



Bioavailability and tolerability of intranasal diazepam in healthy adult volunteers

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Received 10 July 2008; received in revised form 2 December 2008; accepted 11 January 2009

Available online 20 February 2009

KEYWORDS

Seizure emergencies;
Intranasal therapy;
Diazepam;
Glycofurol;
Out-of-hospital

Summary Intranasal therapy has been proposed as an alternative for the management of seizure emergencies. The bioavailability, dose proportionality and tolerability of a supersaturated intranasal formulation of diazepam (DZP) solubilized in a glycofurol–water cosolvent system was investigated. Eight healthy volunteers were randomized into a single-blind, three-way crossover study to compare 5 and 10 mg intranasal DZP doses of the investigational formulation with a 5 mg dose of a DZP solution (DZP injectable, 5 mg/mL) administered intravenously. Treatments were separated by a two-week washout period. Plasma samples for DZP analysis were collected pre-dose and at regular intervals up to 48 h post-dose and assayed by HPLC. Visual analog scales (VAS) were used to assess tolerability (1-tolerable; 10-extremely intolerable) and pain (1-no pain; 4-extreme pain) at predefined time points. Following the 5 and 10 mg doses, the median $t_{\max}$ were 20 and 30 min and the mean $C_{\max}$ were 134.3 ± 62 and 247.6 ± 61 ng/mL. Estimated bioavailability was 75% for both doses. Pain scores of 2 and 2.3 were observed following the 5 and 10 mg doses; tolerability scores were 4.4 and 4.7. Pain and tolerability scores returned to baseline within 10 h. Our formulation provided reasonable bioavailability, but was not well tolerated.

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Introduction

Frequently recurring seizures, prolonged seizures or status epilepticus are recognized as seizure emergencies (Pellock, 2007). Rapid treatment of such emergencies improves outcomes and minimizes associated morbidity (Alldredge et al., 2001). The standard approach to treating seizure emergen-

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cies is intravenous administration of antiseizure 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 (O'Dell et al., 2005, 2007; Driefuss et al., 1998; Kriel et al., 1991).

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 (Lowenstein and Alldredge, 1998). 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 (Driefuss et al., 1998; Cereghino et al., 1998). Nonetheless, older children and adults often refuse therapy because of social objections to this route of administration (Tatum, 2002; O'Regan et al., 1996; Wilson et al., 2004). 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 μ L/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 (Bhattacharyya et al., 2006). 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 (Bhattacharyya et al., 2006; Scheepers et al., 2000; Lahat et al., 2000).

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 (Cloyd, 2007). Our group has developed an investigational intranasal formulation of DZP. In this formulation, DZP dissolved in glycofurool is rapidly mixed with water, which is a poor solvent of DZP but is fully miscible with glycofurool. 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 mucosal membrane (Hou and Siegel, 2006).

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 mg as compared to a 5 mg intravenous dose. Safety and tolerability of this investigational intranasal formulation were also evaluated.

Methods

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 glycofurool 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 h prior to each admission through each 48 h blood draw. They were also instructed to not consume alcoholic beverages 24 h before and after drug administration study days. Subjects were admitted to the CRU where they would remain for 10 h.

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.

Study drugs

The intravenous formulation used in this study was the commercially available parental 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) cosolvent mixture of glycofurool and water (Hou and Siegel, 2006). 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 vol-

