Received: 18 October 2019 | Revised: 25 January 2020 | Accepted: 27 January 2020

DOI: 10.1111/epi.16449

FULL-LENGTH ORIGINAL RESEARCH

Epilepsia

Bioavailability and safety of diazepam intranasal solution compared to oral and rectal diazepam in healthy volunteers

R. Edward Hogan[1] | Barry E. Gidal[2] | Barry Koplowitz[3] | Luana P. Koplowitz[3] | Richard E. Lowenthal[4] | Enrique Carrazana[5]

[1]Department of Neurology, Washington University in St Louis School of Medicine, St Louis, Missouri

[2]School of Pharmacy, University of Wisconsin, Madison, Wisconsin

[3]DUCK FLATS Pharma, Elbridge, New York

[4]Pacific Link Consulting, San Diego, California

[5]Consultant for Neurelis, Miami, Florida

Correspondence
R. Edward Hogan, Department of Neurology, Washington University in St Louis, 4921 Parkview Place Floor 6, Suite C, St Louis, MO 63110.
Email: hoganre@wustl.edu

Funding information
Neurelis, Inc.

Abstract

Objective: The study assesses the bioavailability of diazepam after intranasal administration (diazepam nasal spray) in healthy volunteers. Comparative agents were diazepam rectal gel, which served as the regulatory reference product; and oral diazepam, a product with decades of clinical use. Tolerability of diazepam nasal spray was also assessed.

Methods: This was a phase 1, open-label, randomized, single-dose, three-treatment, three-period, six-sequence crossover study in 48 healthy adult subjects that consisted of a screening period, a baseline period, and an open-label treatment period. Interperiod intervals were at least 28 days.

Results: Forty-eight healthy volunteer subjects were enrolled, two of whom discontinued before receiving study medication. For all routes of administration, the onset of diazepam absorption was rapid, with measurable concentrations of drug present by the first sample time point. The t_{max} (time to reach maximum plasma concentration) was similar for diazepam nasal spray and diazepam rectal gel, both of which were slower than oral diazepam in fasted individuals. Variability (as defined by % coefficient of variation of geometric mean) in peak plasma concentration and area under the curve$_{0\text{-}\infty}$ was lowest with oral diazepam, followed by diazepam nasal spray, with diazepam rectal gel showing the greatest variability. Overall, 131 treatment-emergent adverse events (TEAEs) were considered mild (42 subjects, 91.3%), four TEAEs were considered moderate (four subjects, 8.3%), and no TEAEs were considered severe. The most commonly reported TEAE was somnolence at 56.5% (26/46) during diazepam nasal spray treatment, 89.1% (41/46) with the rectal diazepam gel treatment, and 82.6% (38/46) with oral diazepam treatment. No nasal irritation was observed for the majority of the subjects at any time point after administration, with no score higher than 2 ("minor bleeding that stops within 1 minute").

Significance: Diazepam nasal spray shows predicable pharmacokinetics and represents a potential novel therapeutic approach to control bouts of increased seizure activity (cluster seizures, acute repetitive seizures).

Portions of this work were previously presented at the Annual Meeting of the American Epilepsy Society (New Orleans, Louisiana, November 30 to December 4, 2018).

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2020 The Authors. *Epilepsia* published by Wiley Periodicals, Inc. on behalf of International League Against Epilepsy.

NEURELIS, INC., EX2017, page 1
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00466, U.S. PATENT 11,793,786

KEYWORDS
acute repetitive seizures, bioavailability, diazepam

1 | INTRODUCTION

Bouts of increased seizure activity often referred to as cluster or acute repetitive seizures (cluster seizures) are a challenge to the patient, care partner, and physician. The intravenous formulation of diazepam has been used for >30 years in the treatment of seizure emergencies, including status epilepticus. However, the current standard of care for cluster seizures is a rectal gel formulation of diazepam (Diastat). Although diazepam rectal gel is safe and effective for use in cluster seizures, there are drawbacks to its use.[1,2]

As recently reviewed by Kapoor et al,[3] the intranasal route has many potential benefits for treatment of seizures. To that end, a novel intranasal formulation of diazepam was developed using a nonionic alkylglycoside surfactant (Intravail A3, dodecyl-β-D-maltopyranoside [DDM],[4] and tocopherol). As described by Lipton et al,[5] DDM alters mucosal viscosity and membrane fluidity to loosen cell-cell junctions. DDM induces a rapid and reversible decrease in transepithelial/transendothelial electrical resistance values, enhancing the permeation of the paracellular marker [3H]mannitol, resulting in changes to the tight junctions to facilitate absorption.[6] The present formulation of diazepam, NRL-1 (diazepam nasal spray, Valtoco) is prepared in a container-closure system designed for intranasal delivery. Previous preclinical safety and pharmacokinetic studies of diazepam nasal spray, as well as pharmacokinetic studies in normal volunteers, guided the selection of the optimal formulation for clinical use. Agarwal et al evaluated diazepam nasal spray in a single-dose pharmacokinetic study in healthy volunteers. They found that the solution formulation of intranasal diazepam (the same as NRL-1) had a high bioavailability and was well tolerated.[2]

The objective of the present study was to assess the bioavailability of diazepam after intranasal administration (diazepam nasal spray) in healthy volunteers. Comparative agents were diazepam rectal gel (Diastat), which served as the regulatory reference product, and oral diazepam, a product with decades-long clinical use and essentially complete absorption in fasted individuals.[7] Tolerability of diazepam nasal spray was also assessed.

