Evaluation of plasma diazepam and nordiazepam concentrations following administration of diazepam intravenously or via suppository per rectum in dogs

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Objective—To evaluate the pharmacokinetics of diazepam administered per rectum via compounded (ie, not commercially available) suppositories and determine whether a dose of 2 mg/kg in this formulation would result in plasma concentrations shown to be effective for control of status epilepticus or cluster seizures (ie, 150 to 300 ng/mL) in dogs within a clinically useful interval (10 to 15 minutes).

Animals—6 healthy mixed-breed dogs.

Procedures—Dogs were randomly assigned to 2 groups of 3 dogs each in a crossover-design study. Diazepam (2 mg/kg) was administered IV or via suppository per rectum, and blood samples were collected at predetermined time points. Following a 6- or 7-day washout period, each group received the alternate treatment. Plasma concentrations of diazepam and nordiazepam were analyzed via reversed phase high-performance liquid chromatography.

Results—Plasma concentrations of diazepam and nordiazepam exceeded the targeted range ≤3 minutes after IV administration in all dogs. After suppository administration, targeted concentrations of diazepam were not detected in any dogs, and targeted concentrations of nordiazepam were detected after 90 minutes (n = 2 dogs) or 120 minutes (3) or were not achieved (1).

Conclusions and Clinical Relevance—On the basis of these results, administration of 2 mg of diazepam/kg via the compounded suppositories used in the present study cannot be recommended for emergency treatment of seizures in dogs. (Am J Vet Res 2013;74:611–615)

Status epilepticus and cluster seizures are medical emergencies that require immediate treatment to prevent cerebral damage.1–5 Almost 60% of dogs with idiopathic epilepsy will have at least 1 episode of status epilepticus or cluster seizures during their lifetimes.6 Recurrent trips to the veterinary hospital to obtain emergency treatment for status epilepticus or cluster seizures exact an emotional and financial toll on pet owners. The need for frequent emergency hospital visits may become overwhelming for the owners and may cause them to seek euthanasia for their pet.7,8 Results of 1 study9 indicate that status epilepticus can shorten a dog’s lifespan by 2 years because these animals are more likely to be euthanized, compared with dogs that do not have episodes of status epilepticus. Therefore, effective out-of-hospital emergency treatment for seizures in dogs prone to status epilepticus or cluster seizures can be life-saving.

Diazepam, a benzodiazepine, is a potent, fast-acting, lipid-soluble anticonvulsant that is the mainstay of emergency treatment of seizures.1,2,4,5,7,9 Diazepam is rapidly metabolized into active metabolites (nordiazepam, oxazepam, and temazepam) after administration.9 Nordiazepam rapidly accumulates in plasma at very high concentrations and exceeds diazepam concentrations by an order of magnitude, and oxazepam reaches concentrations between values for nordiazep-...
epam and diazepam. The metabolism of diazepam into temazepam has not been found to be an important pathway in dogs.

Intravenous administration of diazepam provides a rapid clinical effect, but it can be very difficult to perform in a patient during a seizure, even when attempted by trained personnel. Therefore, another route of administration for out-of-hospital emergency treatment would be beneficial. Oral and IM routes of diazepam administration are not appropriate for seizing patients. Commercially available, injectable diazepam solution administered per rectum has been shown to be effective for emergency treatment of seizures in dogs.

Commercially available, injectable diazepam suppositories were compounded at a local commercial pharmacy on the first day of the study; thus, all suppositories were administered within 7 days after compounding. All measurements were performed with an electronic balance that was calibrated daily. All measurements were recorded and stored in a computer to which the balance was connected. The suppositories contained a suspending gel and a fatty acid base as well as diazepam. The base was melted at 30°C and constantly stirred with a magnetic stirrer while the measured diazepam powder was added. The prepared 100-g suspension containing 500 mg of diazepam was then poured into 50 calibrated 2-g suppository molds, so that each 2-g suppository contained 10 mg of diazepam. To allow for precise dosing for each dog, 2-g suppositories were additionally melted and poured into smaller molds (eg, 3 mg of diazepam in a 0.6-g mold).