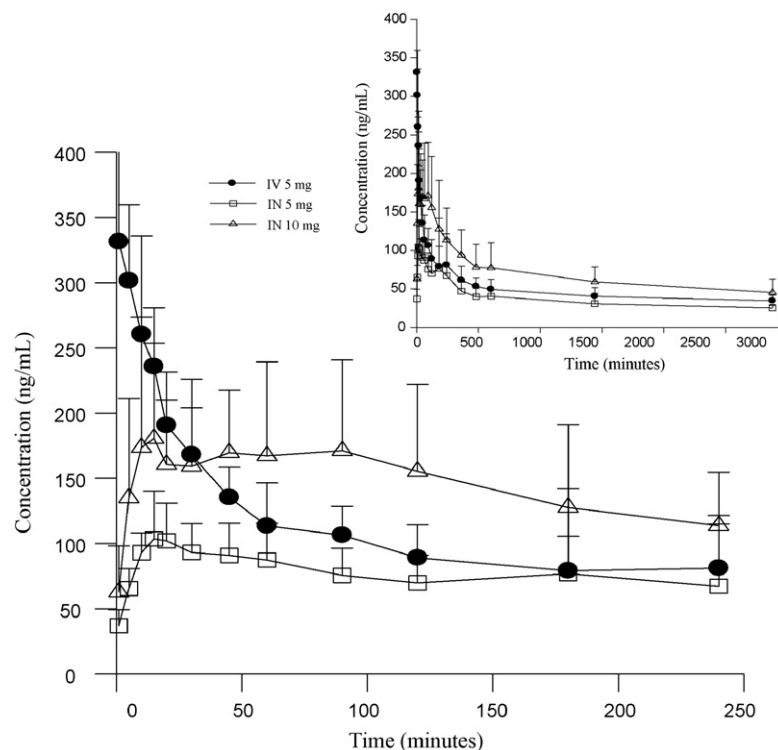


Figure 1 Mean plasma diazepam-concentration time profiles after intravenous and intranasal administration in eight subjects (0–4 h). Inset shows the complete profile (0–48 h).

ume instilled in the other nostril. The 10 mg dose was divided into two equal volumes and instilled into each nostril.

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 mm × 75 mm glass tube. Following addition of NaOH (200 μ L, 0.05 N) and the internal standard lorazepam (200 μ L, 1 mg/mL), the solution was mixed well. DZP and lorazepam 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 μ L 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 μ L of the HPLC mobile phase, consisting of 40% acetonitrile and 60% phosphate buffer (pH 6.0). After 30 s of vortex mixing, 50 μ L 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 μ L.

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 vari-

ation were 5.8% and 6.5%. The mean coefficients of variation for the intraday variation of the DZP quality control samples were 7.7%.

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 (λ_z) 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 (AUC) 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.

Safety and tolerability assessment

Safety was assessed based on the frequency of adverse events and serious adverse events, changes in vital signs, and

Table 1 Mean $\pm$ SD of DZP pharmacokinetic parameters following IV and IN administration.

Pharmacokinetic parameter	5 mg IV dose, mean $\pm$ SD	5 mg IN dose, mean $\pm$ SD	10 mg IN dose, mean $\pm$ SD	Ratio ^a	Dose corrected ratio ^b	90% confidence interval ^b
C_{max} , ng/mL	—	134.3 $\pm$ 61.9	247.6 $\pm$ 60.9	1.84	0.92	0.74, 1.36
AUC_{0-1h} , ng h/mL	187.3 $\pm$ 23.4	88.6 $\pm$ 16.0	157.6 $\pm$ 41.2	1.77	0.88	0.67, 1.45
AUC_{0-last} , ng h/mL	2255.4 $\pm$ 628.9	1710.8 $\pm$ 540.5	3361.8 $\pm$ 916.1	1.96	0.98	0.88, 1.16
$AUC_{0-\infty}$, ng h/mL	4484.0 $\pm$ 1275.4	3295.0 $\pm$ 1134.9	7739.6 $\pm$ 3645.2	2.34	1.17	0.73, 2.07
t_{max} , min (median)	—	20 (15–180)	30 (10–120)	—	—	—
$t_{1/2}$, h	48.3 $\pm$ 17.8	49.1 $\pm$ 20.45	57.0 $\pm$ 28.0	—	—	—
F ($AUC_{0-\infty IN}$) / ($AUC_{0-\infty IV}$)	—	75 $\pm$ 41%	74 $\pm$ 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.**Table 2** Mean AUC_{IN}/AUC_{IV} ratios (%) at different time points after intranasal administration of 5 mg and 10 mg diazepam to eight healthy volunteers.