Key Points

- Diazepam nasal spray demonstrated an acceptable safety profile
- There was less variability in the bioavailability of diazepam by the intranasal route than by the rectal route
- Intravail, an absorption enhancer, provides therapeutic nasal dosing of intranasal diazepam with comparable bioavailability to rectal diazepam with no damage to the nasal mucosa
- Subjects who received diazepam nasal spray experienced less somnolence than those receiving diazepam rectal gel or oral diazepam
- Diazepam nasal spray represents a potential novel therapeutic approach to control acute cluster seizures

2 | MATERIALS AND METHODS

2.1 | Subjects

This was a phase 1, open-label, randomized, single-dose, three-treatment, three-period, six-sequence crossover study in 48 healthy adult subjects that consisted of a screening period, a baseline period, and an open-label treatment period. Interperiod intervals were at least 28 days. Individuals with any contraindication to diazepam, comorbid diseases, or concomitant medications, and those unlikely to be adherent to study procedures were excluded from study participation. The study was approved by Novum Independent Institutional Review Board (Pittsburg, PA), and all subjects gave written informed consent.

2.2 | Pharmacokinetic and statistical analysis

Diazepam nasal spray and diazepam rectal gel (Diastat, Valeant Pharmaceuticals) were dosed based upon the approved doses for diazepam rectal gel and by weight categories, with subjects weighing 51-75 kg ("low") receiving 15 mg of diazepam, and subjects weighing 76-111 kg ("high") receiving 20 mg of diazepam. Diazepam tablets (Valium, Roche/Genentech) at 10 mg were used as a reference comparator.

Subjects were admitted to the study unit no later than 19:00 hours of the evening prior to study drug administration and remained in the unit until after the 24-hour blood sample was collected. All subjects then returned to the study unit for all subsequent assessments for the treatment. At check-in for each treatment period, a urine sample was collected from all subjects to test for drugs of abuse. A urine sample was collected from all

15281167, 2020, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.16449, Wiley Online Library on [16/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

NEURELIS, INC., EX2017, page 2
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00466, U.S. PATENT 11,793,786

female subjects, regardless of childbearing potential, for a urine pregnancy test. A meal was served the evening of check-in. All subjects were then required to fast for at least 10 hours prior to dosing and for 4 hours thereafter to avoid any influence on the pharmacokinetic outcomes. No food intake was permitted after 21:00 hours (9 PM). Drinking water was available and allowed ad libitum during the study except for 1 hour prior to study drug dose administration through 1 hour after dose administration. Standard meals were provided at approximately 4 and 10 hours after drug administration and at appropriate times thereafter.

A Fleet's enema was administered between 22:00 and 24:00 hours the night before dosing diazepam rectal gel. A second Fleet's enema was administered between 07:00 and 09:00 hours in the morning prior to dosing with diazepam rectal gel, which was administered approximately 1 hour after administration of the Fleet's enema and subsequent bowel movement.

Blood samples were collected as follows: baseline (predose), and at 10, 20, 30, and 45 minutes, and at 1, 1.25, 1.5, 1.75, 2, 4, 8, 12, 24, 36, 48, 72, 96, 144, 192, and 240 hours after dosing.

The pharmacokinetic parameters peak plasma concentration (C_{max}), area under the curve $(AUC)_{0\text{-}t}$, and $AUC_{0\text{-}\infty}$ for diazepam and desmethyldiazepam were calculated as follows: C_{max} was obtained directly from the data without interpolation; $AUC_{0\text{–}t}$ was calculated by the linear up log down method; $AUC_{0\text{–}\infty}$ was calculated as $AUC_{0\text{-}t} + C_{last}/\lambda z$, were C_{last} is the last measurable plasma concentration and λz is the terminal first-order elimination rate constant. Pharmacokinetic values were compared among treatments using an analysis of variance model, with treatment, period, sequence, and subject within sequence as the classification variables using the natural logarithms of the data. Confidence intervals (90%) were constructed for the geometric mean ratios, diazepam nasal spray to diazepam rectal gel and diazepam nasal spray to oral diazepam, of C_{max} and AUC using the log-transformed data and the two 1-sided t tests procedure.

The point estimates and confidence limits were converted back to the original scale. Comparability between diazepam nasal spray and diazepam rectal gel, and diazepam nasal spray and oral diazepam, was assessed from the geometric mean ratios and 90% confidence intervals for the three parameters (Phoenix WinNonlin v6.4 or higher, Certara Company). The relative bioavailability of diazepam intranasal relative to the rectal gel is based upon the ratio of the $AUC_{0\text{-}\infty}$.

A minimum of 36 subjects per treatment was determined a priori to detect a 5% difference in means in AUC assuming an intrasubject variability of 13% (half of the previously observed intersubject variability [26%])[2] with 80% power at $\alpha = 0.05$. Subjects who completed at least two treatments, one of which must have been the intranasal product, were included in the primary pharmacokinetic analyses.

The lower limits of quantitation of the liquid chromatography-tandem mass spectrometry bioanalytical assay for diazepam and desmethyldiazepam (also known as nordiazepam) were 1.0 and 0.10 ng/mL, respectively.

