Pharmacokinetic evaluation of novel midazolam gel formulations following buccal administration to healthy dogs

Mohammed F. Aldawsari BS
Vivian W. Lau DVM
Ramapuram J. Babu PhD
Robert D. Arnold PhD
Simon R. Platt BVMS

Received December 22, 2016.
Accepted April 5, 2017.

From the Department of Drug Discovery and Development, Harrison School of Pharmacy, Auburn University, Auburn, AL 36849 (Aldawsari, Babu, Arnold); and the Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, University of Georgia, Athens, GA 30602 (Lau, Platt).

Mr. Aldawsari and Dr. Lau contributed equally to this work.

Address correspondence to Dr. Platt (srplatt@uga.edu).

OBJECTIVE
To determine the physiochemical properties and pharmacokinetics of 3 midazolam gel formulations following buccal administration to dogs.

ANIMALS
5 healthy adult hounds.

PROCEDURES
In phase 1 of a 2-phase study, 2 gel formulations were developed that contained 1% midazolam in a poloxamer 407 (P1) or hydroxypropyl methylcellulose (H1) base and underwent rheological and in vitro release analyses. Each formulation was buccally administered to 5 dogs such that 0.3 mg of midazolam/kg was delivered. Each dog also received midazolam hydrochloride (0.3 mg/kg, IV). There was a 3-day interval between treatments. Blood samples were collected immediately before and at predetermined times for 8 hours after drug administration for determination of plasma midazolam concentration and pharmacokinetic analysis. During phase 2, a gel containing 2% midazolam in a hydroxypropyl methylcellulose base (H2) was developed on the basis of phase 1 results. That gel was buccally administered such that midazolam doses of 0.3 and 0.6 mg/kg were delivered. Each dog also received midazolam (0.3 mg/kg, IV). All posttreatment procedures were the same as those for phase 1.

RESULTS
The H1 and H2 formulations had lower viscosity, greater bioavailability, and peak plasma midazolam concentrations that were approximately 2-fold as high, compared with those for the P1 formulation. The mean peak plasma midazolam concentration for the H2 formulation was 187.0 and 106.3 ng/mL when the midazolam dose administered was 0.6 and 0.3 mg/kg, respectively.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that buccal administration of gel formulations might be a viable alternative for midazolam administration to dogs. (*Am J Vet Res* 2018;79:73–82)

Seizures are the most common manifestation of neurologic disease in small animals.[1] Although most seizures are isolated self-limiting episodes, status epilepticus and cluster seizures are 2 conditions that require urgent medical intervention.[2–4] Status epilepticus is a life-threatening condition characterized by generalized seizures that last > 5 minutes or 2 seizures occurring in tandem without interictal recovery of consciousness.[3] Cluster seizures are defined as ≥ 2 seizures that occur within a 24-hour period, with return to normal mentation between each event.[5]

In a hospital setting, IV administration of a benzodiazepine is considered the first line of treatment for status epilepticus.[6] In nonhospital settings where obtaining IV access in a seizing patient is nearly impossible, alternative routes of drug administration for seizure control are necessary. Rectal administration of diazepam is well described in both human and veterinary medicine.[7–11] Compared with IV administration, rectal administration of diazepam results in lower plasma drug concentrations and bioavailability but achieves reasonable absorption times and duration of detectable concentrations of active metabolites.[12,13] Unfortunately, variable absorption and prolonged onset of action have been reported following rectal administration of both diazepam[14] and midazolam.[15] Intranasal administration of benzodiazepines has also been reported in veterinary patients.[16–18] It is gener-

ABBREVIATIONS

AUC	Area under the concentration-time curve
C_{max}	Peak concentration
HPβCD	Hydroxypropyl β-cyclodextrin
HPLC	High-performance liquid chromatography
HPMC	Hydroxypropyl methylcellulose
LC-MS-MS	Liquid chromatography–tandem mass spectrometry
$t_{1/2}$	Plasma half-life
t_{max}	Time to peak concentration

ally a more pleasant route of drug administration than rectal administration for owners and has the added advantage of avoiding hepatic first-pass effects, which are frequently encountered with drug administration in the rectum.[15,18] Limitations associated with intranasal drug administration include restrictions regarding the volume of a drug that can be administered without pharyngeal runoff and the potential for patients to sneeze following administration.[16–18] Additionally, in veterinary medicine, intranasal drug administration can be difficult because of the small size of the nares of many dogs and cats.

In human patients, buccal administration is another route of administration for midazolam that circumvents the high hepatic first-pass effects associated with rectal or oral administration.[11] Buccal administration of midazolam to epileptic adults and children is reported to be both efficacious and well tolerated.[8,11,19–23] There is 1 report[24] in the veterinary literature that describes oral mucosal absorption of midazolam, but an unknown dose of the drug was administered to anesthetized dogs in that study. Results of that study[24] indicate that oral mucosa absorption of midazolam is pH dependent, with absorption being greatest for formulations with a pH between 3 and 4.

Veterinarians and pet owners need a safe, reliable, and easy treatment for pets that develop acute repetitive seizures in either a clinic or home environment. The primary purpose of the study reported here was to determine the physiochemical properties of 3 novel midazolam gel formulations and evaluate the pharmacokinetics of those formulations following buccal administration to healthy dogs relative to those following IV administration of a commercially available injectable midazolam solution. A secondary objective was to evaluate the effect of buccal administration of various doses of the midazolam gel formulations on plasma drug concentrations. We hypothesized that a suitably formulated midazolam gel would provide rapidly detectable plasma drug concentrations following buccal administration to dogs.

Materials and Methods

Animals

All in vivo study procedures were reviewed and approved by the University of Georgia Institutional Animal Care and Use Committee. Five purpose-bred research hounds (3 females and 2 males) with body weights ranging from 16.9 to 22.4 kg were used for the study. Prior to study initiation, all dogs were determined to be healthy on the basis of results of physical and neurologic examinations and a CBC and serum biochemical profile.

