg intravenous dose and collected blood samples for 60 minutes after drug administration. They reported a ratio of dose normalized AUC_{IN}/ AUC_{IV} in 60 minutes to be 45 and 42 %, C_{max} of 99 and 179 ng/mL and t_{max} of 18 and 42 minutes for the 4 and 7 mg doses, respectively [110]. Slattery and Lau, using a 10 mg dose of DZP dissolved in Cremophor EL, reported a C_{max} of 175 ng/mL and a t_{max} of 1 h [111].

Our preliminary data regarding intranasal DZP administration compares favorably with studies involving rectal administration. Milligan *et al*. administered a 20 mg rectal dose of a DZP solution to 10 adults with epilepsy and then recorded changes in EEG spike counts and measured DZP concentrations [35]. They found that the onset of a reduction in spike counts occurred approximately 15-20 minutes following drug administration and was associated with a DZP concentration of approximately 200 ng/mL. Both DZP concentrations and the pharmacodynamic effect were sustained for 3 hours. A bioequivalence study comparing an 7.5 mg IV dose of DZP injectable solution with a 15 mg dose of a commercial rectal formulation (Diastat®) found that Diastat® had a mean absolute bioavailability of 90 % and reached an initial peak concentration of approximately 375 ng/mL at 45 minutes [77]. In comparison to the studies with rectal DZP, our investigational intranasal formulation attained concentrations greater than 200

ng/mL at 15 minutes following the 10 mg dose, and an estimated relative bioavailability (AUC_{IN}/ AUC_{Rectal}) of approximately 83 %.

All subjects reported swallowing a portion of the dose after intranasal administration. With intranasal administration there is often some fraction of a dose that is swallowed and consequently not absorbed in the nose. As there is a lag time for the gastrointestinal absorption, secondary peaks appear in the concentration-time profile at later times, which in some subjects, were higher than the initial peaks. Such a double peak phenomenon was observed in all subjects, with 3 out of the 8 subjects showing secondary peaks with a C_{max} higher than the initial C_{max}. This contributed to highly variable estimates of t_{max} after nasal administration (Table 3.1-1). The double peaks also occur with rectally administered diazepam [37]. Further modeling of the absorptive processes to assess fractions of dose absorbed via each phase (1^{st} peak and 2^{nd} peak) of absorption would provide information useful in the optimization of a DZP nasal delivery system. Diazepam exhibits highly variable pharmacokinetics [165, 166] . In addition, intranasal administration itself being a delivery route which may provide wide variability in drug disposition together with the method of administration of the medication in our study and small sample size, may have contributed to highly variable pharmacokinetics.

The tolerability scores indicate that the nasal formulation caused considerable, but transient, pain. Similar findings have been reported in other IN DZP studies as well as with IN midazolam [138, 159]. Considering the severity of the clinical situation, the benefit of IN DZP may supersede the discomfort. We think that it is necessary to improve the tolerability in order to ensure acceptance of this mode of therapy.

The formulation used in this study was designed to maximize the concentration of DZP in order to limit the administered volume of solution. No efforts were made to optimize tolerability. Tolerability would probably increase with higher relative water content, using the same dose and total drop volume, as would the driving force for DZP permeation. However, increased water content reduces the kinetic stability of DZP, and could cause earlier precipitation, cancelling the benefit of using a supersaturated

formulation [114]. It is also recognized that the influence of glycofurol on the integrity of the nasal mucosa is not presently understood, and that irritation by glycofurol may affect permeation of DZP across the nasal membrane.

However, our results offer evidence that intranasal administration of DZP exhibits good bioavailability with rapid attainment of DZP concentrations and maintenance of levels for several hours that have previously been shown to be safe and effective when DZP is given rectally. This pilot study supports further development of an intranasal DZP formulation with improved tolerability and bioavailability.

3.2 Relative Bioavailability and Tolerability of Intranasal Diazepam Compared to Rectal Diazepam in Healthy Adult Volunteers

3.2.1 Introduction

Diazepam (Valium®) possesses properties that make it a particularly good candidate for intranasal administration as compared to other benzodiazepines. Its lipid solubility and potency are comparable to midazolam and it also has a substantially longer elimination half-life which may provide a longer duration of action as compared to midazolam [34]. Based on these considerations, our group has undertaken a series of studies investigating the potential of intranasal diazepam as an out-of-hospital treatment for seizure emergencies. We recently reported the pharmacokinetics of an intranasal diazepam formulation in 2 pilot studies [112, 113]. The glycofurol based formulation used in these 2 studies resulted in 75 % & 74 % bioavailability after administration of 5 and 10 mg DZP doses, respectively. The formulations were absorbed rapidly and concentrations were sustained above a therapeutic level of 200 ng/mL for 2-4 hours after drug administration. Our preliminary studies demonstrated that nasal administration of diazepam is feasible, resulting in a C_{max} and t_{max} that are comparable to rectal diazepam, which is FDA-approved for out-of-hospital treatment of seizure emergencies. However, the tolerability of our initial formulation was poor, which made it unsuitable for further development.

Several other formulations have been developed that may be better tolerated than our original formulation. In the present study, we evaluated the tolerability and pharmacokinetics of two new investigational nasal formulations as compared with diazepam rectal gel. The objective of this pilot study was to determine which of the investigational nasal formulations is best tolerated and obtain information on the bioavailability of the two formulations relative to diazepam rectal gel. The results of this study will be used to select a lead nasal formulation and design an adequately powered bioequivalence and safety study.

3.2.2 Methods

3.2.2.1 Subjects and Study Design

This study compared the pharmacokinetics, safety, and tolerability of 2 intranasal formulations of diazepam with a FDA approved rectal diazepam formulation in healthy volunteers. This was a 4-period, 4-way crossover study conducted in 12 healthy subjects. All subjects received a 10 mg rectal diazepam dose (as Diastat) in the first period. Thereafter, subjects randomly received each of the following treatments over the next three periods:

- 10 mg diazepam nasal formulation 10 mg Nas – A
- 10 mg diazepam nasal formulation 10 mg Nas – B1
- 13.4 mg diazepam nasal formulation 13.4 mg Nas – B2

There was at least a 14-day washout period between dosings. For the initial dosing period with rectal diazepam, subjects were confined to the research unit from one day prior to dosing till 2 days after for pharmacokinetic, tolerability, and safety assessments. In order to minimize the number of subjects first exposed to any of the two investigational nasal formulations given as three treatments, only six subjects in period 2 were admitted to the research unit and given one of the three nasal treatments (two subjects each for formulation Nas-A, Nas-B1 and Nas-B2). Stop criteria were defined to decide further dosing; (a) If one of the investigational treatments causes a pain score of 8 or greater for more than 5 minutes or results in continuous nasal bleeding for more than 5 minutes in more than one subject, that treatment would not be administered to any other subjects.(b) If any one subject has pain scores of 8 or more or continuous nasal bleeding for more than 5 minutes following exposure to the first two nasal treatments, that subject would

not receive the third nasal treatment. If the predefined stop criteria were not invoked, the next day the six remaining subjects received one of the three nasal treatments. As the stop criteria were not triggered in the six additional subjects in period 2, thereafter in periods 3 and 4 all subjects (4 subjects each for the 3 treatments) received their designated treatments on the same day.

Prior to each of the three treatments, the subject's eligibility was reviewed. Subjects were instructed to abstain from prescription or over-the-counter medications beginning 24 hours prior to each admission through each 48 hour blood draw. They were also instructed to not consume alcoholic beverages 24 hours before and after drug administration study days.

On the evening before the dose of diazepam was administered, subjects entered the clinical research unit for baseline assessments. Subjects were confined to the clinical research unit for 24 hours following dosing for blood draws and tolerability assessments. Each subject had an indwelling catheter placed in her/his arm. All nasal doses were administered while the subjects were in the supine position. The rectal formulation was administered according to instructions provided in the package insert. All subjects were placed in a bed in supine position and continued to remain so till 4 hours post dose.

3.2.2.2 Study Drugs

All study medications were prepared by a licensed pharmacist at the clinical research unit and dispensed to the study nurse by a licensed physician. Two investigational intranasal diazepam formulations were supplied by DPT Laboratories (Lakewood, NJ) with one of them at two doses (5 mg and 6.7 mg). The formulations of intranasal diazepam include ingredients that are generally recognized as safe (GRAS) and used in other injectable and ophthalmic products, or been shown to be safe in animal studies. Each intranasal formulation was supplied in a single-use, pre-filled bi-dose Pfieffer nasal sprayer device.

A single spray of the device delivered approximately 0.100 mL of the intranasal formulations containing either 5 or 6.7% diazepam. Each subject was administered two sprays and thus, the total intranasal dose received by the subject at each administration was 10.0 or 13.4 mg of diazepam.

The rectal diazepam formulation (Diastat® AcuDial,Valeant Pharmaceuticals North America, Aliso Viejo CA) was provided by the manufacturer.

3.2.2.3 Drug Assay

Five-mL blood samples were collected for determination of plasma diazepam and desmethyldiazepam at the following times: predose (time 0) and 5 ± 1, 10 ± 1, 15 ± 2, 20 ± 2, 30 ± 3, and 45 ± 5 minutes and 1, 1.5, 2, 3, 4, 6, 9, and 12, 24, 48, 72, 96, 144, 192, 240 hours postdose. The bioanalytical assay was completed by MDS Pharma Services.

3.2.2.4 Pharmacokinetic Analysis

DZP concentration-time data were analyzed using a non-compartmental pharmacokinetic approach with WinNonLin software (version 5.2; 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 $\ln 2/(\lambda z)$. 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 to the last non-zero plasma concentration (C_{last}) that was above the lower limit of quantification was calculated as AUC_{last}. The area under the concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{last} + (C_{last}/\lambda z)$. To assess initial exposure after intranasal administration, area under the

concentration-time curve was determined at various time points in the first few hours ($AUC_{0\text{-}t\ h}$).

3.2.2.5 Safety and Tolerability Assessment

Safety assessments included adverse events, clinical laboratory tests (serum chemistry, hematology, and urinalysis), vital signs, 12-lead electrocardiograms, pulse oximetry and physical examinations.

Tolerability was evaluated after both nasal and rectal administration of each dose of diazepam.. The evaluation consisted of three components:

Sedation

A sedation score was used to assess the degree of drowsiness of the subjects after administration of both the rectal and nasal 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 2,3,4,6 & 8 hours post dose. Subjects were also questioned by the trained observer regarding their degree of drowsiness. Scores were recorded on 1-5 scale as follows: 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

Pain Scale

This score was used to assess the subject's overall feeling of pain after the administration of diazepam by either route using a Visual Analog Scale (VAS) that consists of a 10 cm (100mm) horizontal straight line with markings from 1-10 [167]. The ends of the scale are defined as extremes limits of pain sensation: 0-no pain, 10-extreme pain. The subjects

marked a point on the scale which best described their intensity of pain and discomfort just prior to and at 5, 15, 30, 60 minutes and 2,3,4,6 & 8 hours post dose.

Nasal Irritation

Nasal irritation was evaluated after administration of the intranasal formulations. The scoring was done by a trained observer based on an assessment of the nasal mucosa prior to and at 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 144, 192 and 240 hours post dose. Irritation was assessed by evaluating the degree of mucosal inflammation and bleeding. Subjects were also required to report any incident of bleeding or inflammation in-between the actual evaluation time points. Scores were recorded on 1-5 scale as follows: 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 (such as ER trip)

3.2.2.6 Statistical Analysis

Descriptive statistics were used to summarize the data. The study was not designed to have sufficient power to establish bioequivalence of the intranasal formulations compared to the diazepam rectal formulation. However, relative bioavailability of the nasal formulations compared to the rectal formulation was computed and used to obtain an estimate of bioequivalence. For each variable, the data were analyzed using a mixed effects modeling approach in SAS v.9.2 (Cary, NC). For $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, $AUC_{0\text{-}240hr}$, and C_{max} analyses was based on natural log (ln)-transformed values. The ratios between 2 treatments (each nasal formulation versus the rectal formulation) for these parameters (point estimates) and the corresponding 90% confidence intervals (CIs) for the ratios were obtained by exponentiating the differences and the corresponding 90% CIs in logarithms. These data were then used to assess the bioequivalence of the nasal

formulations relative to rectal diazepam. Bioequivalence was concluded if the 90% CIs for the ratio of the geometric means fall completely within the 80% to 125% bioequivalence interval.

3.2.3 Results

3.2.3.1 Pharmacokinetics

12 subjects, 9 male and 3 females were enrolled and assigned to receive all four treatments. Mean diazepam pharmacokinetic parameters are summarized in Table 3.2-1 and the mean profiles are presented graphically in Figure 3.2-1. In general, the nasal spray formulations had an absorption and elimination profile similar to that of the rectal gel formulation. However, there was considerable variability in the profiles of both the nasal spray formulations and of the rectal gel formulation across subjects. There was no consistent pattern of observations that would suggest a failure of any one specific formulation, and in general, there was no indication of an alteration of the elimination profile of drug in any of the groups. The reason for the variability is anticipated to be related to factors leading to incomplete administration and/or absorption of drug.

Median t_{max} values were 0.75 hours for all treatments suggesting that all formulations had a similar rate of absorption. Mean t_{max} values were similar for all three nasal spray doses, ranging from 0.83-1.05 hours. The mean t_{max} value for the rectal dose group was 1.3 hours, reflecting the effect of two subjects with t_{max} values of 3 and 6 hours in this group; remaining subjects (N=10) all had t_{max} values of $\leq$ 1.5 hours. Thus, there was no indication of a more rapid absorption with the nasal spray formulations than that observed with rectal gel dosing.

Table 3.2-1: Mean±SD of DZP pharmacokinetic parameters following rectal and intranasal administration.

PK Parameter	10 mg Rectal Mean ± SD	10 mg Nas-A Mean ± SD	10 mg Nas-B1 Mean ± SD	13.4 mg Nas-B2 Mean ± SD
C_{max} ng/mL	160.9 ± 109.4	181.8 ± 84.16	151.3 ± 108.1	180.7 ± 82.1
t_{max} hr (median)	0.75 [0.3-6.0]	0.75 [0.25-1.5]	0.75 [0.25-3.0]	0.75 [0.25-4.0]
AUC_{0-1} ng.h/mL	112.66 ± 80.35	122.61 ± 52.63	113.46 ± 60.2	100.38 ± 69.02
AUC_{0-4} ng.h/mL	386.51 ± 253.0	387.58 ± 153.42	400.95 ± 187.54	283.99 ± 167.57
$AUC_{0-\infty}$ ng.h/mL	5051.0 ± 3722.7	4450.0 ± 1992.4	3494.7 ± 2179.9	6079.6 ± 4055.6
F ($AUC_{0-\infty IN}$)/($AUC_{0-\infty R}$)	-	~ 88 %	~ 70 %	~ 89 %

As can be seen from the Table 3.2-1, mean peak diazepam concentrations observed with the 10 mg nasal spray formulations were close to those observed with the 10 mg rectal gel formulation. However, median C_{max} values with the 10 mg nasal spray formulations were approximately 15-25% less than the median C_{max} for the 10 mg rectal gel. In contrast, the mean C_{max} values for the 13.4 mg Nas-B1 group was approximately 12 % greater than that of the 10 mg rectal gel, with the median C_{max} values for these two groups being essentially the same. Using a mixed effects modeling approach, the ratios of C_{max} and $AUC_{0-\infty}$ for the nasal spray groups relative to the rectal gel group are listed in Table 3.2-2. The point estimates and the confidence interval for all formulations suggested lack of bioequivalence to the rectal gel.

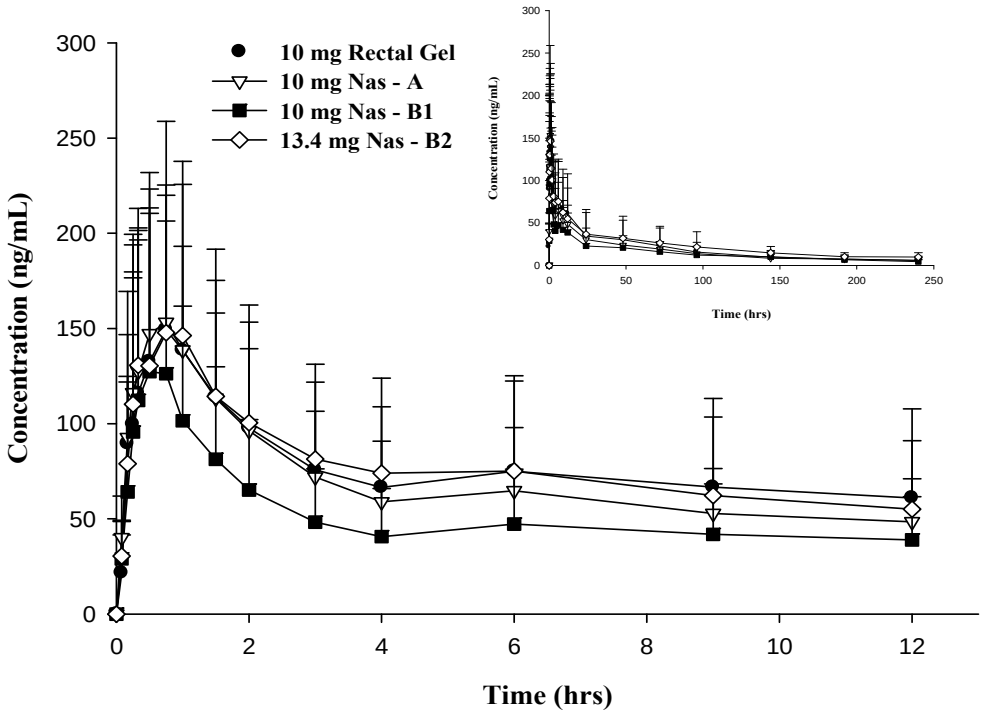


Figure 3.2-1: Mean plasma DZP-concentration time profiles after rectal and intranasal administration in twelve subjects (0—12 h). Inset shows the complete profile (0—240 h).

Table 3.2-2: Mean ratios (%) and 90% confidence intervals of ln-normalized DZP nasal spray pharmacokinetic parameters relative to rectal gel parameters

End Point	10 mg Nas – A	10 mg Nas – B1	13.4 mg Nas –B2	BSV (%CV)	Residual (%CV)
$AUC_{0-\infty}$	0.99 [0.63,1.55]	0.68 [0.44,1.00]	0.87 [0.55,1.36]	53.60	55.33
C_{max}	1.43 [0.77,2.66]	0.86 [0.47,1.60]	0.98 [0.53,1.81]	48.21	80.23

Review of the data and comments by study staff in the case report forms, raised concerns regarding the correct dispensing of the nasal spray formulation during at least the first day of Period 2, and possibly other days. In addition, review of the pharmacokinetic profiles after administration of diazepam rectal gel suggested the potential for missed/inaccurate dosing or incomplete absorption based on non-typical profiles with associated low diazepam concentrations. Furthermore, plasma diazepam concentration profiles in some subjects following nasal spray administration in Periods 3 and 4 were inconsistent with the profiles in other subjects and/or the diazepam profiles of the other nasal spray treatment in the same subjects on other periods, suggestive of administration difficulties, and/or incomplete absorption, and/or nasal leakage. For these reasons, the intent-to-treat data were considered too variable to accurately assess the potential utility of the nasal spray formulations relative to diazepam rectal gel. Considering the primary purpose of the study was to determine the relative potential for the nasal spray formulations to provide an acceptable diazepam pharmacokinetic profile, a pharmacokinetic population was identified representing those pharmacokinetic profiles that were determined to be more representative of the acceptable use of the tested treatments. We identified 10 profiles as potential outliers from the dataset, being contributed by 5 subjects as shown in Figure 3.2-2. We then compared partial AUC values up to 12 hours postdose between the intent-to treat and the pharmacokinetic population, which allowed for the comparison of diazepam exposure over initial time periods following dosing (Figure 3.2-3 a & b). In general, the shorter the time interval postdose, the higher the relative bioavailability of the nasal spray doses relative to the rectal gel dose. For example, the ratio of $AUC_{0\text{-}4}$ hour values for the nasal spray doses relative to the rectal gel dose was between 10-30% greater than respective $AUC_{0\text{-}\infty}$ ratio values. However, on review of the individual subject data, this effect was primarily attributable to 1-2 subjects within each comparison, and not reflective of a consistent formulation effect.

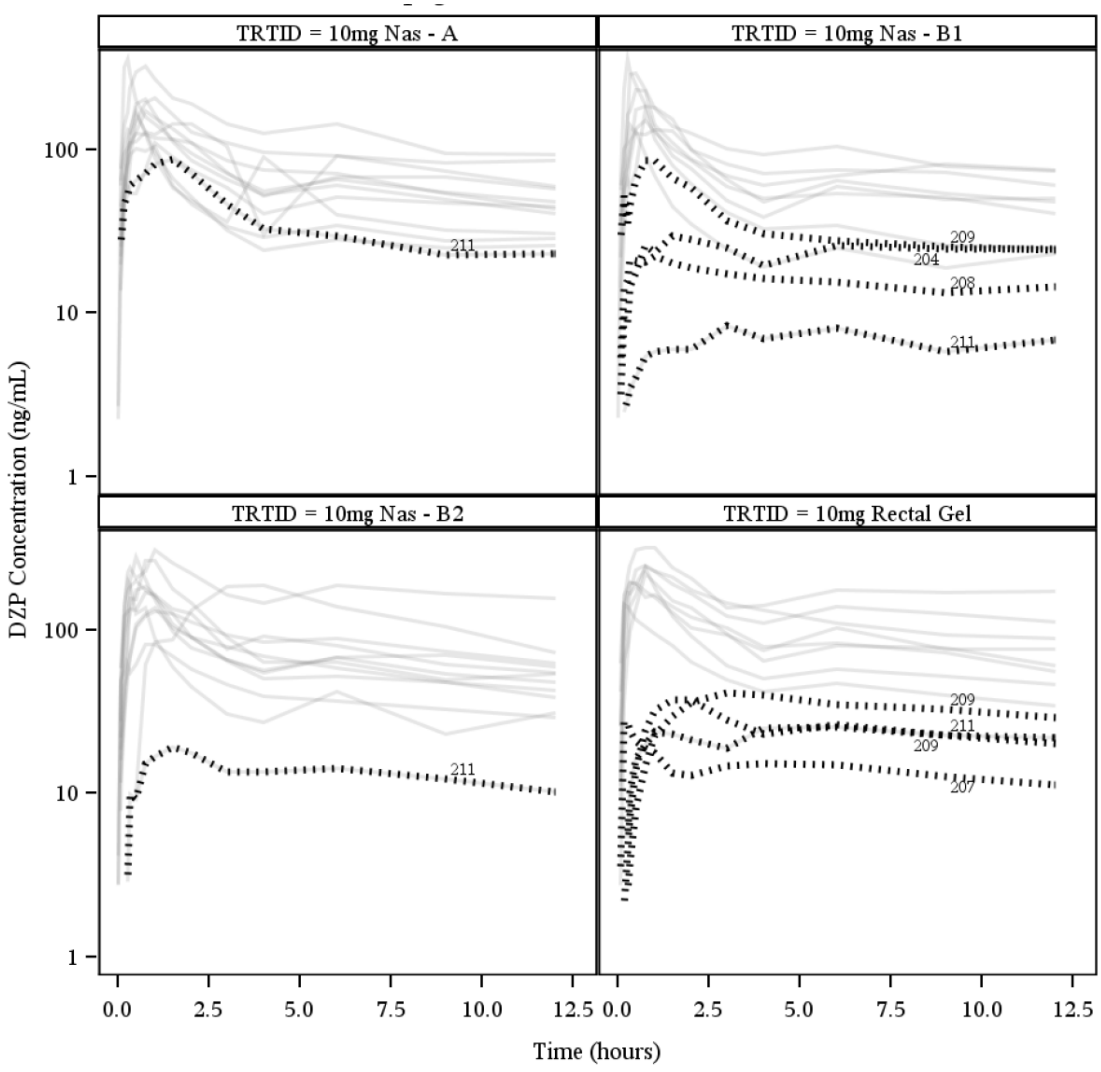


Figure 3.2-2: Plots showing extreme outliers in each treatment group. The concentrations are plotted on a log scale over the first 12 hours of dosing. The dashed grey lines represent individual subjects (with their subject ID's) who were flagged as extreme outliers in each treatment group (TRTID) when compared to the rest of the subjects represented in solid grey.

