Bioavailability of a novel midazolam gel after intranasal administration in dogs

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Objectives—To compare the pharmacokinetics of a novel bioadhesive gel formulation of midazolam after intranasal (IN) administration with that of midazolam solution after IN, IV, and rectal administration to dogs.

Animals—10 (5 males and 5 females) healthy adult Beagles.

Procedures—Dogs were assigned to 4 treatment groups for a crossover study design. Initially, midazolam solution (5 mg/mL) was administered (0.2 mg/kg) IV to group 1, rectally to group 2, and IN to group 3; a 0.4% hydroxypropyl methylcellulose midazolam gel formulation (50 mg/mL) was administered (0.2 mg/kg, IN) to group 4. Each dog received all 4 treatments; there was a 7-day washout period between subsequent treatments. Blood samples were collected before and after midazolam administration. Plasma concentration of midazolam was determined by use of high-performance liquid chromatography.

Results—The peak plasma concentration after IN administration of the gel formulation was significantly higher than that after IN and rectal administration of the solution. Mean ± SD time to peak concentration was 11.70 ± 2.63 minutes (gel IN), 17.50 ± 2.64 minutes (solution IN), and 39 ± 14.49 minutes (solution rectally). Mean bioavailability of midazolam was 70.4% (gel IN), 52.0% (solution IN), and 49.0% (solution rectally). Bioavailability after IN administration of the gel formulation was significantly higher than that after IN and rectal administration of the solution.

Conclusions and Clinical Relevance—IN administration of midazolam gel was superior to both IN and rectal administration of midazolam solution with respect to peak plasma concentration and bioavailability. (Am J Vet Res 2012;73:539–545)
sible. Rectal administration of diazepam is currently the only out-of-hospital treatment that has been evaluated for efficacy in seizures in dogs. In that study, investigators evaluated dogs with a history of cluster seizures; dogs that received diazepam via rectal administration had a significant decrease in the number of cluster seizure events in a 24-hour period as well as a significant decrease in the total cost of emergency care.

Pharmacokinetic studies evaluating benzodiazepines delivered to dogs by routes other than IV are limited. Diazepam administered rectally or IN to dogs reaches plasma concentrations of total benzodiazepine within the anticonvulsant range for humans. Midazolam administered IM to dogs is rapidly absorbed with bioavailability > 90%. However, there was low systemic availability when midazolam was administered rectally to a single dog. Buccal absorption of midazolam has been reported in dogs; however, the dose was not reported and the conditions under which the experiment was conducted were not clinically applicable to dogs with status epilepticus. Midazolam, triazolam, and iltrazolam yielded a maximum plasma concentration within 15 minutes after IN administration in dogs, but the dose was not reported. Another pharmacokinetic study of midazolam administered at 1.5 mg/kg, IN, revealed a bioavailability of only 10%. Finally, administration of lorazepam per rectum in dogs failed to generate plasma concentrations within the lower limit of detection (5 ng/mL).

Results of several studies have indicated that midazolam is effective in the treatment of seizures in humans when administered IM, IN, and via the buccal or sublingual route. The IN route of administration has been investigated extensively in humans; it is more convenient and socially acceptable than is rectal administration of diazepam and also yields equal or better results with regard to anticonvulsant activity and onset of action. Certain preparations of midazolam administered IN have had higher plasma concentrations that were achieved in a shorter amount of time, compared with an equivalent dose administered IM. The effectiveness of midazolam administered IN is such that it has been recommended in consensus guidelines as an alternative rapid treatment for humans with status epilepticus.

Although IN delivery of the parenteral formulation has been effective for treatment of status epilepticus in humans, there are some drawbacks. The most important problem reported is the large volume needed to ensure adequate dose administration. The large volume results in runoff via the nares or the nasopharynx, which decreases the bioavailability and efficacy. The limitations encountered with administration of a large volume prompted a number of studies that were conducted to evaluate novel, highly concentrated formulations of midazolam. Investigations into alternative formulations have yielded products that are more rapidly and completely absorbed than is the commercially available product. However, there are limited data regarding the use of any of these alternative formulations in a clinical setting. Therefore, the primary objectives of the study reported here were to describe the pharmacokinetics of a novel concentrated midazolam gel administered IN to dogs and to compare the pharmacokinetics of the novel gel administered IN with the pharmacokinetics of the commercially available midazolam solution administered IV, IN, or rectally.

