Pharmacokinetics and Pharmacodynamics of a New Intranasal Midazolam Formulation in Healthy Volunteers

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We evaluated the pharmacokinetics and pharmacodynamics of single 5-mg doses of midazolam after administration of a novel intranasal (IN) formula, IM, and IV midazolam in an open-label, randomized, 3-way cross-over study in 12 healthy volunteers. IN doses were delivered as 0.1-mL unit-dose sprays of a novel formulation into both naris. Blood samples were taken serially from 0 to 12 h after each dose. Plasma midazolam concentrations were determined by liquid chromatography/mass spectrometry/mass spectrometry. Noncompartmental analysis was used to estimate pharmacokinetic parameters. The mean midazolam bioavailabilities and % coefficient of variation were 72.5 (12) and 93.4 (12) after the IN and IM doses, respectively. Median time to maximum concentration was 10 min for IN doses. Adverse events were minimal with all routes of administration, but nasopharyngeal irritation, eyes watering, and a bad taste were reported after IN doses. Our results support further development of this novel midazolam nasal spray.

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Midazolam's potency and short duration of clinical activity make it an excellent drug for premedication, and its advantages have been reviewed (1). Although midazolam is marketed only in injectable and oral syrup formulations, it is the most extensively studied intranasal (IN) benzodiazepine. One survey reported that 8% of United States anesthesiologists have used IN midazolam, off-label, to premedicate pediatric patients preoperatively (2). The first clinical investigation of IN midazolam in children was reported by Wilton et al. (3). Another investigation achieved anxiolysis with IN midazolam with a mean onset of 15–20 min (4). Estimates of the bioavailability of midazolam following the IN route of administration have ranged from 50%–83% in human studies (5–11). Most studies have used a dilute aqueous midazolam injection solution that is not suitable for nasal administration because of low pH and suboptimal concentration causing nasal run-off.

The formulations used in the aforementioned trials are generally not appropriate for nasal administration. The formulations will be irritating because the aqueous solution is buffered to pH 3. Moreover, the formulae are too dilute for nasal administration. The adult nasal cavity can only receive and retain about 100–150 μL of liquid, requiring the dose of midazolam be solubilized within this volume. A nasal formula with a concentration of 2.5 mg per 100 μL is necessary to give a single spray per naris. Thus, the formulation methods must be considered when evaluating any estimates of the pharmacokinetics and pharmacodynamics in these reports. Given these findings, there appears to be an unmet medical need to develop an optimal midazolam formulation for nasal delivery.

The objectives of the study were to evaluate the bioavailability of a novel IN midazolam formulation and to compare the pharmacodynamic effects on psychomotor performance and subjective reporting of drug effect after 5 mg doses of midazolam via IN, IM, and IV routes of administration. The specific aims were to: 1) to obtain an IN bioavailability of more than or equal to 70% compared with an IV dose; 2) achieve maximum IN concentration within 10 min of administration; 3) observe sedative properties from IN administration within 10 min; and, 4) demonstrate a nonirritating, well-tolerated formula.

Absolute bioavailability of alternative drug delivery routes is frequently compared with an IV formula for a reference. Area under the concentration-time curve for each route is calculated. Bioavailability is simply the ratio of the IN to IV area under the curve, assuming the same dose was administered and clear-

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assumed that clearance remains constant within each subject across test arm investigations.

METHODS

Twelve nonsmoking, healthy subjects (6 men, 6 women) between the ages of 20 and 29 yr (22.3 ± 2.8 yr, mean ± SD) and weighing 60 to 92 kg (71.1 ± 10.4 kg) participated in this inpatient study. All subjects gave written informed consent for the study which was approved by the Medical IRB of the University of Kentucky. Eleven of the volunteers who enrolled in the study were Caucasian and one was part-Asian. Subjects were within 20% of ideal body weight in relation to height and elbow breadth and weighed at least 60 kg. The subjects were in good health and had no clinically significant previous nasal surgery or polyps or other physical abnormalities of the nose or any systemic medical illness. They abstained from alcohol and caffeine-containing beverages and prescription and nonprescription drugs that might interact with midazolam metabolism or nasal physiology 48 h before the dosing period and during the study. Subjects were admitted to the Clinical Research Center at University of Kentucky on the evening before each study day. The subjects fasted for 8 h before receiving the study drug and continued to fast for 2 h after drug administration.