Study design

The study consisted of 2 phases. During phase 1, 2 gel formulations containing 1% midazolam were formulated for buccal administration to dogs. The 2 formulations underwent rheological and in vitro release analyses. Then, each formulation was buccally administered to each of the 5 dogs such that the total dose of midazolam administered was 0.3 mg/kg. Each dog was also administered midazolam hydrochloride (0.3 mg/kg, IV). Blood samples were collected from each dog immediately before and at predetermined times for 8 hours after drug administration for determination of plasma midazolam concentration and pharmacokinetic analysis. There was a 3-day washout period between treatments. In dogs, the mean half-life of midazolam is 161 minutes following IV administration of a dose of 0.2 mg/kg.[16] Therefore, a 3-day washout period represented > 7 to 10 half-lives, which was considered sufficient to ensure that plasma midazolam concentrations following administration of a particular treatment would not be affected by drug carryover from administration of the preceding treatment.

During phase 2, a third gel formulation containing 2% midazolam was formulated that was more viscous than the formulations developed in phase 1. That formulation was developed in an effort to increase plasma midazolam concentration and AUC following buccal administration to dogs. The formulation was buccally administered to each of 5 dogs twice. The first treatment provided each dog a midazolam dose of 0.3 mg/kg, and the second treatment provided each dog a midazolam dose of 0.6 mg/kg. Each dog was also administered midazolam (0.3 mg/kg, IV). Similar to phase 1, there was a 3-day washout period between treatments, and blood samples were collected before and after midazolam administration for determination of plasma midazolam concentration and pharmacokinetic analysis.

Phase 1

Preparation of midazolam formulations for buccal administration—The 2 initial gel formulations developed consisted of either poloxamer 407 (P1) or HPMC (H1) as the base polymer and had a midazolam concentration of 1% **(Appendix)**. For the P1 formulation, midazolam[a] was obtained from a compounding pharmacy and dissolved in ethanol in a volume sufficient to achieve a 1% midazolam solution. Phosphoric acid (86.6%) diluted 50:50 with deionized water[b] to achieve a 43.3% solution was added to the midazolam-ethanol solution to maintain the solubility of midazolam in the formulation. Then, aqueous poloxamer 407[c] that had been chilled to 4°C was added to the midazolam-ethanol solution in a volume sufficient to formulate a gel with 30% poloxamer 407. The resulting formulation was mixed for 15 minutes.

For the H1 formulation, a 1% midazolam–ethanol solution was prepared in the same manner as that for the P1 formulation. A 50% (wt/wt) HPβCD[d] and water solution was added to the midazolam-ethanol solution under stirring. Then, HPMC K100M[e] dissolved in water in a volume sufficient to achieve a 3% solution was added under stirring to the midazolam-ethanol-HPβCD solution and mixed for 15 minutes.

Rheological analysis of midazolam gel formulations—A flexible rheometer system[f] was used to determine

the rheological properties of each midazolam gel formulation. The same protocol was used for each formulation. The system had a cone-plate geometric arrangement with an angle of 2° and diameter of 40 mm; there was a 1-mm gap between the cone and plate. For each formulation, a 1-mL sample was drawn into a syringe and placed in the cone for evaluation, where it was allowed to stabilize for 5 minutes before measurements were obtained. Rheological measurements were performed with an increasing ramp of shear rates that ranged from 0.1 second^{-1} to 100 seconds^{-1} over 100 seconds, after which the sheer rate was held constant at 150 seconds^{-1} for 120 seconds and then decreased from 100 seconds^{-1} to 0.1 second^{-1} over 100 seconds. All assays were performed in triplicate at 25°, 30°, 35°, 40°, and 50°C. The output shear rate was converted to revolutions per minute by use of the following formula: (shear rate/14.324) × 60.

In vitro release of midazolam—A dialysis membrane diffusion method was used to determine the in vitro release of midazolam from each gel formulation. The diffusion cell apparatus[g] used held up to 6 diffusion cells in series and a motor to rotate magnetic beads. The regenerated cellulose membrane had a molecular weight cutoff of 13,000 g/mol and was soaked in 0.01M PBS solution (pH, 7.4) for 1 hour before use. The membrane was mounted horizontally between the donor and receptor halves of the diffusion cell. The surface area of the dialysis membrane exposed to the formulation in the donor chamber was 0.64 cm^2, and the receptor cell was filled with 5 mL of PBS solution. A water-circulation jacket surrounded the receptor cell to maintain the temperature at a physiologic level (37°C). The donor chamber was covered with a thermoplastic self-sealing film.[h] Approximately 50 mg of the formulation being evaluated was gravimetrically placed on the dialysis membrane with a syringe. The whole receptor medium was collected and replaced with fresh PBS solution at 0, 1, 2, 4, 6, 8, 12, and 24 hours, which ensured that there was a sink condition for midazolam throughout the experiment. The experiment was repeated at least 3 times for each formulation.

Determination of midazolam concentration in in vitro release samples by HPLC analysis—The midazolam concentration in the in vitro release samples was determined by use an HPLC system[i] equipped with a photo diode array–UV detector and a 5-µm, 150 × 4.6-mm C18 column.[j] Separation was performed with an isocratic mode. The mobile phase consisted of a mixture[k] of 10mM sodium acetate trihydrate and acetonitrile (vol/vol, 55:45). The run time was 10 minutes with a flow rate of 1 mL/min at room temperature (approx 22°C). The injection volume was 10 µL, and the UV absorbance of the eluent was determined at a wavelength of 220 nm.

Midazolam administration to dogs—All 5 dogs were buccally administered both of the 2 midazolam gel formulations (P1 and H1) in an amount sufficient to provide a dose of 0.3 mg of midazolam/kg. Each dog also received midazolam[l] (0.3 mg/kg, IV), and there was a 3-day washout period between treatments. All dogs received the same treatment on the same day. Food but not water was withheld from the dogs for 12 hours prior to midazolam administration and throughout the subsequent 480 minutes during which blood samples were collected.

For each buccal treatment, the required amount of gel was calculated for each dog and preloaded into needleless 1-mL plastic syringes. The volume of gel required for the dogs ranged from 1.0 to 1.3 mL. For dogs that required > 1 mL, the remaining amount of gel was preloaded into a second needleless 1-mL syringe. For each dog, the gel was administered along the inner cheek pouch between the teeth and buccal mucosa. Then, the cheeks and lips on the side of gel administration were gently massaged for 5 to 10 seconds, and the dog's head was kept elevated at an angle of at least 30° for 60 seconds.

For the IV treatment, midazolam was injected over a period of 5 to 10 seconds into a cephalic vein either directly or via a catheter depending on the technical skill of the person administrating the drug and on the temperament of the dog. Following injection of the midazolam solution, the needle or catheter was flushed with 0.5 mL of heparinized saline solution to ensure that the entire dose was delivered to each dog.