2.3 | Safety analysis

Safety measures included adverse events, physical examinations, vital sign measurement, electrocardiograms, and clinical laboratory tests. Objective evaluations of nasal irritation were assessed after each administration of study drug using a 6-point (0-5) score by a trained observer as follows: 0 = normal appearing mucosa, no bleeding; 1 = inflamed mucosa, no bleeding; 2 = minor bleeding that 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 that requires medical intervention.[2] Irritation was assessed by evaluating the degree of mucosal inflammation and bleeding. The subjects were also required to report any incident of bleeding or inflammation in-between the actual evaluation time points.

Objective evaluations of sedation were made using a 6-point (0-5) sedation scoring system to assess the degree of drowsiness of the subjects after each administration of study drug at 0.25, 0.5, 1, 2, 4, 6, and 8 hours postdose (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).[2]

A visual analog scale consisting of a 100-mm horizontal line was used to assess acute pain intensity following each administration of study drug.

3 | RESULTS

3.1 | Disposition

Forty-eight healthy volunteer subjects were enrolled, two of whom discontinued before receiving study medication. Forty-six subjects received at least one dose of diazepam nasal spray. An additional two subjects discontinued during the study for reasons unrelated to study medication; thus, 44 subjects completed all assessments.

3.2 | Demographics

Thirty-eight of the dosed subjects enrolled were black (38 of 48 subjects, 79.2%), six were white (six of 48 subjects, 12.5%), one was Asian (1 of 48 subjects, 2.1%), and three

15281167, 2020, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.16449, Wiley Online Library on [16/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

NEURELIS, INC., EX2017, page 3
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00466, U.S. PATENT 11,793,786

were other (three of 48 subjects, 6.3%). Eight subjects were Hispanic or Latino (eight of 48 subjects, 16.7%) and 40 were non-Hispanic or Latino (40 of 48 subjects, 83.3%). Subjects ranged in age from 18 to 55 years. There were 52.1% (25 of 48) male subjects and 47.9% (23 of 48) female subjects. Twenty subjects with a body weight of 51-75 kg and 28 subjects with a body weight of 76-111 kg were included in the safety analysis data set (Table 1).

3.3 | Pharmacokinetics

Mean blood levels of diazepam for each treatment are shown in Figure 1 (hours 0-2).

For both nasal and rectal routes of administration, the onset of diazepam absorption appeared to be rapid, with measurable concentrations of drug being observed by the first sample time point. The t_{max} (time to reach maximum plasma concentration) for diazepam nasal spray was comparable to that of diazepam rectal gel delivered under "ideal" conditions, that is, in a fasting state with an enema the night prior to dosing and a second enema 1 hour prior to dosing the following morning. $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ were similar, and thus only the latter is presented. Variability (as defined by % coefficient of variation of geometric mean) in C_{max} and $AUC_{0-\infty}$ was lowest with oral diazepam, followed by diazepam nasal spray, with diazepam rectal gel showing the greatest variability. Despite delivery under "ideal" conditions, diazepam rectal gel demonstrated significantly greater variability, with a number of outliers on both the high and low end of the plasma range. In all three routes of administration, the high-weight subjects showed greater variability compared to the low-weight subjects.

Compared to diazepam rectal gel, intranasal administration of diazepam nasal spray resulted in comparable $AUC_{0-\infty}$ values at the 20-mg dose but was slightly lower than diazepam rectal gel at the 15-mg dose. The slightly lower exposure at the 15-mg dose of diazepam nasal spray relative to diazepam rectal gel was possibly due to the variability in absorption of diazepam rectal gel. The 15-mg dose for diazepam rectal gel had higher exposure than the 20-mg dose. The range of exposures for diazepam nasal spray at both the 15-mg and 20-mg doses was well within the range of that from diazepam rectal gel. Diazepam rectal gel showed the greatest variability of exposures, accounting for both the highest and lowest exposures in both the 15-mg and 20-mg groups Oral diazepam, a positive control in this study, after a 10-hour fast, resulted in an $AUC_{0\text{-}\infty}$ between that of the nasal spray and the rectal gel, and a C_{max} greater than either of the two other routes (Table 2, Figure 2A-C).

TABLE 1 Demographics: safety population

	Low-weight group, 51-75 kg, N = 18[a]	High-weight group, 76-111 kg, N = 28[b]	Total, N = 46[c]
Sex, n (%)			
Female	10 (55.6)	13 (46.4)	23 (50.0)
Male	8 (44.4)	15 (53.6)	23 (50.0)
Age, y			
Mean (SD)	35.9 (10.19)	38.8 (9.58)	37.7 (9.81)
Race, n (%)			
White	3 (16.7)	3 (10.7)	6 (12.5)
Black or African American	12 (66.7)	24 (85.7)	36 (78.3)
Asian	1 (5.6)	0 (0.0)	1 (2.1)
Other	2 (11.1)	1 (3.6)	3 (6.3)
Ethnicity, n (%)			
Hispanic or Latino	5 (27.8)	3 (10.7)	8 (17.4)
Non-Hispanic or Latino	13 (72.2)	25 (89.3)	38 (82.6)

Abbreviations: N, total number of subjects within population; n, number of subjects with nonmissing information.

[a]All subjects in the low-weight group received treatment with 15 mg diazepam nasal spray (intranasal), 15 mg diazepam rectal gel, and 10 mg oral diazepam in a crossover design.