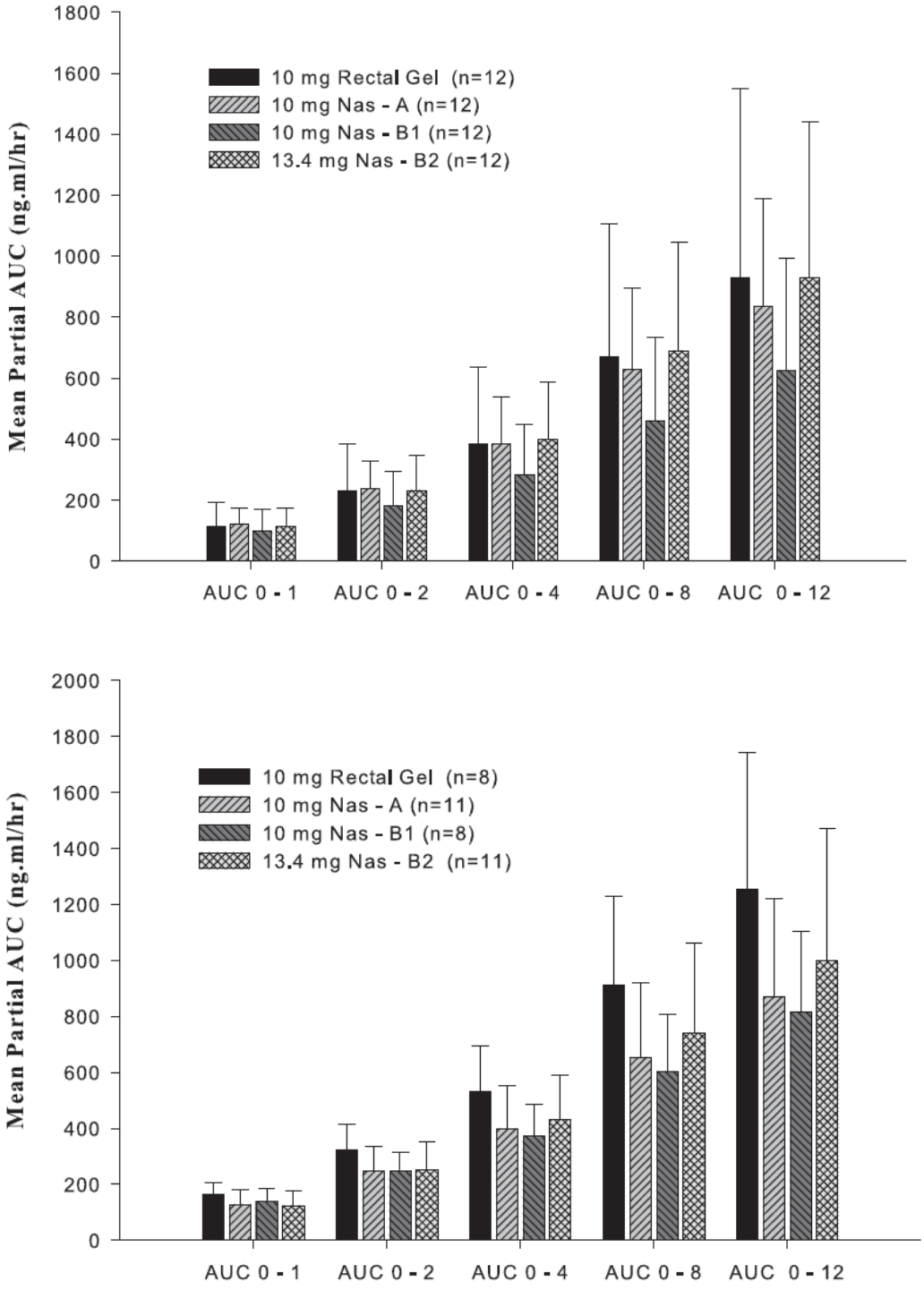


Figure 3.2-3: Comparative partial AUC plots for (a) intent-to-treat population and (b) pharmacokinetic population

3.2.3.2 Safety and Tolerability

No unanticipated adverse events were reported by subjects following nasal administration of diazepam. All subjects, however, reported swallowing a portion of the nasal dose. There were no observed clinically significant abnormalities in vital signs or ECG measurements and no clinically relevant changes in laboratory parameters during the study.

Pain and tolerability scores were reported on a scale of 1-10 with 1 being no pain and 10, extreme. Figure 3.2-4 shows that rectal drug administration was tolerated well by all the subjects. Immediately after nasal drug administration, subjects reported irritation and pain which lasted for a short time before returning to baseline. Only three subjects reported scores above 7 immediately after administration which lasted for not more than 5 minutes. Considering that the ingredients of the formulation are being used in marketed ophthalmic formulations, we did not expect any lasting irritation or side effects. Subjects did however report the feeling of a bad after-taste minutes after administration which was consistent with reports of swallowing of some part of dose after administration. The after-taste was partially relieved by a few sips of water.

Subjects reported increasing sedation which reached a maximum effect about 2 hours after dosing (Figure 3.2-5). Sedation did not correlate well with peak concentrations. Even the highest 13.4 mg dose did not cause significant sedation.

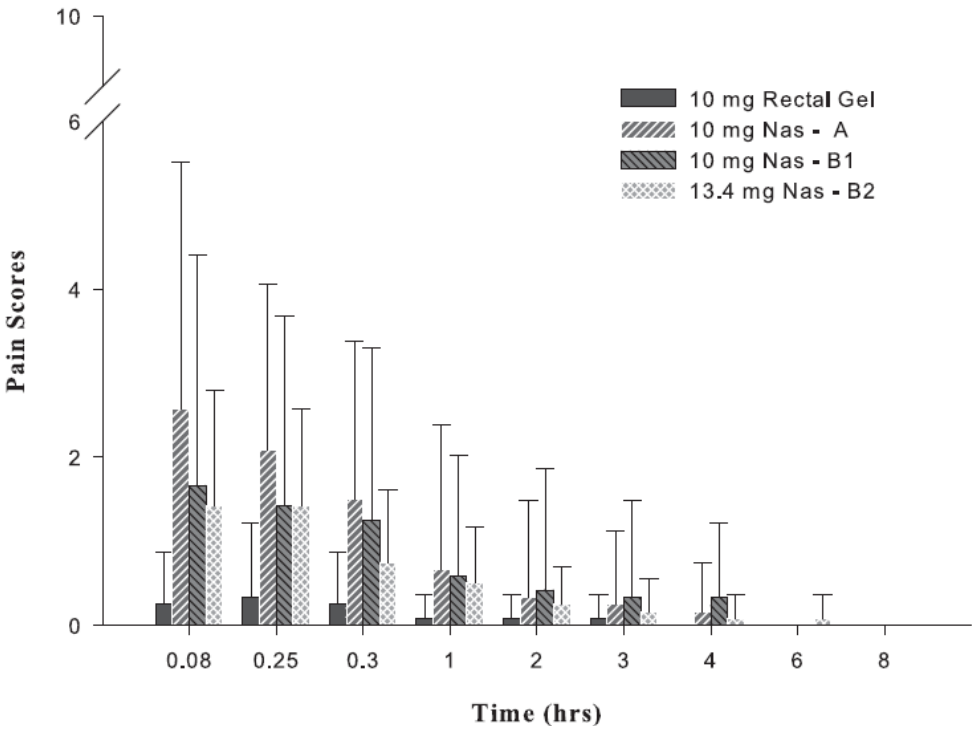


Figure 3.2-4: Comparison of mean global pain and tolerability scores (1-no pain; 10-extreme pain) after rectal and IN administration (n = 12). Baseline scores were zero (0) for all subjects.

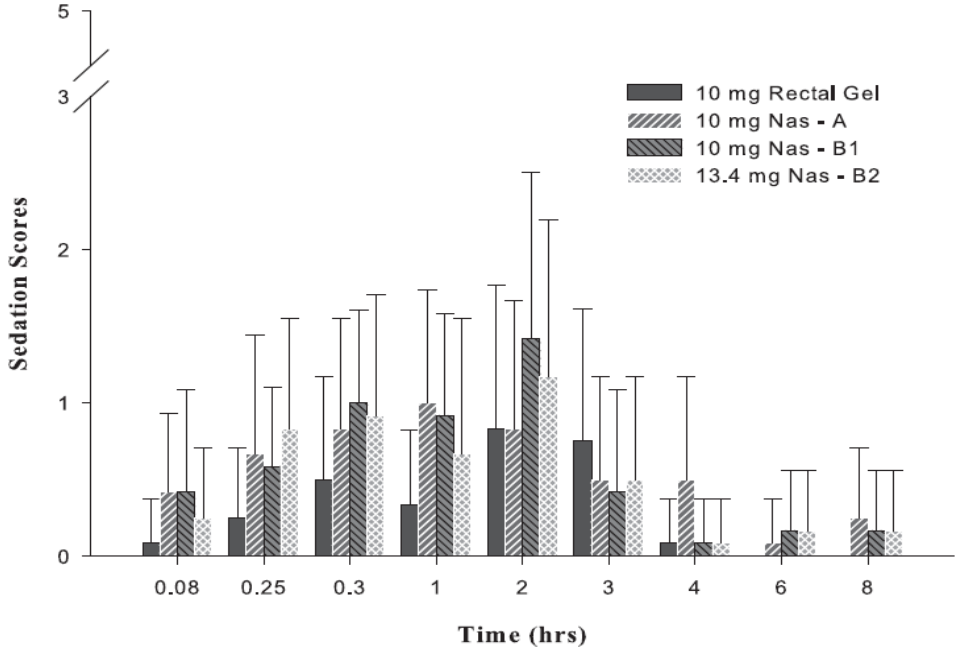


Figure 3.2-5: Comparison of sedation scores (1-minimum; 5-maximum) after rectal and IN administration (n = 12)

3.2.4 Discussion

This is the first study which directly compares the pharmacokinetics of three different diazepam intranasal treatments relative to that of diazepam rectal gel. The data suggest that either Nas-A or Nas-B(1/2) have the theoretic potential to provide a pharmacokinetic profile very similar to that of Diastat rectal gel, including attainment of a t_{max} within approximately 0.5-1 hours postdose, and maintenance of diazepam concentrations above a threshold at various time periods within the first 12 hours of dosing. Unfortunately, extensive variability of the formulations both within and across subjects was observed. No clear pharmacokinetic advantage was observed for either Nas-A or Nas-B. Although the single Nas-A dose appeared to have AUC parameters more similar to the rectal gel than the Nas-B formulation, it should be noted that there was considerable variability among all the nasal treatments and that the confidence intervals for all parameters are quite large. The variability was typically associated with a general reduction in relative exposure to a specific formulation within a subject, suggestive of incomplete absorption of the intended dose, incomplete dosing, or through loss of drug through nasal discharge (nasal products) or rectal discharge (rectal gel).

When compared to our previous investigations using a supersaturated formulation of diazepam [112, 113], the intranasal formulations used in this study were also rapidly absorbed with good, but incomplete bioavailability. However, unlike the supersaturated formulation, which caused considerable discomfort immediately following administration and was poorly tolerated, the formulations used in this study were well tolerated with minimal discomfort and/or irritation. The sedation scores for the subjects after intranasal administration were no different than those after the rectal gel administration. In response to a subjective questionnaire regarding preference of intranasal or rectal route of administration outside a hospital setting for the given indication of seizure emergencies, all subjects preferred the intranasal route.

We've made significant improvements in our efforts to pursue the development of an intranasal formulation of diazepam as an alternate treatment option to rectal diazepam for seizure emergencies outside a hospital setting. Our previous formulation, the supersaturated glycofurol solution exhibited rapid absorption and maintenance of therapeutic levels for a while but the formulation was poorly tolerated probably due to the large amount of organic solvents used to solubilize diazepam. The formulations used in this study have ingredients that are used in marketed ophthalmic preparation with a considerably higher aqueous component. This change was reflected in the pain, tolerability and irritation scores as shown in Figure 3.2-4. The only issue reported by subjects with respect to the formulation was the presence of a bad after taste, possibly due to swallowing a portion of the dose which is common in intranasal dosing and an issue which can be easily addressed. In comparison to our previous study where a 1 mL syringe was used to drip the formulation into the nose, we used a metered single-use, pre-filled bi-dose nasal sprayer device. This allowed more efficient administration of doses. However, we encountered problems in dosing in a few subjects due to improper administration. When we subsequently checked the devices we found residual solution left in the device. Unfortunately, even after exclusion of subject data that were confirmed to be misdosed, the variability was still high across all the nasal formulations and the rectal dosing. Additional controls for dosing and potential loss of administered drug after dosing will be required in subsequent studies.

Although not extensively discussed in the literature, there seems to be considerable variability in rectally administered diazepam pharmacokinetics. This is consistent with the inherent variability in diazepam pharmacokinetics when administered by other routes pharmacokinetics [165, 166]. In the course of development, the rectal diazepam clinical trials focused on efficacy, without a supplementary pharmacokinetic component. However, looking closely at some controlled data in healthy volunteers [77] and an unpublished study from the original sponsors (Excel Pharmaceuticals) which more rigorously characterized rectal diazepam pharmacokinetics, we conclude that rectal diazepam exhibits similar variability. A population analysis of rectal diazepam gel

(Diastat®) in 75 healthy volunteers pooled across 5 different studies (manuscript under preparation) shows considerable between-subject variability ranging from 30 -70 % in different pharmacokinetic parameters. Considering that these estimates reflect variability in controlled pharmacokinetic studies in healthy adult volunteers, variability could be expected to be even greater when being administered to patients (children and adults) in an out-of-hospital setting by a care-giver in a stressful condition at the time of a seizure. This suggests rectal diazepam is effective despite considerable variability in exposure and that, exposure levels in the early time periods (0-4 hours) mainly contributing to the efficacy. Future clinical studies with rectal diazepam should explore the relationship of response to drug concentrations attained within the first 2-4 hours after dosing. One can then hypothesize that the main considerations during the development of nasal product would be the maximum plasma concentrations C_{max} which are required to produce a therapeutic effect and also rapidity of onset of pharmacological action represented by t_{max} [168]. Keeping this in mind, it may be irrelevant to show the absolute bioavailability of a potential nasal product as long as the therapeutic concentrations are achieved rapidly and sustained for a period of time before emergency care is provided. This however may not be acceptable from a regulatory point of view.

The intranasal doses that we evaluated here, 10 and 13.4 mg, provided maximum concentrations in the range of 150-190 ng/mL that can be easily optimized to provide higher concentrations to rapidly achieve the therapeutic benchmark of 200 ng/mL [35]. Furthermore, these concentrations are being attained at the same time or earlier than via the rectal route (0.75 h). Acknowledging that this observation may be sensitive to sampling and design issues, conservatively it is possible to achieve therapeutic levels by giving a second nasal dose 5 or 10 minutes after the first. This would not pose any safety issues as diazepam is known to have a wide therapeutic and safety window as shown with rectal diazepam [25, 95, 169]. In a scenario of misdosing due to improper technique, leakage or other reasons, it is practically easier to repeat a nasal dose than a rectal dose to achieve therapeutic levels. The bioavailability of the two nasal formulations relative to rectal diazepam was in the range of 70-90 %. Considering that initial exposure levels

after treatment are more critical than looking at bioavailability estimated using the complete area under the curve from 0-inf, the partial AUC's shown in Figure 3.2-3 clearly indicate that the rate and extent of absorption is similar during early time periods for both the intranasal and rectal gel formulations. Our intranasal formulations also fulfill most of the requirements of an ideal intranasal benzodiazepine formulation outlined by Wermeling [168].

There was no formulation which was a clear winner due to the high variability in this study, which is primarily reflected in the relative ratios of log transformed dose normalized C_{max} and $AUC_{0-\infty}$ which have very wide confidence intervals. The partial AUC's reflecting exposures during early times after dosing and minimal to no tolerability issues clearly suggest that the nasal spray formulations used in this study have the potential to be developed as an alternative to rectal diazepam gel. However, considerable planning and care should be taken in subsequent studies to ensure decreased variability, especially contributed due to improper administration related issues and also in the choice of the most appropriate parameters for evaluation of the formulation.

3.3 Population Pharmacokinetics of Intranasal Diazepam

3.3.1 Introduction

Seizure emergencies are defined as frequently recurring or prolonged seizures which require immediate medical attention and treatment to minimize mortality and morbidity. The standard treatment for a seizure emergency is intravenous administration of a benzodiazepine (BZD), preferably lorazepam or diazepam. When given within 30 minutes of seizure onset, IV BZDs are effective in more than 80% of patients [8]. Intravenous administration, however, requires skilled personnel and transport to a medical facility that can delay initiation of therapy [156]. Treatment delay is associated with longer seizure duration, greater difficulty in terminating the seizure, prolonged hospitalization, higher mortality, and reduced quality of life [8, 12, 90, 93].

Most seizure emergencies occur at home, work, or school. Studies over the last 15-20 years have demonstrated that out-of-hospital therapy is highly effective and can be safely administered by family members or emergency medical technicians [16, 45]. As a result of this research, a FDA-approved rectal diazepam gel (Diastat®) is now considered the standard out-of-hospital treatment for acute repetitive seizures, prolonged seizures, and status epilepticus. Despite the safety and efficacy of rectal diazepam, it remains underutilized. Many patients, particularly older children and adults, as well as caregivers object to rectal administration [93, 94, 117]. This has created a need for a fast, more convenient, and socially acceptable delivery route for effective management of seizures emergencies.

For many years, the potential for an intranasal benzodiazepine as an alternative to Diastat® in the treatment of out-of-hospital seizure emergencies has been considered. Of the various benzodiazepines, midazolam, diazepam, lorazepam and clonazepam, in this specific decreasing order have been studied most extensively [107, 112, 140, 150]. The availability of a fast acting intranasal treatment that can be easily and safely administered by the patient or a caregiver would improve the management of seizure emergencies.

Diazepam however possesses properties that make it a particularly good candidate for intranasal administration. Its lipid solubility and potency are comparable to midazolam [34]. Diazepam has a substantially longer elimination half-life which may provide a longer duration of action as compared to midazolam. Based on these considerations, our group has undertaken a series of studies investigating the potential of intranasal diazepam as an alternate treatment for seizure emergencies.

The primary aim of the study was to evaluate the population distribution of pharmacokinetic parameters of different formulations of intranasal diazepam in healthy adult volunteers and to assess the influence of different covariates on its absorption and disposition.

3.3.2 Methods

Study Design

Data from 3 Phase 1 clinical trials comprised the final population PK database. Study design information, including subject populations, formulations, doses, and PK sampling, can be found in Table 3.3-1. The phase 1 clinical trials included single-dose intranasal (IN) and intravenous (IV) data from healthy adult, mostly Caucasian and nonsmoking subjects. These studies were approved by local Institutional Review Boards. Forty one subjects, (18 – IV; 23 – IN) from 4 trials for a total of 29 IV and 55 IN diazepam administrations were included in this population pharmacokinetic analysis. All plasma samples with quantifiable concentrations of diazepam were used for the population pharmacokinetic analysis. Measurements below the quantification limit and missing values were excluded from the analysis. Data for the analysis were merged and formatted for analyses, using SAS version 9.2 (SAS®).

Diazepam was supplied as a sterile solution for intranasal administration for the different formulations used. Specific details regarding study aspects, formulations and doses used

can be found in earlier published non-compartmental analyses of these studies. Briefly, two formulation technologies were used; supersaturated glycofurol based (study S01 & S02) and microemulsion based (study S03). The former is a supersaturated solution of diazepam in gycofurol and water at 5 % concentration to deliver 5 mg or 10 mg doses. Two variants of the microemulsion based formulations were used, Nas-A (5%) and Nas-B (5% & 6.7%) where the difference was in the presence of an extra permeation enhancer in the later, compared to Nas-A. The formulations were administered as a nasal drip using a syringe in S01 and S02 for the glycofurol based formulations whereas for the microemulsion formulations, a pre-filled Pfeiffer bi-dose nasal spray device was used to deliver 100 μL solutions into each nostril. Two sprays into each nostril for Nas-A provided a 10 mg dose whereas Nas-B was studied at two doses of 10 mg and 13.4 mg. The population pharmacokinetic analysis was used to evaluate the systemic exposure of these three formulations relative to IV.

Table 3.3-1: Studies included in the IN DZP population pharmacokinetic database

Study	Design	Subjects	PK Sampling	Doses	Formulation
S01	Single blind, single dose, phase 1	Females (n=3)	0-48 h	IV - 5 mg IN - 5 mg	IV-Valium® IN-Glycofurol
S02	Single blind, single dose, phase 1	Males (n=6) Females (n=2)	0-48 h	IV - 5 mg IN - 5 mg & 10mg	
S03	Open label, single dose, phase 1	Males (n= 9) Female (n= 3)	0-240 h	10 mg (Nas-A & Nas-B1) 13.4 mg (Nas-B2)	IN only Microemulsion Nas-A Nas-B
L01	Single blind, 2 way crossover, phase 1	Males (n=18)	0-240 h	IV - 7.5 mg	IV-Valium®

Data Analysis

The population PK analysis for repeated measures was conducted via nonlinear mixed-effects modeling in NONMEM version 7 using PdxPOP version 4 [170, 171] as an interface and for post-processing. The first-order conditional estimation method with η-ε interaction (FOCE-INT) was used for all model runs.

Model selection was guided by various goodness-of-fit criteria, including diagnostic scatter plots, plausibility and precision of parameter estimates, and the correlation between model parameter less than |0.95|. Final model parameter estimates were reported with a measure of estimation uncertainty based on the nonparametric bootstrap 95% confidence intervals [172].

Structural Model

An integrated pharmacokinetic model which simultaneously fit data from both IV and IN administrations was used. A sequence of compartmental models was tested and the results compared. An open two-compartment pharmacokinetic model with first-order absorption and elimination was finally chosen for model fitting. Because the input function of diazepam after intranasal administration from the current formulations into the systemic circulation is complex with double peaks in most of the individuals, a series of dual-input models was also tested to describe the intranasal absorption of diazepam. The double peaks were mostly due to a 2^{nd} phase of absorption probably resulting from swallowing a portion of the nasally administered dose, which is common for this route of administration. As subjects in study S03 did not have an IV arm, the bioavailability of formulations in this study was estimated by using the typical values of the disposition parameters from the pooled IV data. A logit transformation as shown in equation 1 was used to constrain the bioavailability parameter, *Fn* between 0 and 1 to improve model stability.

$$Fn = \frac{\exp(LOG(\frac{\theta}{1-\theta}) + \eta_j)}{1 + \exp(LOG(\frac{\theta}{1-\theta}) + \eta_j)} \qquad \textbf{Eq 1}$$

where, θ represents the population estimate of Fn and η_j represents the interindividual variability in F, the representation of which is explained below.

Statistical model

All interindividual error terms were described by an exponential error model, or log-normal parameter distribution (Equation 2). An attempt was made to define a full block covariance matrix for the interindividual random effects (Ω) when possible.

$$Pi = \hat{P}.\exp(\eta_{Pi}) \qquad \textbf{Eq 2}$$

where, Pi is the estimated parameter value for individual *i*, $\hat{P}$ is the typical population value (geometric mean) of the parameter, and η_{Pi} are individual-specific interindividual random effects for subject i and parameter P, and are assumed to be distributed: η~N(0, ω^2) with covariance matrix for the interindividual random effects (Ω). Interindividual variability was included on clearance (CL), central volume of distribution (V1), peripheral volumes of distribution (V2), intercompartmental clearance (Q), absorption rate constant (ka) and bioavailability of drug (F). The approximate standard deviation (SD) from the logit transformations reflecting the variability in *Fn* was computed as equation 3 [173, 174]:

$$SD\,Fn = sqrt(\omega)^2 \bullet \theta \bullet (1-\theta) \qquad \textbf{Eq 3}$$

Residual variability was explained by a proportional error model:

$$C_{ij(obs)} = C_{ij(pred)} + C_{ij(pred)} * \varepsilon_{ij} \qquad \textbf{Eq 4}$$

where, $C_{ij\ (obs)}$ and $C_{ij(pred)}$ are the observed and predicted diazepam values in the i^{th} individual at the j^{th} time point respectively, and $\varepsilon_{ij} \sim N(0, \sigma^2)$. The residual variability on concentrations was accounted for any variability due to different assay methodologies used across studies.