Data and blood sample collection—Physiologic variables monitored for all dogs included heart rate, respiratory rate, and indirect blood pressure. Heart rate was measured by means of thoracic auscultation or palpation of a peripheral pulse just before and every 5 minutes for the first 30 minutes after midazolam administration. Respiratory rate and indirect blood pressure were measured immediately before and at 15 and 30 minutes after midazolam administration.

Peripheral blood samples (2 mL) were collected into standard blood collection tubes that contained lithium heparin as an anticoagulant via a catheter that was aseptically placed in a cephalic vein or via jugular venipuncture immediately before and at 3, 6, 9, 12, 15, 20, 30, 60, 120, 240, and 480 minutes after midazolam administration. Following collection, blood samples were stored on ice for a maximum of 2 hours prior to centrifugation. After centrifugation, plasma was harvested from each sample, placed in a polypropylene vial, and stored at –70°C until analysis. The plasma midazolam concentration for each sample was measured within 14 days after collection.

Determination of plasma midazolam concentration—For each sample, the plasma midazolam concentration was determined by means of LC-MS-MS that involved the use of an ultra-HPLC system[m] coupled to a triple quadrapole mass spectrometer.[n] Chromatographic quantification was achieved with a 2.1 × 50-mm, 1.8-µm C8 column[o] and use of a mo-

bile phase composed of 10mM ammonium formate (A) and acetonitrile (B), with a gradient elution of 40% A and 60% B at minute 0 and 20% A and 80% B at minute 1. The flow rate was 0.5 mL/min, and the column temperature was set at 40°C. The injection volume was 1 µL, and samples were introduced into the mass spectrometer with electrospray ionization. Analysis time was 1 minute, and mass detection was achieved by use of multiple reaction monitoring. For midazolam, the qualifying (precursor) ion had an m/z of 326.1, the quantifying (product) ion had an m/z of 249.1 (m/z, 291.1 → 249.1), the fragmentor was set at 160 V, and collision energy was set at 26 and 40 V. For diazepam (internal standard), the precursor ion had an m/z of 285.1, the product ion had an m/z of 154.0 (m/z, 193.1 → 154), the fragmentor was set at 140 V, and collision energy was set at 32 and 26 V.

To validate the LC-MS-MS method used to measure plasma midazolam concentration, 10 mg of midazolam was weighed and dissolved in 100 mL of methanol to obtain a stock solution with a concentration of 100 µg of midazolam/mL. A portion of that solution was diluted further with methanol to achieve a solution with a midazolam concentration of 6 µg/mL. To construct a calibration curve, an equal volume of plasma obtained from each of the 5 study dogs prior to midazolam administration was pooled and then separated into aliquots. Those aliquots were spiked with midazolam to achieve samples with 1,000, 500, 250, 125, 62.5, 31.3, 15.6, 7.81, 3.91, 1.95, 0.977, 0.488, and 0.244 ng of midazolam/mL. A blank aliquot (midazolam concentration, 0 ng/mL) was also included for each calibration curve. A new calibration curve was generated each time plasma samples were assayed. A stock solution with a diazepam concentration of 150 ng/mL was prepared for use as the internal standard. The limit of detection for midazolam was determined by injection of serially diluted standard solutions into the system until a signal-to-noise ratio of 3 was obtained. The lower limit of quantification was defined as the lowest plasma concentration of midazolam that could be quantified with acceptable precision and accuracy under experimental conditions. The volume of internal standard (diazepam) added to the samples was 50 µL. The analyte response at the lower limit of quantitation was 5 times the analyte response for the blank aliquot. Depending on the lower limit of quantification for midazolam for the calibration curve of interest, 4 midazolam concentrations (0.244, 3.906, 62.5, and 500 ng/mL) were selected as quality control samples. All quality control samples were analyzed in triplicate. The measured plasma midazolam concentrations in those samples had a precision that ranged from 85% to 110% and accuracy that ranged from 88% to 115%. The percentage of midazolam recovery from all quality control samples was within 80% to 120% of the expected amount.

Following validation of the LC-MS-MS method, it was used to determine the midazolam concentration in individual plasma samples collected from dogs immediately before and after midazolam administration. Briefly, 50 µL of the internal standard (diazepam concentration, 150 ng/mL) was added to 100 µL of each plasma sample and vortexed for 1 minute. Fifty microliters of 0.1M sodium hydroxide was added to the resulting mixture and vortexed for 30 seconds, which was followed by the addition of 0.8 mL of diethyl ether and vortexing for another 10 minutes. Samples were centrifuged at 3,000 × *g* for 5 minutes at room temperature, and the vials were transferred to a freezer set at –80°C for 20 minutes. Then, the organic layer within each vial was transferred into a 5-mL radioimmunoassay vial and evaporated under a gentle stream of nitrogen gas at 40°C. The resulting residue was reconstituted in 100 µL of acetonitrile, and the midazolam concentration was determined by the LC-MS-MS method previously described.

Pharmacokinetic analysis—For each of the 3 treatments, the plasma midazolam concentration over time was plotted for each dog. The AUC was calculated by use of the linear trapezoidal rule. Relative bioavailability of midazolam following buccal administration was calculated by use of the mean AUC and results for the IV treatment as the referent. The C_{max} was calculated on the basis of observed data, and the t_{max} was defined as the time of the first occurrence of C_{max}. The $t_{1/2}$ and other pharmacokinetic values were calculated by use of standard equations.

Phase 2

Phase 2 took place 2 months after completion of phase 1, and the same 5 dogs were used in both phases. During phase 2, a third gel formulation (H2) was developed that contained 2% midazolam in an HPMC base. The H2 formulation was prepared in the same manner as the H1 formulation developed in phase 1, except the amount of midazolam was doubled to yield a 2% solution. Likewise, the H2 formulation underwent rheological and in vitro release analyses as described in phase 1. That formulation was then buccally administered to each of the 5 dogs twice. The first treatment ($H2_{0.3}$) provided each dog a midazolam dose of 0.3 mg/kg, and the second treatment ($H2_{0.6}$) provided each dog a midazolam dose of 0.6 mg/kg. Each dog was also administered midazolam[1] (0.3 mg/kg, IV). As in phase 1, all dogs received the same treatment on the same day, and there was at

Table 1—Physical properties of gel formulations containing 1% midazolam in a poloxamer 407 base (P1), 1% midazolam in an HPMC base (H1), and 2% midazolam in an HPMC base (H2) that were created to administer midazolam buccally to dogs.