[b]All subjects in the high-weight group received treatment with 20 mg diazepam nasal spray (intranasal), 20 mg diazepam rectal gel, and 10 mg oral diazepam in a crossover design.

[c]Two low-weight subjects enrolled in the study, but withdrew prior to receiving diazepam nasal spray. Therefore, per protocol, the subjects were not included in the safety population.

15281167, 2020, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.16449, Wiley Online Library on [16/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

NEURELIS, INC., EX2017, page 4
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00466, U.S. PATENT 11,793,786

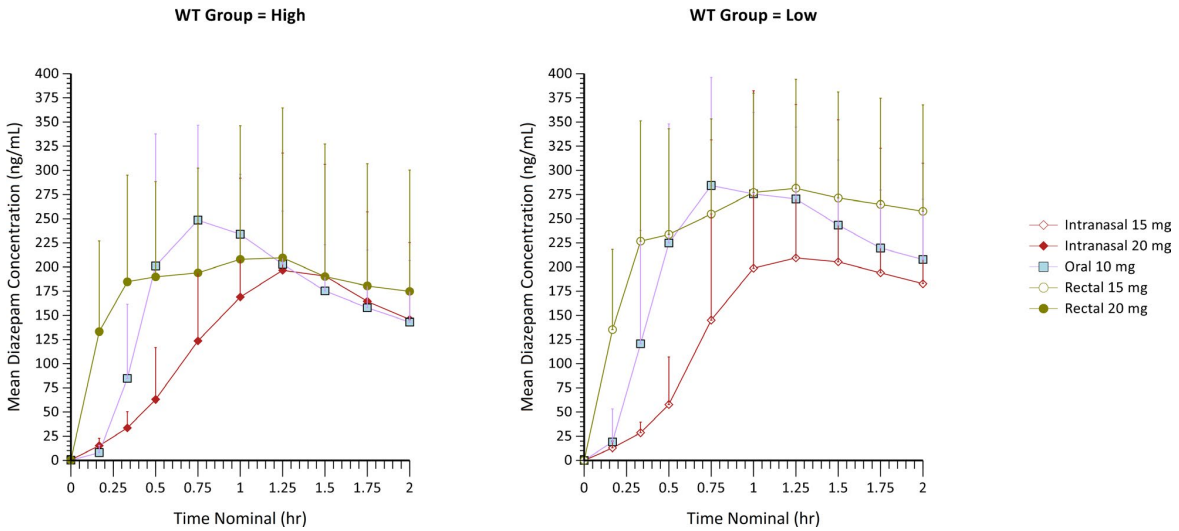

FIGURE 1 Mean plasma concentration-time profiles of diazepam (0-2 hours), mean ± SD. WT, weight

TABLE 2 Pharmacokinetic measures

	Nasal spray, 15 mg	Nasal spray, 20 mg	Rectal gel, 15 mg	Rectal gel, 20 mg	Oral, 10 mg-low	Oral, 10 mg-high
C_{max}, ng/mL						
n	17	28	17	27	17	28
Geometric mean	225.66	185.53	280.28	163.63	338.36	286.15
GCV%	60	84	109	229	25	30
90% CI	85-598	54-643	60-1314	16-1645	219-523	174-571
t_{max}, h						
n	17	28	17	27	17	28
Geometric mean	1.33	1.37	1.19	0.79	1	0.81
GCV%	47	63	117	92	63	40
Min	0.75	0.5	0.33	0.17	0.33	0.5
Median	1.25	1.25	1.25	1	1	0.75
Max	4	12	8	4	4	2
$AUC_{0-\infty}$, h ng/mL						
n	17	28	17	27	17	28
Geometric mean	6999	8069	9953	7855	8268	7850
GCV%	44	81	83	170	44	38
90% CIs	3376-14 508	2410-27 011	2815-35 193	1075-57 403	3376-14 508	4168-14 782

Note: The proportion of data extrapolated for geometric means for $AUC_{0-\infty}$ ranged from 4% to 15%.

Abbreviations: AUC, area under the curve; CI, confidence interval; C_{max}, peak plasma concentration; GCV, geometric coefficient of variation; Max, maximum; Min, minimum; t_{max}, time to reach maximum plasma concentration.

A statistical comparison of nasal diazepam to rectal and oral diazepam in provided in Table 3. The relative bioavailability for the nasal product is similar to or greater than the rectal product in high-weight subjects, and less than the rectal product in low-weight subjects, and less than the oral product in both weight groups.