Covariate model

The only covariates that were available and common across these healthy volunteer studies included age, weight, height, race, gender, formulation and dose. The relationships between individual pharmacokinetic parameters and covariates were explored using the software Xpose [175]. A generalized additive model (GAM) was used to analyze the relationship between covariates and individual parameter estimates from the base model in a stepwise fashion [176]. This procedure, based on the Akaike information criteria, will search for significant relationships between each of the parameters and the candidate covariates. These models guided the inclusion of covariates into the population model. The identified candidate covariates were evaluated in the base model by testing each covariate individually on each parameter and then by testing combinations of covariates on the parameters. The P level for inclusion of a covariate into the final population model was 0.01. This test was based on the objective function value produced by NONMEM, which is minus twice the Log Likelihood value. The difference in the objective function value between hierarchical models is approximately chi-squared distributed.

Model Evaluation

The final population PK model was evaluated using a nonparametric bootstrap and a predictive check. For the nonparametric bootstrap procedure, 100 replicate data sets were generated by random resampling from the original data set with replacement, using the individual as the sampling unit [172, 177]. Population parameters for each data set were

subsequently estimated using NONMEM, and empirical 95% CIs were constructed by observing the 2.5th and 97.5th quantiles of the resulting parameter distributions for all bootstrap runs.

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 [178, 179].

3.3.3 Results

The IN & IV diazepam PK database consisted of 41 subjects contributing a total of 1568 plasma diazepam concentrations. There were 11 subjects receiving both IV and IN diazepam, 12 receiving only IN and 18 receiving only IV diazepam contributing to a total of 55 IN profiles and 29 IV profiles. Subject demographics are summarized in Table 3.3-2.

The data were best described by an integrated 2-compartment model that served as the base model with first-order absorption implemented using the subroutine ADVAN6. The base 2-compartment model was revised to account for the effect of different formulations on absorption rate constant and bioavailability. One of the most common issues with intranasal administration is the swallowing of a portion of dose soon after administration, or via mucociliary clearance through the back of the throat. Swallowed drug generally results in absorption of this portion of dose at a later time. A series of models were thus implemented to capture the evident double peaks in most of the individual's concentration time profile (Figure 3.3-1). Brief descriptions of a few are given here.

Table 3.3-2: Summary of subject demographics in the population pharmacokinetic database for IN DZP

Categorical Covariates		
	No of Subjects	%
Sex		
Male	33	80.5
Female	8	19.5
Race		
Caucasian	38	92.7
African American	3	3
Continuous Covariates		
	Mean (Median)	Range
Weight, kg	75.34 (74.6)	48-115
Age, years	31.15 (26)	19-64

First, a change point model where the absorption rate would be altered at a specific time point, which was estimated using the MTIME function in NM7 was tested. Second, we tested a catenary chain transit compartment model which was used earlier for intranasal diazepam [180]. Here some portion of the drug was assumed to enter a second depot (transit) compartment from the main absorption depot while some drug is lost via nasal leakage. Drug from this transit compartment was then allowed to enter the systemic circulation with a different Ka after a time lag, along with drug entering from the main compartment. Finally, we also tested a variety of dual input models, where a fraction of the dose was expected to enter the systemic circulation simultaneously from two different

depot compartments with different absorption rate constants. The effect of lag time from one of the depot's was tested to facilitate drug entry at a delayed time to capture the double peak. Further, we also tested a transit compartment model [181] from one of the depot's of the dual input model. Of all the models tested above, the dual input model with an absorption lag from the second depot fared best with a 230 point drop in objective function value for the addition of 4 parameters (fixed and random effects on F and Ka). However, the model run time was significantly increased (from 2 hours to nearly 7 hours even without the implementation of the covariance step) and there were sufficient hints of overparametrization based on run times and lack of ability to implement the covariance step even after fixing or not estimating any of the interindividual variability parameters. Hence, we settled with the base model (one absorption depot), considering the objectives of this analysis.

The above model was revised to account for the use of different assay techniques in each study for the plasma diazepam concentration data on the residual error. The correlation and scatterplots between random effects did not indicate the necessity for inclusion of a covariance between the random effects. Also, after GAM analysis and diagnostically checking for relations with parameters, we did not identify any significant covariates affecting the absorption and disposition. As mentioned earlier, each formulation was assigned its own bioavailability and absorption rate parameter. Table 3.3-3 provides the parameter estimates of the structural and random variance parameters from the final model. Estimates for the structural and random variance parameters demonstrated good precision with the exception of the interindividual variability estimates of the absorption rate parameters.

Thus, the final model included a two compartment model with interindividual variability on clearance (CL), central and peripheral volume of distribution (V1, V2), intercompartmental clearance (Q), and with a proportional residual error model for residual variability. The bioavailability parameter (F) and first-order absorption rate constant (Ka) were estimated separately for each formulation. Diagnostic plots of the observed and population model predicted concentrations after nasal administrations,

stratified by formulation are shown in Figure 3.3-2. The diagnostics for structural and error model are shown in Figure 3.3-3.

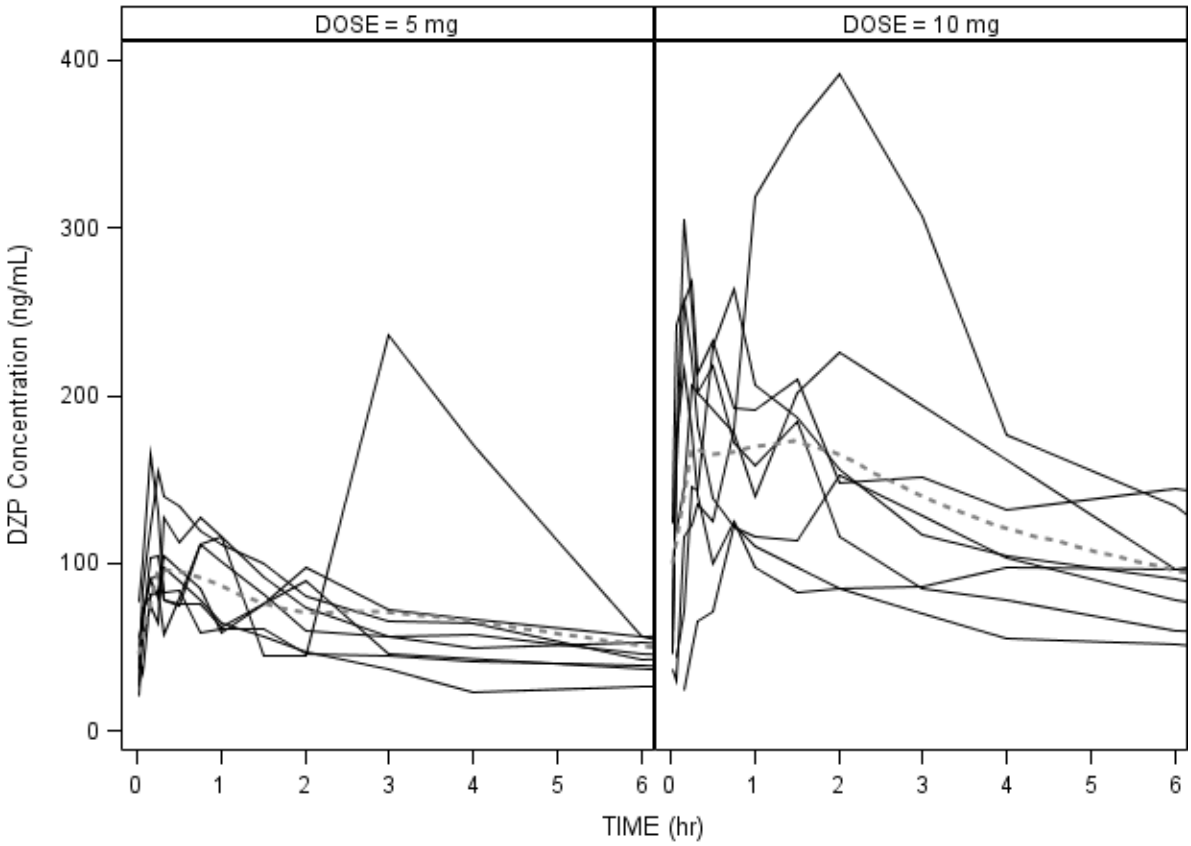


Figure 3.3-1: Representative concentration-time plots (0-6 hours) showing double peaks after IN administration of 5 and 10 mg doses.

Diagnostic plots revealed that the model was consistent with the observed data with some under-prediction for concentrations greater than 250 ng/mL (usually C_{max}) especially for the glycofurol based formulation. The bioavailability after intranasal administration of glycofurol was found to be 0.77 with RSE 13.4% and the interindividual variability was estimated to be 0.23 (expressed as SD based on equation 2). In contrast, the bioavailability of the microemulsion based formulations was a low 0.38 with an RSE of 9.16 % and an interindividual variability of 0.07. The precision of the absorption rate constant was low for both formulations.

Table 3.3-3: Population pharmacokinetic parameters for the typical individual after intravenous or nasal administration of DZP.

Parameter	NONMEM Estimate (95% CI)	Bootstrap Median (95^{th} percentile)
Clearance (L/h)	1.06 (0.94, 1.18)	1.04 (0.91, 1.15)
V1 (L)	17.40 (15.7, 19.1)	17.47 (15.8, 19.0)
V2 (L)	50.60 (43.3, 57.9)	50.93 (45.1, 56.8)
Q (L/h)	11.50 (9.74, 13.3)	10.48 (8.16 , 12.4)
IN Ka (h^{-1}) *Glycofurol formulation*	8.62 (3.23, 14.0)	8.83 (4.28, 14.0)
IN Ka (h^{-1}) *Microemulsion formulation*	2.82 (2.0, 3.64)	2.92 (2.11, 3.70)
IN F *Glycofurol formulation*	0.77 (0.57, 0.97)	0.78 (0.56, 0.95)
IN F *Microemulsion formulation*	0.381 (0.31, 0.45)	0.37 (0.006, 0.43)
IN Ka - LOBA	0.67 (0.29, 1.11)	0.82 (0.37, 1.44)
IN F - LOBA	0.10 (0.07, 0.12)	0.107 (0.063, 0.20)
IIV_{CL}	34.5 (27.1, 40.4)	34.2 (25.05, 41.47)
IIV_{V1}	29.9 (18.4, 38.07)	27.98 (15.1, 39.8)
IIV_{V2}	42.4 (32.8, 50.1)	41.5 (31.4, 53.1)
IIV_{Q}	39.4 (27.5, 48.37)	38.4 (27.0,49.4)
IIV_{Ka} *Glycofurol formulation*	94.4 (47.6, 124.9)	90.6 (0.2, 126.4)
IIV_{F} (SD) *Glycofurol formulation*	0.23	0.26
IIV_{Ka} *Microemulsion formulation*	42.9 (8.25, 60.3)	37.53 (0.2, 58.9)
IIV_{F} (SD) *Microemulsion formulation*	0.07	0.15
RUV (% CV) *Study S01 and S02*	28.8 (21.4, 34.64)	29.2 (21.8, 35.5)
RUV (% CV) *Study S03*	36.5 (31.25, 41.0)	36.7 (32.4, 42.1)
RUV (% CV) *Study L01*	15.6 (13.41, 17.49)	15.4 (13.3, 17.1)

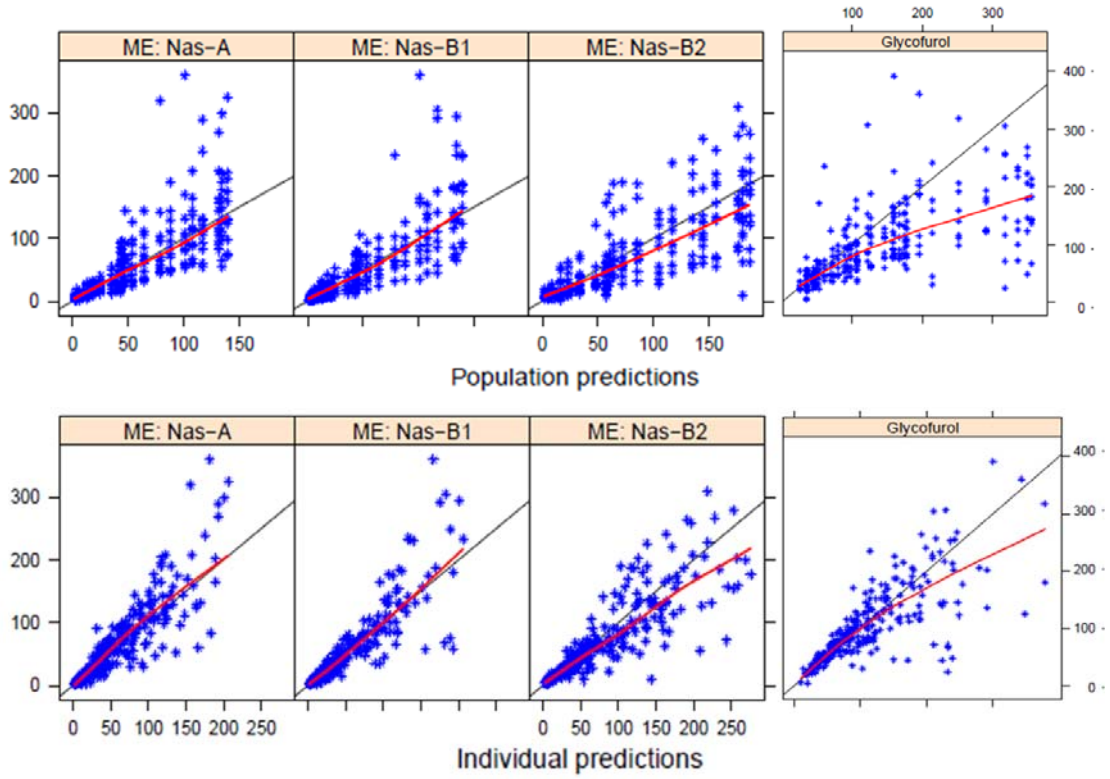


Figure 3.3-2: Model based population predicted and individual predicted versus observed DZP concentrations after intranasal administration of the two different formulation technologies (ME – Microemulsion; Glycofurol). In the top panel predictions are based on the parameters of the typical individual whereas in the bottom panel predictions are based on individual parameter estimates.

3 subjects, representing four profiles (3 from Nas-A and 1 from Nas-B) were flagged for low bioavailabilty (LOBA) as they were identified to differ significantly from the rest in terms of the exposure levels after drug administration. All these subjects were part of study 3 and had unusually low drug levels which may have been due to improper drug administration or formulation leakage from site of administration. The bioavailability and absorption rate constant for these subjects was identified to be 0.10 and 0.67 h^{-1} respectively. Finally, the residual unexplained variability was partitioned for different studies to account for assay differences.

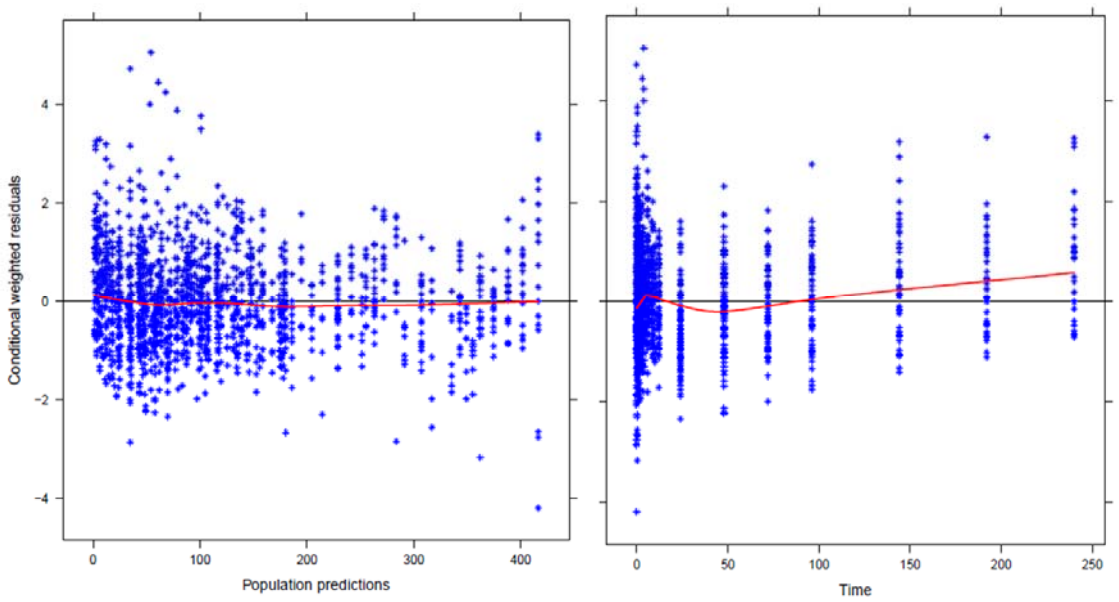


Figure 3.3-3: Conditional weighted residuals vs population predicted DZP concentration (ng/mL) and vs time (h). Values are indicated by solid stars with a smoothing spline trend line through the data. A solid black line at y = 0 is included as a reference.

Model Evaluation

The intranasal diazepam population PK model evaluation results, which included the results of a predictive check and a nonparametric bootstrap which was truncated to just 100 replications due to computational limitations, revealed 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 3.3-4), with the exception of the glycofurol based formulation, especially at C_{max}. The nonparametric bootstrap procedure resulted in 95% CIs for population PK parameter estimates, which are presented in the final model parameter table (Table 3.3-3). Overall, typical structural model parameters and random variance terms were estimated with good precision, while the absorption rate parameters were poorly estimated.

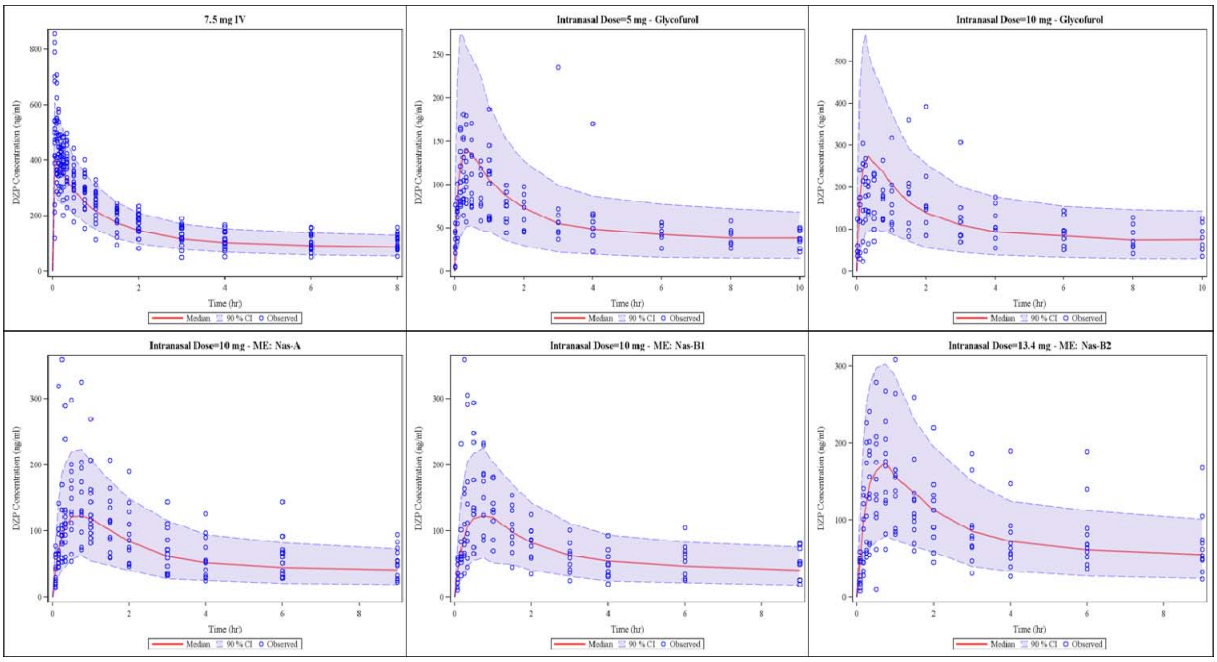


Figure 3.3-4: Formulation and dose stratified visual predictive check plots for DZP concentrations over time (0-8h) for both routes of administration. The solid line represents the median and the dotted line represents the 5th and 95th percentile for model simulated concentrations. The open circles denote the observed concentrations.

3.3.4 Discussion

This is the first reported population pharmacokinetic analysis of intranasal diazepam. Currently, most pharmacokinetic studies for intranasal diazepam as well as midazolam, lorazepam and clonazepam have used noncompartmental analysis with subjects receiving single doses and the analysis completed independently on each occasion.

Population values of CL, V1, V2, Q, Ka, and bioavailability have been quantified from this pooled data for 2 different intranasal formulations and intravenous diazepam (Table 3.3-3). Additionally, the between-subject variability of these pharmacokinetics parameters have been characterized. A 2-compartment model best described the data which is in agreement with previously published individual pharmacokinetic modeling reports [182, 183]. It is acknowledged that the Ka for both formulations have particularly

large interindividual variability and also that the residual unexplained variability is particularly large for studies which included intranasal administration. There were no predictors affecting absorption or disposition in this analysis. Model structural parameters and random variance parameters were estimated with good precision. Goodness-of-fit criteria revealed that the final model was consistent with the observed data. The model evaluation results provided evidence that both the fixed and random effects components of the final model were reflective of the observed data and that the individual predicted concentrations would be suitable for further simulations.

Intranasal diazepam was rapidly absorbed from both formulations indicated by the high absorption rate constants compared to rectal diazepam (Chapter 4) for which this alternative nasal product is being developed. The median time to maximum concentrations were 20 and 45 minutes for the glycofurol and microemulsion based formulations respectively. There is sufficient evidence of a second peak of absorption which is common after intranasal administration. We tried to implement several models to capture this consistent phenomenon, but were not able to establish an efficient model with a good balance between performance and predictability. The clinical implication of the presence of double peak for preventing seizures in an emergency situation is unknown at this time. While the second peak may be important to sustain concentrations above a therapeutic level for a period of time before emergency services arrive, on the other hand it may be detrimental if a large fraction of the drug enters the systemic circulation through the second peak at which point this rescue therapy may be futile. Ideally, it would be preferable to reduce this variability and complexity in absorption by optimizing administration procedures and use of an efficient nasal spray device. For the glycofurol based formulation, a syringe was used to drip the solution into the nose where as the microemulsions were administered using a Pfiffer bi-dose nasal spray device. This may have contributed to the wide variability and poor precision of the absorption parameters.

The bioavailability of the glycofurol formulation was estimated to be 77 % which is close to the noncompartmental value of 75 % [112]. In contrast, the microemulsion

formulations performed poorly with a low bioavailability of ~ 40 %. Such low values were not expected for these formulations as their bioavailability relative to the rectal gel was about 80 % [184] and the absolute bioavailability of rectal diazepam is known to be about 90 % [77]. Taking this into consideration, we expected a bioavailability of at least greater than 60 %. Only three other studies evaluated the bioavailability of intranasal diazepam using different formulation than used here. Gizurarson *et al.* used a co-solvent mixture of glycofurol and polyethylene glycol 200 and studied a 2 mg IN dose compared to a 2 mg IV dose. Sampling till just 5 hours post dose, they reported a bioavailability of 50 ± 23.3 % [109]. Lindhardt *et al.* using a polyethylene glycol 300 based formulation reported a bioavailability of 45 and 42 % for 4 and 7 mg dose [110]. Here also, the sampling was limited to just one hour post dose. Finally, Lau *et al.* reported a bioavailability of 78 % using a Cremophor based formulation after sampling for just one hour post-dose [111]. Given that the half-life of diazepam ranges from 20-60 hours, the bioavailabilities reported were likely an underestimate of the actual extent of absorption. It should be noted that subjects receiving the microemulsions in study S03 did not have a cross-over IV arm, and so the bioavailability was estimated using the population estimates for the IV disposition parameters. This lack of cross-over data may have contributed to the low estimates here. Considering that initial/partial exposure levels after treatment are more critical than overall bioavailability, the low values of bioavailability are not of much concern. The rate and extent of absorption is similar during early time periods, from 0 to 4 hours, for the intranasal formulations as compared to the rectal gel formulations (data not shown). Our intranasal formulations also fulfill most of the requirements of an ideal intranasal benzodiazepine formulation outlined by Wermeling [168].