	Formulation		
Property	**P1**	**H1**	**H2**
Appearance	Clear	Clear	Clear
pH	3.5	3.2	3.8
Density at room temperature (g/mL)	0.938	0.938	1.03
Assay (%)	101.2	101.3	100.8
Viscosity (Pa•s)	124	3.92	12.5

least a 3-day interval between treatments. Food but not water was withheld from the dogs for 12 hours prior to midazolam administration and throughout the subsequent 480 minutes during which blood samples were collected. Buccal and IV administration of midazolam; monitoring of heart rate, respiratory rate, and indirect blood pressure; collection and processing of blood samples; determination of plasma midazolam concentrations; and pharmacokinetic analysis were performed as described for phase 1.

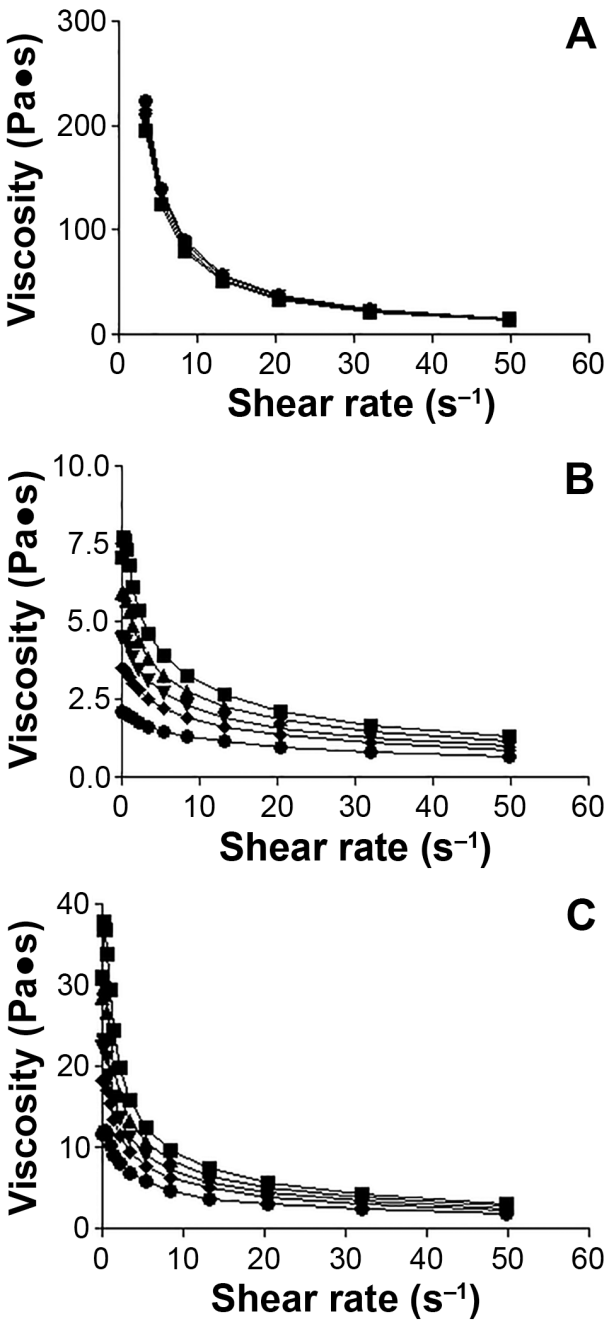

Figure 1—Viscosity versus shear rate for gel formulations containing 1% midazolam in a poloxamer 407 base (P1; A), 1% midazolam in an HPMC base (H1; B), and 2% midazolam in an HPMC base (H2; C) that were created to administer midazolam buccally to dogs at ambient temperatures of 25°C (squares), 30°C (triangles), 35°C (inverted triangles), 40°C (diamonds), and 50°C (circles). Notice the y-axis scale varies among the 3 panels.

Statistical analysis

For each phase and pharmacokinetic parameter evaluated, the mean and SE were calculated for each of the 3 treatments. Within each phase, pharmacokinetic parameters were compared among the 3 treatments by use of a 1-way ANOVA. All analyses were performed with commercially available statistical software,[p] and values of $P < 0.05$ were considered significant.

Results

Dogs

All buccal and IV treatments were well tolerated by all 5 dogs. Two dogs developed excessive salivation that was self-limiting following administration of the $H2_{0.6}$ treatment (phase 2), and one of those dogs similarly developed transient excessive salivation following the IV treatment during phase 2. The heart rate, respiratory rate, and indirect blood pressure remained within the respective reference ranges for all dogs throughout the observation period for all treatments and did not differ significantly among the treatments of phase 1 or phase 2 at any time.

Midazolam gel formulations

All 3 midazolam formulations (P1, H1, and H2) created were clear gels with pHs that ranged between 3.2 and 3.8 **(Table 1)**. The viscosity of the P1 formulation was 30- and 10-fold that of the H1 and H2 formulations, respectively, and did not change appreciably when the ambient temperature was varied from 25° to 50°C **(Figure 1)**. Conversely, the viscosity of the H1 formulation decreased 3-fold and that of the H2 formulation decreased 4-fold when the ambient temperature was varied over the same range. However, at room temperature, all 3 formulations had optimum

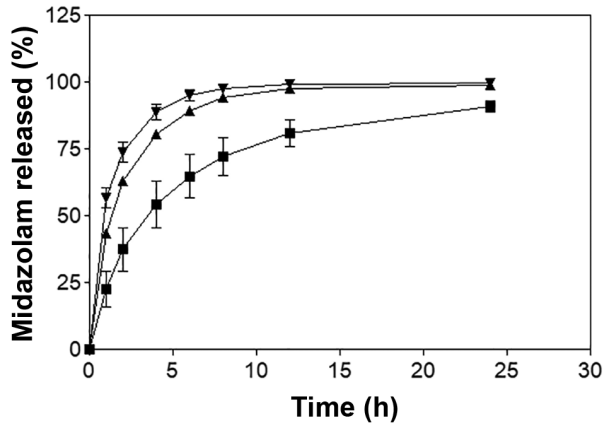

Figure 2—Mean ± SE percentage of midazolam released across a dialysis membrane over time for the P1 (squares), H1 (triangles), and H2 (inverted triangles) gel formulations of Figure 1. ***See*** Figure 1 for remainder of key.

rheological properties and physical consistencies suitable for administration through a 1-mL needleless syringe.