3.4 | Safety

Overall, 131 treatment-emergent adverse events (TEAEs) were mild (42 subjects, 91.3%), four TEAEs were moderate (four subjects, 8.3%), and no TEAEs were severe. No TEAEs were life-threatening and no SAEs or deaths occurred in the

15281167, 2020, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.16449, Wiley Online Library on [16/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

NEURELIS, INC., EX2017, page 5
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00466, U.S. PATENT 11,793,786

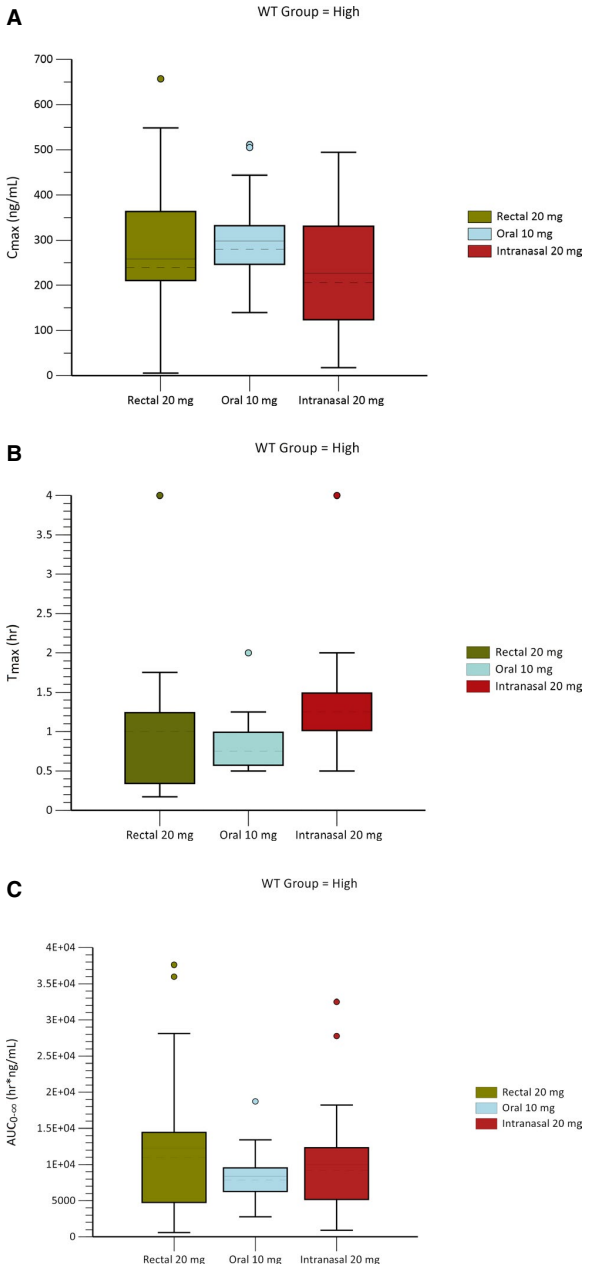

FIGURE 2 Comparative pharmacokinetic measures: box and whiskers plots, mean ± SD. AUC, area under the curve; C_{max}, peak plasma concentration; T_{max}, time to reach maximum plasma concentration; WT, weight

study. Although 100% of the subjects in the study experienced at least one TEAE during the study, the frequency of TEAE occurrences was less in the diazepam nasal spray treatment group (28 subjects, 60.9%) compared to diazepam rectal gel (43 subjects, 93.5%) and oral diazepam (39 subjects, 84.8%; Table 4). No subjects were discontinued from the study due to a TEAE.

The most commonly reported TEAE was somnolence: 56.5% (26/46) with diazepam nasal spray treatment, 89.1% (41/46) with rectal diazepam gel treatment, and 82.6% (38/46) with oral diazepam treatment. For a full listing of all reported events, see Table 4.

There was no nasal irritation for the majority of the subjects at any time point after administration, with no score higher than 2 ("minor bleeding that stops within 1 minute").

For diazepam nasal spray and oral diazepam, all sedation scores were 0 at baseline, and 2 ("napping or sleeping, but easily awakened") or less (scale of 0-5) at all posttreatment visits. For diazepam rectal gel, the maximum sedation score was up to 4 ("sleeping, very difficult to awaken, promptly returns to sleep"). The mean sedation scores for diazepam nasal spray were approximately 0.5-1.0 units less than for diazepam rectal gel or oral diazepam.

There were no reports of pain after administration of diazepam nasal spray.

4 | DISCUSSION

Diazepam is a well-established medication, with proven efficacy in the treatment of epileptic seizures. However, there is a need for an ideal formulation of diazepam for administration in the acute setting. In the practical setting treating cluster seizures, the diazepam delivery method should have a broad spectrum of activity, intermediate duration of action, and rapid onset of action for cessation of seizure, and be easy and safe to use, with minimal discomfort. Other important characteristics include low inter- and intrapatient variability, availability of both adult and pediatric formulations, a long shelf life,[8] practical administration in the outpatient setting, good tolerability, and predictable pharmacokinetics. With respect to routes of administration, each has its advantages and disadvantages. For example, intravenous administration is rapidly delivered to the brain and can terminate a seizure, but most cluster seizures occur outside the hospital, precluding or delaying intravenous administration.[8] Oral administration may not be viable during an acute seizure due to slow absorption, choking hazard, or inability of the patient to swallow.[8] Rectal administration has administration challenges in the outpatient setting, especially in public settings. There is recent emphasis on development of intranasal delivery for drugs with poor solubility in the gastrointestinal system or extensive hepatic first-pass elimination.[9] The high vascularization of the nasal cavity provides potential rapid absorption, which avoids first-pass hepatic metabolism.[9–11] In a small retrospective study, the onset of antiseizure activity in treating status epilepticus in patients with stroke was faster with intranasal diazepam than with intravenous diazepam (3 vs 9.5 minutes).[9,12,13] Intranasal delivery also may be easier

15281167, 2020, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.16449, Wiley Online Library on [16/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

NEURELIS, INC., EX2017, page 6
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00466, U.S. PATENT 11,793,786