The intranasal diazepam population PK modeling provided a simultaneous, comprehensive analysis across a range of doses and formulations in healthy volunteers. The rapid absorption of these formulations clearly indicated that a nasal spray of diazepam is a feasible alternative to rectal administration. This utility of this model to

establish, via simulations, a relationship between exposure metrics and response is currently being evaluated.

CHAPTER 4

POPULATION PHARMACOKINETICS OF RECTAL DIAZEPAM IN HEALTHY ADULT VOLUNTEERS

4.1 Introduction

Diazepam is a benzodiazepine derivative commonly used in the treatment of anxiety, insomnia, seizures, muscle spasms, restless legs syndrome and many other neurological conditions. It possesses anxiolytic, anticonvulsant, hypnotic, sedative, skeletal muscle relaxant, and amnestic properties [166]. The disposition of diazepam after IV administration is best explained by a 2-compartment open model where the drug distributes rapidly into lipoid tissues and quickly crosses the blood-brain barrier. Females and elderly persons have a larger volume of distribution as compared to males and younger persons respectively [161]. Diazepam exhibits high protein binding in the range of 97-99 % and is metabolized in the liver via oxidation catalyzed by CYP3A4 and CYP2C19. The primary metabolite is desmethlydiazepam which is equipotent with diazepam and the two minor, active metabolites are temazepam and oxazepam. Desmethyldiazepam is oxidized to oxazepam which is glucuronidated and renally excreted.

A rectal diazepam gel is marketed in the United States as Diastat® by Valeant Pharmaceuticals. Diastat is the first and only FDA-approved, at-home medication for patients two years and older on stable antiepileptic medications (AEDs) who experience bouts of increased seizure activity, sometimes called serial or cluster seizures or breakthrough seizures [10]. Diastat was introduced as a preferred alternative to intravenous diazepam as it is easier to use, is available in pre-measured doses (unlike rectal diazepam solution) and can be administered outside the hospital by non-medical personnel such as family or caregivers who are properly trained by healthcare providers [9]. Administration of diazepam (as Diastat) in the rectal cavity facilitates rapid absorption due to the small surface area and rich vascularisation of the rectum, with plasma concentrations exceeding 200 ng/mL, 15 minutes after dose administration and a bioavailability of 90% (range 70–110%) [77]. The safety and efficacy of this product was evaluated in two placebo-controlled trials [16, 92] which were later followed up by an

open-labeled study in the same patient population [95]. Although many studies have evaluated the diazepam pharmacokinetics via various formulations and routes including rectal administration, there are no published reports on a population pharmacokinetic analysis of diazepam rectal gel (based on pubmed search with various combination of the following keywords; “rectal diazepam population pharmacokinetics”)

The primary aim of the study was to evaluate and report, the population distribution of pharmacokinetic parameters of rectal diazepam gel product (Diastat) in healthy adult volunteers and to assess the influence of different covariates on the pharmacokinetic parameters.

4.2 Methods

Study Design

All studies were conducted with the approval of the local Institutional Review Boards. For the population pharmacokinetic analysis, drug concentration data after IV and rectal administration of diazepam in a total of 75 healthy subjects were pooled from 5 different Phase-I studies (Table 4-1). Concentration time data after IV administration was obtained from 3 studies (S01, S02, L01), the details of which have been published earlier [77, 112, 113]. Rectal diazepam concentration time data were pooled from 3 studies (S03, L01, L02), of which only L01 has been published [77]. All studies involved single doses of either IV or rectal diazepam and in the case of L01, both. Only the details of the unpublished studies will be outlined briefly here.

Study -S03. This study compared the pharmacokinetics, safety, and tolerability of 2 investigational intranasal formulations of diazepam with Diastat in 12 healthy adult volunteers. The two intranasal formulations were administered as 3 treatments with one of the formulation being studied at two different doses. The study was an active-control (diazepam rectal gel), double-blinded, four period crossover study of single doses of

diazepam formulations administered in succession with a washout period of at least 14 days between each dose. Only the rectal diazepam data from this study was used for this analysis. [184].

Study-L02. This study evaluated the bioequivalence of rectal diazepam gel from three different batches manufactured at different time periods. The study was a single-dose, open-label, randomized, comparative three-way crossover bioavailability study. 34 healthy adult volunteers (17 males and 17 females) completed the crossover study of a 15 mg single dose of diazepam rectal gel.

Population pharmacokinetic analysis

Nonlinear mixed effect modeling was applied for the pharmacokinetic analysis of the data using the software NONMEM7 [170] with PdxPOP4 interface [171] using an Intel Fotran Compiler v 11.1 on a 32 bit Windows 7 environment. SAS® v 9.2 (Cary, NC), Xpose4 and PdxPOP4 were used for data exploration and visualization, model diagnostics, candidate covariate identification and model comparison.

Structural pharmacokinetic model

The model building strategy involved the development of an integrated model that simultaneously fit the data from both IV and rectal routes of administration. Thus, the estimates from this model of the population disposition parameters and interpatient variabilities are based on data from all subjects receiving diazepam by either administration route. Two- and three compartment disposition models were evaluated for diazepam. A first-order absorption model, characterized by the absorption rate constant (ka) with estimated bioavailability (F) was used for extravascular administration. The bioavailability parameter, *Fn* was constrained between 0 and 1 by using a logit transform as shown in equation 1:

$$Fn = \frac{\exp(LOG(\frac{\theta}{1-\theta}) + \eta_j)}{1 + \exp(LOG(\frac{\theta}{1-\theta}) + \eta_j)} \qquad \textbf{Eq 1.}$$

where, θ represents the population estimate of *Fn*. The approximate standard deviation (SD) from the logit transformations reflecting the variability in *Fn* was computed as equation 2 [173, 174]:

$$SD\,Fn = sqrt(\omega)^2 \bullet \theta \bullet (1-\theta) \qquad \textbf{Eq 2.}$$

A parallel absorption model to capture double peaks in some cases was also investigated for rectal administration. The analyses were made using the first order conditional estimation method with interaction in NONMEM7.

Table 4-1: Studies included in the rectal DZP population pharmacokinetic database

Study	**Design**	**Inclusion Population**	**PK Sampling**	**Doses**	**Reference**
S01	Single blind, single dose, phase 1	Healthy adult females	0-48 h	IV - 5 mg	[113]
S02	Single blind, single dose, phase 1	Healthy adult male and female subjects	0-48 h	IV - 5 mg	[112]
S03	Open label, single dose, phase 1	Healthy adult male and female subjects	0-240 h	Rectal - 10 mg	NA
L01	Single blind, 2 way crossover, phase 1	Healthy adult male subjects	0-240 h	IV - 7.5 mg Rectal - 15 mg	[77]
L02	Single blind, 3 way crossover, phase 4	Healthy adult male and female subjects	0-240 h	Rectal - 15 mg, in 3 periods	NA

Statistical model

The interpatient variability for all parameters except *Fn* was described using exponential models. Interpatient variability was included on clearance (CL), central volume of

distribution (V1), peripheral volumes of distribution (V2), intercompartmental clearance (Q2), absorption rate constant (ka) and bioavailability of drug (F). Covariances between interpatient variabilities in different pharmacokinetic parameters were investigated.

Residual variability, a combination of assay variability and model misspecification, were described as a proportional. The residual errors were assumed to be symmetrically distributed. Further, it was assumed that the residual error may not be constant across the individuals since data were pooled from different studies conducted over a period of time using different assay's. To account for such a variation in the residual variability, an individual's contribution to the residual error was tested for by including an interpatient variability in the residual error model [185].

Covariate model

Potential covariates that could influence the individual pharmacokinetic parameters were explored using the software Xpose4 [175]. The only covariates that were common across these healthy volunteer studies included age, weight, height, race, gender and dose. The covariate model was built in a stepwise fashion within the population model. A generalized additive model (GAM) was used to analyze the relationship between covariates and individual parameter estimates from the base model [186]. Significant relationships between each of the parameters and the candidate covariates are based on the Akaike information criteria (AIC), where the model with the lower AIC is chosen. These models guided the inclusion of covariates into the population model. The potential covariates based on the above assessment were then evaluated in the base model by testing each covariate individually on each parameter and then by testing combinations of covariates on the parameters. The P level for inclusion of a covariate into the final population model was 0.01 that is based on the objective function value produced by NONMEM, which is minus twice the Log Likelihood value. The difference in the objective function value between hierarchical models is approximately chi-squared distributed.

Model diagnostics and validation

The basic goodness of fit plots for diagnostic purposes included population and individual predictions versus observed concentrations, as well as diagnostics for structural and error model misspecifications. The population predictions are based on the typical population parameters in the final population model, and the individual predictions on the empirical Bayes estimates of individual parameters. The final model was evaluated by two methods. A nonparametric bootstrap by sampling each subject with replacement was done to evaluate the model stability and performance [172, 177]. Only 100 such iterations were done due to computational limitations, and the final population PK model was fitted to each bootstrap dataset. Estimates of all model parameters from 100 runs were then rank ordered to get the 2.5 and 95th percentile. A visual predictive check of the final model was also done to evaluate the adequacy of both fixed and random effects [179]. Here the final model was used to simulate concentrations from the original study design. Concentrations at each unique time point obtained from 100 predictive check runs were rank ordered and summarized to get the 2.5, 50 and 95th percentile.

4.3 Results

The population pharmacokinetic analysis of diazepam was based on 3100 plasma concentrations obtained from 75 individuals. Subject demographics, both categorical and continuous covariates are summarized in Table 4-2. The pooled dataset had a predominantly high Caucasian population and the ratio of males to females was approximately two. Of the 29 subjects receiving IV diazepam 18 received rectal diazepam also (Study L01).

An integrated two compartment structural model best described the time course of plasma concentrations of diazepam for all subjects and was therefore chosen as the base model

for the present analysis. Then, a parallel absorption model (dual input) was explored in order to capture the presence of double peak phenomena in the concentration time profile after rectal administration which was seen in a few individuals. However, the final model included a single input compartment as the presence of the second input function did not drastically change the model performance with a modest 50 points drop in OFV for the addition of 4 parameters from the parallel component (typical values and interindividual variability parameters on F and KA). Of all the covariates tested using the GAM procedure, only age turned out to be a significant predictor of the peripheral volume of distribution. The final disposition model is described as follows: a two compartment model with interindividual variability on clearance (CL), central and peripheral volume of distribution (V1, V2), intercompartmental clearance (Q), age as an influential covariate on peripheral volume (V2), inclusion of covariance between random effects of both volume's and intercompartmental clearance, bioavailability parameter (F), first-order absorption rate constant (Ka), and a proportional error model for residual variability. Although the inclusion of an interindividual variability on residual variability parameter (epsilon) reduced the objective function value by more than 100 points, there was a significant increase in run time of model. Moreover, there was no substantial change in the precision or variability of the parameter estimates and therefore we chose the model without this additional variability on epsilon. The final population parameter estimates based on the model are given in Table 4-3. The clearance in the typical subject was calculated to be 1.2 L/h, with relative standard error (RSE) of 4.07 % while the interindividual variability, as expressed by the coefficient of variation, was 29.7%. Diagnostic plots of the observed and population model predicted concentrations after IV and rectal administrations are shown in Figure 4.3-1. The diagnostics for structural and error model are shown in Figure 4.3-2.

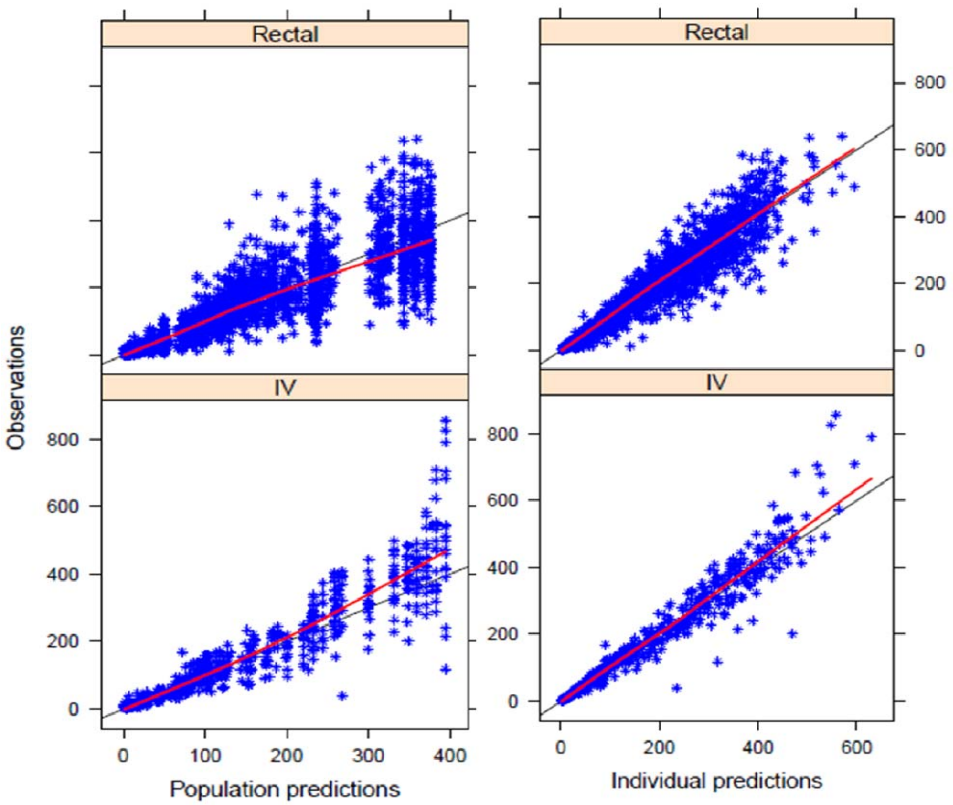


Figure 4.3-1: Model based population predicted and individual predicted versus observed DZP concentrations after intravenous and rectal administration. In the left panel predictions are based on the parameters of the typical individual whereas in the right panel predictions are based on individual parameter estimates.

The rectal diazepam pharmacokinetics was best described with a first order absorption without lag time. The bioavailability was found to be 0.86 with RSE 2.3% and the interindividual variability was estimated to be 0.09 (expressed as SD based on equation 2). The estimated value for the absorption rate constant was 1.44 h^{-1} with an RSE of 8.85%. 4 subjects were flagged for low bioavailabilty (LOBA) as they were identified to differ significantly from the rest in terms of the exposure levels after drug administration. All these subjects were part of study 3 and had unusually low drug levels which may be due to improper drug administration or formulation leakage from site of administration. The bioavailability and absorption rate constant for these subjects was identified to be 0.19 and 0.42 h^{-1} respectively.

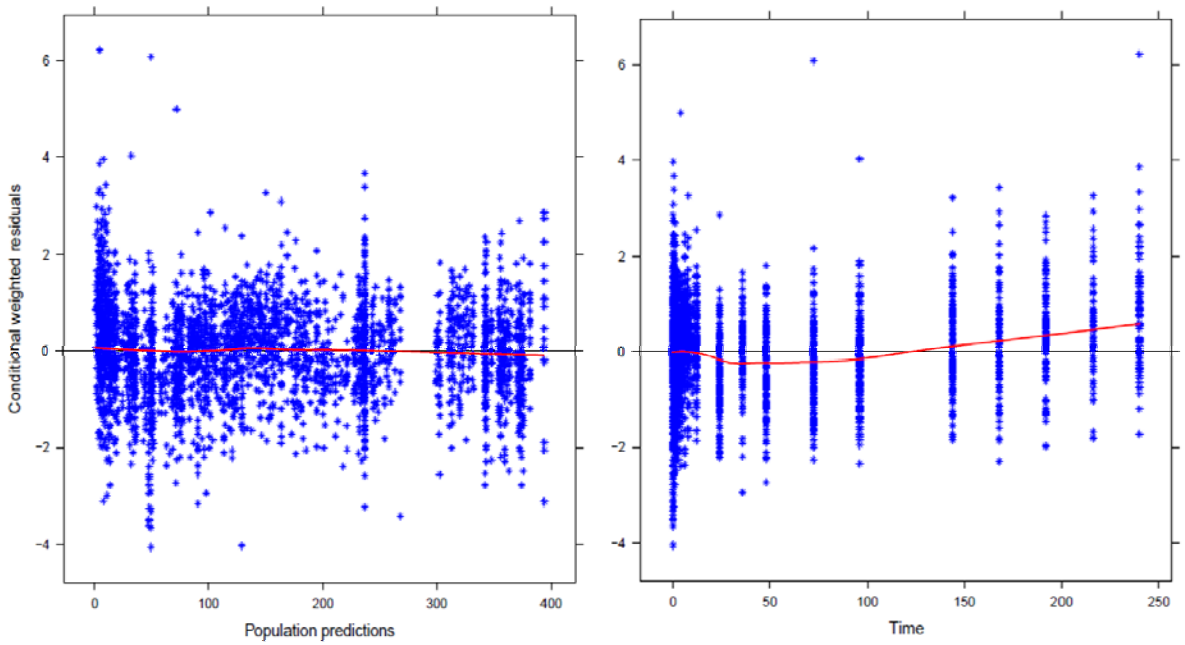


Figure 4.3-2: Conditional weighted residuals vs population predicted DZP concentration (ng/mL) and vs time (h). Values are indicated by solid stars with a smoothing spline trend line through the data. A solid black line at y = 0 is included as a reference.

Model Evaluation

The results for the population PK model evaluation of diazepam, which included results of a predictive check and a nonparametric bootstrap, showed that the final model provided a reliable description of the data with good precision of the structural model and variance parameter estimates. The predictive check demonstrated that the simulated distributions of median concentrations values were in agreement with the observed values (Figure 4.3-3), with the exception of noticeable inflated variances in study S03, especially at C_{max}. The nonparametric bootstrap procedure (truncated to 100 replicates) resulted in 95% CI's for population PK parameter estimates, which are presented in the final model parameter table (Table 4-3). The parameter estimates from the bootstrap procedure are similar to those obtained from NONMEM, with the exception of interindividual variability on Q, the intercompartmental clearance.

Table 4-2: Summary of subject demographics in the population pharmacokinetic database for rectal DZP

Categorical Covariates		
	No of Subjects	%
Sex		
Male	50	66.7
Female	25	33.3
Race		
Caucasian	70	93.3
African American	5	6.7
Treatment		
Intravenous	29	28.0
Rectal	64	72.0
Continuous Covariates		
	Mean (Median)	Range
Weight, kg	70.81 (69)	45.3-114.9
Age, years	32.44 (30)	19-64

Table 4-3: Population pharmacokinetic parameters for the typical individual after intravenous or rectal administration of DZP.

Parameter	NONMEM Estimate (95% CI)	Bootstrap Median (95^{th} percentile)
Clearance (L/h)	1.22 (1.12, 1.32)	1.21 (1.13, 1.27)
V1 (L)	18.40 (15.6, 21.2)	18.56 (15.8, 21.5)
V2 (L)	50.00 (44.8, 55.2)	49.75 (45.4, 54.9)
Q (L/h)	10.60 (8.7, 12.5)	10.48 (8.16 , 12.4)
Rectal Ka (h^{-1})	1.44 (1.10, 1.78)	1.47 (1.16, 1.74)
Rectal F	0.86 (0.80, 0.96)	0.85 (0.81, 0.89)
Rectal Ka – LOBA	0.42 (0.05, 0.80)	0.58 (0.14, 1.64)
Rectal F – LOBA	0.19 (0.13, 0.27)	0.20 (0.13, 0.32)
Age on V2	0.96 (0.53, 1.49)	0.95 (0.49, 1.33)
RUV (% CV)	21.1 (19.6, 22.6)	21.0 (19.3, 22.5)
IIV_{CL}	29.7 (23.2, 35.0)	29.0 (24.6, 33.6)
IIV_{V1}	36.5 (21.4, 46.9)	35.4 (25.3, 47.1)
IIV_{V2}	40.4 (30.9, 47.9)	39.8 (28.6, 48.3)
IIV_{Q}	71.1 (21.5, 98.1)	65.7 (0.6, 90.7)
COV_{V1-V2}	-0.0745 (r = -0.506)	-0.065 (-0.164, -0.0185)
COV_{Q-V1}	-0.159 (r = -0.639)	-0.163 (-0.369, -0.083)
COV_{Q-V2}	0.191 (r = 0.694)	0.20 (0.093,0.36)
IIV_{Ka}	42.2 (27.8, 52.8)	40.7 (24.0, 52.9)
IIV_{F} (SD)	0.08	0.08

IIV – Interindividual variability; RUV – Residual unexplained variability; COV – Covariance; LOBA – Low bioavailability subjects;

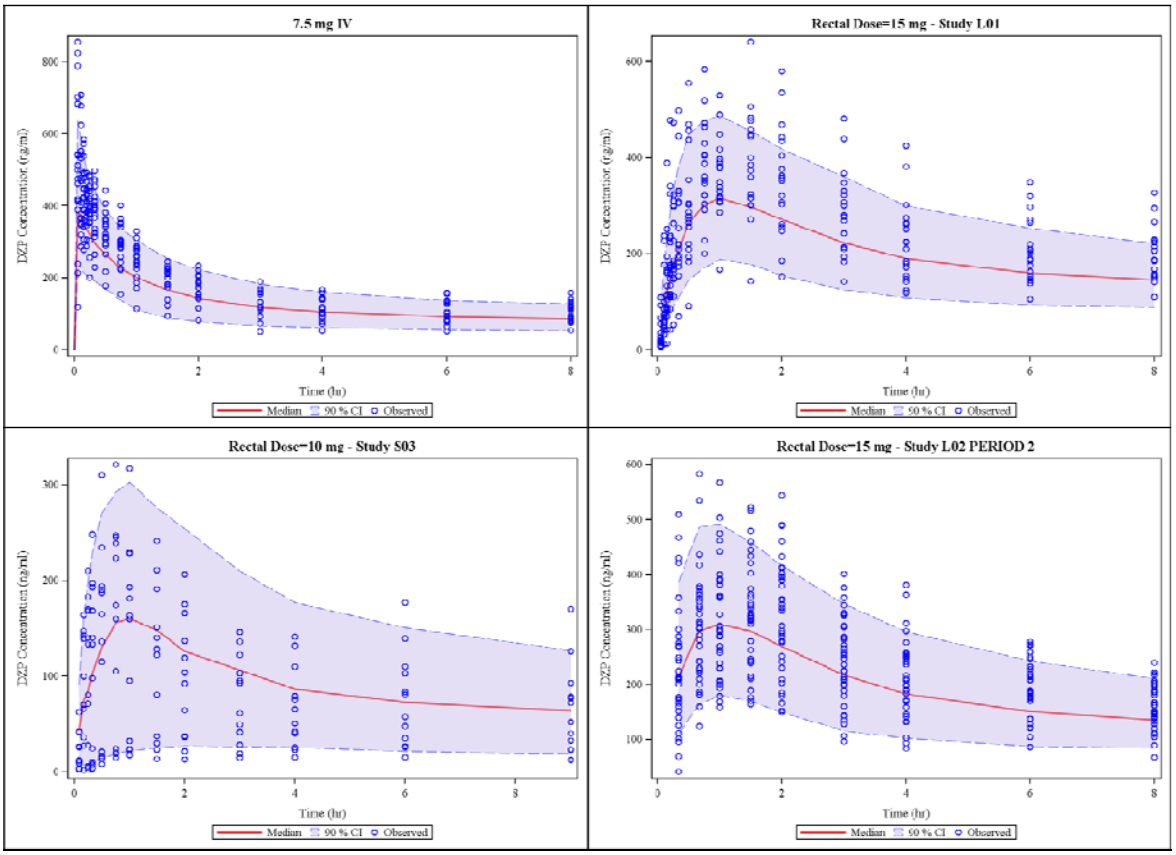


Figure 4.3-3: Stratified (on study) visual predictive check plots for DZP concentrations over time (0-8h) for both routes of administration. The solid line represents the median and the dotted line represents the 5th and 95th percentile for model simulated concentrations. The open circles denote the observed concentrations.

4.4 Discussion

We developed a population PK model of rectal diazepam in healthy adult volunteers, that, to our knowledge is the first such analysis for this mode of administration. Majority of clinical investigations involving Diastat, during and after its introduction have been

focused on efficacy as the primary endpoint. Only a few of reports have focused on pharmacokinetic analysis, which is understandable considering the high efficacy rate of this product. A population model based approach enables the utilization of data collected across different studies to determine subject specific characteristics which may affect drug exposure.