The rate of midazolam diffusion across a dialysis membrane (ie, in vitro release analysis) for the H1 and H2 formulations was significantly ($P < 0.001$) greater than that for the P1 formulation. For example, at 4 hours after buccal administration, the mean ± SE percentage of midazolam released was 80.5 ± 1.2% for the H1 formulation and 91.1 ± 0.0% for the H2 formulation, compared with only 54.2 ± 8.8% for the P1 formulation **(Figure 2)**. This suggested the H1 and H2 formulations had better midazolam-release properties than the P1 formulation and therefore were likely better alternatives for buccal administration of midazolam in vivo.

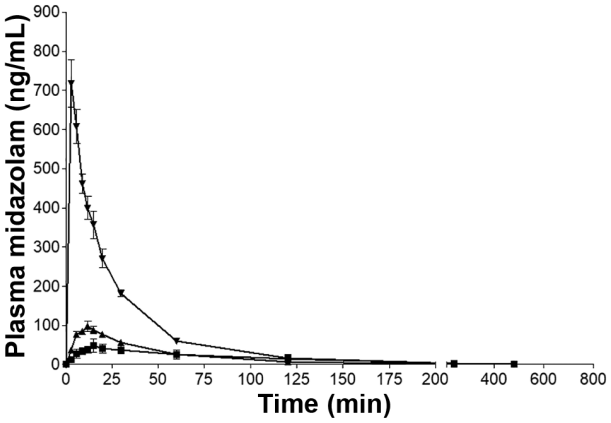

Figure 3—Mean ± SE plasma midazolam concentrations over time for 5 healthy adult purpose-bred hounds following buccal administration of the P1 (squares) and H1 (triangles) formulations described in Figure 1 in a volume sufficient to deliver a dose of 0.3 mg of midazolam/kg to each dog and IV administration of midazolam (0.3 mg/kg; inverted triangles; phase 1). Each dog received each treatment, and there was at least 3 days between treatments. ***See*** Figure 1 for remainder of key.

Phase 1

The mean ± SE plasma midazolam concentrations over time for the 3 midazolam treatments (P1, H1, and IV) administered in phase 1 were plotted **(Figure 3)**, and the pharmacokinetic parameters were summarized **(Table 2)**. Although the mean $t_{1/2}$ of midazolam for the P1 (58.7 minutes) and H1 (63.8 minutes) treatments was similar to that for the IV treatment (68.7 minutes), the mean C_{max} achieved for the P1 (47.7 ng/mL) and H1 (98.3 ng/mL) treatments was only 6.7% and 13.7%, respectively, of that achieved for the IV treatment (717 ng/mL). Additionally, the bioavailability of midazolam following buccal administration was 25.2% and 28.4% for the P1 and H1 treatments, respectively.

Phase 2

The mean ± SE plasma midazolam concentrations over time for the 3 midazolam treatments ($H2_{0.3}$, $H2_{0.6}$, and IV) administered in phase 2 were plotted **(Figure 4)**, and the pharmacokinetic parameters were summarized **(Table 3)**. Although the mean $t_{1/2}$ of midazolam for the IV treatment (90.7 minutes) was 1.3 times that for the IV treatment of phase 1 (68.7 minutes), the mean C_{max} for the IV treatment (608.5 ng/mL) was only 85% of that for the IV treatment of phase 1 (717 ng/mL). The mean C_{max} (106.3 ng/mL) and bioavailability (40.8%) of midazolam for the $H2_{0.3}$ treatment were greater than those for the P1 and H1 treatments of phase 1. Furthermore, the mean C_{max} for the $H2_{0.6}$ treatment (187.0 ng/mL) was significantly greater than that for the $H2_{0.3}$ treatment.

Discussion

Results of the present study indicated that the 3 midazolam gel formulations (P1, H1, and H2) developed for buccal administration of the drug to dogs were absorbed effectively, with C_{max} achieved within 15 minutes after midazolam administration and bio-

Table 2—Pharmacokinetic parameters for midazolam following buccal administration of a compounded gel formulation that contained 1% midazolam in a poloxamer 407 (P1 treatment) or HPMC (H1 treatment) base in a volume sufficient to deliver a dose of 0.3 mg of midazolam/kg, and IV administration of midazolam (0.3 mg/kg; IV treatment) to each of 5 healthy adult purpose-bred research hounds (phase 1).

	Treatment		
Parameter	**P1**	**H1**	**IV**
$t_{1/2}$ (min)	58.7 ± 16.6	63.8 ± 44.9	68.7 ± 36.7
t_{max} (min)	15.0	12.0	—
C_{max} (ng/mL)	47.7 ± 38.5	98.3 ± 26.5	717 ± 138
AUC_{0-t} (ng/mL•min)	4,620 ± 4,530	5,170.1 ± 330	18,400 ± 2,400
$AUC_{0-\infty}$ (ng/mL•min)	4,630 ± 4,550	5,230 ± 300	18,400 ± 240
$AUMC_{0-\infty}$ (ng/mL•min^2)	402,037 ± 535,400	365,947.7 ± 54,907.6	719,912 ± 230,301
$MRT_{0-\infty}$ (min)	86.8 ± 28.0	69.9 ± 11.6	39.1 ± 12.0
V_z/f (mg/[ng/mL])	0.183 ± 0.164	0.176 ± 0.138	0.054 ± 0.030
Bioavailability (%)	25.2	28.4	—

Values represent the mean ± SE. If an SE is not reported, the value was the same for all 5 dogs. The study had a crossover design; each dog received each of the 3 treatments with a 3-day washout period between treatments.