TABLE 3 Relative bioavailability: probability paired comparisons between treatment groups, C_{max} and $AUC_{0-\infty}$

Comparison	C_{max} Ratio	C_{max} 90% CI	$AUC_{0-\infty}$ Ratio	$AUC_{0-\infty}$ 90% CI
Low weight: nasal (15 mg) vs rectal (15 mg)	85%	57-126	74%	53-102
High weight: nasal (20 mg) vs rectal (20 mg)	118%	69-202	100%	65-102
Low weight: nasal (15 mg) vs oral (10 mg)	45%	33-59	60%	47-76
High weight: nasal (20 mg) vs oral (10 mg)	32%	25-41	50%	42-60

Note: Ratios and CIs were calculated using natural logarithmic transformations. Low weight = 51-75 kg; high weight = 76-111 kg.

Abbreviations: C_{max}, peak plasma concentration; AUC, area under the curve; CI, confidence interval.

TABLE 4 Summary of treatment-emergent adverse events by treatment reported by two or more subjects: safety population

System organ class preferred term	Nasal spray, n (%)	Rectal gel, n (%)	Oral, n (%)	Total, n (%)
Subjects with at least 1 adverse event	28 (60.9)	43 (93.5)	39 (84.8)	46 (100.0)
Nervous system disorders				
Somnolence	26 (56.5)	41 (89.1)	38 (82.6)	46 (100.0)
Headache	2 (4.3)	1 (2.2)	0 (0.0)	3 (6.5)
Investigations				
Urine analysis abnormal	1 (2.2)	0 (0.0)	4 (8.7)	5 (10.9)
Blood pressure decreased	0 (0.0)	3 (6.5)	1 (2.2)	4 (8.7)
Blood glucose increased	1 (2.2)	2 (4.3)	0 (0.0)	3 (6.5)
Blood pressure increased	1 (2.2)	0(0.0)	1 (2.2)	2 (4.3)
Reticulocyte count increased	0 (0.0)	0 (0.0)	2 (4.3)	2 (4.3)
Gastrointestinal disorders				
Nausea	2 (4.3)	0 (0.0)	0 (0.0)	2 (4.3)
Renal and urinary disorders				
Hematuria	1 (2.2)	1 (2.2)	0 (0.0)	2 (4.3)

Note: N = 46. MedDRA version 19.0 used for coding. Subjects with two or more adverse events in the same system organ class (or with the same preferred term) are counted only once for that system organ class (or preferred term).

than other routes for patients or caregivers.[12] Intranasal administration requires little training, is easily performed, and carries little risk of injury.[11]

Like any locally applied drug product, formulations of intranasal drugs must be carefully developed to optimize drug absorption and therapeutic efficacy and minimize unwanted side effects.[9] The volume feasible is about 100-150 μL per nostril, requiring a relatively potent molecule and highly bioavailable drug product.[9] One way to maximize the area over which drug is absorbed is to use a spray, which allows for solutions to be distributed on nonciliated mucosal areas (whereas drops are primarily distributed on ciliated surfaces). Therefore, positioning of the head is not crucial (drops require patient to hold their head back so as not to lose drug to environment or throat).[10]

Although intranasal delivery provides a potentially attractive route for diazepam delivery, the solubility and nasal mucosal permeability of diazepam present unique challenges for consistent drug delivery. Historically, after extensive pharmacokinetic evaluation, another diazepam nasal formulation showed inadequate bioavailability when compared to other diazepam delivery formulations.[14,15] Our study assesses the

15281167, 2020, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.16449, Wiley Online Library on [16/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

NEURELIS, INC., EX2017, page 7
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00466, U.S. PATENT 11,793,786

pharmacokinetics of diazepam after intranasal administration (diazepam nasal spray) in healthy volunteers, which is an important step in assessing its potential use for cluster seizures. We also directly compare pharmacokinetics of diazepam nasal spray with rectal diazepam (the currently US Food and Drug Administration–approved treatment for clusters) in the same subject cohort.

In a previous study,[2] diazepam nasal solution (NRL-1) was 97% bioavailable compared to intravenous diazepam (and no stratification by weight was given). In the present study, oral diazepam provided predictable bioavailability as judged by all three primary pharmacokinetic measures, particularly by t_{max}. There are some differences in bioavailability between studies that may be due, in part, to differences in subject body mass index (BMI). Accumulation of diazepam in subjects with higher body mass index has been reported. Diazepam is a lipophilic drug and thus absorbed into the fat and then later released slowly into the systemic circulation. Therefore, the overall AUC values between subjects of low BMI and high BMI in the current study were not meaningfully different.[16,17]

There was predictable bioavailability of diazepam nasal spray, although slightly less than the relevant comparison product, diazepam rectal gel. Although the mean exposures were slightly less with diazepam nasal spray, the range of exposures was more consistent among the subjects, and also within the range of exposures observed with diazepam rectal gel. There was less variability in the bioavailability of diazepam by the intranasal route than by the rectal route (% geometric coefficient of variation of AUC = 42%-66% compared to 87%-172%). Therefore, although the overall bioavailability of nasal delivery was approximately 60% compared to oral delivery, the overall consistency of bioavailability as measured by AUC suggests that nasal delivery of diazepam will enable reliable and predictable dosing.