A randomized, open-label, 3-way cross-over design was used. On three different occasions, separated by 1 wk, the subjects received a single dose of each of the following three treatments in random order, counterbalanced so that an equal number of subjects received each treatment first, second, or third:

- Treatment A: 5 mg IV of midazolam infused over 15 min
- Treatment B: 5 mg of IM midazolam
- Treatment C: 5 mg IN midazolam solution (2.5 mg/100 μL per sprayer/naris)

The 25 mg/mL IN midazolam formulation was prepared aseptically, creating a sterile product, under Good Manufacturing Practices conditions in the University of Kentucky College of Pharmacy Center for Pharmaceutical Science and Technology. The IN formulation, a nonaqueous solution containing midazolam 25 mg/mL, polyethylene glycol 400, butylated hydroxytoluene, saccharin, and propylene glycol, provided 2.5 mg of midazolam in 0.1 mL spray from a modified version of a commercially available unit-dose spray pump (Pfeiffer of America, Princeton, NJ, unit dose system). Commercially available midazolam (Versed® Injection; Roche Laboratories, Nutley, NJ) was purchased for comparative IV and IM administration.

Before study drug administration, subjects gently blew their noses. A physician administered a spray to each naris and the subjects remained in a semi-recumbent position, with the head of the bed elevated

midazolam in 10 mL sterile saline solution, was administered by infusion over a period of 15 min in an antecubital vein of the contralateral arm for blood sampling.

Serial blood samples were obtained through an indwelling venous catheter according to the following schedule: 0 (pre-dose), 5, 10, 20, 30, and 45 min, and 1, 1.5, 2, 3, 4, 8, and 12 h after drug administration. Venous blood samples were collected in 10-mL heparinized Vacutainer® tubes. After collection, the blood was centrifuged at 4°C, and the plasma was transferred to polypropylene tubes. The plasma was stored at or below −20°C at the study site until shipped to AAI Development Services, Inc., Kansas City Facility in Shawnee, KS for midazolam assay.

Plasma samples were analyzed by AAI International, a Good Laboratory Practices compliant laboratory, using liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) to determine the concentrations of midazolam, 1-hydroxymidazolam, and the added internal standard triazolam-d_4. Analytes were extracted from plasma using liquid phase extraction. They were separated using reverse phase high performance liquid chromatography on a 3-micron C18 column. The analytes were detected using a PE/Sciex API III+ LC/MS/MS system in multiple reaction monitoring (MRM) mode for the following precursor/products (m/z): midazolam, 326 and 291; 1-hydroxymidazolam, 342 and 203; and triazolam-d_4, 347 and 312; with retention times of 1.75, 1.53, and 1.50 min, respectively. The lower limit of detection for midazolam and 1-hydroxymidazolam was 0.50 ng/mL. The method was linear over a concentration range of 0.50–500.0 ng/mL using a 0.5-mL sample volume. Overall accuracy (inter-batch) was 89.3%–106.0% for midazolam and 93.3%–108.0% for 1-hydroxymidazolam. The inter batch overall precision (%CV) was 2.9%–9.4% for midazolam and 3.4%–113.0% for 1-hydroxymidazolam. The specificity, linearity, sensitivity, accuracy, precision, and stability of the data were within the Food and Drug Administration guidelines for Bioanalytical Method Validation. Freeze/thaw stability (3 cycles) and long-term sample storage (13 mo, −20°C) were tested and were acceptable.

A physician was in attendance for at least 4 h after each dose, and subjects were observed throughout the study session by a research nurse. Vital signs (arterial blood pressure, heart rate, respiratory rate) were measured before and at 10, 20, 30, 45, 60, 75, 90, 105 min and 2, 3, 4, 6, 8, and 12 h after each dose. Continuous pulse oximetry monitoring was done for all subjects for the first 6 h and as clinically indicated thereafter. Any observation of oxygen saturation <90% was recorded as an adverse event. In addition to spontaneously reported subjective symptoms, which were allowed at any time, subjects were also questioned as to their adverse event experience each time that vital

An otolaryngologist examined the nasal passages to evaluate development of local mucosal irritation, inflammation, bleeding, and excoriation or ulceration at screening, before dosing on each study day, at 2–4 h after each dose, and within 72 h after treatment.