Drug administration—Diazepam was administered IV via a butterfly infusion set placed into a cephalic vein after preparation of the skin with 70% alcohol solution. The butterfly infusion set was flushed with heparinized saline (0.9% NaCl) solution immediately after diazepam administration. For suppository administration, fecal material was removed from the distal portion of the rectum with a gloved index finger before inserting the suppositories. After administration of the suppositories, the dogs’ tail was held firmly again the anus for 10 minutes to prevent expulsion.

Blood sample collection—Hair was clipped over an external jugular vein, and an IV catheter was placed following aseptic preparation of the skin. Blood samples (4.0 mL) were collected from the catheter immediately prior to (ie, baseline) and at 3, 5, 10, 15, 20, 30, 45, 60, 90, 120, 240, and 420 minutes after diazepam administration via each route. Samples were transferred into tubes containing lithium heparin and centrifuged, and plasma was collected and stored in polypropylene vials at −80°C for at least 72 days until the plasma diazepam and nordiazepam concentrations were determined.

Materials and Methods

Animals—Six (5 female and 1 male) healthy mixed-breed dogs, with a mean age of 4.9 years (range, 4 to 10 years) and mean body weight of 20.2 kg (range, 16 to 24 kg), were used for the study. Each dog was considered healthy on the basis of results of a complete physical examination (including neurologic examination), CBC, serum biochemical analysis, and electrolyte evaluation before the start of the study. The dogs were part of a research colony at the College of Veterinary Medicine at the University of Tennessee and were housed in individual runs in that institution’s laboratory animal care facility. All procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee and the Michigan State University Institutional Animal Care and Use Committee.

Experimental protocol—Food was withheld for 8 hours before and during the sample collection period, but all dogs had free access to water. Dogs were randomly assigned, via a coin flip, to 2 groups (n = 3/group) in a crossover-design study. In the first phase of the study, 3 dogs were administered diazepam (2 mg/kg IV), and the remaining 3 received diazepam (2 mg/kg) suppositories per rectum. Blood samples were collected immediately before and at predetermined intervals after drug administration. After a 6- or 7-day washout period, the process was repeated, with each dog receiving the alternate treatment in the second phase of the study.

Diazepam suppositories—Diazepam suppositories were compounded at a local commercial pharmacy on the first day of the study; thus, all suppositories were administered within 7 days after compounding. All measurements were performed with an electronic balance that was calibrated daily. All measurements were recorded and stored in a computer to which the balance was connected. The suppositories contained a suspending gel and a fatty acid base as well as diazepam. The base was melted at 30°C and constantly stirred with a magnetic stirrer while the measured diazepam powder was added. The prepared 100-g suspension containing 500 mg of diazepam was then poured into 50 calibrated 2-g suppository molds, so that each 2-g suppository contained 10 mg of diazepam. To allow for precise dosing for each dog, 2-g suppositories were additionally melted and poured into smaller molds (eg, 3 mg of diazepam in a 0.6-g mold).

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Blood sample collection—Hair was clipped over an external jugular vein, and an IV catheter was placed following aseptic preparation of the skin. Blood samples (4.0 mL) were collected from the catheter immediately prior to (ie, baseline) and at 3, 5, 10, 15, 20, 30, 45, 60, 90, 120, 240, and 420 minutes after diazepam administration via each route. Samples were transferred into tubes containing lithium heparin and centrifuged, and plasma was collected and stored in polypropylene vials at −80°C for at least 72 days until the plasma diazepam and nordiazepam concentrations were determined.