AUC_{IN}/AUC_{IV} (%), time (h)				
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.

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 min prior to drug administration and 0, 5, 15, 60 min and 10 h 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 (Dingemanse et al., 2000). 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, 0, 5, 15 and 600 min. A composite pain score was calculated as the average of scores for the 3 pain characteristics.

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.

Results

Pharmacokinetics

Eight subjects (6 male and 2 female; mean age, 28.3 years) were enrolled and completed the study. As shown in Fig. 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 1). Both t_{max} (median range, 20–30 min) and $t_{1/2}$ (mean range, 49.1–57.0 h) were comparable following the intranasal doses. Table 2 shows the ratio, expressed as a percent, of drug exposure after intranasal administration relative to intravenous dose at different time points.

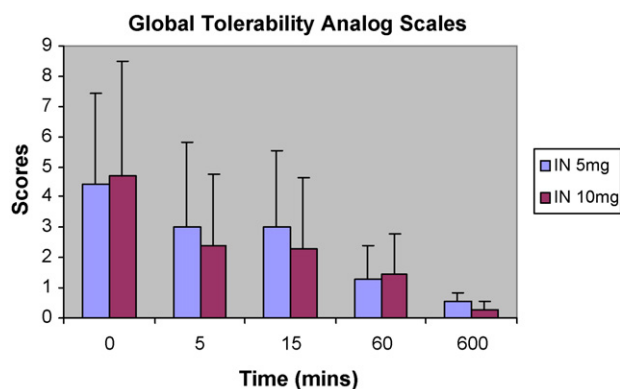


Figure 2 Comparison of mean global tolerability scores after IN administration ($n=8$). Baseline scores were zero (0) for all subjects.

Crossover, sequence or period effects were not significant.

Safety and tolerability

No unanticipated adverse events were reported by subjects following intranasal administration of DZP. All subjects, however, reported swallowing a portion of the intranasal 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 min, scores decreased to 3.0 and 2.5 respectively, and returned to baseline by 10 h (Fig. 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.

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. Posterior nasal drainage and watery eyes were reported by all subjects.

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 min, indicating significant discomfort.

Given limited resources, we chose to maximize the number of subjects and limit blood sample collection to 48 h,

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 (Cloyd et al., 1998). 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 1). The average bioavailability, F , was 75 and 74% for the 5 and 10 mg doses respectively. Although intranasal 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 2. The results for exposure in the first 60 min were comparable with the results demonstrated previously by Lindhardt et al. (2001).

The pharmacokinetic parameters obtained following intranasal 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. (1999) 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. Blood samples were collected for 5 h following drug administration. The mean bioavailability was $50.4 \pm 23.3\%$ with a t_{max} of 18 ± 11 min. Gizurarson reported that all subjects complained of nasal discomfort immediately following drug administration which resolved within 30 min. Lindhardt et al. evaluated an intranasal DZP formulation in polyethylene glycol 300 in 7 healthy volunteers. Using a crossover design, they compared 4 and 7 mg intranasal doses with a 5 mg intravenous dose and collected blood samples for 60 min after drug administration. They reported a ratio of dose normalized AUC_{IN}/AUC_{IV} in 60 min to be 45 and 42%, a C_{max} of 99 and 179 ng/mL and a t_{max} of 18 and 42 min for the 4 and 7 mg doses, respectively (Lindhardt et al., 2001). 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 (Lau and Slattery, 1989).