Pharmacokinetic parameters were determined using standard noncompartmental methods (13) with log-linear least square regression analysis (weighting factor 1/Y) to determine the elimination rate constants (λ_Z) (WinNonlin version 3.2; Pharsight Corp., Palo Alto, CA). Time to and maximum plasma concentration (T_{max} and C_{max}), elimination half-life ($t_{1/2}$), area under the plasma concentration-time curve from time zero to infinity ($AUC_{0-\infty}$) were also calculated. The absolute bioavailability (F) for the IN and IM dosage forms was determined by the formula $F = AUC_{IN,0-\infty}/AUC_{IV,0-\infty}$ for the IN dose and $F = AUC_{IM,0-\infty}/AUC_{IV,0-\infty}$ for the IM dose.

The subjects completed assessments of drug-induced impairment including a Digit-Symbol Substitution Task (DSST), Visual Analog self-measures and the Stanford Sleepiness Scale (SSS) at 0 (1 h before dosing as a practice session), 10, 20, 30, and 45 min and 1, 1.5, 2, 3, 4, 6, 8, and 12 h (14,15).

The Visual Analog Scale (VAS) consisted of 10 statements ("Stimulated," "Sedated," "High," "Anxious," "Fatigued," "Hungry," "Headache," "Feel a drug effect," "Like the drug effect," and "Willing to take the drug again") that were presented sequentially above a 100-mm line labeled "not at all" on the left end and "extremely" on the right end.

For the SSS, subjects described their current level of sleepiness among the following options: "Feeling active and alert, wide awake," "Functioning at high level, but not peak, able to concentrate," "Relaxed, awake, not at full alertness, responsive," "A little foggy, not at peak, let down, " "Fogginess, beginning to lose interest in remaining awake, slowed down," "Sleepiness, prefer to be lying down, fighting sleep, woozy," or "Almost in reverie, sleep onset soon, lost struggle to remain awake" (15).

DSST performance was analyzed according to total trial rate, correct trial rate, incorrect trial rate, and percentage of trials that were correct. Ratings on the VAS were scored based on the number of discrete units between the subject's rating and the left endpoint on each 100-unit scale. When subjects failed to initiate the DSST or the SSS scales because they were asleep, they were assigned a "0" for total response rate and a "7" for the sleep rating. No other substitutions for missing values were possible given the nature of the other quantitative measures.

Dependent variables were analyzed as a function of route and time after dose. Analyses of peak effects, time to peak effects, and area under the curve for response (AUCR), using linear trapezoidal rules, were also evaluated. Separate AUCR analyses were completed between baseline and 4 h after dose ($AUCR_{0-4}$; between baseline and 12 h after dose ($AUCR_{0-12}$, i.e., over the full time course).

Statistical analyses were performed using Proc GLM with PC SAS (version 6.12; SAS Institute, Cary, NC). An analysis of variance model with factors sequence, subject nested within sequence, treatment, and period, was performed for peak effects, time to peak effects, and AUCR. Gender and route were used to analyze peak effects, time to peak effects, and AUCR. For VAS analysis, given the number of missing values because of subjects being asleep, degrees of freedom were inconsistent across variables and conditions. The least square means for each treatment group and pairwise comparisons between treatment groups were presented. To assess the gender effect and gender-by-route interaction, an analysis of variance with factors gender, period, route, and gender-by-route interaction was performed.

Descriptive statistics, mean and standard deviation, were calculated for the pharmacokinetic parameters. The statistical tests were two-sided with a critical level of 0.05. An analysis of variance with factors sequence, subject nested within sequence, treatment, and period was performed for log-transformed AUC and C_{max}. The carryover effect for the three treatments was analyzed using an analysis of variance of log-transformed AUC and C_{max}. Analysis of variance with factors sequence, subject nested within sequence, treatment and period for sequence ($P > 0.1$) indicated that carryover effects were not significant. The difference in T_{max} values between the IN and IM treatment was compared using an analysis of variance of rank-transformed T_{max}.