Materials and Methods

Animals—Ten Beagles (5 males and 5 females) weighing between 7.0 and 12.0 kg were used in the study. A physical examination was performed on all dogs prior to the experiment to ensure that they were healthy. Complete blood counts, serum biochemical analyses, and urinalyses were performed on all dogs. All procedures were approved by the University of Georgia Institutional Animal Care and Use Committee.

Drug formulation—Commercially available midazolam hydrochloride (5 mg/mL) was used for IV, rectal, and IN administration. The midazolam gel formulation (50 mg/mL) was aseptically prepared under good manufacturing practices at the Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, University of Georgia; thus, a sterile product was created. The gel formulation was an aequous solution with a final volume of 10 mL that consisted of purified United States Pharmacopeia-grade water containing 0.5 g of midazolam, 0.2% sodium hydroxide, 0.08% hydrochloric acid, and 0.01% benzalkonium chloride combined with 0.4% hydroxypropyl methylcellulose powder. The pH of the gel formulation was 2.98.

Experimental protocol—Dogs were assigned to 4 treatment groups on the basis of the numeric order of their ear tattoo number in a crossover design. The first 3 dogs (with the lowest ear tattoo numbers) were assigned to group 1, the next 3 dogs were assigned to group 2, the next 2 dogs were assigned to group 3, and the last 2 dogs (with the highest ear tattoo numbers) were assigned to group 4. Midazolam solution (5 mg/mL) was initially administered IV to group 1, rectally to group 2, and IN to group 3; 0.4% hydroxypropyl methylcellulose midazolam gel (50 mg/mL) was administered to group 4 (0.2 mg/kg, IN). All dogs received each treatment, with a 7-day washout period between subsequent treatments. Food was withheld from all dogs throughout sample collection, but water was available.

Blood samples were collected into lithium heparin tubes from an external jugular vein before and 3, 6, 9, 12, 15, 20, 30, 60, 120, 240, and 480 minutes after midazolam administration. Blood samples were immediately centrifuged, with the resultant plasma stored in polypropylene vials and frozen at −80°C for later determination of midazolam concentration. Heart rate was measured before and 5, 10, 15, 20, 25, and 30 minutes after midazolam administration. Respiratory rate, rectal temperature, and indirect blood pressure were measured before and 15 and 30 minutes after drug administration. A subjective assessment of sneezing, run off of medication via the nares, sedation, and ataxia was performed, and the results were recorded.

Drug administration—A catheter was inserted in a cephalic vein for IV administration of midazolam. The catheter was flushed with 0.5 mL of heparinized saline (0.9% NaCl) solution immediately after drug ad-
ministration. For rectal administration, the midazolam solution was injected via a 1-mL syringe through an 8F red rubber catheter inserted 2 cm into the rectum and flushed with 5 mL of sterile saline solution immediately after drug administration. Both the solution and gel formulation were administered IN by use of a 1-mL syringe. Approximately half the dose was administered into each nostril during a 20-second period. The head of each dog was held extended at approximately a 30° angle for 1 minute to prevent nasal runoff of midazolam from the nares. The 1-mL syringe used for drug administration had marked gradations at intervals of 0.01 mL. The volume of gel administered was rounded to the nearest 0.005 mL, which was estimated at the midpoint between 2 gradations. This method of volume estimation resulted in a mean ± SD dose range of 0.2 ± 0.02 mg/kg in a 7-kg dog, which was the smallest dog in the study.

Determination of plasma midazolam concentration—A stock solution of midazolam (1.0 mg/mL) was prepared in methanol. Standard solutions of midazolam were prepared by serial dilution. The final concentrations of the standard solutions were 100, 50, 25, 10, 5, 2.5, 1, 0.5, and 0.1 µg/mL. Stock solution was refrigerated when not in use and replaced on a biweekly basis. Fresh standard solutions were prepared for each day of analysis.