Variability in exposure is a concern with rectal diazepam gel, with some doses producing very low plasma diazepam concentrations. This may lead to incomplete seizure treatment and the need for repeated dosing.[18,19]

We selected oral diazepam as a control in this study for pharmacokinetic purposes. This route provided a reference bioavailability as judged by all three primary pharmacokinetic measures, particularly t_{max}. Thus, one could consider that oral diazepam might be a good therapeutic alternative for the treatment of cluster seizures. However, the therapeutic utility of oral diazepam for the treatment of cluster seizures is limited. The subjects in the present study were fasted for 10 hours, which is an unlikely scenario in most patients with cluster seizures. The time to maximal absorption of oral diazepam in the fed state is delayed relative to the fasted state.[20] Furthermore, oral access during cluster ictal states is not reliable, especially if there is associated impairment of awareness during seizures that impairs swallowing of medication. Oral delivery in a patient in a confused state also risks aspiration. Thus, there is a need for a readily accessible alternative delivery routes.[17] Overall, in the study, diazepam nasal spray demonstrated an acceptable safety profile. The most commonly reported TEAE was somnolence. Subjects who received diazepam nasal spray experienced less somnolence and sedation than those receiving diazepam rectal gel or oral diazepam judged by both adverse event reports and somnolence scores (magnitude and duration).

Somnolence was less common with diazepam nasal spray (56.5%), as compared to rectal (89.1%) and oral delivery (82.6%). Clinical effects of diazepam relate to many factors, including previous exposure,[21] rate of escalation of serum concentration levels,[22] and age.[23] Because of these factors, as well as interindividual tolerance of diazepam side effects, there is no fixed diazepam plasma level associated with central nervous system depression.[23] Given the multiple factors related to clinical effects of diazepam, the etiology of less somnolence in the diazepam nasal spray group is uncertain. However, better consistency of absorption in the nasal spray group, as compared to the rectal gel delivery group, could result in better overall tolerability. Comparing the nasal spray with the oral delivery group, nasal delivery showed a longer t_{max}, which may be related to better tolerability due to less rapid escalation of serum concentrations.

The study design sought to provide the most favorable pharmacokinetic profile and variability attainable by oral and rectal administration by requiring pretreatment fasting (ideal for oral administration) and enemas (ideal for rectal administration). In everyday use, there is greater pharmacokinetic variability if oral diazepam is taken with food.[20,24,25] Also, rectal administration could be affected by the fecal content of the rectum, with a full rectum resulting in leakage of the medication. Despite employing ideal conditions for administration of rectal diazepam, our study shows marked variability in rectal diazepam pharmacokinetics. This is consistent with past studies of rectal diazepam, with some subjects showing negligible diazepam plasma levels after rectal diazepam administration.[18,19]

Safety and tolerability data from this study demonstrated that the use of intranasal diazepam (diazepam nasal spray) with a nonionic alkylglycoside surfactant could provide an alternative to rectal administration, which some patients regard as cumbersome, particularly in adults. In addition, in this healthy volunteer study, the safety and tolerability of diazepam nasal spray likewise compare favorably to oral diazepam, another diazepam formulation with a well-known and established safety profile.

As noted, the formulation of the diazepam nasal product was designed to decrease pharmacokinetic variability, and

15281167, 2020, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.16449, Wiley Online Library on [16/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

therefore provide a potential treatment with reliable bioavailability, in a convenient dosing form. Based upon the pharmacokinetic results, overall tolerability, and nasal safety, we conclude that this formulation may be a good addition to the armamentarium for the treatment of cluster seizures. Thus, diazepam nasal spray represents a potential novel therapeutic approach to control cluster seizures.

ACKNOWLEDGMENTS

This study was supported by Neurelis.

CONFLICT OF INTEREST

R.E.H. has received personal compensation for consulting, serving on a scientific advisory board, speaking, or other activities with SK Chemical. R.E.H. has received research support from UCB Pharmaceuticals, Neurelis, Biogen, and Engage Therapeutics. B.E.G. has received compensation for speaking from Eisai, Sunovion, Aquestive, and Greenwich, compensation for consulting for Eisai, Sunovion, Zogenix, SK Bioscience, and Greenwich, and research support from UCB. B.K. has received compensation as a consultant to Neurelis. L.P.K. has received compensation as a consultant to Neurelis. R.E.L. has received compensation as a consultant to Neurelis. E.C. has received personal compensation for consulting, serving on a scientific advisory board, speaking, or other activities from Neurelis, Marinus, Zogenix, Adamas, and Aeromics. E.C. has received compensation for serving on the Board of Directors of Marinus Pharmaceuticals and Hawaii Biotech. Gary D. Novack, PhD provided medical writing support, sponsored by Neurelis. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