RESULTS

All 12 subjects completed the study without clinically significant or serious adverse events. There were no clinically relevant changes in physical examination, nasal evaluations, or laboratory tests. Doses of the study drug were well tolerated and events were mild to moderate and temporary (2–90 min). The most common adverse events seen with nasal administration were ones frequently associated with midazolam and nasal administration, e.g., eye watering (58% of IN doses), dizziness (25%, 25%, and 17% for IV, IM, and IN doses, respectively), bad taste (25% after IN doses), and nasal congestion/feeling nasopharyngeal irritation (100% after IN doses). Three of 12 subjects reported a bad taste immediately after IN administration of midazolam that lasted 2–20 min. Four of 12 subjects noted blurred vision, one each in the IV and IM groups and 2 in the IN group. No subjects experienced respiratory depression, apnea, laryngospasm, bronchospasm, or wheezing.

Mean midazolam plasma concentration versus time curve profiles ($n = 12$) over the first 2 h after IV, IM, and IN administration are shown in Figure 1. Mid-

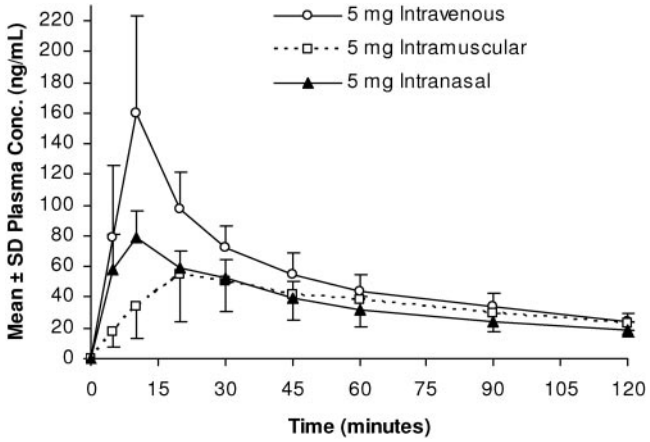

Figure 1. Plasma concentrations of midazolam after 5-mg IV, IM, and IN midazolam administration. Values are mean (± SD) for 12 subjects for each dose.

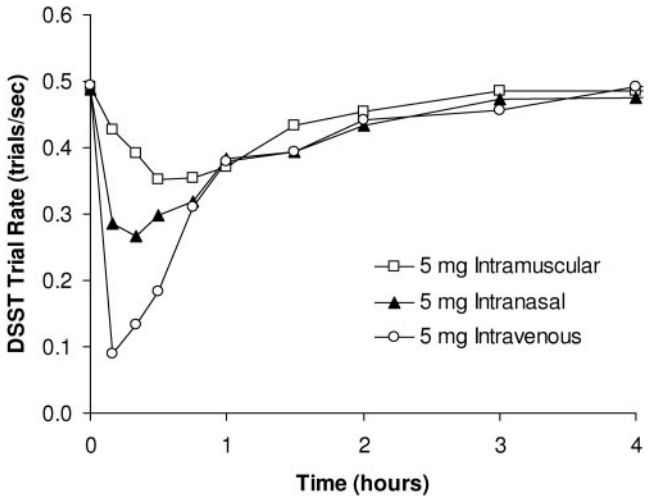

Figure 2. Mean (n = 12) digit symbol substitution test (DSST) trial rating as a function of time over 4 h after 5-mg midazolam doses administered via IV, IM, and IN routes of administration.

with concentrations reaching a peak in 2 individuals at 5 min and in 75% of the individuals in ≤10 min (median T_{max} = 10 min). Mean pharmacokinetic parameters are presented in Table 1. C_{max} values after the IN dose were higher than those after the IM dose. A significantly shorter T_{max} was observed for the IN formulation compared with the IM formulation (P = 0.0001). Levels of 1-hydroxymidazolam were very low, and as such, are not reported. The ratios of metabolite to parent AUCs were 0.16 to 0.22 for the 3 routes of administration.

DSST

Differences in drug-induced psychomotor and cognitive impairment were observed across routes of administration. Subjects were awakened, when possible, to initiate performance tasks at all scheduled time points but were unable to complete all tasks on numerous occasions. Three subjects failed to complete performance tasks at 6 time points, as the result of sleepiness, after IM dose administration; 7 subjects failed to complete performance tasks at 13 time points after IN dose administration; and 11 subjects failed to complete performance tasks at 29 time points after IV dose administration. Figure 2 presents trial rate on the DSST as a function of time after dose administration. No gender or gender-by-route interactions were observed, except for $AUCR_{0-4}$ (for gender, P = 0.0375). The carryover effect was not significant ($P > 0.1$) for all 8 DSST and parameters.