Determination of plasma benzodiazepine concentration—Analysis of diazepam and nordiazepam in plasma samples was conducted by means of HPLC. This system consisted of a separations module and a UV detector. The modified method of Odou et al was used. Briefly, previously frozen plasma samples were thawed and vortexed. One milliliter of plasma was placed in a 15-mL screw cap tube followed by 75 µL of midazolam (internal standard, 10 µg/mL). Two hundred microliters of 7.5M NaOH and 5 mL of ethyl acetate–hexane (40:60) solution were then added, and samples were vortexed for 1 minute followed by centrifugation for 20 minutes at 1,050 × g. The organic layer was removed, placed into a clean glass tube, and evaporated with nitrogen gas. The residue was reconstituted in 250 µL of the mobile phase and placed in HPLC vials; 100 µL was injected into the HPLC system.

The compounds were separated on a reversed phase C18 (4.6 × 250-mm, 5-µm) column with a guard
column. The mobile phase was a mixture of 10mM sodium acetate buffer (pH 4.7) and acetonitrile (50:50). The flow rate was 1.0 mL/min, and the column temperature ambient (approx 22°C). Absorbance was measured at 220 nm.

Standard curves for plasma analysis were prepared by spiking untreated canine plasma with diazepam and nordiazepam to produce a linear concentration range of 5 to 2,500 ng/mL. Spiked standards were treated in the same manner as plasma samples. Mean recoveries ranged from 82% to 93% for diazepam and nordiazepam. Intra- and interassay variability was < 10%. The lower and upper limits of quantitation were 5 ng/mL and 2,500 ng/mL, respectively.

Pharmacokinetic analysis—Pharmacokinetic parameters were calculated via noncompartmental analysis with commercially available software. Selected pharmacokinetic parameters included the area under the concentration–time curve from time zero to infinity, total systemic clearance, terminal half-life, and Cmax. The area under the curve was calculated via the log-linear trapezoidal rule.

Results

All dogs became sedated and ataxic and were unable to stand immediately after injection when diazepam was administered IV. Sedation and ataxia completely resolved in all dogs within 45 minutes after diazepam injection. One dog struggled during the injection, and some of the diazepam was given perivascularly. Two dogs each had 1 minor episode of vomiting (at 5 and 35 minutes after the injection). All dogs had plasma concentrations of diazepam and nordiazepam > 300 ng/mL within 3 minutes after IV drug administration.

No dog had signs of sedation at any time after receiving diazepam suppository. Two dogs defecated (one at 75 minutes and another at 240 minutes) after suppository administration. No adverse effects associated with diazepam administration via this route were detected during the study. One sample at the 30-minute time point was excluded because the sample was lost during the extraction process.

When detected, plasma diazepam concentrations after suppository administration ranged from 10 to 99 ng/mL; thus, the targeted concentration was not achieved in any dog during the study. Diazepam was first detected at 10 minutes in 1 dog, at 45 minutes in 3 dogs, at 90 minutes in 1 dog, and at 240 minutes in 1 dog. Plasma nordiazepam concentrations were first detected at 10 to 30 minutes and ranged from 5 to 581 ng/mL following diazepam suppository administration. Targeted concentrations of nordiazepam were detected after 90 minutes in 2 dogs and after 120 minutes in 3 dogs. Targeted plasma concentrations of nordiazepam were not achieved in the dog that defecated at 75 min-

![Figure 1](image-url)