AUC_{0-t} = AUC from time 0 to the final sample collection. $AUC_{0-\infty}$ = AUC from time 0 extrapolated to infinity. $MRT_{0-\infty}$ = Mean resonance time extrapolated to infinity. V_z/f = Apparent volume of distribution during the terminal phase. — = Not observed or calculated.

availability ranging from approximately 25% to 41%. The minimum plasma midazolam concentration required to effectively eliminate seizure activity in dogs has not been established. However, commonly recommended doses of midazolam to control seizures in dogs range from 0.06 to 0.5 mg/kg, IV, or 0.2 mg/kg, intranasally, and achieve mean ± SE plasma midazolam concentrations of 860 ± 360 ng/mL to 450 ± 90 ng/mL, respectively.[15,16,25,26] Although the mean C_{max} of midazolam following IV administration was superior to that following buccal administration in the present study, the mean ± SE C_{max} achieved following administration of the $H2_{0.6}$ treatment (187.0 ± 104.3 ng/mL) was comparable to the mean ± SE C_{max} following intranasal administration of a commercial midazolam solution at a dose of 0.2 mg/kg (210 ± 20 ng/mL) and greater than that following rectal administration of a commercial midazolam solution at a dose of 0.2 mg/kg (150 ± 10 ng/mL).[16,27] However, the mean ± SE C_{max} of midazolam following intranasal administration of midazolam in an HPMC gel (450 ± 90 ng/mL) in that study[16] was greater than that following buccal administration of any of the midazolam gels formulated for the present study. The AUC of midazolam, a reflection of its bioavailability and clearance, following buccal administration of the drug in the present study was similar to or greater than the AUC following IM administration of midazolam to healthy dogs of another study.[15]

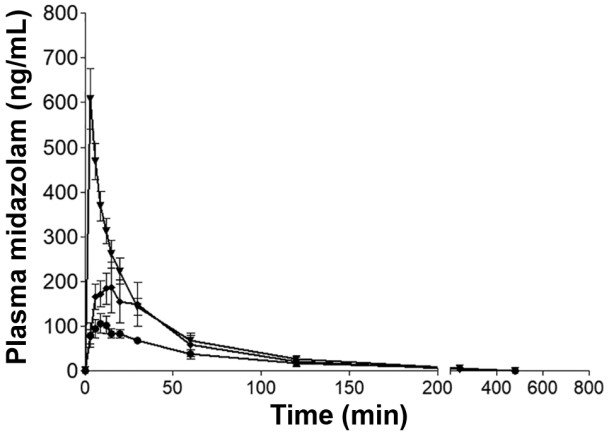

Figure 4—Mean ± SE plasma midazolam concentrations over time for the dogs of Figure 3 following buccal administration of the H2 formulation described in Figure 1 in a volume sufficient to deliver a dose of 0.3 ($H2_{0.3}$ treatment; circles) or 0.6 ($H2_{0.6}$; diamonds) mg/kg to each dog and IV administration of midazolam (0.3 mg/kg; inverted triangles; phase 2). ***See*** Figures 1 and 3 for remainder of key.

The fact that the C_{max} was achieved within 15 minutes after buccal administration of the midazolam gels formulated in the present study suggested that buccal administration is a viable alternative for midazolam administration to dogs in status epilepticus or with cluster seizure activity when IV access is not possible. Buccal administration of midazolam to human patients causes electroencephalographic changes within 5 minutes and before the drug becomes detectable in venous blood samples.[28] The authors of that study[28] theorized that midazolam absorbed buccally distributes rapidly from arterial blood to the brain and fat, resulting in low venous midazolam concentrations within the first few minutes after administration. However, the peak venous midazolam concentration for the human patients of that study[28] (86.7 ng/mL) was substantially lower than the C_{max} achieved following buccal administration of 0.6 mg of midazolam/kg ($H2_{0.6}$ treatment) to the dogs of the present study (187 ng/mL). Electroencephalographic evaluation and determination of arterial midazolam concentrations are necessary to determine whether buccal administration of the drug to dogs has similar effects to those observed in human patients. The possibility that buccal administration of midazolam causes CNS effects prior to achievement of the C_{max} is intriguing.

Following buccal administration of the midazolam formulations developed in the present study, the mean C_{max} was greater and the mean t_{max} was faster than those reported following rectal administration of midazolam to dogs.[15] Absorption of midazolam following rectal administration is dependent on the technical skill of the person administering the drug and the amount of fecal material present in the rec-

Table 3—Pharmacokinetic parameters for midazolam following buccal administration of a compounded gel formulation that contained 2% midazolam in an HPMC base in a volume sufficient to deliver a dose of 0.3 ($H2_{0.3}$ treatment) or 0.6 ($H2_{0.6}$ treatment) mg of midazolam/kg, and IV administration of midazolam (0.3 mg/kg; IV treatment) to the dogs of Table 2 (phase 2).

	Treatment		
Parameter	**$H2_{0.3}$**	**$H2_{0.6}$**	**IV**
$t_{1/2}$ (min)	75.3 ± 18.7	71.3 ± 13.8	90.7 ± 15.4
t_{max} (min)	9.0	15.0	—
C_{max} (ng/mL)	106.3 ± 35.2	187.0 ± 104.3	608.5 ± 150.9
AUC_{0-t} (ng/mL•min)	7,805.8 ± 3,598.8	12,101.9 ± 8,902.8	19,001.4 ± 4,578.2
$AUC_{0-\infty}$ (ng/mL•min)	7,873.9 ± 3,626.9	12,162.4 ± 8,954.1	19,312.3 ± 4,641.1
$AUMC_{0-\infty}$ (ng/mL•min^2)	677,800 ± 663,759	788,994 ± 704,905	1,261,242 ± 408,388
$MRT_{0-\infty}$ (min)	86.1 ± 33.0	64.9 ± 9.4	65.3 ± 8.8
V_z/f (mg/[ng/mL])	0.138 ± 0.071	0.085 ± 0.053	0.068 ± 0.016
Bioavailability (%)	40.8	—	—

Phase 2 took place 2 months after completion of phase 1.
See Table 2 for remainder of key.

tum. Additionally, midazolam is hydrophilic at low pHs, which decreases its absorption across the rectal mucosa.[15,24] In epileptic children, the duration between midazolam administration and onset of its antiseizure effects is substantially shorter when the drug is administered buccally versus rectally.[22,29]

Drugs can be administered in greater volumes by the buccal route than by the intranasal route. Thus, buccal administration of midazolam may be preferable to intranasal administration, particularly in large or heavy patients that are actively seizing and require high doses of the drug because the oral cavity is considerably larger and more easily accessible than the nasal cavity.[16] Alternatively, midazolam can be administered to dogs by the IM route when IV access is not possible, but IM drug administration may be beyond the technical ability or comfort of some owners.[15]