A two compartment structural model best described the time course of plasma diazepam concentrations, which is in agreement with previously published individual pharmacokinetic modeling [182, 183, 187], although there are also reports of a three compartment models in the literature [165]. A three compartment model was tested, but it did not have any improvement in the objective function value in comparison to the two compartment model. Diazepam administered as a rectal gel was rapidly absorbed and the absorption process was best described by a first-order process. We evaluated the utility of a dual input function model with parallel first-order absorption processes to capture the presence of double peaks in some individuals. However, this model did not provide any improvement of fit, either due to the close proximity of the two peaks or because the number of subjects clearly showing two peaks was small relative to the total sample size.

Diastat bioavailability was estimated to be around 86 % which is slightly less (90%) when compared to the estimate obtained from the only published comprehensive pharmacokinetic analysis of the product [77]. Moreover, this value was similar to the 80% bioavailability observed with the rectally administered diazepam solutions with average peak concentration (C_{max}) occurring 10-60 minutes post-dose [35, 70-76]. Even diazepam suppositories which are more slowly absorbed than liquid formulations show a bioavailability averaging 65 - 85% [73, 81]. The interindividual variability in bioavailability in our study was just 9.3 % (CV) which was similar to the previous published study. Only eighteen subjects had data following both intravenous and rectal administration. Due to the absence of cross over data with intravenous diazepam for the remaining subjects, we estimated their bioavailability based on the population disposition parameters. This sparsity in cross over data may have contributed to the small estimate of variability in bioavailability [188]. However it should be noted that all these studies were

conducted in controlled settings in clinical research centers, whereas the actual administration procedure outside a hospital settings in patients with seizures includes a series of steps [189] that would be expected to typically inflate this variability.

The absorption rate constant of the rectally administered diazepam was about 1.44 h^{-1}, a relatively high value that produced a median t_{max} of 1 – 1.5 h. However, as suggested earlier, the presence of double peaks resulted, in most cases, in the first peak having a lower or similar concentration than that of the second peak. This has certainly contributed to the high estimate for median t_{max}, although there was an initial steep rise in concentrations. Plasma concentrations associated with clinical effectiveness ($\geq$ 200 ng/mL) [35] typically occur within five to ten minutes, similar to both IV and intramuscular diazepam administration. In comparison, diazepam suppositories are more slowly absorbed, with therapeutically effective concentrations attained 20 to 120 minutes post-dose or later and C_{max} occuring 45 minutes to 3 hours after administration, depending on the formulation [35, 50, 73-75, 78-83]. All subjects included in this analysis were healthy volunteers pretreated with an enema to ensure an empty rectum. This may have also contributed to faster absorption and greater bioavailability than would occur in patients, which may subsequently affect efficacy. However, similar results were reported even in studies where subjects were not pretreated to enema [74, 81].

As shown in Figure 4.3-1, the diagnostic plots for the model underpredicted concentrations greater than 500 ng/mL especially with IVadministration. The plots of visual predictive check show that the model can be used for further simulations. An underperformance of the model simulations can however be noticed in Figure 4.3-3 for rectal diazepam administered in Study S03. As discussed earlier, 4 subjects from this study exhibited unusually low concentrations throughout, which were attributed to improper drug administration resulting in leakage from the site of administration. This may have attributed to the wide variability in simulations for this particular study. The only predictor of diazepam's distribution, as determined by change in the peripheral volume of distribution was age. This is consistent with earlier reports of effect of age on volume which is the primary reason for change in half-life rather than clearance [183].

We present here the first population pharmacokinetic model for diazepam administered rectally, Diastat®. The results from this model based analysis are consistent with earlier individual pharmacokinetic analysis. There were no significant covariates affecting the absorption or disposition of rectal diazepam in this analysis. Moreover, this model has the advantage that it may be used and built upon to simulate profiles in children in whom dosing has been established without any pharmacokinetic assessment of Diastat in that population.

CHAPTER 5

BRIEFING DOCUMENT WITH EXPLORATORY ANALYSIS AND FUTURE DIRECTIONS

5.0 Executive Summary

This chapter will provide the reader an overall summary of all research conducted towards the development of an intranasal diazepam product. The contents of this chapter may be used as the basis for future study proposals in the development of the intranasal diazepam product. The chapter will first emphasize the need and potential benefit, in terms of patient care and regulatory advantages over other competitors, of developing an intranasal product. Then a brief background of the current rectal diazepam therapy is provided which will be followed by a summary of the clinical pharmacology findings from literature and research conducted as part of this thesis. The chapter will conclude with an exploratory pharmacokinetic comparison of rectal and intranasal diazepam which lay foundation for future clinical development of an intranasal diazepam product.

5.0.1 Value Proposition for Unmet Medical Need

Diazepam is an anticonvulsant which acts at the $GABA_A$ receptors. In epilepsy, it is commonly used as a rescue therapy to treat seizure emergencies. It is given IV when emergencies occur in hospital settings or when trained medical personnel are available. In the absence of trained individuals and when seizures occur outside a hospital setting, a rectal diazepam gel product is most often used. In 1997, the FDA approved a rectal diazepam gel, Diastat®, for use in the treatment of increased bouts of seizure activity in patients on a stable regimen of an antiepileptic drug(s). Diastat quickly became the drug of choice for treating seizure emergencies outside the hospital in young children. Diastat is available as a pre-filled ready-to-use delivery system available in adult and pediatric sizes in various dosage strengths (2.5, 5, 7.5, 10, 12.5 and 15 mg) based on weight.

Although some older children and adults also use Diastat, many patients in these age groups as well as physicians object to the route of administration and as a result the

benefit of this very effective treatment is lost. Nonetheless, as many as 70-80,000 patients receive a Diastat prescription every year. The widespread objection to rectal therapy suggests that an alternative route to administer rescue therapy is needed.

5.0.2 Current Therapy

The management of acute repetitive seizures (ARS) outside a hospital setting in the absence of medical supervision has been improved by the availability of Diastat® (Valeant Pharmaceuticals International), a formulation of diazepam for rectal administration, which has shown to be safe and effective for treatment of this condition in two placebo controlled and open-label extension trials. Diastat is the only approved treatment for ARS in the United States.

Administration of rectal diazepam by caregivers has been largely successful in the treatment of ARS in terms of both safety and efficacy in clinical trials and in the community setting. Pellock and Shinnar identified 9 respiratory events and 3 deaths reviewing data reported to MedWatch and the FDA [23]. They found that none of the 3 deaths were associated with respiratory complications, although one was unwitnessed. All 9 subjects with respiratory events had returned to baseline. The authors concluded that diazepam rectal gel had very low rate of morbidity and mortality, especially considering that over 2 million doses had been prescribed and over 1.5 million administered at the time of their review. The results of 2 randomized double blind controlled trials were published by Kapur [24] in which children with status epilepticus received 20 mg of rectal diazepam by caregivers. No serious respiratory events were noted in any of the 185 children in the study. Rectal diazepam effectively controlled recurring seizures and although some degree of prolonged somnolence was observed, the method of treatment appeared to be safe in the hands of medical caregivers. Brown *et al* reported on 149 cases in which 10 patients received 51 overdosages of rectal diazepam by caregivers [25]. Three patients received accidental overdoses and 7 patients received

intentional overdoses due to inadequate efficacy of previously standard doses (without side effects). In all cases, no patient experienced respiratory complications even though doses were 188 to 256 % higher than the recommended dose. Even when caregivers administered large doses, these data indicate that the risk of respiratory adverse events may be low, particularly in patients who do not respond to the standard recommended dose.

5.0.3 Existing Unmet Need

Despite the availability of Diastat which some older children and adults use, many patients in these age groups as well as physicians object to the route of administration and instead use other therapies not approved for this purpose, receive no treatment, or use emergency medical services or acute care systems.

This report evaluates an alternative route for treatment of seizure emergencies as the approved rectal product is not socially acceptable by older children, adults and females especially in an out-of-hospital setting. We developed and evaluated various nasal spray formulations which can be easily administered with rapid absorption characteristics intended as an alternative to rectal administration.

5.0.4 Clinical Superiority

Pursuant to *21 CFR 316.3(b)(3)(iii)*, a nasal spray that delivers diazepam and that carries the same indication as diazepam rectal gel, would mark a major contribution to patient care and is, therefore, clinically superior to diazepam rectal gel. Older children, adults, and their respective caregivers, prefer nasal over rectal administration [106, 129, 164]. Many patients decline therapy because of their objection to rectal administration. As a result, effective treatment of seizure emergencies is delayed until arrival of paramedics or

admission to an emergency department. The availability of a diazepam nasal spray would provide a treatment option for these patients who eschewed diazepam rectal gel.

In addition to providing a preferred route of administration, a diazepam nasal spray would enhance compliance over that of the diazepam rectal gel. After placing a seizing patient on their side and retrieving the medication, the Diastat label instructs the caregiver to perform the following steps [189]:

1. Push up with thumb and pull to remove cap from syringe.
2. Lubricate rectal tip with lubricating jelly.
3. Turn person on side facing you.
4. Bend upper leg forward to expose rectum
5. Separate buttocks to expose rectum
6. Gently insert syringe tip into rectum
7. Slowly count to 3 while gently pushing plunger in until it stops.
8. Slowly count to 3 before removing syringe from rectum.
9. Slowly count to 3 while holding buttocks together to prevent leakage

For the Diazepam Nasal Spray product, the instructions to the caregiver will be simple, brief, and more intuitive as compared to diazepam rectal gel. In addition to avoiding the need for removal of clothing, most individuals are familiar with nasally administered products and would likely be more comfortable administering the product to a patient having a seizure. This greater ease of use would result in the patient receiving the rescue medication much sooner after seizure onset. These factors will contribute to greater ease of use and compliance than with diazepam rectal gel.

Greater utilization and a better ease of use of Diazepam Nasal Spray will result in improved outcomes for the population at risk, which is evidence of its clinical superiority over diazepam rectal gel. Furthermore, for those patients who refuse to use diazepam rectal gel due to fear of embarrassment, Diazepam Nasal Spray will provide them with an acceptable treatment option for this indication.

5.0.5 Overview of Phase-1 Diazepam Nasal Spray Program

The different nasal spray formulations of diazepam evaluated in our studies included 5, 10 and 13.4 mg doses in small volumes of co-solvent systems (100-300 μL). The development program for a nasal spray formulation has consisted of 3 studies in healthy volunteers. The first study was S01, and compared the absorption characteristics of a supersaturated diazepam formulation in glycofurol/water based co-solvent system to intravenously administered diazepam and intranasally administered midazolam in 4 subjects. The second study, S02, was conducted after confirmation of safety (in terms of irritability and pain after nasal administration) and good absorption characteristics of the glycofurol-based supersaturated formulation from S01. S02 was a 3-way crossover bioavailability, tolerability and dose ranging study of 5 and 10 mg nasal spray formulations compared with 5 mg diazepam given intravenously in 8 subjects. Although the results of this study exhibited rapid absorption and comparable pharmacokinetic characteristics to rectal diazepam, the nasal spray formulation caused moderate irritability and was not tolerated well by subjects. Moreover, glycofurol was not acceptable as GRAS (Generally Regarded As Safe) excepient in the FDA inactive ingredient database. Therefore, further evaluation of this product was halted. For the third study, S03, we tested 2 new microemulsion-based formulations. The ingredients in these formulations were previously used in approved ophthalmic products and were, therefore, considered as safe alternative for nasal administration as compared to the glycofurol based systems. S03 compared the pharmacokinetics, safety, and tolerability of 2 intranasal formulations of diazepam with Diastat in healthy volunteers. This was a 4-period, 4-way crossover study conducted in 12 healthy subjects. The nasal doses tested in this study were 10 (Nas-A & Nas-B1) and 13.4 mg (Nas-B2) whereas a 10 mg dose was used for rectal administration.

For the purpose of comparison and evaluation of the nasal products relative to the approved rectal gel, pharmacokinetic data were also gathered from 2 previously conducted rectal diazepam studies. Of these 2 studies, L01 was single-blind crossover

study of diazepam rectal gel and intravenously administered diazepam in 18 healthy adult volunteers. Study L02 evaluated the bioequivalence of rectal diazepam gel from three different batches manufactured at different time periods. The study was a single-dose, open-label, randomized, comparative three-way crossover bioavailability study in 34 healthy adult volunteers.

The primary findings from the comparative evaluation of the nasal spray formulations to the rectal product were: (i) Presence of high variability in exposure profiles after nasal administration across all formulations tested (ii) Although there was high variability, the dose normalized partial AUC's after nasal administration ($AUC_{0\text{-}tmax}$ to $AUC_{0\text{-}4}$ hr) at initial times when the drug levels are most important are 60-80 % of that when given via the rectal route. (iii) Proper optimization of the nasal spray (microemulsions) device and the subsequent administration procedure supported by appropriate sample size would reduce the variability and give more power for statistically testing non-inferiority or equivalence to the rectal product depending on the pathway chosen for further development of the nasal product.

5.0.6 Proposed Clinical Development Program

These are recommendations after review of all the available nasal and rectal diazepam data.

Although there is no discernable difference between the two microemulsion based formulations, Nas-B seems to be a better alternative than Nas-A. This recommendation is solely based on subjective assessment of various parameters such as partial AUC's and also the fact that Nas-B formulation could be modified to get higher doses in the same volume. Between the two Nas-B treatments, 10 mg Nas-B1 is definitely superior to 13.4 mg Nas-B2. Moreover, even with extreme profiles (very low exposure levels) for 4

subjects in the Nas-B1 group the median exposure levels are better than the other 2 nasal treatments, Nas-A and Nas – B2.

The following are the recommendations for future clinical studies:

(i) **Biopharmaceutical Optimization**: The taste and flavor of the formulation should be improved as subjects reported the feeling of a bad after taste when the nasal spray was administered.

(ii) **Delivery and Pharmacy Optimization**: Every effort must be made in training the study staff to administer the drug in the most appropriate way to minimize leakage, either out of the nostril or via mucociliary clearance to be swallowed into the gut. This would require an easy-to-use nasal spray device with a good delivery mechanism and a formulation with optimal viscosity to improve nasal residence time and prevent leakage due to run-off.

(iii) **PK-PD Model Based Clinical Development**: We propose to conduct for the approval of this drug, dose ranging characterization of the Nas-B formulation and one bioequivalence study followed by an active controlled Phase-3 study to show non-inferiority of the intranasal product relative to the rectal product. The phase-3 study will be planned to extend into an open-labeled phase where patients can be monitored for further safety and efficacy data.

5.1 Background

Diazepam is a benzodiazepine having potent inhibitory activity at the $GABA_A$ receptor with potent anticonvulsant properties in addition to the treatment of anxiety, insomnia, seizures, muscle spasms, restless legs syndrome and many other neurological conditions. The pharmacokinetics, safety and tolerability of diazepam given via different routes (IV, oral, rectal) have been studied extensively and are well understood [161]. This section

summarizes the pharmacokinetics and tolerability of diazepam administered intranasally and rectally.

Diazepam possesses properties that make it a particularly good candidate for intranasal administration. Its lipid solubility and potency are comparable to midazolam. Diazepam has a substantially longer elimination half-life which may provide a longer duration of action as compared to midazolam [34].

There are 3 reports describing intranasal diazepam administration to healthy volunteers. Gizurarson *et al.* prepared an intranasal formulation containing 20 mg/mL diazepam dissolved in 5% glycofurol in polyethylene glycol 200. They compared a 2 mg IN dose of this formulation with the same dose given IV [109]. Blood samples were collected for 5 hours following drug administration. The mean bioavailability was 50.4 ± 23.3% with a time to peak of 18 ± 11 minutes. All subjects complained of nasal discomfort immediately following drug administration, but the discomfort had resolved within 30 minutes. Lindardt *et al.* evaluated an intranasal formulation of diazepam dissolved in polyethylene glycol 300 in 7 healthy volunteers [190]. Using a crossover design, they compared 4 and 7 mg intranasal doses with a 5 mg intravenous dose and collected blood samples for 60 minutes after drug administration. The intranasal formulation had a relative bioavailability of 45 and 42 %, a C_{max} of 99 and 179 ng/ml, and t_{max} of 18 and 42 minutes for the 4 and 7 mg doses. Given that the half-life of diazepam ranges from 20 to 50 hrs, their bioavailability values are likely an underestimate of the actual extent of absorption. Slattery and Lau, using a 10 mg dose of diazepam dissolved in Cremophor EL and a longer period for collection for blood samples, reported a bioavailability of 78 % with a C_{max} of 175 ng/mL and a t_{max} of 1 hr in two subjects [111].

Diastat is a formulation of diazepam rectal gel developed for rectal administration with predictable absorption, metabolism and stability that is convenient and portable [77]. Here a brief summary of the pharmacokinetics of rectal diazepam from three clinical studies is provided. The details of pharmacokinetics of a 10 mg rectal dose studied in S03 will be discussed in the intranasal diazepam section 5.2.3. The pharmacokinetics of the

other two studies will not be discussed in detail as they are primarily being used for comparison to intranasal diazepam and to emphasize the presence of high variability in the approved rectal gel formulation. A population pharmacokinetic model has been developed for the pooled data from these studies and is presented in Chapter 4. Figure 5.1-1 shows the mean pharmacokinetic profiles (0-4 h) from the three studies.

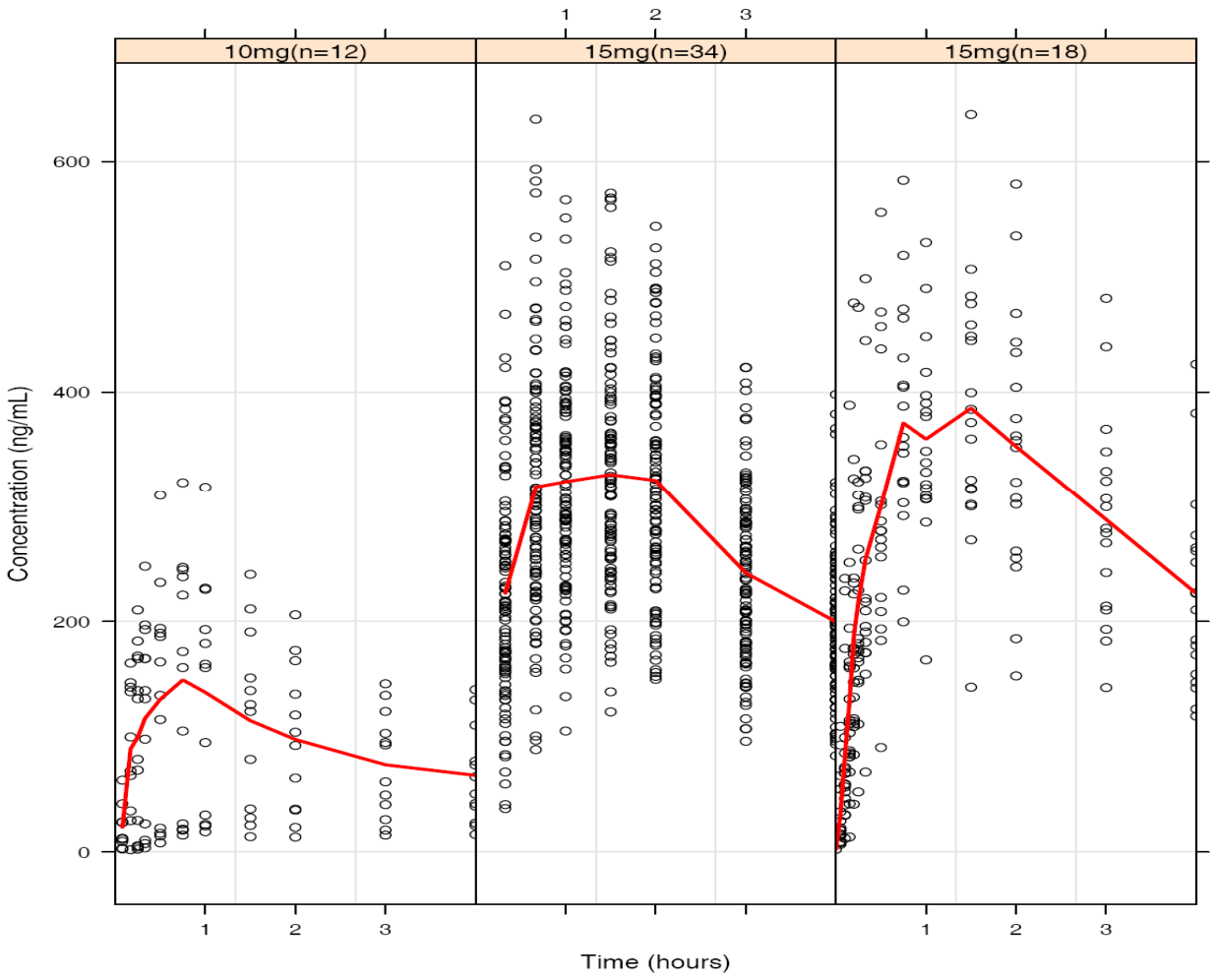


Figure 5.1-1: Concentration time profiles rectal diazepam (0-4 h) from 3 studies, S03, L02 and L01 going from left to right. The solid lines represent the mean profile.

5.1.1 Study L01

A single-blind, crossover comparison of the pharmacokinetics and cognitive effects of a new diazepam rectal gel with intravenous diazepam [77]

This study compared the pharmacokinetics and cognitive effects of the diazepam rectal gel product (Diastat) with intravenous diazepam. Twenty healthy volunteers were enrolled in a single-blind, randomized, double-dummy, two-period, crossover study. Subjects received either 15 mg of diazepam rectal gel or 7.5 mg of diazepam by intravenous infusion. Blood samples for diazepam and desmethyldiazepam analysis were obtained before the dose and from 3 min to 240 h after the dose. Heart rate and blood pressure were measured over the first 24-h period. Subjects also completed five repetitions of a neuropsychological test battery over the first 8 h period.

Mean diazepam pharmacokinetic parameters are summarized in Table 5-1 and the mean profiles are presented graphically in Figure 5.1-1. Diazepam rapidly appeared in plasma after rectal administration, exceeding 200 ng/mL within 15 min and reaching maximum concentrations of 447 ± 91.1 ng/mL at approximately 70 min. The absolute bioavailability of diazepam rectal gel was 90.4%. Alterations in the cognitive effects measured using the neuropsychological test battery over 8 hour period after rectal drug administration were mild and dissipated within 4 hours. Details of the results can be found in the original publication by Cloyd *et al*. [77].

5.1.2 Study L02

Randomized, single-dose, 3 way crossover bioavailability study of three formulations of Athena Diazepam viscous solution for rectal administration in healthy volunteers

This study evaluated the bioequivalence of rectal diazepam gel from three different batches manufactured at different time periods. The study was a single-dose, open-label, randomized, comparative three-way crossover bioavailability study. 34 healthy adult

volunteers (17 males and 17 females) completed the crossover study of a 15 mg single dose of diazepam rectal gel. Blood samples were collected for pharmacokinetic analysis before dosing and from 3 minutes to 240 hours after dosing.

Figure 5.1-1 shows the mean pharmacokinetic profile (0-4 h) compared with the profiles from the other 2 studies discussed here. Mean pharmacokinetic parameters are presented in Table 5-1. Although the mean C_{max} and the median t_{max} differ slightly across the studies, the results from this study compare well with L01.

Table 5-1: Mean pharmacokinetic parameters for rectal DZP across 3 studies

Study		**$t_{1/2}$ (hrs)**	**t_{max} (hrs)**	**C_{max} (ng/mL)**	**$AUC_{0\_1.5}$ ng*hr/mL**	**AUC_{last} ng*hr/mL**	**$AUC_{0-\infty}$ ng*hr/mL**
S03	*N*	8	8	8	8	8	8
	Mean	58.99	0.62	224.87	249.99	5813.66	6353.49
	SD	28.41	0.21	52.68	68.55	3297.82	3768.77
	Median	53.83	0.75	231.00	239.08	4551.18	4666.29
L01	*N*	18	18	18	18	18	18
	Mean	49.52	1.18	447.13	454.96	10805.19	11381.46
	SD	19.05	0.55	91.11	103.58	3179.42	3714.01
	Median	51.04	1.25	453.24	433.48	10214.35	10799.60
L02	*N*	101	101	101	101	101	101
	Mean	49.97	1.35	390.02	396.53	9970.75	10490.88
	SD	24.01	0.64	96.76	109.83	2796.81	3103.51
	Median	46.35	1.50	382.14	399.39	9735.26	10497.29

5.2 Summary of Clinical Pharmacology Findings

Three clinical studies have been conducted to evaluate the safety and pharmacokinetics of diazepam administered intranasally in healthy adult volunteers. Studies S01 and S02 evaluated a supersaturated glycofurol formulation at 5 and 10 mg doses whereas study

S03 evaluated 2 microemulsion based formulations Nas-A at 10 mg and Nas-B at 10 and 13.4 mg.