**Figure 1**—Mean ± SD plasma concentrations of diazepam and nordiazepam versus time in 6 healthy mixed-breed dogs after administration of diazepam (2 mg/kg) IV or via suppository (compounded at a commercial pharmacy with a suspending gel and fatty acid base) per rectum. Circles and diamonds indicate diazepam and nordiazepam concentrations, respectively, following IV administration. Squares and triangles indicate diamepam and nordiazepam concentrations, respectively, following administration per rectum. The 2 bold lines on the Y-axis indicate limits of the targeted concentration for benzodiazepines.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diazepam concentration following IV administration</th>
<th>Nordiazepam concentration following IV administration</th>
<th>Nordiazepam concentration following per rectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal half-life (h)</td>
<td>2.26 ± 0.41</td>
<td>2.96 ± 0.94</td>
<td>3.22 ± 1.67</td>
</tr>
<tr>
<td>Concentration at time 0 (ng/mL)</td>
<td>2,429.17 ± 522.08</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>T_max (h)</td>
<td>NA</td>
<td>0.23 ± 0.20</td>
<td>1.75 ± 0.50</td>
</tr>
<tr>
<td>C_max (ng/mL)</td>
<td>1,386.67 ± 353.28</td>
<td>4,850 ± 698</td>
<td>252.0 ± 184.25</td>
</tr>
<tr>
<td>AUC_0-∞ (h•mg/mL)</td>
<td>1,169 ± 1,580</td>
<td>4,850 ± 698</td>
<td>1,111 ± 1,446</td>
</tr>
<tr>
<td>Total body clearance (mL/L/kg)</td>
<td>0.09 ± 0.2</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Vdss (L/kg)</td>
<td>0.29 ± 0.08</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Vd (L/kg)</td>
<td>0.15 ± 0.03</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>1.72 ± 0.31</td>
<td>4.67 ± 1.70</td>
<td>6.14 ± 1.59</td>
</tr>
</tbody>
</table>

Terminal half-life is reported as harmonic mean ± SD; remaining parameters are given as mean ± SD. *The number of samples with detectable diazepam concentrations was too small to calculate pharmacokinetic parameters following administration per rectum.

AUC_0-∞ = Area under the time-concentration curve from time 0 to infinity. MRT = Mean residence time from time 0 to infinity. NA = Not applicable. T_max = Time of C_max. Vdss = Apparent volume of distribution. Vd = Apparent volume of distribution at steady state. Suppositories were compounded at a commercial pharmacy with a suspending gel and fatty acid base.
utes, and diazepam was detectable in this dog at only 2 time points (45 and 60 minutes), although concentrations of diazepam and nordiazepam were similar to those of most other dogs until the 90-minute sample.

The mean plasma concentration–time profiles of diazepam and nordiazepam after administration of diazepam IV and via suppositories per rectum were shown graphically (Figure 1), and the associated pharmacokinetic parameters were summarized (Table 1). Terminal half-life and concentration at time 0 of diazepam after IV administration were 2.26 ± 0.41 hours and 2,429.17 ± 522.06 ng/mL, respectively; terminal half-life and C_{\text{max}} of nordiazepam were 2.96 ± 0.94 hours and 1,366.67 ± 353.28 ng/mL, respectively. Pharmacokinetic parameters for diazepam after rectal administration were not calculated because of an insufficient number of samples that contained detectable drug concentrations. Terminal half-life and C_{\text{max}} of nordiazepam after administration of diazepam suppositories were 3.22 ± 1.67 hours and 252.0 ± 184.25 ng/mL, respectively.

Discussion

In the present study, we tested the hypothesis that a dose of 2 mg of diazepam/kg administered via suppositories per rectum would result in plasma concentrations of diazepam and nordiazepam expected to be effective for control of status epilepticus or cluster seizures in dogs (150 to 300 ng/mL)\(^8\) within a clinically useful interval (10 to 15 minutes)\(^12\), and thus have potential for out-of-hospital treatment of these conditions in dogs. We used similar numbers of dogs, the same random crossover design, and similar blood sample collection times as were used in pharmacokinetic studies\(^8,9,11,12,15\) performed to evaluate other routes and methods of administration for diazepam (ie, IV, intranasal, and per rectum administration of injectable diazepam). Three dogs in our study had a 7-day washout period, and the other 3 dogs had a 6-day washout period. The reason for this difference is because the 2 groups were treated on consecutive days for the first treatment, but it was feasible to treat both groups in 1 day for the second treatment.