The 3 optimized midazolam gel formulations created in the present study had similar pHs but different viscosity to assess bioadhesion and absorption of the drug. Of those 3 formulations, the P1 formulation (1% midazolam in a poloxamer 407 base) had the greatest viscosity and lowest C_{max} and bioavailability. Results of the in vitro drug release analysis indicated that the P1 formulation also had the lowest percentage of midazolam diffuse across the dialysis membrane, which suggested that the midazolam in that formulation was not readily available for absorption by the buccal mucosa in vivo. The H1 formulation (1% midazolam in an HPMC base) had the lowest viscosity, better midazolam release characteristics and bioavailability, and a greater mean C_{max} than the P1 formulation. Unlike the P1 formulation, both the H1 and H2 (2% midazolam in an HPMC base) formulations contained HPβCD, a drug solubilizer and buccal permeation enhancer, at concentrations of 17.6% and 22%, respectively, which helped to improve buccal absorption of midazolam. Hydroxypropyl β-cyclodextrin has been described as a mucosal permeation enhancer for other drugs such as ropinirole and bupivacaine.[30,31]

Standard injectable solutions and commercially available oral syrups have been used for buccal administration of midazolam to human patients.[8,11,22,28,29] In dogs, midazolam absorption following intranasal administration was better when administered in a 0.4% HPMC gel rather than in a standard injectable solution.[16] The investigators of that study[16] theorized that, compared with the injectable solution, the gel formulation improved midazolam retention and mucosal contact time in the nasal cavity. A similar HPMC gel formulation was developed for buccal administration in the present study to improve retention of midazolam in the oral cavity. Absorption of midazolam from the oral mucosa of dogs is pH dependent, with ideal absorption occurring when the pH is between 3 and 4.[24] An acidic environment is necessary to maintain the solubility of midazolam; however, a pH < 3, which has been reported for some commercially available injectable or oral midazolam solutions, can impair mucosal absorption of the drug.[24] The gels developed in the present study were carefully formulated to maintain the pH between 3 and 4.

Concerns associated with buccal or sublingual administration of any medication to patients during status epilepticus include the patient swallowing or aspirating the drug and inadvertent bite wounds to the person administrating the drug.[11] The C_{max} may be inhibited in patients that inadvertently swallow midazolam owing to hepatic first-pass metabolism following gastric absorption of the drug.[15,24] One dog of the present study developed excessive, albeit transient, salivation following administration of the $H2_{0.6}$ and IV treatments of phase 2. Salivation can cause swallowing or otherwise contribute to loss of a buccally administered drug from the oral cavity and adversely affect drug absorption. Many dogs with generalized seizures salivate excessively when seizing; however, the present study was not designed to investigate whether buccal administration of midazolam will yield therapeutic plasma drug concentrations in dogs with excessive salivation. Although aspiration of buccally or orally administered drugs is a concern, especially in seizing patients, < 2 mL of the H2 formulation developed in this study was required to deliver a midazolam dose of 0.3 to 0.6 mg/kg, even in patients that weighed ≥ 50 kg. Therefore, the risk of aspiration for patients following buccal administration of midazolam gel formulations is expected to be minimal. Bite wounds sustained by caregivers during drug administration are another concern. The oral mucosa is readily accessible, arguably more so than the nasal mucosa. Buccal administration of a drug simply requires insertion of the tip of a syringe into the space between the teeth and lips; it does not require insertion of anything between teeth. Buccal administration of midazolam is well tolerated by children and adults, even when their teeth are clenched during a seizure.[11,20] Results of the present study indicated that buccal administration of midazolam was quick and well tolerated by dogs and did not pose a technical challenge for the people administering the drug.

In human medicine, the social stigma associated with rectal drug administration has resulted in poor compliance with that route of drug administration and a trend toward the use of intranasal and buccal routes for administration of benzodiazepines to human patients with epilepsy.[8,11,20–23,32] In children and adults, buccal administration of midazolam results in similar, if not superior, drug absorption and onset of efficacy, compared with rectal administration of diazepam.[8,10,11,22,29] The relative ease of drug administration and social acceptability of administering solutions orally, rather than rectally, make the buccal route of drug administration a preferable option for pet owners and veterinary personnel when IV access is not possible in a seizing patient.

To our knowledge, the present study was the first to investigate the dose-dependent pharmacokinetics of midazolam in dogs following buccal administration. Further research is necessary to determine the

gel formulation, midazolam concentration, and dosage required to optimize drug absorption and plasma drug concentrations as well as to determine long-term stability and shelf life of midazolam gel formulations prior to recommendation of their use in clinical settings. Pharmacokinetic studies in patients receiving long-term anticonvulsant therapy are also warranted because chronic administration of phenobarbital can affect plasma concentrations of benzodiazepines.[33] Nevertheless, results of the present study indicated that buccal administration of a midazolam gel formulation may be a viable alternative for administration of the drug to dogs, especially those in which IV administration is not possible.

Acknowledgments

Supported in part by the Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, University of Georgia. Dr. Aldawsari was supported by a fellowship from the Saudi Cultural Mission. Mass analysis was completed by the Auburn University Specialized Pharmaceutical and Experimental Center for Translational Research and Analysis and supported in part by the Auburn University Major Grants Program.

The authors thank Ben Nie for development of the LC-MS-MS assay and quantification of midazolam concentrations.

Footnotes

a. Professional Compounding Centers of America, Houston, Tex.
b. Milli-Q water purifier system, EMD Millipore Corp, Darmstadt, Germany.
c. Pluronic F127, Letco Medical, Decatur, Ala.
d. Trapasol HPβCD, CTD Inc, Alachua, Fla.
e. Methocel K100M HPMC, Colorcon Inc, Montgomeryville, Pa.
f. CVO 100 Bohlin Rheometer, Malvern Instruments, Southborough, Mass.
g. PermeGear Inc, Bethlehem, Pa.
h. Parafilm M, Bemis Co Inc, Neenah, Wis.
i. Alliance 2695 Separation module and 2998 PDA detector, Waters Corp, Milford, Mass.
j. Luna C18 column, Phenomenex Inc, Torrance, Calif.
k. VWR International, Suwannee, Ga.
l. Bedford Laboratories, Bedford, Ohio.
m. 1290 UHPLC system, Agilent Technologies, Santa Clara, Calif.
n. 6460 triple quadrapole mass spectrometer, Agilent Technologies Inc, Santa Clara, Calif.
o. Zorbax SB-C8 column, Agilent Technologies Inc, Santa Clara, Calif.
p. Prism, version 3, GraphPad Software Inc, La Jolla, Calif.