Table 2 presents pharmacodynamic parameters for DSST Trial Rate. It was obvious that differences among routes of administration occurred during the first 30 min after drug administration. On all measures, the order of magnitude of effects were identical with IV producing larger effects with a faster onset of action than IN, which in turn produced larger effects with a faster onset than IM. Significant effects of route were obtained on $AUCR_{0-4}$. Follow-up tests indicated a significant difference between the IV and IM routes only, with the IV route engendering significantly greater $AUCR_{0-4}$ than the IM route (P = 0.002). No significant gender, route, or gender-by-route interactions were obtained on time to peak effect or $AUCR_{0-12}$.

Figure 3 presents the relationship between midazolam concentrations from each route of administration in relation to DSST trial rates. The data parallel conclusions from Figure 2 and 4 showing a rapid affect of each route with an IV>IN>IM orientation. A shallow clockwise hysteresis is present for each route of administration.

Figure 4 presents SSS ratings as a function of time after dose administration. Table 2 presents pharmacodynamic parameters for the SSS. As with the DSST task, the order of magnitude of effects on most pharmacodynamic outcome measures were identical, with

Table 1. Pharmacokinetic Parameters of Midazolam After IV Infusion Over 15 min, IM and IN Administration of 5 mg Midazolam in Healthy Volunteers

Formulation	T_{max} (min)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng · h/mL)	$t_{1/2}$ (h)	F (%)
5 mg IV	12.4 (6.8)	167 (48)	186 (31)	3.14 (0.7)	assume 100%
5 mg IM	29.2 (10.9)	59 (29)	175 (39)	4.17 (2.09)	93.4 (12)
5 mg IN	10.3 (5.0)	80 (17)	134 (26)	3.25 (0.97)	72.5 (12)

T_{max} = time to maximum plasma concentration; C_{max} = maximum plasma concentration; $t_{1/2}$ = elimination half-life; $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time zero to infinity; F = bioavailability; IV = intravenous; IM = intramuscular; IN = intranasal

Data are mean (SD), n = 12.

Table 2. Mean Single-Dose Midazolam Pharmacodynamic (PD) Parameters and Multiple Comparisons After IV Infusion Over 15 min, IM and IN Administration of 5 mg Midazolam in Healthy Volunteers

PD Parameter	Treatment A 5 mg IV	Treatment B 5 mg IM	Treatment C 5 mg IN	IN vs IV	IN vs IM	IV vs IM	Gender
DSST							
Peak effect (trials/s)	0.55 (0.06)	0.53 (0.06)	0.55 (0.08)	0.6689	0.3233	0.1635	1.000
Time to minimum (min)	12.5 (6.2)	133 (23)	59 (133)	0.4318	0.2129	0.0497	0.8451
$AUCR_{0-4}$ (rating × h)	−0.41 (0.19)	−0.20 (0.18)	−0.30 (0.21)	0.0731	0.1116	0.0020	0.0375
$AUCR_{0-12}$ (rating × h)	−0.30 (0.36)	−0.21 (0.57)	−0.16 (0.35)	0.4635	0.7692	0.6575	0.0986
Stanford Sleepiness Scale							
Peak effect (rating)	6.3 (1.2)	5.0 (1.8)	5.4 (1.9)	0.0568	0.3690	0.0081	0.0037
Time to peak (min)	27 (27)	49 (25)	45 (47)	0.2232	0.7997	0.1456	0.9035
$AUCR_{0-4}$ (rating × h)	7.1 (4.2)	6.1 (2.8)	7.4 (3.7)	0.8514	0.4703	0.5908	0.1658
$AUCR_{0-12}$ (rating × h)	4.4 (8.8)	9.2 (8.7)	7.1 (6.6)	0.4117	0.5990	0.1858	0.1878

Values in parentheses for parameters are SD. DSST = digit symbol substitution test