5.2.1 Study S01

Glycofurol based formulation of IN DZP vs IV DZP, IN and IV MDZ in 4 healthy volunteers [113]

The objective of this pilot study was to evaluate the absorption characteristics of an investigational intranasal formulation of diazepam in comparison with the intranasal midazolam.

The study was a four-way, randomized, single-blind, crossover design in which subjects received 5 mg doses of IN diazepam, IN midazolam, IV diazepam and IV midazolam. Subjects were admitted to the clinical research unit located at Hennepin County Medical Center and remained there for 8 h on four separate occasions after a minimum 1-week washout period. Commercial formulations were used for IV administration of diazepam and midazolam. The IN diazepam formulation consisted of an investigational supersaturated solution containing 40 mg/mL of diazepam, glycofurol and water. The injectable midazolam formulation was also used for intranasal administration. The intranasal doses of 5 mg were dripped in using a 1.0 mL syringe. Blood sample for pharmacokinetic analysis were taken pre-dose and at regular intervals up to only 48 hours post-dose.

Pharmacokinetic and Tolerability Results

Mean PK parameters are presented in Table 5-2 for 3 subjects as one of the subjects dropped out after completion of the IN DZP arm in the first period due to personal schedule conflicts.

Table 5-2: Mean (± S.D.) pharmacokinetic parameters of DZP and MDZ in healthy volunteers following IV and IN administration of 5mg dose.

PK Parameter	IV DZP	IN DZP	IV MDZ	IN MDZ
t_{max} (min)	-	28.8 ± 20.96	-	21.6 ± 7.63
C_{max} (ng/mL)	344.0 ± 92.81*	179.2 ± 8.85	165.2 ± 96.42*	62.8 ± 14.51
Half life (hrs)	59.1 ± 7.76	22.4 ± 3.45	0.9 ± 0.60	3.0 ± 0.74

**** Concentration 5 minutes after injection.***
***** AUC & Bioavailability not reported due to insufficient sampling in the terminal phase***

The results of this study showed that the peak plasma concentrations were achieved rapidly for intranasal diazepam. The 5 mg dose resulted in average maximum concentrations of 179 ng/mL which is close to the commonly accepted therapeutic level for diazepam of 200 ng/mL [35]. Hence, doubling the dose would probably increase the concentrations to above this therapeutic level. Subjects rated both formulations as causing considerable pain, each with a maximum average score of 3.2 on a scale of 1-4 immediately following nasal administration. Fifteen minutes later, the mean pain score for both drugs was 1.2 indicating that as the volume of the formulation in the nose is cleared the pain decreases. Posterior nasal drainage and watery eyes were reported by all subjects. Due to limited sampling (0-48 h, approximately one half-life), it was unrealistic to estimate absolute bioavailability. However, the mean relative ratio for the partial area (AUC_{0-48} hr) of the 5 mg IN DZP and 5 mg IV DZP was more than 100 % (not shown in the table) suggesting that a good amount of the drug is entering the system relative to the IV treatment. Another observation was the fact that the t_{max} after IN DZP administration was 28 minutes which is longer than the nasal residence time of formulations. This indicates that absorption of the drug is still in process either through a depot compartment or after swallowing of the dose to produce high concentrations at such a later time.

5.2.2 Study S02

Dose ranging and tolerability study of 5 and 10 mg glycofurol based IN DZP vs IV DZP in 8 healthy volunteers [112]

The objective of this study was to evaluate using a dose ranging approach, the pharmacokinetics, safety and tolerability of the investigational supersaturated formulation of diazepam used in study S01.

Eight healthy volunteers were randomized into a single-blind, three-way crossover study to compare 5 and 10 mg intranasal diazepam doses of the supersaturated glycofurol formulation with a 5 mg dose of a diazepam solution (DZP injectable, 5 mg/mL) administered intravenously. Treatments were separated by a two-week washout period. Visual analog scales were used to assess tolerability (1-tolerable; 10-extremely intolerable) and pain (1-no pain; 4-extreme pain) at predefined time points.

Pharmacokinetic and Tolerability Results

As shown in Figure 5.2-1, DZP concentrations rise rapidly and are maintained for several hours following intranasal administration. There was a linear increase in C_{max} and exposure (AUC_{0-t}) with DZP dose reflecting approximate dose proportionality (Table 5-3). As hypothesized in S01, doubling of the 5 mg dose raised the concentrations to above 200 ng/mL which are known to be levels required for pharmacological action. The results demonstrate that the formulation is rapidly absorbed with good, but incomplete bioavailability. Once C_{max} is attained, concentrations plateau for several hours before declining. Both t_{max} (median range, 20-30 minutes) and $t_{1/2}$ (mean range, 49.1 - 57.0 h) were comparable following the intranasal doses. Estimated bioavailability was 75% for both doses.

No unanticipated adverse events were reported by subjects following nasal administration of DZP. All subjects, however, reported swallowing a portion of the nasal dose. There were no observed clinically significant abnormalities in vital signs or ECG measurements

and no clinically relevant changes in laboratory parameters during the study. Immediately after intranasal administration, subjects reported an average global tolerability score of 4.4 and 4.7 on a scale of 0 to 10 for the 5mg and 10 mg DZP doses, respectively (Figure 5.2-2). Within 15 minutes, scores decreased to 3.0 and 2.5 respectively, and returned to baseline by 10 h. Subjects rated the formulation at both the 5 mg and 10 mg doses as causing considerable pain with scores of 2.0 and 2.3 out of 4 respectively, immediately following intranasal administration. Fifteen minutes later, the composite pain score for both doses were 1.8 and 1.3 respectively, and the scores returned to baseline by 10 h. This formulation provided reasonable bioavailability, but was not well tolerated.

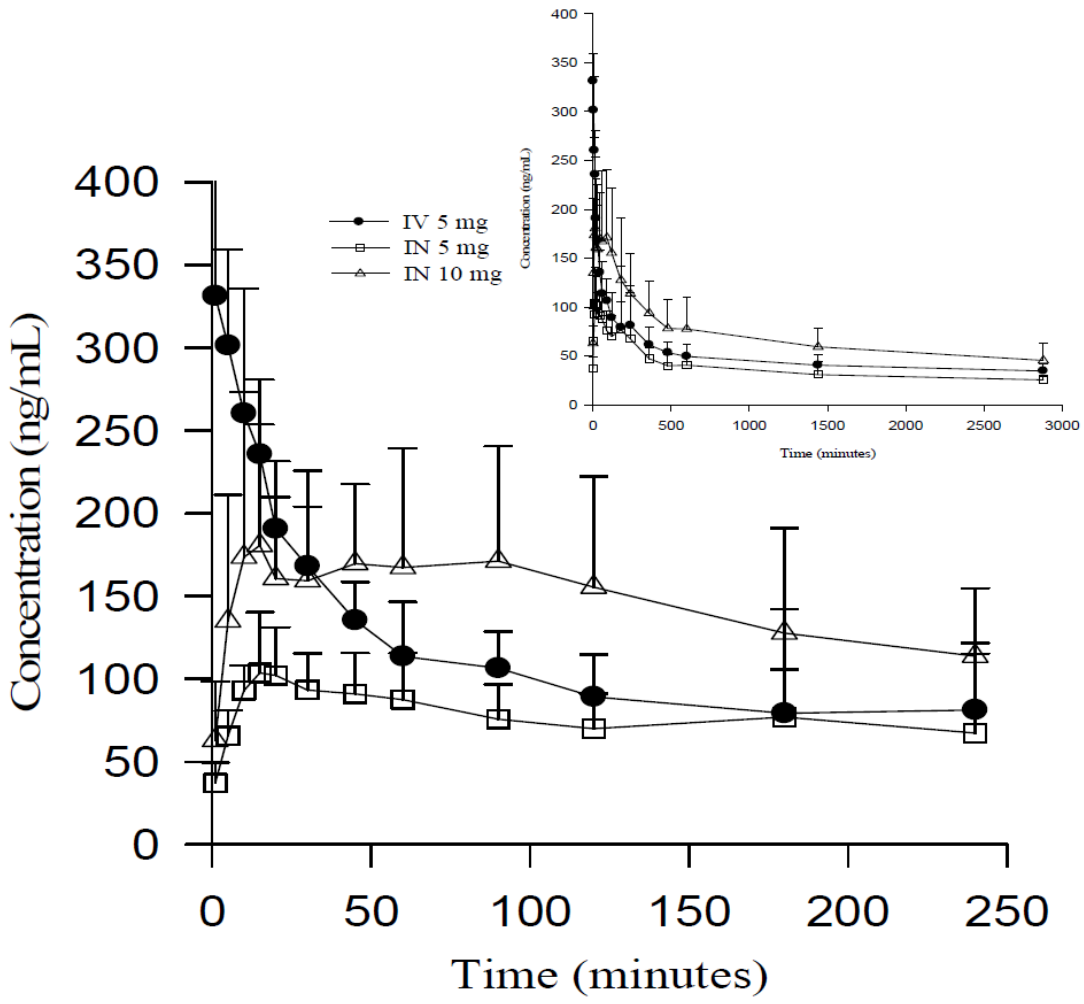


Figure 5.2-1: Mean concentration time profile after intravenous and intranasal administration of DZP in eight healthy adult volunteers. Inset shows the complete profile (0-48 h).

Table 5-3: Mean ± SD of DZP pharmacokinetic parameters following IV and IN administration.

Pharmacokinetic Parameter	5 mg IV Mean ± SD	5 mg IN Mean ± SD	10 mg IN Mean ± SD	Dose Corrected Ratio[b]	90 % Confidence Interval[b]
C_{max}, ng/mL	-	134.3 ± 61.9	247.6 ± 60.9	0.92	0.74,1.36
$AUC_{0\text{-}1h}$, ng.h/mL	187.3 ± 23.4	88.6 ± 16.0	157.6 ± 41.2	0.88	0.67, 1.45
$AUC_{0\text{-}last}$, ng.h/mL	2255.4 ± 628.9	1710.8 ± 540.5	3361.8 ± 916.1	0.98	0.88, 1.16
$AUC_{0\text{-}\infty}$, ng.h/mL	4484.0 ± 1275.4	3295.0 ± 1134.9	7739.6 ± 3645.2	1.17	0.73, 2.07
t_{max}, min	-	20 (15-180)	30 (10-120)	-	-
$t_{1/2}$, h	48.3 ± 17.8	49.1 ± 20.45	57.0 ± 28.0	-	-
F ($AUC_{0\text{-}\infty IN}$)/($AUC_{0\text{-}\infty IV}$)	-	75 ± 41 %	74 ± 53 %	-	-

[a] Ratio of 10 mg intranasal diazepam to 5 mg intranasal diazepam based on arithmetic means.[b] Ratios of dose normalized parameters, calculated by arithmetic means, 90% confidence intervals about the difference of the log-transformed parameters.

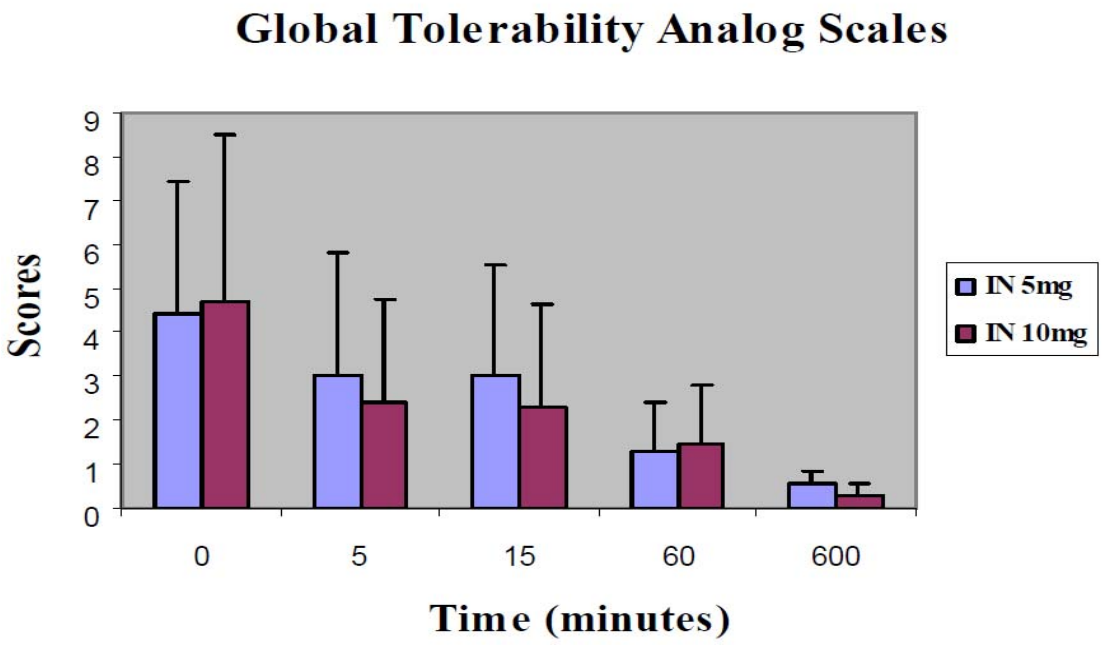


Figure 5.2-2: Comparison of Mean Global Tolerability Scores after IN administration (n = 8). Baseline scores were zero (0) for all subjects.

5.2.3 Study S03

Relative Bioavailability and Tolerability of Two Microemulsion Based Intranasal Diazepam Compared to Rectal Diazepam in Healthy Adult Volunteers [184]

Studies S01 and S02 demonstrated that nasal administration of diazepam is feasible, resulting in C_{max}, and t_{max}, that are comparable to rectal diazepam, which is FDA-approved for out-of-hospital treatment of seizure emergencies. However, the tolerability of the formulation was poor, which made it unsuitable for further development. Moreover, glycofurol is not listed as GRAS (generally regarded as safe) in the FDA inactive ingredient database which further supported our decision to move to a different formulation.

Several other formulations have been developed that may be better tolerated than the glycofurol based formulation. Two such microemulsion based formulations have been tested in study S03.

This study compared the pharmacokinetics, safety, and tolerability of 2 microemulsion based intranasal formulations (Nas-A and Nas-B) of diazepam with a FDA approved rectal diazepam formulation in healthy volunteers. Nas-A was formulated at 5 % strength to deliver 10 mg of drug in 2 sprays of 5 mg each and Nas-B was formulated at 5 & 6.7 % strength to deliver 10 mg and 13.4 mg of drug in 2 sprays. This was a 4-period, 4-way crossover study conducted in 12 healthy subjects. All subjects received a 10 mg rectal diazepam dose (as Diastat) in Period 1 in order to enhance subject continuation in the study. Thereafter, subjects randomly received each of the following treatments over the next three periods:

- 10 mg diazepam nasal formulation 10 mg Nas – A
- 10 mg diazepam nasal formulation 10 mg Nas – B1
- 13.4 mg diazepam nasal formulation 13.4 mg Nas – B2

There was at least a 14-day period between dosing. Safety and tolerability were assessed using sedation, pain and nasal irritation scores.

Pharmacokinetic and Tolerability Results

Mean diazepam pharmacokinetic parameters are summarized in Table 5-4 and the mean profiles are presented graphically in Figure 5.2-3. In general, the nasal spray formulations had an absorption and elimination profile similar to that of the rectal gel formulation. However, there was considerable inter-subject variability in the profiles of both the nasal spray formulations and of the rectal gel formulation.

The median t_{max} value was 0.75 hours for all treatments suggesting that all formulations had a similar rate of absorption. Mean t_{max} values were similar for all three nasal spray doses, ranging from 0.83-1.05 hours. The mean t_{max} value for the rectal dose group was 1.3 hours, reflecting the effect of two subjects with t_{max} values of 3 and 6 hours in this group; remaining subjects (N=10) all had t_{max} values of $\leq$ 1.5 hours. Thus, there was no indication of a more rapid absorption with the nasal spray formulations than that observed with rectal gel dosing.

As can be seen from the Table 5-4, mean peak diazepam concentrations observed with the 10 mg nasal spray formulations were close to those observed with the 10 mg rectal gel formulation. However, median C_{max} values with the 10 mg nasal spray formulations were approximately 15-25% less than the median C_{max} for the 10 mg rectal gel. In contrast, the mean C_{max} values for the 13.4 mg Nas-B1 group was approximately 12 % greater than that of the 10 mg rectal gel, with the median C_{max} values for these two groups being essentially the same.

As it is highly desirable to treat seizure emergencies in the early phase of the attack, AUC values over a range of time intervals up to 4 hours postdose were evaluated for the comparison of diazepam exposure over initial time periods following dosing (Figure 5.2-4). In general, the shorter the time interval postdose, the higher the relative bioavailability of the nasal spray doses as compared to the rectal gel dose. For example,

the ratio of AUC_{0-4} hour values for the nasal spray doses relative to the rectal gel dose was between 10-30% greater than respective $AUC_{0-\infty}$ ratio values. However, on review of the individual subject data, this effect was primarily attributable to 1-2 subjects within each comparison, and not reflective of a consistent formulation effect.

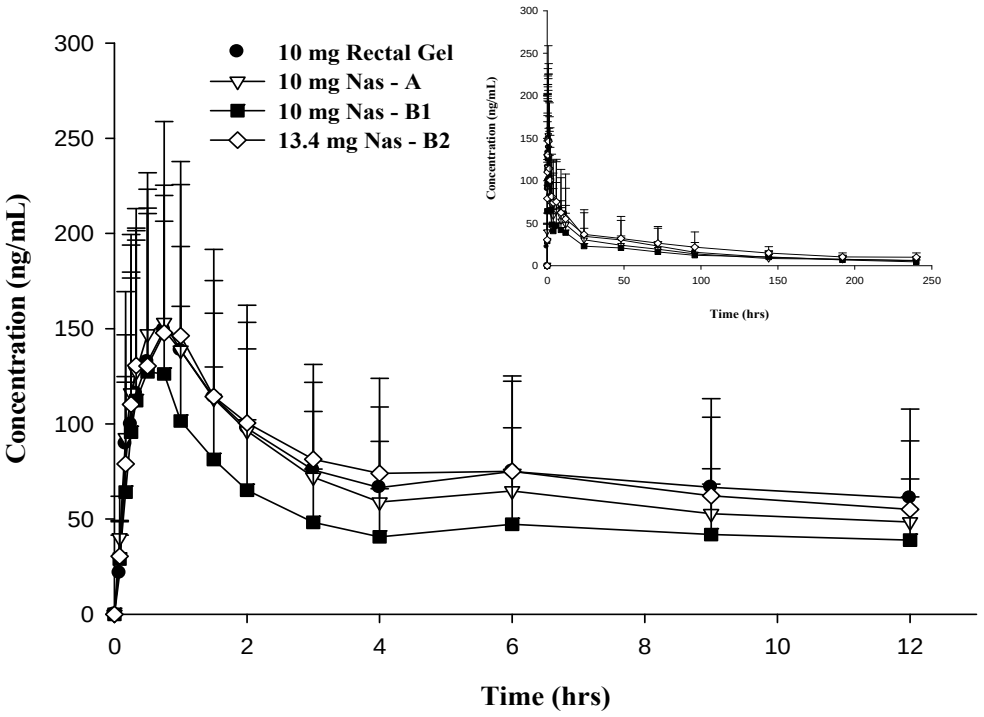


Figure 5.2-3: Mean plasma DZP-concentration time profiles after rectal and intranasal administration in twelve subjects (0—12 h). Inset shows the complete profile (0—240 h).

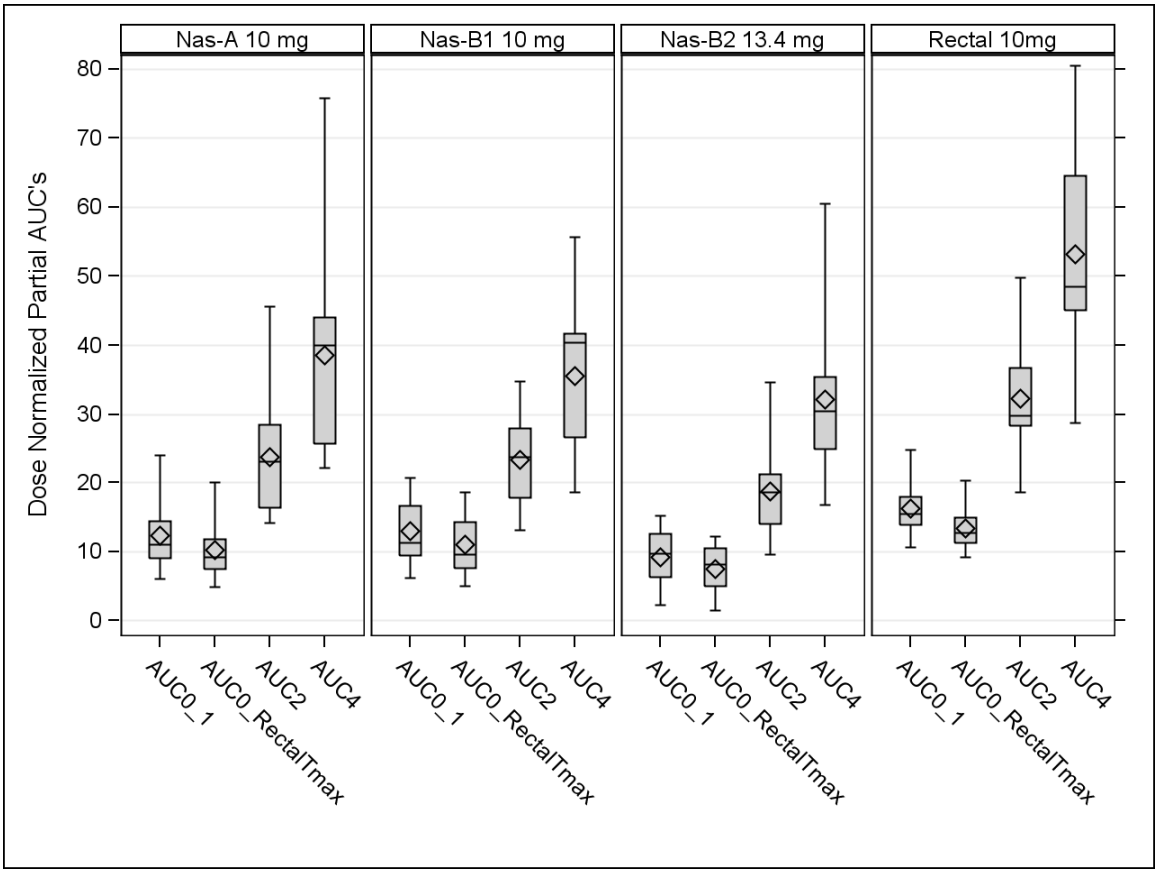


Figure 5.2-4: Dose normalized partial AUC's for the nasal and rectal treatments

Figure 5.2-5 shows that rectal drug administration was tolerated well by all the subjects. Immediately after nasal drug administration, subject reported irritation and pain which lasted for a short time before returning to baseline. Only three subjects reported scores above 7 immediately after administration which lasted for not more than 5 minutes. Considering that the ingredients of the formulation are being used in marketed opthalmic formulation, we did not expect any lasting irritation or side effects. Subjects did however report the feeling of a bad after taste due to which they consumed a few sips of water minutes after administration which was consistent with reports of swallowing of some part of dose after administration.