References

1. Muñana KR. Update: seizure management in small animal practice. *Vet Clin North Am Small Anim Pract* 2013;43:1127–1147.
2. Monteiro R, Adams V, Keys D, et al. Canine idiopathic epilepsy: prevalence, risk factors and outcome associated with cluster seizures and status epilepticus. *J Small Anim Pract* 2012;53:526–530.
3. Lowenstein DH, Bleck T, Macdonald RL. It's time to revise the definition of status epilepticus. *Epilepsia* 1999;40:120–122.
4. Saito M, Muñana KR, Sharp NJ, et al. Risk factors for development of status epilepticus in dogs with idiopathic epilepsy and effects of status epilepticus on outcome and survival time: 32 cases (1990–1996). *J Am Vet Med Assoc* 2001;219:618–623.
5. Mariani CL. Terminology and classification of seizures and epilepsy in veterinary patients. *Top Companion Anim Med* 2013;28:34–41.
6. Dewey CW. Anticonvulsant therapy in dogs and cats. *Vet Clin North Am Small Anim Pract* 2006;36:1107–1127.
7. Podell M. The use of diazepam per rectum at home for the acute management of cluster seizures in dogs. *J Vet Intern Med* 1995;9:68–74.
8. Baysun A, Aydin ÖF, Atmaca E, et al. A comparison of buccal midazolam and rectal diazepam for the acute treatment of seizures. *Clin Pediatr (Phila)* 2005;44:771–776.
9. Bhattacharyya M, Kalra V, Gulati S. Intranasal midazolam vs rectal diazepam in acute childhood seizures. *Pediatr Neurol* 2006;34:355–359.
10. Nakken KO, Lossius MI. Buccal midazolam or rectal diazepam for treatment of residential adult patients with serial seizures or status epilepticus. *Acta Neurol Scand* 2011;124:99–103.
11. Scott RC, Besag FM, Neville BG. Buccal midazolam and rectal diazepam for treatment of prolonged seizures in childhood and adolescence: a randomised trial. *Lancet* 1999;353:623–626.
12. Mealey KL, Boothe DM. Bioavailability of benzodiazepines following rectal administration of diazepam in dogs. *J Vet Pharmacol Ther* 1995;18:72–74.
13. Papich MG, Alcorn J. Absorption of diazepam after its rectal administration in dogs. *Am J Vet Res* 1995;56:1629–1636.
14. Probst CW, Thomas WB, Moyers TD, et al. Evaluation of plasma diazepam and nordiazepam concentrations following administration of diazepam intravenously or via suppository per rectum in dogs. *Am J Vet Res* 2013;74:611–615.
15. Schwartz M, Muñana KR, Nettifee-Osborne J, et al. The pharmacokinetics of midazolam after intravenous, intramuscular, and rectal administration in healthy dogs. *J Vet Pharmacol Ther* 2013;36:471–477.
16. Eagleson JS, Platt SR, Strong DL, et al. Bioavailability of a novel midazolam gel after intranasal administration in dogs. *Am J Vet Res* 2012;73:539–545.
17. Platt SR, Randell SC, Scott KC, et al. Comparison of plasma benzodiazepine concentrations following intranasal and intravenous administration of diazepam to dogs. *Am J Vet Res* 2000;61:651–654.
18. Musulin SE, Mariani CL, Papich MG. Diazepam pharmacokinetics after nasal drop and atomized nasal administration in dogs. *J Vet Pharmacol Ther* 2011;34:17–24.
19. Camfield PR. Buccal midazolam and rectal diazepam for treatment of prolonged seizures in childhood and adolescence: a randomised trial. *J Pediatr* 1999;135:398–399.
20. Kutlu NO, Dogrul M, Yakinci C, et al. Buccal midazolam for treatment of prolonged seizures in children. *Brain Dev* 2003;25:275–278.
21. Wiznitzer M. Buccal midazolam for seizures. *Lancet* 2005;366:182–183.
22. McIntyre J, Robertson S, Norris E, et al. Safety and efficacy of buccal midazolam versus rectal diazepam for emergency treatment of seizures in children: a randomised controlled trial. *Lancet* 2005;366:205–210.
23. Talukdar B, Chakrabarty B. Efficacy of buccal midazolam compared to intravenous diazepam in controlling convulsions in children: a randomized controlled trial. *Brain Dev* 2009;31:744–749.
24. Zhang J, Niu S, Zhang H, et al. Oral mucosal absorption of midazolam in dogs is strongly pH dependent. *J Pharm Sci* 2002;91:980–982.
25. Horikawa H, Tada T, Sakai M, et al. Effects of midazolam on the threshold of lidocaine-induced seizures in the dog. *J Anesth* 1990;4:265–269.
26. Patterson EN. Status epilepticus and cluster seizures. *Vet Clin North Am Small Anim Pract* 2014;44:1103–1112.
27. Court MH, Greenblatt DJ. Pharmacokinetics and preliminary observations of behavioral changes following administration of midazolam to dogs. *J Vet Pharmacol Ther* 1992;15:343–350.
28. Scott RC, Besag F, Boyd SG, et al. Buccal absorption of mid-

azolam: pharmacokinetics and EEG pharmacodynamics. *Epilepsia* 1998;39:290–294.
29. Ashrafi MR, Khosroshahi N, Karimi P, et al. Efficacy and usability of buccal midazolam in controlling acute prolonged convulsive seizures in children. *Eur J Paediatr Neurol* 2010;14:434–438.
30. Kontogiannidou E, Andreadis DA, Zografos AL, et al. Ex vivo buccal drug delivery of ropinirole hydrochloride in the presence of permeation enhancers: the effect of charge. *Pharm Dev Technol* 2017;22:1017–1021.
31. Jug M, Maestrelli F, Bragagni M, et al. Preparation and solid-state characterization of bupivacaine hydrochloride cyclodextrin complexes aimed for buccal delivery. *J Pharm Biomed Anal* 2010;52:9–18.
32. Chakupurakal R, Sobithadevi D, Choules A, et al. Buccal midazolam: are we ready yet? *Seizure* 2010;19:310.
33. Wagner SO, Sams RA, Podell M. Chronic phenobarbital therapy reduces plasma benzodiazepine concentrations after intravenous and rectal administration of diazepam in the dog. *J Vet Pharmacol Ther* 1998;21:335–341.