ORCID

R. Edward Hogan https://orcid.org/0000-0003-2272-5005

REFERENCES

1. Haut SR, Seinfeld S, Pellock J. Benzodiazepine use in seizure emergencies: a systematic review. Epilepsy Behav. 2016;63:109–17.
2. Agarwal SK, Kriel RL, Brundage RC, Ivaturi VD, Cloyd JC. A pilot study assessing the bioavailability and pharmacokinetics of diazepam after intranasal and intravenous administration in healthy volunteers. Epilepsy Res. 2013;105:362–7.
3. Kapoor M, Cloyd JC, Siegel RA. A review of intranasal formulations for the treatment of seizure emergencies. J Control Release. 2016;237:147–59.
4. Maggio ET, Pillion DJ. High efficiency intranasal drug delivery using Intravail(R) alkylsaccharide absorption enhancers. Drug Deliv Transl Res. 2013;3:16–25.
5. Lipton RB, Munjal S, Brand-Schieber E, Rapoport AM. DFN-02 (sumatriptan 10 mg with a permeation enhancer) nasal spray vs placebo in the acute treatment of migraine: a double-blind, placebo-controlled study. Headache. 2018;58:676–87.
6. Munjal S, Brand-Schieber E, Allenby K, et al. A multicenter, open-label, long-term safety and tolerability study of DFN-02, an intranasal spray of sumatriptan 10 mg plus permeation enhancer DDM, for the acute treatment of episodic migraine. J Headache Pain. 2017;18:31.
7. Divoll M, Greenblatt DJ, Ochs HR, Shader RI. Absolute bioavailability of oral and intramuscular diazepam: effects of age and sex. Anesth Analg. 1983;62:1–8.
8. Maglalang PD, Rautiola D, Siegel RA, et al. Rescue therapies for seizure emergencies: new modes of administration. Epilepsia. 2018;59(Suppl 2):207–15.
9. Pires A, Fortuna A, Alves G, Falcão A. Intranasal drug delivery: how, why and what for? J Pharm Pharm Sci. 2009;12:288–311.
10. Kalviainen R. Intranasal therapies for acute seizures. Epilepsy Behav. 2015;49:303–6.
11. Kapoor M, Winter T, Lis L, Georg GI, Siegel RA. Rapid delivery of diazepam from supersaturated solutions prepared using prodrug/enzyme mixtures: toward intranasal treatment of seizure emergencies. AAPS J. 2014;16:577–85.
12. Benfield J, Musto A. Intranasal therapy to stop status epilepticus in prehospital settings. Drugs R D. 2018;18:7–17.
13. Inokuchi R, Ohashi-Fukuda N, Nakamura K, et al. Comparison of intranasal and intravenous diazepam on status epilepticus in stroke patients: a retrospective cohort study. Medicine (Baltimore). 2015;94:e555.
14. Henney HR, Sperling MR, Rabinowicz AL, Bream G, Carrazana EJ. Assessment of pharmacokinetics and tolerability of intranasal diazepam relative to rectal gel in healthy adults. Epilepsy Res. 2014;108:1204–11.
15. Sperling MR, Haas KF, Krauss G, et al. Dosing feasibility and tolerability of intranasal diazepam in adults with epilepsy. Epilepsia. 2014;55:1544–50.
16. Abernethy DR, Greenblatt DJ, Divoll M, Shader RI. Prolonged accumulation of diazepam in obesity. J Clin Pharmacol. 1983;23:369–76.
17. Tanimoto SA, Koplowitz LP, Lowenthal RE, et al. Evaluation of the pharmacokinetics and dose proportionality of diazepam after intranasal administration of NRL-1 to healthy volunteers. Clin Pharmacol Drug Dev. 2020. https://doi.org/10.1002/cpdd.767 [Epub ahead of print].
18. Garnett WR, Barr WH, Edinboro LE, Karnes HT, Mesa M, Wannarka GL. Diazepam autoinjector intramuscular delivery system versus diazepam rectal gel: a pharmacokinetic comparison. Epilepsy Res. 2011;93:11–6.
19. Ivaturi V, Kriel R, Brundage R, Loewen G, Mansbach H, Cloyd J. Bioavailability of intranasal vs. rectal diazepam. Epilepsy Res. 2013;103:254–61.
20. Genentech. Valium(R) (diazepam) package insert, 2016.
21. Hillestad L, Hansen T, Melsom H. Diazepam metabolism in normal man. II. Serum concentration and clinical effect after oral administration and cumulation. Clin Pharmacol Ther. 1974;16:485–9.
22. Hillestad L, Hansen T, Melsom H, Drivenes A. Diazepam metabolism in normal man. I. Serum concentrations and clinical effects after intravenous, intramuscular, and oral administration. Clin Pharmacol Ther. 1974;16:479–84.

15281167, 2020, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.16449, Wiley Online Library on [16/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

23. Reidenberg MM, Levy M, Warner H, et al. Relationship between diazepam dose, plasma level, age, and central nervous system depression. Clin Pharmacol Ther. 1978;23:371–4.
24. Greenblatt DJ, Allen MD, MacLaughlin DS, Harmatz JS, Shader RI. Diazepam absorption: effect of antacids and food. Clin Pharmacol Ther. 1978;24:600–9.
25. Yamazaki A, Kumagai Y, Fujita T, et al. Different effects of light food on pharmacokinetics and pharmacodynamics of three benzodiazepines, quazepam, nitrazepam and diazepam. J Clin Pharm Ther. 2007;32:31–9.

How to cite this article: Hogan RE, Gidal BE, Koplowitz B, Koplowitz LP, Lowenthal RE, Carrazana E. Bioavailability and safety of diazepam intranasal solution compared to oral and rectal diazepam in healthy volunteers. *Epilepsia*. 2020;61:455–464. https://doi.org/10.1111/epi.16449

15281167, 2020, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/epi.16449, Wiley Online Library on [16/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License