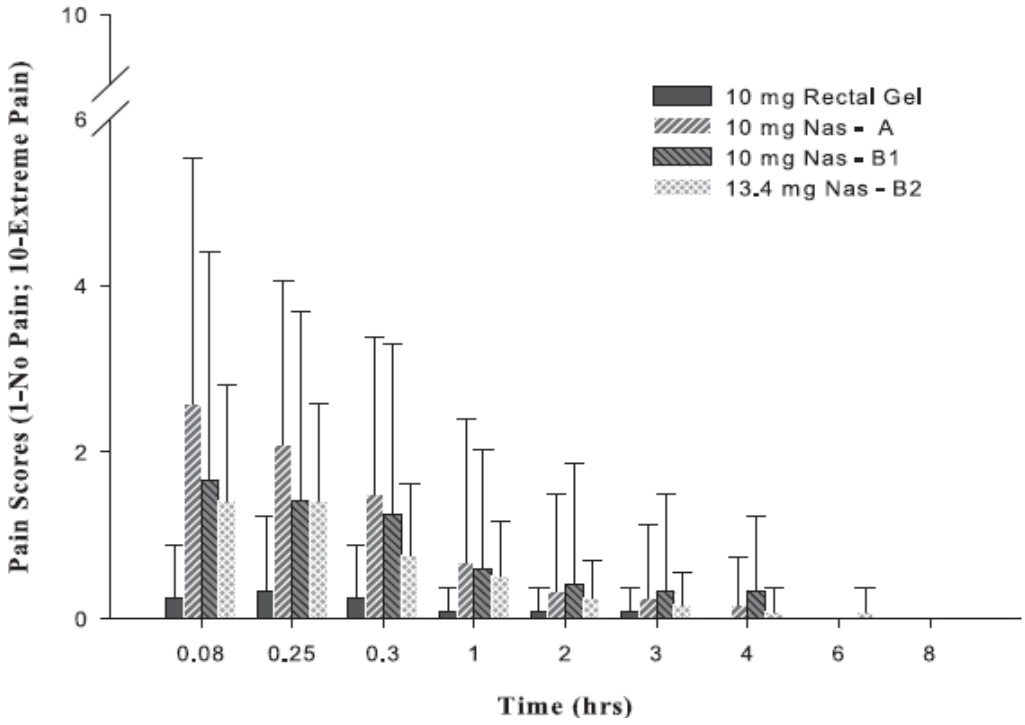


Figure 5.2-5: Comparison of mean global tolerability scores after rectal and IN administration (n = 12). Baseline scores were zero (0) for all subjects

Conclusions

Diazepam administered intranasally (all tested formulations) is rapidly absorbed with peak concentrations at 20-45 minutes after administration. Dose proportionality was not conclusive in S02 or S03 probably due to the high variability. Mean terminal elimination half-lives ($t_{1/2}$) appear to be consistent among the various doses and formulations used. The partial AUC's reflecting exposures during early times after dosing and minimal to no tolerability issues in S03 clearly suggest that the nasal spray formulations used in this study have the potential to be developed as an alternative to rectal diazepam gel. Of the two formulations tested, Nas-B appears to have superior relative partial bioavailability to rectal diazepam at early time points when exposure is most critical. However, considerable planning and care should be taken in subsequent studies to minimize variability, especially contributed due to improper administration related issues. Finally, a population pharmacokinetic model has been developed for intranasal diazepam using all

data available from the above studies [Section 3.3]. This model could be useful in the design and analysis of subsequent pharmacokinetic studies involving intranasal diazepam.

Table 5-4: Mean±SD of DZP pharmacokinetic parameters following rectal and intranasal administration.

PK Parameter	10 mg Rectal Mean ± SD	10 mg Nas-A Mean ± SD	10 mg Nas-B1 Mean ± SD	13.4 mg Nas-B2 Mean ± SD
C_{max} ng/mL	160.9 ± 109.4	181.8 ± 84.16	151.3 ± 108.1	180.7 ± 82.1
t_{max} hr (median)	0.75 [0.3-6.0]	0.75 [0.25-1.5]	0.75 [0.25-3.0]	0.75 [0.25-4.0]
AUC_{0-1} ng.h/mL	112.66 ± 80.35	122.61 ± 52.63	113.46 ± 60.2	100.38 ± 69.02
AUC_{0-4} ng.h/mL	386.51 ± 253.0	387.58 ± 153.42	400.95 ± 187.54	283.99 ± 167.57
$AUC_{0-\infty}$ ng.h/mL	5051.0 ± 3722.7	4450.0 ± 1992.4	3494.7 ± 2179.9	6079.6 ± 4055.6
F ($AUC_{0-\infty IN}$)/($AUC_{0-\infty R}$)	-	~ 88 %	~ 70 %	~ 89 %

5.3 Placebo Controlled Efficacy Trials of Rectal Diazepam

The National Institute of Neurological Disorders and Stroke and Athena Neurosciences conducted a clinical trial (RADARS I) to assess the efficacy and safety of a diazepam rectal gel delivery system (Diastat) in children and adults to assess the value of home

treatment of Acute Repetitive Seizures (ARS). Subsequently, a second study was sponsored by Athena Neurosciences (RADARS II). The results of these studies have been published in separate reports [16, 92]. For the purpose of this communication, we combine the data from these two studies for analysis.

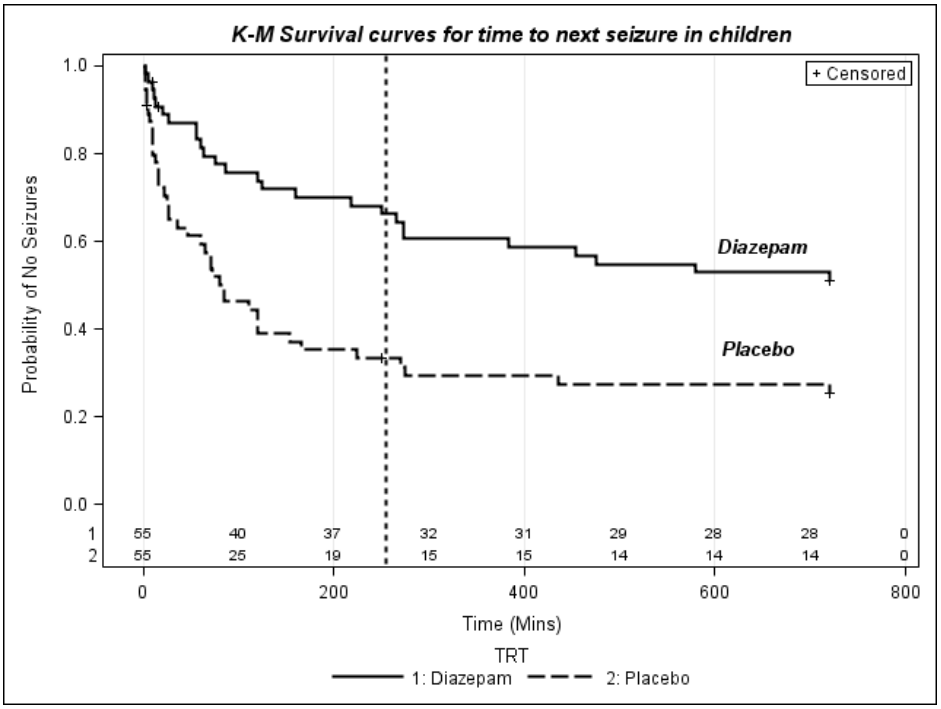
ARS was defined as an episode of multiple seizures of complex partial or generalized type (tonic, clonic, tonic-clonic, atypical absence, or myoclonic) occurring within a specified time period and distinguishable by the patient's caregiver as distinct from the patient's usual seizure pattern. The time period of observation for children was 12 hours in both the studies whereas for adults it was 24 hours in RADARS I and 12 hours in RADARS II. Eligible patients enrolled included males and females who were 1-76 years of age, with a maximal body weight of 114 kg. Patients needed to have had at least four (study one) or two (study two) ARS episodes within the previous year, with one episode within the previous 6 months. A prospective, double-blind, placebo-controlled design was used in both studies. Patients were randomized by age and site. Investigators, nurse coordinators, patients, caregivers, and clinical monitors were unaware of whether the patient was receiving diazepam or placebo. The caregiver initiated treatment when an ARS episode was identified. During the treatment period a study nurse or physician was available by pager and for telephone consultation and clinical monitoring. The observation period for seizures and safety assessments began after the first dose and continued for 12 or 24 hours. The dose for each patient was based on age and weight. The targeted doses were 0.5 mg/kg for ages 2-5 years, 0.3 mg/kg for ages 6-11 years, and 0.2 mg/kg for those 12 years and older. In RADARS I, children received a second dose 4 hours after the initial treatment and adults received 2 additional doses at 4 and 12 hours after the first dose. All patients in RADARS II received only one dose.

Efficacy variables were selected from those common to both original studies: seizure frequency, time to next seizure, and caregiver's global evaluation of outcome. For the purpose of this analysis the seizure count was taken from the time after the first dose was administered. In the original study, the seizure counts were recorded after 15 minutes of the first dose. Here the results of the pooled data for these two trials are summarized for

children and adults separately as was reported in two earlier publications [162, 191]. Following that, some exploratory analysis that was not already reported in the original publications will be used to come up with possible new hypothesis regarding importance of early exposure times for treatment success.

There were 110 intent-to-treat children from these two studies of which 55 were randomized into the diazepam treatment arm and 55 into the placebo arm. There was no statistical difference between the two treatment groups in terms of etiology or prior seizure history. The only demographic difference was the distribution of males and females in the two groups. There was a significant reduction in seizure frequency, expressed as seizures/hour, in children who received diazepam (median = 0) compared with placebo (median =0.25). Further, 59 % of the diazepam treated children remained seizure free compared to 31 % in the placebo group. Kaplan-Meier analysis (Figure 5.3-1) shows that the time to next seizure was significantly longer in the diazepam treated group compared to placebo. As mentioned earlier, the seizure count recorded immediately after the first dose was used here as opposed to 15 minutes after the first dose which was used in the publication. The difference in this approach resulted in the slight difference of the survival graph reported here compared to the one in the original publication, however, with no change in conclusions or inference. There were no significant covariates explaining the differences between the two groups.

Out of the 95 adults from the two studies, 46 were randomized into the diazepam rectal gel treatment arm and 49 were in the placebo arm. There were no statistically different demographic variables between the two groups. Similar to the children population, there was a significant difference in seizure frequency in patients who received the diazepam rectal gel (median =0) compared to placebo (median =0.13). Time to next seizure (first seizure immediately after administration) was significantly longer in the diazepam treated group compared to placebo (Figure 5.3-1).



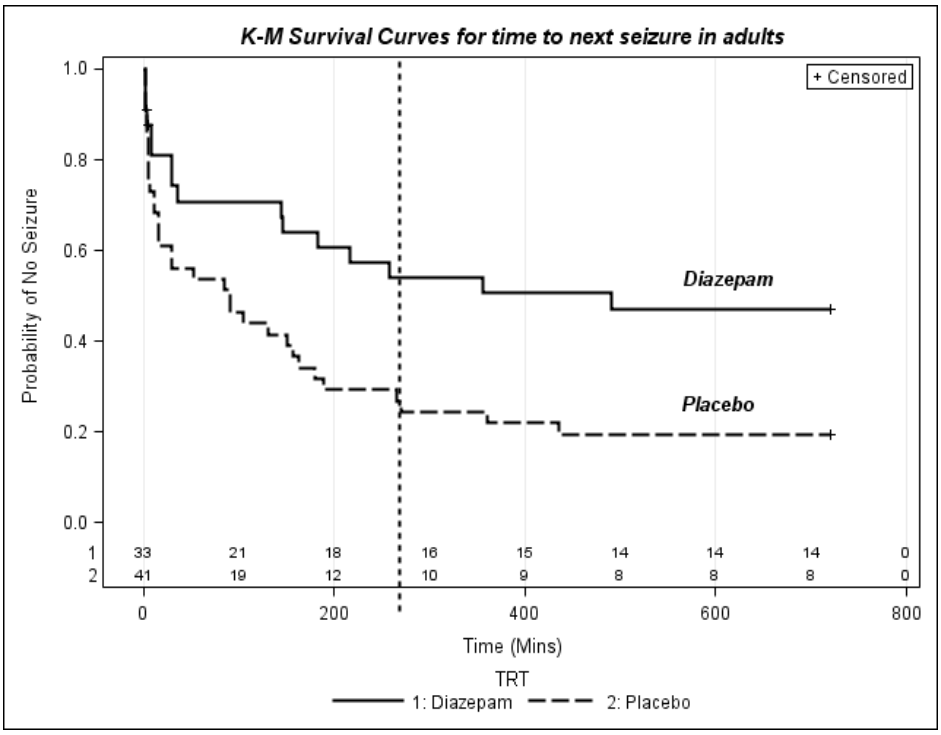


Figure 5.3-1: Kaplan-Meier survival curves representing time to first seizure in children (top) and adults (bottom).

5.3.1 Exploratory Analysis

Investigators of the original publications reported better performance of the sub-group of patients which received 2 or more doses in the observation period as compared to those who received a single dose. Kriel *et al.* reported that children who received two doses of diazepam had a lower seizure frequency than those receiving one diazepam dose for 4-12 and 0-12 hour observation periods. Further, children who received a single diazepam dose did not demonstrate a significant difference in remaining seizure free during the 0-4 h vs 4-12 hour periods [162]. Summarizing the adult data, Cereghino *et al.* show that the proportion of patients who remained seizure free in RADARS I (multiple doses) was much higher in the diazepam treated group compared to the placebo resulting in a treatment effect of 58 %. In RADARS II (single dose) this treatment effect was estimated at 38 % [191].

Although, comparison of subgroups receiving a single dose versus those receiving two doses was exploratory in nature as suggested by the authors, certain counter arguments may be presented which may question the eventual inference of two doses being better than one. In the analysis of both children and adults, seizure frequency was computed as the total number of seizures divided by the observation period. Use of this metric would definitely support the better performance of diazepam relative to placebo, but on the other hand would confound the actual time of seizure occurrence when comparing the performance of two doses versus one. It can be seen from Figure 5.3-2 that given that a patient is a treatment failure (having at least one seizure after the first dose), the likelihood of a patient experiencing a higher seizure count (frequency) for a child or adult is greater when the time to first seizure is within 2 hours after the first dose. Very few patients (less than 5 % in children and adults) experience their first seizure after 4 hours when the first dose was given. Considering that a second dose was administered only in RADARS I, Figure 5.3-2 clearly shows that only 5 patients experience their first seizure after 4 hours. For the remaining subjects who experienced their first seizure within four hours of the first dose, it is not known whether they experience the remaining number of

seizures within the first four hours or if their seizures are spread over the entire 12 hour observation period. It is also unclear in original analysis whether the subgroup analysis of 2 doses vs 1 dose was done on those subjects who were treatment failures or on all subjects. If the latter, then the results would be highly biased as a large proportion of patients may not have experienced a seizure in the observation period which will pull down the mean seizure frequency rate for the later part of the observation period (after 4 hours). Thus, no conclusive evidence can be drawn regarding the utility of second dose 4 hours after the first one.

Considering the above discussion, a hypothesis can be generated to test the utility of the second dose of diazepam at four hours given that a patient is a treatment failure (at least one seizure after treatment initiation). It may be possible that the second dose should be administered within the first 2 hours considering most of the first events occur within that time frame. Such a hypothesis could only be tested if the time to every event (not just the first) data is available. At this time we only have the time to first seizure data.

An important inference of interest from the observation that most of the time to first seizure events occur early is the fact that the few hours after the first dose (may be the first 2, 3 or 4 hours at maximum) are the most crucial times when adequate drug exposure should be attained to achieve optimal seizure control. It is unclear whether the relapse of seizure is due the refractory nature of the epilepsy in the individual patient or the loss of drug effect in the subgroup of subjects who experience a seizure after four hours (may be first or subsequent seizures after relapse) especially in RADARS II where only a single dose was given.

Exposure response relationship has been defined for seizure control with diazepam in the seminal work of Milligan. A therapeutic level of 200 ng/mL for adequate seizure control is usually considered as the benchmark. 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. Rectal diazepam significantly reduced EEG spike frequencies within 20 minutes at a mean serum diazepam level of 210

ng/mL. The mean C_{max} of diazepam was 413 ng/mL and the mean t_{max} was 32 minutes [35]. Based on these results, subsequent controlled clinical trials using similar doses, and presumably similar plasma diazepam concentrations, have demonstrated that rectal diazepam is effective in treating acute repetitive seizures [16].

The conclusions from the exploratory analysis are that adequate exposures are important primarily within the first few hours of dosing and about 200 ng/mL concentrations should be preferably achieved for seizure control. The utility of the second dose can be tested only if more data regarding time of every event for all individuals is made available. The latter is important because the patients and their caregivers can be informed to switch to an alternative treatment or visit an emergency room when adequate seizure control is not attained after a first dose depending on the nature of the ARS.

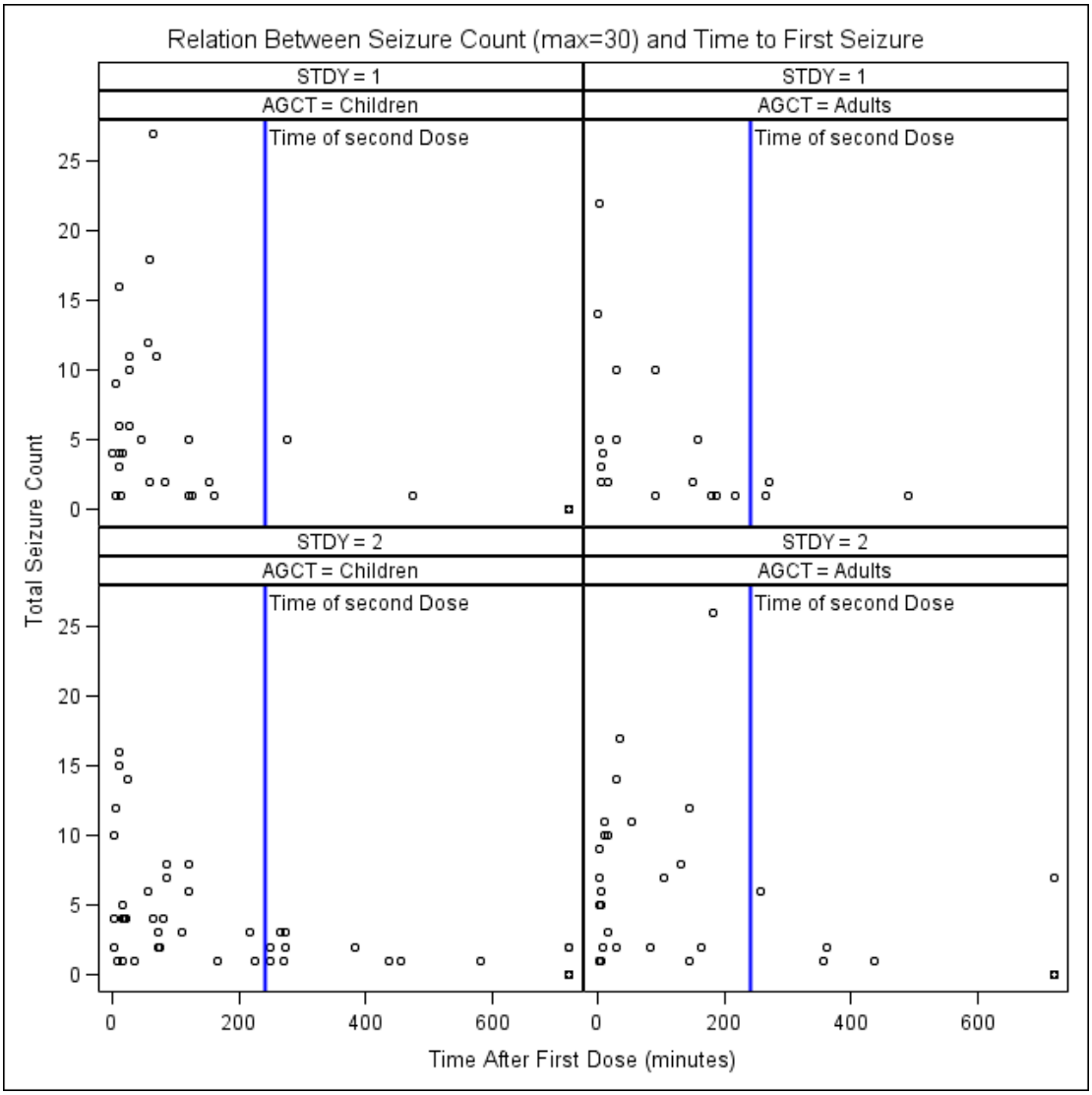


Figure 5.3-2: Seizure count related to time after first DZP dose for adults and children

5.4 Nasal vs Rectal Diazepam

As discussed in the section of clinical superiority of nasal to rectal diazepam (Section 5.0.4), the availability of a fast acting, easy-to-use nasal diazepam spray would definitely improve patient and caregiver compliance and avoid the embarrassment of having to take a rectal product in a public setting especially for older children, adults and females. 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. This traditional pathway would utilize the 505 (b) (1) application provided by the Hatch-Waxman act. The other, possibly quicker pathway, would be to use either 505 (b) (2) or 505 (j) of the same act.

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 may 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. As such, repeating all the clinical studies required for a 505(b)(1) application would be expensive and time-consuming. So, 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 pharmacokinetics and safety studies for intranasal diazepam. This would save both valuable time and resources for a sponsor.

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 demonstrated the generic product to be bioequivalent to the innovator product. Thus, for intranasal diazepam, the ANDA path may be chosen by conducting a single bioequivalence comparison with rectal diazepam.

In either of the above cases, orphan designation may be achieved by using the clause of clinical superiority provided in *21 CFR 316.3(b)(3)(iii)* for orphan drug products [153] which states the following:

"Clinically superior means that a drug is shown to provide a significant therapeutic advantage over and above that provided by an approved orphan drug (that is otherwise the same drug) in one or more of the following ways: (i) Greater effectiveness than an approved orphan drug (as assessed by effect on a clinically meaningful endpoint in adequate and well controlled clinical trials). Generally, this would represent the same kind of evidence needed to support a comparative effectiveness claim for two different drugs; in most cases, direct comparative clinical trials would be necessary; or (ii) Greater safety in a substantial portion of the target populations, for example, by the elimination of an ingredient or contaminant that is associated with relatively frequent adverse effects. In some cases, direct comparative clinical trials will be necessary; or (iii) ***In unusual cases, where neither greater safety nor greater effectiveness has been shown, a demonstration that the drug otherwise makes a major contribution to patient care.****"*

Considering the last point, a nasal product may be given orphan status which provides a major contribution to patient care in terms of providing better compliance and acceptability by all age groups.

In either case it is essential initially to establish comparable exposure levels of intranasal diazepam product relative to rectal diazepam. There are a few important points to be considered while making this evaluation. As discussed in the previous section it is essential to target and achieve adequate exposure in the initial few hours of an ARS episode by achieving target concentrations known to produce pharmacological effects (200 ng/mL). It is also important to attain these concentrations rapidly enough to prevent seizure recurrence. Moreover, considering the fact that seizures often recur for 4-6 hours after initiation of an episode, only the partial exposure levels in terms of area under the curve (AUC) up to 4 or 6 hours after drug administration may be important for

comparison with rectal diazepam. The bioavailability, representing the extent of absorption compared to an IV dose or the relative exposure in terms of $AUC_{0-\infty}$ compared to that of rectal drug may not be as relevant. The FDA guidance on bioequivalence for orally administered immediate release products suggests that in the scenario when early exposure measures may be informative on the basis of appropriate clinical efficacy/safety or PK/PD studies, partial AUC's may be used as a measure of early exposure. Here, partial AUC is defined as the partial area truncated at the population median t_{max} of the reference formulation assuming adequate samples have been collected to establish t_{max}. Further the guidance recommends that for drugs with long half-life ($t_{1/2}$ of diazepam = 40-60 hrs) and low intrasubject variability in distribution and clearance, truncated AUC up to 72 hours can be used in place of AUC_{0-t} or $AUC_{0-\infty}$ [192]. Although this guidance is specific for immediate release orally administered drugs, the nature of the nasal spray (rapid action product) may be considered on similar grounds.

Using the above considerations a summary of comparative pharmacokinetics of nasal diazepam with respect to rectal diazepam is presented here.

For the purpose of this comparison, all the nasal and rectal diazepam data from 5 studies discussed above have been pooled and a noncompartmental analysis was conducted to calculate various exposure measures of partial AUC's and total AUC. C_{max} and t_{max} were determined from observed data. The partial AUC's considered here were AUC 0 to median population t_{max} of rectal formulation ($AUC_{0-Rectaltmax}$), AUC 0 to median population t_{max} of all nasal formulations ($AUC_{0-Nasaltmax}$), AUC_{0-1}, AUC_{0-2} and AUC_{0-4} hrs.

