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Pharmacokinetics of a Single Transdermal Dose of Mirtazapine in Rhesus Macaques (*Macaca mulatta*)

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Decreased appetite is a common clinical problem in captive rhesus macaques (*Macaca mulatta*). Mirtazapine, a tetracyclic antidepressant originally developed for humans, has shown promise as a safe and effective promoter of weight gain and appetite in several veterinary species including rhesus and cynomolgus macaques. Although mirtazapine is available as oral formulations, transdermal delivery in macaques with reduced appetite would allow quick, painless, topical application. Here we describe the pharmacokinetics of a single application of a widely available veterinary transdermal mirtazapine formulation in 6 rhesus macaques. A dose of 0.5 mg/kg of transdermal mirtazapine ointment that has proven to be effective in rhesus was applied to the caudal pinnae of 3 female and 3 male young adult macaques. Serum was collected at 0, 0.5, 1, 3, 6, 8, 12, 24, 36, 48, and 72 h after administration. Our data indicate transdermal mirtazapine is absorbed at a lower level in rhesus as compared with published values in domestic cats (rhesus peak serum concentration: 1.2 ± 0.3 ng/mL), while drug half-life is longer than that reported in cats (rhesus: 33 ± 7 h). Mirtazapine reaches peak plasma concentrations in rhesus at 16 ± 10 h after administration; our model indicates that up to 5 d of serial dosing may be necessary to reach steady state. Our preliminary data also suggest that sex differences may contribute to efficacy and/or indicate sex-based differences, as male macaques reached T_{max} more quickly than females (19 ± 2 h in females and 8 ± 3 h in males) and showed higher variation in half-life (33 ± 4 h in females and 34 ± 11 h in males). While previous work indicates clinical efficacy of the 0.5 mg/kg dosage in macaques, further investigation is warranted to determine if rhesus may benefit from higher recommended doses than companion animal species.

Abbreviations and Acronyms: CNPRC, California National Primate Research Center; TDMTZ, transdermal mirtazapine

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Introduction

Decreased appetite is a common clinical problem in captive rhesus macaques (*Macaca mulatta*) and is attributable to a wide variety of causes including illness, social or husbandry-related stress, and, in the research environment, experiment-related manipulations.[2,24] Decreased appetite can negatively affect animal health and welfare and may influence research outcomes due to resultant changes in metabolism and physiology. Pharmacologic stimulation of appetite, when not contraindicated by health or research needs, is therefore of interest to clinicians and scientists who work with this common nonhuman primate (NHP) species in research, zoo, and wildlife rehabilitation environments.

Mirtazapine, a tetracyclic antidepressant, was synthesized in 1989 and first received medical approval in the Netherlands in 1994. In the United States, it was initially licensed by the Food and Drug Administration (FDA) in 1996 to treat major depressive disorder (MDD) in humans.[19] Mirtazapine is a noradrenergic and specific serotonergic antidepressant. It acts as an antagonist of adrenergic α-2 autoreceptors and heteroreceptors, 5-HT2 and 5-HT3 serotonin receptors, and H1 histamine receptors.[3,19] In addition to the antidepressant activity of these actions, potentially beneficial side effects that include anxiolysis, appetite stimulation, sedation, analgesia, and antiemetic activity have led to several common off-label uses in human medicine and psychiatry.[19] Appetite stimulation is thought to be related to antagonism of serotonin and H1 histamine receptors,[9,29] while weight gain may occur due to pharmacologic effects on leptin and tumor necrosis factor-α,[9] metabolism of insulin and glucose,[17] and lipid processing.[22]

Based on this information, a veterinary transdermal mirtazapine (TDMTZ) formulation was approved by the FDA in 2018 for the management of weight loss in domestic cats (*Felis catus*).[25] On- and off-label use for appetite stimulation is now common in companion animal medicine, particularly as supportive care for chronic illnesses such as renal disease,[30,31] hepatic disease,[13] and neoplasia,[12] which often feature decreased appetite or anorexia. Previous work indicates this transdermal formulation is effective in treating hyporexia and promoting weight gain in rhesus and cynomolgus macaques at a dosage of 0.5 mg/kg when applied topically to the pinna.[24] This formulation and dose is now in regular clinical use at the California National Primate Research Center (CNPRC) with encouraging anecdotal