Plasma calibration points were prepared by spiking 400 µL of the blank plasma with 40 µL of each midazolam standard solution. The calibration curve of the plasma was for the range of 0.01 to 10 µg/mL with calibration points of 10, 5, 1, 0.5, 0.1, 0.05, and 0.01 µg/mL.

Plasma concentrations of midazolam were analyzed by use of an HPLC method with UV detection at 220 nm. Plasma aliquots of 0.4 mL were mixed with 400 ng of the internal standard diazepam and 0.1 mL of sodium hydroxide (0.5M). After liquid-liquid extraction with 1 mL of cyclohexane-dichloromethane (55:45 [vol/vol]), the organic layer was evaporated. The residue was dissolved in 100 µL of water-acetonitrile (95:5 [vol/vol]), and 50 µL of the resulting solution was injected onto the chromatograph.

Chromatographic system—A chromatographic system coupled with a UV detector was used for biosample analysis. Separation was achieved on a C18 (250 × 4.6 mm; 5µM) column with a C18 guard column. The isocratic mobile phase was 25mM KH2PO4, buffer (pH, 7.0) and acetonitrile (56:44 [vol/vol]). Mobile phase flow rate was 1 mL/min. The UV detector wavelength was set at 220 nm.

Validation of the HPLC analytic method—Linearity of the HPLC method for the determination of midazolam was evaluated by use of a calibration curve in the range of 0.01 to 10 µg/mL. The calibration curve was obtained by plotting the ratio of the peak area of each analyte to the internal standard versus the theoretical midazolam concentration. Least squares linear regression analysis was used to determine the slope, intercept, and correlation coefficient. The calibration curve required an R² ≥ 0.99, which was considered appropriate for a validated method. To evaluate precision, at least 5 quality-control samples at each of 3

concentrations were prepared and injected on a single day (intraday) and on different days (interday). The variability of midazolam determination was expressed as the coefficient of variation, which should be ≤ 15% for all concentrations. Accuracy was expressed as the percentage bias of theoretical versus calculated concentrations, and it should be within 15% for all concentrations of midazolam. The absolute recoveries of midazolam from plasma were determined at several standard concentrations by spiking the drug into the corresponding fresh blank plasma. The percentage of recovery was calculated by comparing the peak area of extracted samples with that for samples in which the same amount of compound was diluted with acetonitrile. The recoveries for 3 quality-control concentrations of midazolam in plasma were examined at least 5 times for each concentration.

Pharmacokinetic analysis—Pharmacokinetic values were determined for the plasma midazolam concentration-time profiles. Plasma concentration-time data were analyzed with software by use of noncompartmental methods. Observed Cmax, Tmax, bioavailability, elimination half-life, and AUC were determined. Pharmacokinetic values were calculated by use of standard equations.

Statistical analysis—A 1-way ANOVA for repeated measures was used to compare concentrations among groups and time points. A 2-factor model was used that included the time, group, and time-by-group interaction. A 1-way ANOVA for repeated measures was also used to compare Cmax, Tmax, bioavailability, and AUC among groups. A single factor model that included a group factor was used to compare pharmacokinetic values between groups. An unstructured covariance structure was used in all repeated-measures models. Hypothesis tests were 2 sided with α < 0.05. Statistical analysis was performed by use of commercially available software. Data were reported as mean ± SD.

Results

Calibration curves were linear in the range from 0.01 to 10 µg/mL (r² > 0.99). Interday and intraday coefficients of variation were < 7%. Absolute recovery was > 95%.

The IV administration of midazolam solution yielded the highest mean ± SD peak plasma midazolam concentration of 1.82 ± 0.278 µg/mL (Figure 1). Mean plasma concentrations of midazolam were significantly (P < 0.001) higher at 3, 6, 9, and 12 minutes after IV administration than those achieved at the same times after administration via other methods of delivery. At 15 and 20 minutes after drug administration, the mean plasma concentrations for both the IV administration of the solution and IN administration of the gel formulation were significantly higher than those for IN and rectal administration of the solution; however, the mean plasma concentrations for the IV administration of the solution and IN administration of the gel formulation did not differ significantly.