IV producing larger effects with a faster onset than IN, which in turn produced larger effects with a faster onset than IM. An exception was for $AUCR_{0-4}$ in which the order was IN, IV, IM, suggesting that IN had an overall greater effect than IV in the first 4 h. There were no statistical differences in time to peak sleep ratings, $AUCR_{0-4}$, or $AUCR_{0-12}$ values for the different routes. Significantly greater peak sleep ratings were observed following the IV route, which were significantly greater than following the IM route ($P = 0.0081$) but no different compared to the IN route. No significant gender, route or gender-by-route interactions were obtained on time to peak effect, $AUCR_{0-4}$, or $AUCR_{0-12}$ values. Gender differences were significant for peak effect of sleep ratings. Females had significantly higher sleep ratings than males for peak effect ($P = 0.0037$ in follow-up analysis). Significant gender-by-route interactions were observed for $AUCR_{0-12}$ in main effects analysis ($P = 0.0145$). A possible explanation for this finding is that the female subjects had a significantly lower weight than their male counterparts, providing a higher dose on a mg/kg basis.

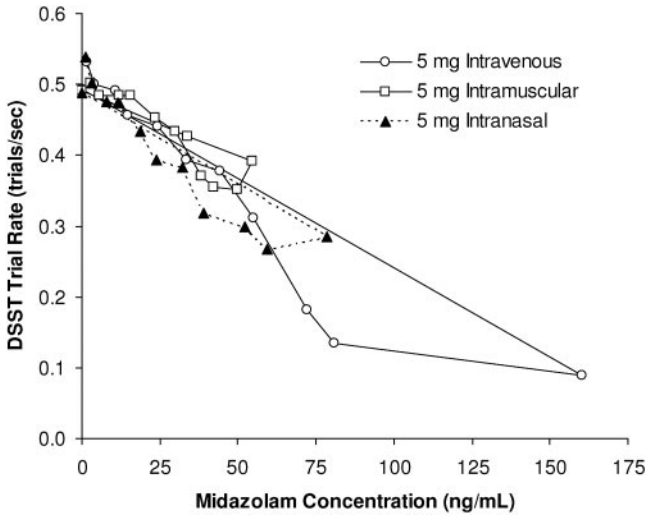

Figure 3. Digit symbol substitution test (DSST) trial rating as a function of midazolam concentration after 5-mg midazolam doses administered via IV, IM, and IN routes of administration.

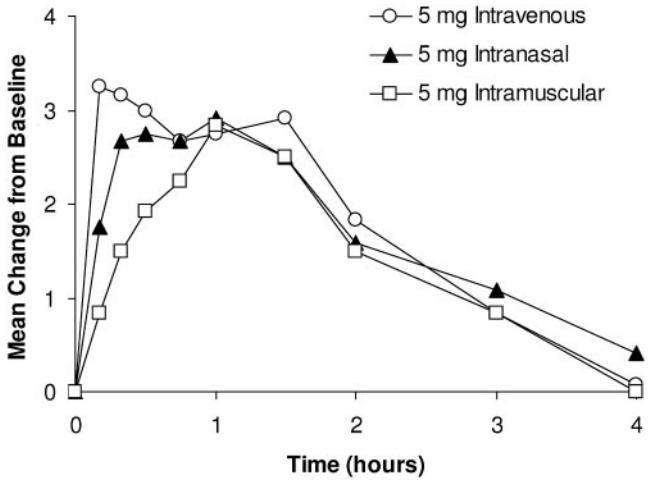

Figure 4. Mean change from baseline ($n = 12$) Stanford Sleepiness Scale rating as a function of time over 4 h after

Statistical comparisons for IM and IN to the IV route were precluded because of the relatively large number of missing values from the IV route. However, significant differences in ratings were observed across time for all VAS scales ($P < 0.01$), with the exception of Anxious. Differences in ratings of High were observed as a function of route ($P < 0.05$), with ratings after IN doses significantly larger than after IM doses. Route by time interactions were obtained on ratings of Sedated ($P < 0.005$), High ($P < 0.0001$), Headache ($P < 0.05$), and Feel Drug ($P < 0.01$). Simple effects analyses of these interactions indicated significant time effects during both the IN and IM routes, and significant differences between the IN and IM routes 10 (Sedated, High, Feel drug), 20 (High, Headache), and 60 (Headache) min after dose.

DISCUSSION

The earliest clinical studies of midazolam nasal

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