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results. The appetite-stimulating and analgesic properties of mirtazapine have been studied in several other animal species including dogs,[10,15,28] mice,[20,38] rats,[14,34] rabbits,[29] and horses.[33]

To our knowledge, pharmacokinetic information regarding TDMTZ use in macaques has not been published to date. Our study measured serum levels after a single-dose transdermal application of mirtazapine to rhesus macaques. We hypothesized that our results would be similar to those reported in cats given the same dose.[7] Our data provide information on how macaques metabolize this drug, potentially allowing more appropriate dosing and encouraging further investigation of the use of mirtazapine in NHPs.

Materials and Methods

Animals. A total of 6 young adult rhesus macaques (*Macaca mulatta*) were enrolled in this study. All 6 animals were obtained from the CNPRC breeding colony and housed in accordance with the Institute of Laboratory Animal Resources' *Guide for the Care and Use of Laboratory Animals*,[27] the Public Health Service Policy, and the Animal Welfare Act[1] and Animal Welfare Regulations.[2] This project was conducted in accordance with the University of California, Davis' Institutional Animal Care and Use Committee (IACUC) and its status as an AAALAC-accredited and USDA-registered facility.

The study used 3 male and 3 female macaques with similar age, weight, and body condition score (BCS) within same-sex groups (Table 1). All subjects received measles and tetanus vaccinations at or after 6 mo of age and had consistently screened negative for simian immunodeficiency virus, simian T-lymphotropic virus, and simian retrovirus type D. In addition, animals 5 and 6 were maintained within our SPF colony and tested negative for macacine herpesvirus-1 (MHV-1). All testing for pathogen exclusion was done via established methods at the CNPRC.[39] Preproject health screening included a complete physical exam by a CNPRC clinical veterinarian, serum chemistry, and complete blood count. Routine colony veterinary care includes annual physical examinations and semiannual tuberculosis screening. In our study population, veterinary intervention was minimal and related to blood collection: one animal received a single dose of intramuscular ketoprofen (Ketofen, Zoetis, Parsippany-Troy Hills, NJ; 5 mg/kg) approximately 30 h after mirtazapine dosing due to forearm contusions, which were considered secondary to serial venipuncture. Although blood collection volumes in all animals were within established University of California, Davis IACUC limits, animals 1 and 3 received 40 to 60 mL/kg subcutaneous lactated Ringer solution (Lactated Ringers Injection USP, Baxter International, Deerfield, IL) approximately 9 h after TDMTZ administration to provide prophylactic volume replacement due to their smaller size.

Macaques were housed indoors for the duration of the study, with a minimum 2-wk acclimation period before experimental manipulations. Husbandry staff trained the macaques for nonsedated cage-side blood collection before experimental collections. Macaques were provided with hanging mirrors, a rotation of manipulanda and enrichment objects, and constant visual and auditory contact with conspecifics. If socially paired, animals were temporarily separated for sedation and recovery and for blood collection, but otherwise remained in full social contact with the partner. All animals were housed in standard quad-unit stainless steel caging under a 12:12-h light-dark cycle (0600 to 1800 h), a 20 to 26 °C temperature range, and 30% to 70% humidity. All animals were fed a commercial chow (LabDiet Monkey Diet 5047, Purina Mills International, St. Louis, MO) twice daily and were supplemented with approximately one-fourth cup of mixed dried oats and peas as forage once per day. Fresh produce was offered twice a week per CNPRC husbandry standards, treats were provided at experimental collections and welfare checks, and animals had unrestricted access to potable water from the University of California, Davis campus water system.