Observed mean ± SD peak plasma midazolam concentration after IN administration of the gel formulation (0.43 ± 0.09 µg/mL) was significantly (P < 0.001)
higher than that achieved after IN administration of the solution (0.21 ± 0.02 µg/mL) and rectal administration of the solution (0.15 ± 0.01 µg/mL). Mean plasma concentrations of midazolam after IN administration of the gel formulation were significantly (P < 0.001) higher at 6, 9, and 12 minutes after delivery, compared with the concentrations achieved at those same times after IN and rectal administration of the solution (Figure 1).

Mean ± SD Tmax was significantly (P < 0.001) longer after rectal administration of the solution (39 ± 14.49 minutes) than that after IN administration of the gel formulation (11.70 ± 2.63 minutes) or IN administration of the solution (17.50 ± 2.64 minutes; Figure 1). However, the Tmax for IN administration of the gel and solution did not differ significantly. The Tmax after IV administration of the solution was < 3 minutes.

Mean bioavailability after IN administration of the gel formulation (70.4%) was significantly (P < 0.001) higher than that after IN (52.0%) and rectal (49.0%) administration of the solution. The AUC from 0 to 30 minutes after IN administration of the gel formulation was significantly (P < 0.001) higher than that after IN and rectal administration of the solution. The AUC was significantly (P = 0.005) higher 0 to 30 minutes after IN administration of the solution than the AUC after rectal administration of the solution (Table 1).

The elimination half-life was similar among all other methods of administration (Table 1). Mean resident time after IV administration of the solution was significantly (P < 0.001) less than that for other methods of administration. Clearance after IN administration of the gel formulation was significantly (P < 0.001) lower than the clearance after IN and rectal administration of the solution. The volume of distribution after rectal administration of the solution was significantly (P < 0.021) higher than that after IN administration of the gel formulation.

Dogs tolerated all forms of treatment well. Sneezing was not apparent after IN administration of the gel or solution. Some degree of runoff from the nares was seen after administration of the midazolam solution in all dogs despite elevation of the head. Subjectively, the amount of runoff appeared to be minimal. After IV administration, dogs were ataxic and sedated almost immediately, with this effect lasting for up to 15 minutes. The degree of ataxia and sedation was subjectively assessed to be less among the other methods of administration, compared with the degree after IV administration of the solution, with an onset of approximately 5 minutes and a duration of up to 15 minutes for the other 3 methods of administration. Heart rate, respiratory rate, and blood pressure remained within the physiologic ranges after midazolam administration for all methods of administration.

**Discussion**

Midazolam has great potential as a short-acting anticonvulsant in dogs because it possesses twice the affinity for the benzodiazepine receptor and 4 times the hypnotic potency of diazepam. The precise midazolam plasma concentration needed to stop seizure activity is not known in dogs or humans. Knowledge...

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**Table 1—Mean ± SD (range) values for pharmacokinetic variables determined after IV, IN, and rectal administration of midazolam solution (0.2 mg/kg) and IN administration of midazolam gel (0.2 mg/kg) to 10 (5 males and 5 females) healthy adult Beagles.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>IV gel</th>
<th>IN solution</th>
<th>Rectal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (µg/mL)</td>
<td>1.82 ± 0.278 (1.47–2.32)</td>
<td>0.45 ± 0.09 (0.32–0.60)</td>
<td>0.21 ± 0.02 (0.18–0.25)</td>
</tr>
<tr>
<td>Tmax (min)</td>
<td>&lt; 3.00 (NA)</td>
<td>11.70 ± 2.63 (9.00–15.00)</td>
<td>17.50 ± 2.64 (15.00–20.00)</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>121.09 ± 25.79 (91.80–161.00)</td>
<td>119.64 ± 22.78 (94.00–161.00)</td>
<td>118.70 ± 18.78 (102.00–162.00)</td>
</tr>
<tr>
<td>Total AUC (min•µg/mL)</td>
<td>71.11 ± 13.59 (50.50–90.40)</td>
<td>50.34 ± 7.60 (38.10–61.00)</td>
<td>27.56 ± 5.31 (20.50–47.50)</td>
</tr>
<tr>
<td>AUC for 0 to 30 min (min•µg/mL)</td>
<td>22.47 ± 3.85 (17.40–29.20)</td>
<td>8.35 ± 1.70 (6.20–10.80)</td>
<td>4.53 ± 0.46 (3.90–5.40)</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>NA</td>
<td>70.4</td>
<td>52.0</td>
</tr>
</tbody>
</table>