Our preliminary data regarding intranasal DZP administration compares favorably with studies involving rectal administration. Milligan et al. (1982) 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. They found that the onset of a reduction in spike counts occurred approximately 15–20 min 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 h. A bioequivalence study comparing an 7.5 mg IV dose of DZP injectable solution with a 15 mg dose of a commercial rectal formulation (Diasat®) found that Diasat® had a mean absolute bioavailability of 90% and reached an initial peak concentration of approximately 375 ng/mL at 45 min (Cloyd et al., 1998). In comparison to the studies with rectal DZP, our investigational intranasal formulation attained concentrations greater than 200 ng/mL at 15 min 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 1). The double peaks also occur with rectally administered diazepam (Remy et al., 1992). Further modeling of the absorptive processes to assess fractions of dose absorbed via each phase (1st peak and 2nd peak) of absorption would provide information useful in the optimization of a DZP nasal delivery system. Diazepam exhibits highly variable pharmacokinetics (Kaplan et al., 1973; Schmidt, 1995). 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 intranasal formulation caused considerable, but transient, pain. Similar findings have been reported in other intranasal DZP studies as well as with intranasal midazolam (Bjorkman et al., 1997; Ivaturi et al., 2009; Loftsson et al., 2001). Considering the severity of the clinical situation, the benefit of intranasal 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 (Hou and Siegel, 2006). It is also recognized that the influence of glycofurool on the integrity of the nasal mucosa is not presently understood, and that irritation by glycofurool 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.

Acknowledgements

We would like to thank Usha Mishra for her help with the DZP assay. We would also like to acknowledge Parents Against

Childhood Epilepsy (PACE), the Epilepsy Foundation and Valeant Pharmaceuticals for funding this project.

References

- Allredge, B.K., Gelb, A.M., Isaacs, S.M., et al., 2001. A comparison of lorazepam, diazepam, and placebo for the treatment of out-of-hospital status epilepticus. *N. Engl. J. Med.* 345, 631–637.
- Bhattacharyya, M., Kalra, V., Gulati, S., 2006. Intranasal midazolam vs rectal diazepam in acute childhood seizures. *Pediatr. Neurol.* 34, 355–359.
- Bjorkman, S., Rigemar, G., Idvall, J., 1997. Pharmacokinetics of midazolam given as an intranasal spray to adult surgical patients. *Br. J. Anaesth.* 79, 575–580.
- Cereghino, J.J., Mitchell, W.G., Murphy, J., Kriel, R.L., Rosenfeld, W.E., Trevathan, E., 1998. Treating repetitive seizures with a rectal diazepam formulation: a randomized study. The North American Diastat Study Group. *Neurology* 51, 1274–1282.
- Cloyd, J.C., 2007. Pharmacologic considerations in the treatment of repetitive or prolonged seizures. *J. Child Neurol.* 22, 475–525.
- Cloyd, J.C., Lalonde, R.L., Beniak, T.E., Novack, G.D., 1998. A single-blind, crossover comparison of the pharmacokinetics and cognitive effects of a new diazepam rectal gel with intravenous diazepam. *Epilepsia* 39, 520–526.
- Dingemans, J., Soubrouillard, C., Paris, J., Pisano, P., Blin, O., 2000. Pronounced effect of caprylocaproyl macrogolglycerides on nasal absorption of IS-159, a peptide serotonin 1B/1D-receptor agonist. *Clin. Pharmacol. Ther.* 68, 114–121.
- Driefuss, F., Rosman, N., Cloyd, J., et al., 1998. A comparison of rectal diazepam gel and placebo for acute repetitive seizures. *N. Engl. J. Med.* 338, 1869–1875.
- Gizurarson, S., Gudbrandsson, F.K., Jonsson, H., Bechgaard, E., 1999. Intranasal administration of diazepam aiming at the treatment of acute seizures: clinical trials in healthy volunteers. *Biol. Pharmaceut. Bull.* 22, 425–427.
- Hou, H., Siegel, R.A., 2006. Enhanced permeation of diazepam through artificial membranes from supersaturated solutions. *J. Pharmaceut. Sci.* 95, 896–905.
- Ivaturi, V., Riss, J., Kriel, R., Cloyd, J., 2009. Pharmacokinetics and tolerability of intranasal diazepam and midazolam in healthy adult volunteers. *Acta Neurologica Scandinavica*, in press.
- Kaplan, S.A., Jack, M.L., Alexander, K., Weinfeld, R.E., 1973. Pharmacokinetic profile of diazepam in man following single intravenous and oral and chronic oral administrations. *J. Pharmaceut. Sci.* 62, 1789–1796.
- Kriel, R., Cloyd, J., Hadsall, R., Carlson, A., Floren, K., Jones-Saete, C., 1991. Home use of rectal diazepam for cluster and prolonged seizures: efficacy, adverse reactions. Quality of life and cost analysis. *Pediatr. Neurol.* 7, 13–17.
- Lahat, E., Goldman, M., Barr, J., Bistrizter, T., Berkovitch, M., 2000. Comparison of intranasal midazolam with intravenous diazepam for treating febrile seizures in children: prospective randomised study. *BMJ* 321, 83–86.
- Lau, S., Slattey, J., 1989. Absorption of diazepam and lorazepam following intranasal administration. *Int. J. Pharmaceut.* 54, 171–174.
- Lindhardt, K., Gizurarson, S., Stefansson, S.B., Olafsson, D.R., Bechgaard, E., 2001. Electroencephalographic effects and serum concentrations after intranasal and intravenous administration of diazepam to healthy volunteers. *Br. J. Clin. Pharmacol.* 52, 521–527.
- Loftsson, T., Gudmundsdottir, H., Sigurjonsdottir, J.F., et al., 2001. Cyclodextrin solubilization of benzodiazepines: formulation of midazolam nasal spray. *Int. J. Pharm.* 212, 29–40.
- Lowenstein, D.H., Alldredge, B.K., 1998. Status epilepticus. *N. Engl. J. Med.* 338, 970–976.