The therapeutic range of benzodiazepines in dogs is not well established, but circulating concentrations between 150 to 300 ng/mL are reported to be therapeutic.\(^8\) All dogs in our study had plasma concentrations of diazepam and nordiazepam above this range immediately (ie, in the first sample obtained after administration at 3 minutes) following IV administration. The highest plasma concentration of diazepam after administration via suppository per rectum was 99 ng/mL, and this was detected at 120 minutes. Targeted concentrations of nordiazepam were detected 90 minutes after per rectum administration in 2 dogs and after 120 minutes in 3 dogs. One dog did not have targeted concentrations of diazepam or nordiazepam at any time during the study after per rectum administration. This dog had defecated 75 minutes after suppository administration (the suppositories had melted, and the feces had waxy streaks present). However, plasma concentrations of diazepam and nordiazepam in this dog were similar to those of most other dogs until the 90-minute sample.

In 3 studies\(^8,11,12\) performed to evaluate diazepam administered per rectum, a parenteral product was administered into the terminal rectum of dogs and not given in a suppository formulation. This difference in drug delivery system may explain why targeted plasma concentrations of benzodiazepines were rapidly reached in those studies but not in the present study. Another possible explanation for the difference in our results from those of previous studies is the method of sample analysis. The authors of 2 studies\(^8,12\) used a commercially available assay for benzodiazepine analysis that is designed for nonspecific identification of benzodiazepines in human urine or serum, and the manufacturer suggests that the assay is only semiquantitative.\(^11\) This method is used to examine total benzodiazepine concentrations and does not distinguish between diazepam and its metabolites. The actual diazepam concentrations in the previous studies may have been low, similar to those found in the present study. Other factors that could have impacted our results include absorption of the drug in feces, degradation of the drug, defecation after drug administration, and suppository composition. Although more complete removal of feces could have been achieved with enema administration, this was not done because it would not approximate the situation of home treatment for a dog in status epilepticus. Absorption across the rectal mucosa is expected for a drug that has high lipid solubility such as diazepam. Although the diazepam suppositories used in our study were prepared by a commercial pharmacy, this formulation may not be appropriate for use in dogs.

The diazepam dose of 2 mg/kg (given IV or per rectum) is currently the maximum recommended single dose for treating status epilepticus.\(^8\) We did not know the pharmacokinetics of diazepam administered via suppository per rectum, and we did not want to exceed this dose. Additionally, we did not administer diazepam at a lower dose because we wanted to maximize the likelihood of reaching therapeutic plasma concentrations in a timely manner after per rectum administration.

The pharmacokinetics of the commercially available injectable preparation of diazepam administered IV or per rectum in dogs have been well established.\(^7,8,10,11,12,13\) The results of our study for IV administration are similar to those previously described. Rectal administration of a diazepam solution is accepted as effective in the emergency treatment of cluster seizures and status epilepticus when IV access is not possible or trained personnel are unavailable (eg, at-home treatment by dog owners).\(^1,2,4,5,8,11,13\) The distal portion of the rectum is drained by hemorrhoidal veins directly into the vena cava, avoiding portal vein transport to the liver where the drug can be rapidly metabolized.\(^8,11,13\) Thus, direct access to the systemic circulation is possible and therapeutic benzodiazepine concentrations can be reached relatively rapidly via this route.

Although compounding diazepam into rectal suppositories provided a convenient and apparently safe formulation for administration, the results of the present study did not support the hypothesis that administration of diazepam via suppositories (as formulated for this study) would result in presumed therapeutic plasma concentrations of diazepam or nordiazepam within
an acceptable time frame to be effective as emergency
treatment for cluster seizures or status epilepticus.

a. Valium (5 mg/mL), Hospira Inc, Lake Forest, Ill.
b. Community Pharmacy, Knoxville, Tenn.
c. Supposibase F, Letco, Decatur, Ala.
e. Surflo winged infusion set, Terumo Medical Products, Somerset, NJ.
g. 2695 Separations Module, Waters Corp, Milford, Mass.
h. 2487 UV Detector, Waters Corp, Milford, Mass.
i. Symmetry Column, Waters Corp, Milford, Mass.
j. WinNonlin, version 5.2, Pharsight Corp, Mountain View, Calif.

References