All dogs received each treatment; there was a 7-day washout period between subsequent treatments. t1/2 = Elimination half-life. NA = Not applicable.
of midazolam’s effectiveness as an anticonvulsant in dogs is limited to a single study in which investigators determined that the administration of 0.2 mg of midazolam/kg, IV, had a stronger suppressive effect against lidocaine-induced seizures than did the same dose of diazepam. The dose used in most studies on the successful treatment of seizures with IN administration of the parenteral solution of midazolam in humans is 0.2 mg/kg, which is a dose derived from other sedation and anesthesia studies. Pharmacokinetic studies in humans conducted to evaluate IN administration of the parenteral solution formulation at doses of 0.2 mg/kg have revealed a mean Cmax between 104 and 182 ng/mL within 12 minutes after administration, which is much higher than the 40 ng/mL considered to be the minimum therapeutic concentration for sedation in adults. Estimated bioavailability of the parenteral solution formulation administered at a dose of 0.2 mg/kg, IN, in humans ranges from 50% to 83%. Midazolam administered at a dose of 0.2 mg/kg, IN, has been found to penetrate the human brain in 2 to 5 minutes, as indicated by the appearance of β activity in the electroencephalogram and suppressed epileptic activity. This suggests that early entry of modest concentrations of midazolam into the brain compartment has anticonvulsant effects and that the plasma midazolam concentration needed to stop a seizure could be far less than the concentration needed to cause sedation.

The present study revealed that 0.4% hydroxypropyl methylcellulose midazolam gel is readily absorbed when administered IN, with a pharmacokinetic profile superior to that of the parenteral solution when administered IN or rectally to dogs. More specifically, from 0 to 30 minutes after IN administration of the gel, AUC was significantly (P < 0.001) higher than that after IN or rectal administration of the solution; AUC is a key variable when evaluating a drug for use in a setting in which the time to clinical effect is important. The mean peak plasma concentration of 450 ng/mL is more than twice the peak plasma concentration for human studies in which the parenteral solution was administered at the same dose via the IN route. The Tmax and Cmax for IN administration of the gel formulation are similar to the mean ± SD values for IM administration of 0.5 mg/kg in dogs (Cmax, 549 ± 121 ng/mL; Tmax, 8 ± 2 minutes).

Alternative formulations of midazolam administered IN have favorable pharmacokinetics in humans. The common feature for all of the formulations is a solubility enhancer, which results in a more concentrated product and allows delivery of a much smaller volume. The objective is to create a product that could deliver an effective dose in a volume of 0.1 to 0.2 mL, which is an accepted retention volume in the human nose. Because of the extreme phenotypic variation in domestic dogs (size of the dog and conformation of the head), a mean nasal retention volume in dogs is difficult to define. Nonetheless, a highly concentrated, low-volume product would be advantageous in brachycephalic and large-breed dogs. We evaluated the highest concentration (50 mg/mL) currently reported, with 30 mg/mL being the next highest concentration previously reported. It is difficult to make comparisons between the gel formulation in the present study and other formulations in human studies. All pharmacokinetic studies in humans on the use of alternative midazolam formulations have used doses < 0.1 mg/kg. The highest peak plasma concentration reported in these studies was 80 ng/mL, which was reached within 7.2 and 10.3 minutes, respectively. Reported bioavailability of the alternative formulations ranged from 64% to 92%.