- Milligan, N., Dhillon, S., Oxley, J., Richens, A., 1982. Absorption of diazepam from the rectum and its effect on interictal spikes in the EEG. *Epilepsia* 23, 323–331.
- O'Dell, C., Shinnar, S., Ballaban-Gil, K.R., et al., 2005. Rectal diazepam gel in the home management of seizures in children. *Pediatr. Neurol.* 33, 166–172.
- O'Dell, C., Wheless, J.W., Cloyd, J., 2007. The personal and financial impact of repetitive or prolonged seizures on the patient and family. *J. Child Neurol.* 22, 615–705.
- O'Regan, M.E., Brown, J.K., Clarke, M., 1996. Nasal rather than rectal benzodiazepines in the management of acute childhood seizures? *Dev. Med. Child Neurol.* 38, 1037–1045.
- Pellock, J.M., 2007. Overview: definitions and classifications of seizure emergencies. *J. Child Neurol.* 22, 95–135.
- Remy, C., Jourdil, N., Villemain, D., Favel, P., Genton, P., 1992. Intrarectal diazepam in epileptic adults. *Epilepsia* 33, 353–358.
- Scheepers, M., Scheepers, B., Clarke, M., Comish, S., Ibitoye, M., 2000. Is intranasal midazolam an effective rescue medication in adolescents and adults with severe epilepsy? *Seizure* 9, 417–422.
- Schmidt, D., 1995. Benzodiazepines: diazepam. In: Levy, R.H., Mattson, R.H., Meldrum, B.S. (Eds.), *Antiepileptic Drugs*. Raven Press, New York, pp. 705–724.
- Tatum, W., 2002. Adult perceptions of emergency rectal medications for refractory seizures. *Epilepsy Behav.* 3, 535–538.
- Wilson, M.T., Macleod, S., O'Regan, M.E., 2004. Nasal/buccal midazolam use in the community. *Arch. Dis. Child* 89, 50–51.