Table 5-5 shows the total number of concentration-time profiles per each formulation and route of treatment. The comparison is subjective and performed using graphical measures. No attempt was made to statistically fit models for comparison.

Table 5-5: Number of profiles from each formulation used for comparative analysis

Formulation	Number of Profiles
Glycofurol 5/10 mg	19
Nas-A 10 mg	12
Nas-B1 10mg	9
Nas-B2 13.4 mg	11
Rectal 10/15 mg	127

The mean dose normalized parameter of each nasal formulation was divided by the corresponding mean dose normalized parameter of the rectal formulation to get the relative ratio as a percentage. In order to get variability around the estimate of the ratio of means, the original dataset with individual AUC's was bootstrapped (stratified sampling with replacement) to produce 1000 datasets. Relative ratios of nasal to rectal were then computed for each of the 1000 datasets to obtain 1000 ratios which were used to calculate the variability around the mean ratio.

Figure 5.4-1 shows a plot of % nasal response for different partial dose normalized AUC's relative to rectal. The population median t_{max} of the rectal formulation was 1.5 h compared to 0.75 h for all the nasal formulations together.

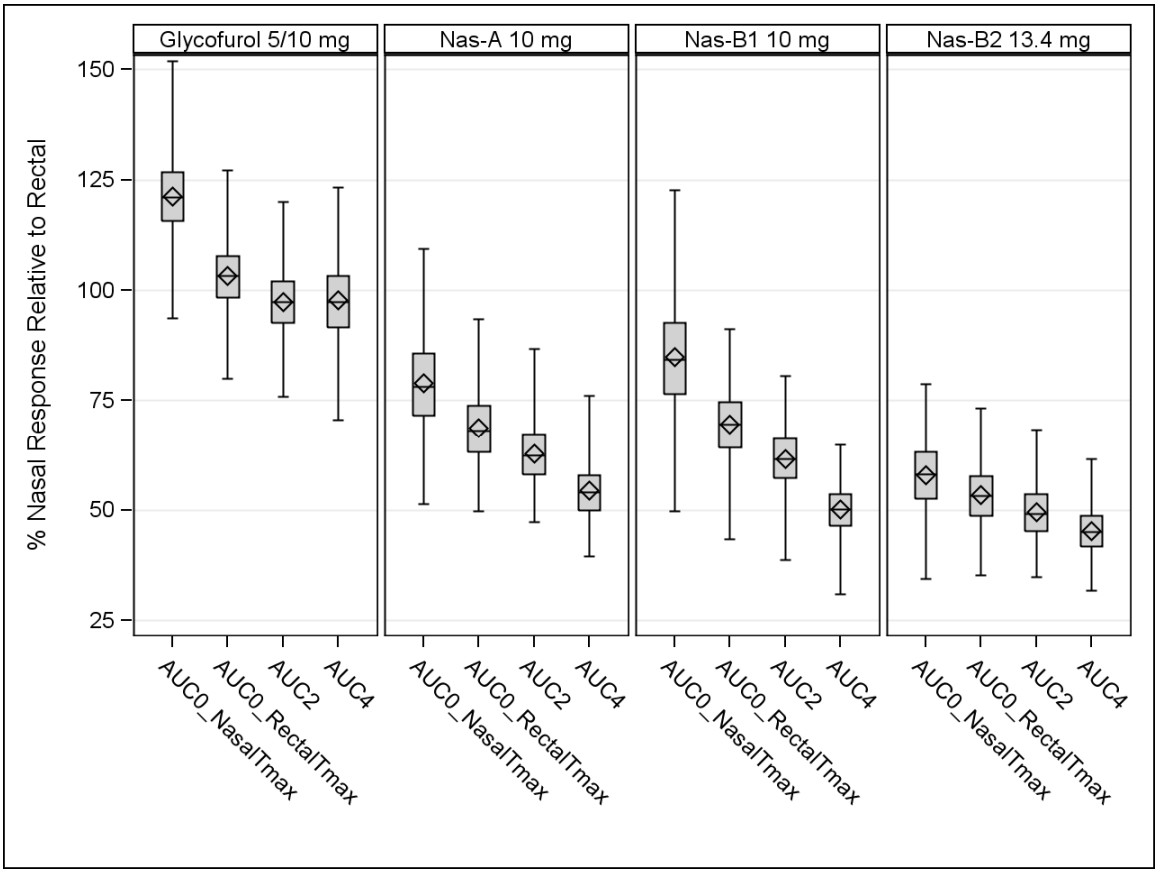


Figure 5.4-1: Relative dose normalized partial areas of each of the nasal formulations to the rectal DZP gel product (Diastat)

Of the three formulations, glycofurol (S01 & S02), Nas-A and Nas-B (S03), it can be clearly seen from Figure 5.4-1 that supersaturated glycofurol based formulation is superior to the other two formulations with relative partial areas almost 100 % of the rectal formulation. However, as discussed earlier, the glycofurol based formulation was not tolerated well by any of the volunteers and this ingredient is not considered as GRAS by the FDA's inactive ingredient database. Hence, with the aim of further development, this formulation will be dropped from the discussion. The two microemulsion based formulations, Nas-A and Nas-B were tolerated well by subjects and the ingredients of the formulation are accepted by the FDA.

Of these two formulations, Nas-B1 (10mg) seems to have a relative ratio better than Nas-B2 (13.4mg) and Nas-A especially at $AUC_{0\text{-}Nasaltmax}$. Although, visually Nas-B1 is better than Nas-B2, the confidence intervals are very wide and it would be difficult to distinguish one from the other. As Nas-B can be formulated at higher strengths, it may be a useful choice for further development. An interesting trend observed in all formulations is that, shorter the time interval postdose, the higher the relative bioavailability of the nasal spray doses relative to the rectal gel dose. For example, the ratio of $AUC_{0\text{-}Nasal}/AUC_{Rectaltmax}$ of 0.75 and 1.5 hours values for the nasal spray relative to the rectal gel was between 10-30% greater than their respective $AUC_{0\text{-}4}$ ratio values. This trend continues till $AUC_{0\text{-}\infty}$. It is possible that the nasal formulations are being absorbed much faster and thus producing peak plasma concentrations much earlier than the rectal formulation. This is supported by the absorption rate constant values which were estimated for the intranasal formulations (Section 3.3) and rectal formulation (Chapter 4) in the population pharmacokinetic analysis. The absorption rate constants were 8.82, 2.68 and 1.48 h^{-1} for the glycofurol, microemulsions and rectal gel formulations, respectively. Although the greater relative bioavailability at early time points can be explained by higher absorption rate constants, the decreasing trend of these relative partial areas with time may be explained by a combination of biopharmaceutical factors affecting drug absorption. The absorption surface are for the rectal route (200-400 cm^2) is only slightly greater compared to the intranasal route (200 cm^2), both values depending on the exact site at which drug is deposited. However, the volume of the rectal cavity is much larger allowing up 10 mL of drug solution to be administered without any discomfort compared to the nasal cavity where 100-300 μL of solution at maximum can be administered into each nostril. It is possible that although there is rapid absorption from the highly vascularized nasal membrane soon after administration, the shorter residence time of the drug formulation at the site of absorption along with nasal drainage contributes to a decrease in amount of drug available for absorption over time. For the rectal route, the absorption is rapid (but relatively less than nasal) and the residence time of formulation is longer at the site of absorption with relatively less leakage due to larger volume of the rectal cavity as compared to the nasal cavity.

Figure 5.4-1 shows that the nasal formulations are absorbed almost 50-80 % relative to that of the rectal gel at early time points. On the other hand the dose normalized C_{max} of the nasal formulations are about 60-80 % of the rectal gel as seen in Figure 5.4-2. Considering that the average C_{max} of the rectal product is about 400 ng/mL for a 15 mg dose, the nasal formulations at a 10 mg dose are producing concentrations greater than 200 ng/mL (60-80 %) which are greater than the concentrations required for pharmacological action. Furthermore, these concentrations are being attained earlier than via the rectal route (0.75 vs 1.5 h). Acknowledging that this observation may be sensitive to sampling and design issues, conservatively it is possible to achieve therapeutic levels by giving a second nasal dose 5 or 10 minutes after the first. This would not pose any safety issues as diazepam is known to have a wide therapeutic and safety window as shown with rectal diazepam. In a scenario of misdosing due to improper technique, leakage or other reasons, it is practically easier to repeat a nasal dose than a rectal dose to achieve therapeutic levels.

The relatively poor performance of the 13.4 mg Nas-B2 relative to 10 mg Nas-B1 is intriguing because, even though the dose normalized partial AUC's at early time points are much lower for the 13.4 mg dose than the 10 mg dose (Figure 5.4-1), the dose normalized $AUC_{0-\infty}$ seems to be the same (Figure 5.4-2). This observation may imply a delayed absorption for the 13.4 mg dose which is not evident in comparison of the median t_{max} of this formulation compared to the others as seen in Table 5-4 (Section 5.2.3) and also contradicts with the explanation of nasal residence time of the formulation. Hence any dose dependent absorption and bioavailability has to be investigated in subsequent studies. However, it is acknowledged that the confidence intervals for the 10 mg and 13.4 mg overlap each other making them indistinguishable statistically. The relative C_{max} and partial areas and the variability around these estimates (obtained by bootstrapping) can only be improved than what we estimated in our study (S03). If problems relating to inaccurate dosing due to device familiarity issues can be overcome in subsequent studies by prior training of study staff, the mean relative C_{max} and partial areas may be increased with much less variability than what was seen here.

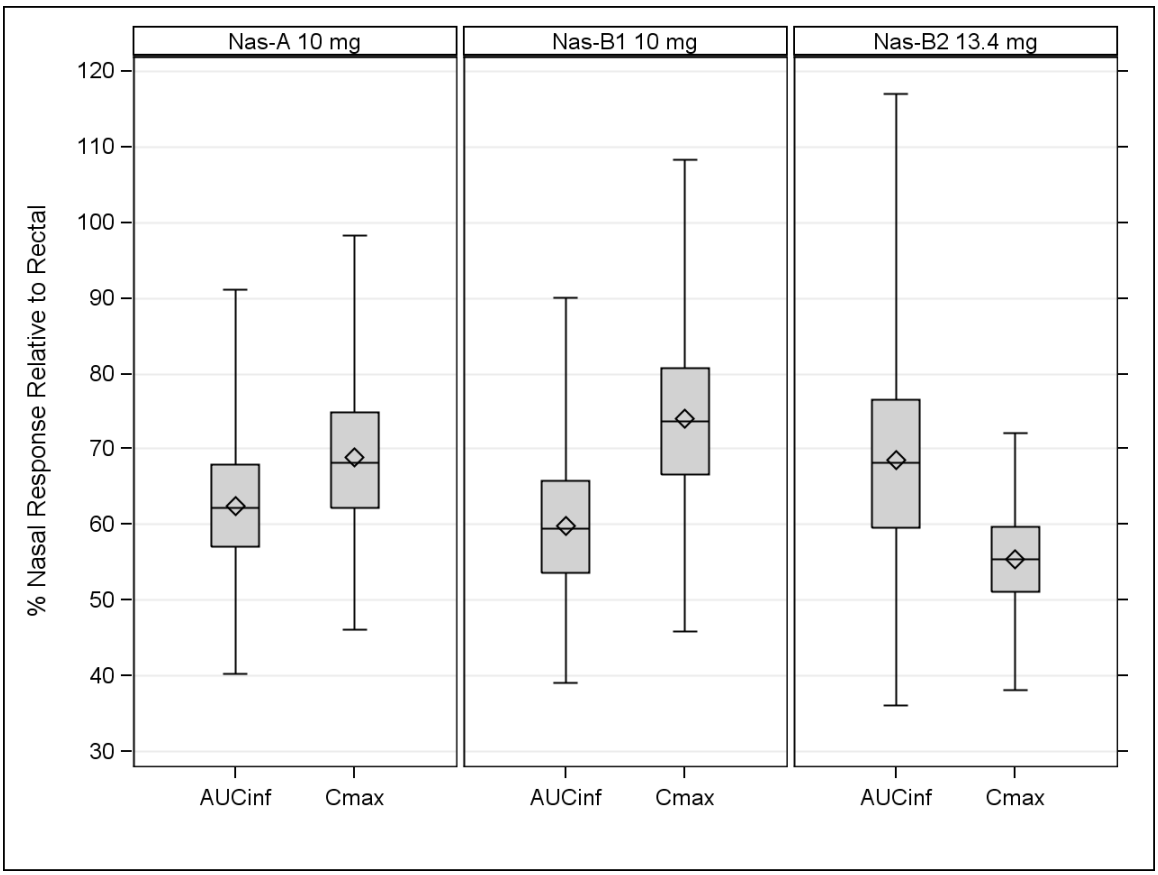


Figure 5.4-2: Relative dose normalized $AUC_{0-\infty}$ and C_{max} of each of the nasal formulations to the rectal DZP gel product (Diastat)

5.5 Proposed Clinical Development Plan

The ability to be formulated at 2 strengths clearly gives an advantage for the selection of Nas-B formulation. With no safety issues even at the high dose, the subsequent development plan should consider the following studies;

a. Dose ranging characterization and also evaluation of the pharmacokinetics after 2 doses given minutes apart.

b. One adequately powered bioequivalence study with rectal diazepam**.**

c. One phase-3 active controlled trial with the aim of showing non-inferiority and the possibility of extending this phase-3 trial to an open-labeled study to continue monitoring safety and efficacy.

CHAPTER 6

CONCLUSIONS

There is clearly a need for additional therapies and/or alternative treatments for patients with ARS. Despite the availability of Diastat, most adolescent and adult patients do not use this product, instead using off-label remedies, foregoing therapy, or engaging emergency medical services or acute care hospital systems.

We successfully studied the potential of a fast acting intranasal diazepam spray as an alternate route to rectal diazepam. Of the two formulation technologies that were tested, glycofurol based and microemulsion based, only the microemulsions can be used for further development as there are certain tolerability, regulatory and design issues with the glycofurol formulation. Of the two microemulsion formulations evaluated, Nas-B which can be formulated at higher strengths is clearly preferable for future studies.

Although further analysis is warranted, there is evidence on several lines regarding the futility of a second dose of Diastat at four hours after the seizure episode begins.

Appropriate planning and choice of meaningful end points such as partial AUC's and an efficient development path will be key factors for the successful development of an intranasal diazepam product.

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APPENDIX

A. NONMEM Code for Intranasal Diazepam Model

```
;Model Desc: IN DZP -  2 IN Formulations
;;; LBAF IS A FLAG FOR SUBJECTS WITH LOW BIOAVAILABILITY
;;; LBAF=0 NORMAL LBAF=1 FOR POOR ABSORBERS
;;; ID 15 F4-10MG ID 19-F4-10MG ID-22 F4-10MG ID 22 F5 13.4MG
$PROB RUN# 241
$INPUT C ID TIME AMT DV EVID CMT OID TRT FORM STDY PERI DOSE AGE SEX RACE WT
LBAF
$DATA dzp_pooled_lbaf.csv IGNORE=C
$SUBROUTINES ADVAN6 TRANS1 TOL=5
$MODEL
  COMP=(CENTRAL)
  COMP=(PERIP)
  COMP=(NASAL1)
  COMP=(NASAL2)
$PK
  TVCL=THETA(1)
  CL=TVCL*EXP(ETA(1));        CLEARANCE FROM CENTRAL COMPARTMENT
  TVV1=THETA(2)
  V1=TVV1*EXP(ETA(2));        VOLUME OF CENTRAL COMPARTMENT
  TVV2=THETA(3)
  V2=TVV2*EXP(ETA(3));        VOLUME OF PERIPHERAL COMPARTMENT
  TVQ=THETA(4)
  Q=TVQ*EXP(ETA(4)); DISTRIBUTION CLEARANCE TO & FROM CENTRAL COMPARTMENT
  S1=V1/1000 ;   SCALE FACTOR FOR CENTRAL COMPARTMENT
  F1=1

  ;formulation 2
  IF (FORM.EQ.2) THEN
  TVF3=THETA(5)
  F3=EXP(LOG(TVF3/(1-TVF3))+ETA(5))/(1+EXP(LOG(TVF3/(1-TVF3))+ETA(5)))
  KN1 =THETA(6)*EXP(ETA(6))
  ENDIF

  ;formulation 3
  IF (FORM.GE.3.AND.LBAF.EQ.0) THEN
  TVF3=THETA(7)
  F3=EXP(LOG(TVF3/(1-TVF3))+ETA(7))/(1+EXP(LOG(TVF3/(1-TVF3))+ETA(7)))
  KN1 =THETA(8)*EXP(ETA(8))
  ENDIF

  ;POOR ABSORBERS
  IF (LBAF.EQ.1) THEN
  TVF3=THETA(9)
  F3=EXP(LOG(TVF3/(1-TVF3)))/(1+EXP(LOG(TVF3/(1-TVF3))))
  KN1 =THETA(10)
  ENDIF

  F4=0 ; ; NO DUAL ABSORPTION PROFILES EXPECTED FOR THIS POPULATION
```

```
 KN2=0
 ALAG4=0

 K31=KN1
 K41=KN2

 TAD=TIME ; for a single dose only
 SID=ID

$DES
  DADT(1)=K31*A(3)+K41*A(4)+Q/V2*A(2)-CL*A(1)/V1-Q/V1*A(1)
  DADT(2)=Q/V1*A(1)-Q/V2*A(2)
  DADT(3)=-K31*A(3)
  DADT(4)=-K41*A(4)

$ERROR
 ST12=0
 IF(STDY.LE.2) ST12=1
 ST3=0
 IF(STDY.EQ.3) ST3=1
 ST4=0
 IF(STDY.EQ.4) ST4=1
 IPRED=F
 W=F
 IRES= DV-IPRED
 IWRES=IRES/W
 Y = F + W*ST12*ERR(1) + W*ST3*ERR(2) + W*ST4*ERR(3)

$THETA
(0,1.07);[CL]
(0,15.3);[V1]
(0,54.1);[V2]
(0,17.5);[Q]
;;FORM 2
  (0,0.7,1); [F3-2/5]
  (0,4)    ; [KN1-2/5]
;;FORM 345
  (0,0.6,1); [F3-345]
  (0,6)    ; [KN1-345]
;;FORM 5
  (0,0.2,1); [F3-LBAF]
  (0,1)    ; [KN1-LBAF]

$OMEGA
  0.04 ;[P] omega(1,1)
  0.04 ;[P] omega(2,2)
  0.04 ;[P] omega(3,3)
  0.04 ;[P] omega(4,4)
  0.04 ;[A] omega(5,5)
  0.04 ;[P] omega(6,6)
  0.04 ;[A] omega(7,7)
  0.04 ;[P] omega(8,8)

$SIGMA
```

```
 1 ;[P] sigma(1,1)
 1 ;[P] sigma(2,2)
 1 ;[P] sigma(3,3)

$EST METHOD=1 INTERACTION PRINT=5 MAX=2000 NSIG=2 SIGL=6 MSFO=241.MSF
$COV PRINT=E
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F3 F4 ALAG4 IWRES CWRES IPRED ONEH NOPR FILE=241.tab
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$TABLE ID TRT FORM STDY DOSE SEX RACE ONEHEADER NOAPPEND NOPRINT
FILE=CATAB241
$TABLE ID CL V1 V2 Q KN1 KN2 F3 F4 ALAG4 ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 ETA8
ONEH NOAP NOPR FILE=PATAB241
$TABLE ID TIME IPRED IWRES CWRES ONEHEADER NOPRINT FILE=SDTAB241
$TABLE ID CL V1 V2 Q KN1 KN2 F3 F4 ALAG4 NOAPPEND NOPRINT FILE=241.par
$TABLE ID ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 ETA8 FIRS NOAPP NOPR FILE=241.eta
```

B – NONMEM Code for Rectal Diazepam Model

```
;Model Desc: Rectal Diazepam
;; LBAF DATASET; SUBJECTS 18,20,21 AND 22 FROM STUDY 3

$PROB RUN# 143
$INPUT C ID TIME AMT DV EVID CMT OID TRT FORM STDY PERI DOSE AGE SEX RACE WT
LBAF
$DATA REC_IV_DZP_LN.CSV IGNORE=C IGNORE=(CMT.EQ.6)
$SUBROUTINES ADVAN6 TRANS1 TOL=5
$MODEL
  COMP=(CENTRAL)
  COMP=(PERIP1)
  COMP=(RECTAL1)
$PK

  TVCL=THETA(1)
  CL=TVCL*EXP(ETA(1));          CLEARANCE FROM CENTRAL COMPARTMENT
  TVV1=THETA(2)
  V1=TVV1*EXP(ETA(2));          VOLUME OF CENTRAL COMPARTMENT
  TVV2=THETA(3)+THETA(10)*(AGE-30)
  V2=TVV2*EXP(ETA(3));          VOLUME OF PERIPHERAL COMPARTMENT
  TVQ=THETA(4)
  Q=TVQ*EXP(ETA(4)); DISTRIBUTION CLEARANCE TO & FROM CENTRAL COMPARTMENT
  S1=V1/1000 ;   SCALE FACTOR FOR CENTRAL COMPARTMENT

 F1=1
 IF(TRT.EQ.3.AND.LBAF.EQ.0) THEN
 TVF3=THETA(5)
 F3=EXP(LOG(TVF3/(1-TVF3))+ETA(5))/(1+EXP(LOG(TVF3/(1-TVF3))+ETA(5)))
 KN1 =THETA(6)*EXP(ETA(6))
 ENDIF

 IF(TRT.EQ.3.AND.LBAF.EQ.1) THEN
 TVF3=THETA(7)
 ;;F3=TVF3*EXP(ETA(5))
 F3=EXP(LOG(TVF3/(1-TVF3)))/(1+EXP(LOG(TVF3/(1-TVF3))))
 KN1 =THETA(8)
 ENDIF

 K31=KN1

$DES
  DADT(1)=K31*A(3)+Q/V2*A(2)-CL*A(1)/V1-Q/V1*A(1)
  DADT(2)=Q/V1*A(1)-Q/V2*A(2)
  DADT(3)=-K31*A(3)

$ERROR
 IPRED=F
 W=SQRT(F*F*THETA(9)*THETA(9))
 Y = F + W*ERR(1)
 IRES= DV-IPRED
 IWRES=IRES/W
```

```
$THETA
  (0, 1);[CL]
  (0, 10);[V1]
  (0, 40);[V2]
  (0, 10);[Q]
;; LBAF=0
  (0,0.7,1); [F3-STDY3,4,5]
  (0,4)    ; [KN1-STDY3,4,5]
;;LBAF=1
  (0,0.2,1); [F3--LBAF]
  (0,1)    ; [KN1-LBAF]
;; W ON F
  1 ; [W ON F]
  0.5 ; [age ON v3]

$OMEGA
0.1 ;[P] omega(1,1)
$OMEGA BLOCK (3)
0.121; [P]
0.01; [F]
0.14; [P]
0.04; [F]
0.04; [F]
0.35; [P]
$OMEGA
0.04;[P] omega(5,5)
0.04;[P] omega(6,6)

$SIGMA
 1 FIX; USING THETA TRICK FOR IWRES

$EST METHOD=1 INTERACTION PRINT=5 MAX=2000 NSIG=2 SIGL=6 MSFO=143.MSF
$COV PRINT=E
$TABLE ID TIME TRT FORM STDY PERI AGE DOSE SEX RACE WT LBAF CL V1 V2 Q KN1 F3
CWRES IWRES IPRED ONEH NOPR FILE=143.tab
$TABLE ID TRT FORM STDY AGE DOSE SEX RACE WT ONEHEADER NOPRINT
FILE=COTAB143
$TABLE ID CL V1 V2 Q KN1 F3 ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ONEHEADER NOAPPEND
NOPRINT FILE=PATAB143
$TABLE ID TIME IPRED IWRES CWRES ONEHEADER NOPRINT FILE=SDTAB143
$TABLE ID TRT FORM STDY PERI CL V1 V2 Q KN1 F3 FIRSTONLY NOAPPEND NOPRINT
FILE=143.par
$TABLE ID ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ONEH FIRS NOAP NOPR FILE=143.eta
```