Pharmacokinetics of drugs administered IN can be improved by use of bioadhesive gels that prolong contact time with the nasal mucosa and metered spray delivery systems that deposit medication more rostrally in the nasal antrum so that there is slower clearance from the nasopharynx. The methylcellulose used in the present study is a viscosity-enhancing agent that can promote retention in the nasal cavity by slowing the ciliary movement of mucus. Although the present study indicated that the gel had superior pharmacokinetics, compared with pharmacokinetics for the parenteral solution, the amount of the improved pharmacokinetic profile secondary to the lower volume versus the use of methylcellulose cannot be determined. Nasal spray delivery systems are used in most studies in humans. In dogs, the use of an atomizer can improve the pharmacokinetics of midazolam administered IN. An inexpensive metered nasal delivery system was evaluated in the present study; however, the device failed to deliver the gel in an effective manner. Thus, we did not report results for that method of delivery.

In most humans, transient nasal irritation, tearing, and a raw-throat sensation are reported in pharmacokinetic studies conducted with both the parenteral solution and alternative formulations. Midazolam is water soluble; however, to remain in solution, it must be buffered to a pH of approximately 3.0. It is thought that the low pH is the cause of most of the discomfort reported; however, alternative formulations with a pH of 4.0 to 4.2 also cause discomfort, which suggests that it may be the midazolam that is the irritant. However, the importance of the adverse effects is questionable because most patients treated for a seizure with midazolam via IN administration do not report any adverse effects. It is difficult to know whether there were similar adverse effects in the dogs of the present study. The pH of the gel (2.98) was similar to that of the parenteral solution. No nasal discharge, epistaxis, or behavior that could be interpreted as nasal irritation was detected in any of the dogs receiving either formulation of midazolam via IN administration.

The parenteral midazolam solution did not perform as well as the gel but still resulted in plasma concentrations well within an anticonvulsant range (on the basis of studies in humans). The mean ± SD Tmax of 17.30 ± 2.64 minutes for the parenteral solution is similar to that reported in a human study conducted to evaluate the parenteral solution at similar doses. Analysis of these results suggests that IN administration of the parenteral midazolam solution could be effective in treating seizures in dogs.

Rectal administration of the parenteral midazolam solution also resulted in concentrations that would be considered therapeutic in humans; however, the mean ± SD Tmax was prolonged (39 ± 14.49 minutes).
bioavailability of 49% is an improvement from the almost total lack of absorption found in another study.\textsuperscript{10} Results of pharmacokinetic studies\textsuperscript{12,13,63} on midazolam administered rectally in humans vary, with bioavailability ranging from 18% to 52% and a T\textsubscript{max} of 12.1 to 31 minutes. Diazepam administered rectally in dogs can result in mean ± SD bioavailability ranging from 31.7 ± 21.8% to 79.9 ± 20.7% and a mean T\textsubscript{max} of 14.3 ± 3.7 minutes.\textsuperscript{17,18} On the basis of these pharmacokinetics, diazepam administered rectally as an anticonvulsant would seem to be superior to midazolam administered rectally.

For the study reported here, we concluded that the novel midazolam gel was rapidly absorbed in dogs, with a pharmacokinetic profile similar to that of many of the other alternative midazolam formulations evaluated in humans. Although the midazolam gel provided the best results, both the gel formulation and the parenteral solution formulation had the potential to be effective in the treatment of seizures when administered IN. Future investigations into the stability of the gel when stored at ambient temperatures and in a refrigerator must be performed before clinical trials can be performed with the gel. Until clinical trials are performed, effective and safe dosing recommendations for IN use of the parenteral solution formulation or the gel formulation cannot be made.

a. Midazolam HCl (5 mg/mL), Bedford Laboratories, Bedford, Ohio.
b. Midazolam powder, Pharmaceutical Compounding Center of America, Houston, Tex.
d. Agilent 1100 series HPLC, Agilent Technologies, Wilmington, Del.
g. WinNonlin, version 5.2, Pharsight Corp, Mountain View, Calif.
h. SAS, version 9.1.3, SAS Institute Inc, Cary, NC.

References