Experimental design. This study used a veterinary ointment of 2% wt/wt mirtazapine (Miratаz, Dechra Veterinary Products, Overland, KS) formulated for topical/transdermal administration. The product is supplied in a 5-g aluminum multiuse tube and was stored below 25 °C prior to use, per manufacturer instructions.[25] The macaques were sedated with ketamine hydrochloride (Zetamine, MWI Veterinary Supply, Boise, ID) at 9 to 11 mg/kg to facilitate accurate drug administration and dosing. All macaques were dosed on the same day between 0700 and 0900. After sedation, macaques were weighed and baseline blood samples were collected. The caudal region of one pinna of each macaque was then shaved, gently cleaned using 70% ethanol-soaked gauze, and wiped dry. Each animal then received a single dose of 0.5 mg/kg of TDMTZ that was applied to the external surface of the pinna and gently massaged into the skin. Whole blood samples were drawn into zero-additive tubes by trained personnel, first under ketamine sedation at $t = 0$ and $t = 0.5$, and afterward by nonsedated cage-side

Table 1. Signalment and transdermal mirtazapine dosage of study animals

ID (sex)	Age	Weight (kg)	Body condition score (out of 5.0)	Housing status[a]	Pathogen exclusion status[b]	Mirtazapine dose (mg)
1 (F)	5 y, 1 mo	5.68	2.5	Paired, full contact	SIV, SRV, STLV negative	2.84
2 (F)	6 y, 11 mo	7.58	3.0	Visual, auditory contact	SIV, SRV, STLV negative	3.80
3 (F)	6 y, 11 mo	5.28	2.0	Paired, full contact	SIV, SRV, STLV negative	2.64
4 (M)	5 y, 11 mo	10.57	3.0	Paired, full contact	SIV, SRV, STLV negative	5.28
5 (M)	6 y, 11 mo	10.72	2.5	Visual, auditory contact	SIV, SRV, STLV, MHV-1 negative	5.36
6 (M)	5 y, 0 mo	8.41	2.5	Visual, auditory contact	SIV, SRV, STLV, MHV-1 negative	4.21

[a]All animals were maintained in constant visual and auditory contact with conspecifics for the duration of the study.
[b]MHV-1, macacine herpesvirus-1; SIV, simian immunodeficiency virus; SRV, simian retrovirus type D; STLV, simian T-lymphotropic leukemia virus.

collection after recovery from ketamine sedation. Additional blood collection was performed at 1, 3, 6, 8, 12, 24, 36, 48, and 72 h after dosing. Samples were allowed to clot at room temperature for a minimum of 30 min. Clotted samples were then centrifuged at a minimum of 850 *g* for 15 min, and serum was withdrawn and frozen at −80 °C until analysis.

Mirtazapine assay. Serum concentrations of mirtazapine were determined using a modification of methods previously established for measuring mirtazapine in cats.[7] The analytical system consisted of a Sciex 6500+ QTRAP triple quadrupole mass spectrometer with a turbo ionspray source coupled to a Sciex Exion UPLC system with a cooled autosampler. Samples were chromatographed on a Luna Phenyl-Hexyl, 3 µm, 2.0 × 50-mm reverse-phase chromatography column (Phenomenex, Torrance, CA). The liquid chromatography gradient had mobile phase A consisting of 10 mM ammonium acetate and mobile phase B consisting of acetonitrile. Chromatographic separation was achieved by holding mobile phase B steady at 20% from 0 to 1.0 min, increasing linearly from 20% to 98% between 1.0 and 2.0 min, holding steady at 98% until 3.5 min, and then decreasing linearly to 20% between 3.5 and 4.0 min followed by equilibration at 20% until 5.0 min. The sample injection volume was 3 µL, flow rate was 800 µL/minute, and total analysis run time was 5.0 min. The mass spectrometer settings were optimized as follows: turbo ion spray temperature, 550 °C; ion spray voltage, 5,500; source gas 1 and 2, 45 and 35 units, respectively; curtain gas, 35 units; and collision gas, medium. Compound parameters for mirtazapine were optimized as follows: declustering potential, 58 V; entrance potential, 10 V; collision energy, 35 V; and collision cell exit potential, 24 V. Sample concentrations of mirtazapine were quantified by the internal standard reference method in the multiple reaction monitoring mode with ion transitions at mass-to-charge ratio (m/z) 266.2→195.1 atomic mass units (amu) for mirtazapine and m/z 372.1→176.2 for the internal standard trazadone. Analytical standards of mirtazapine and trazadone were purchased from Millipore Sigma (St. Louis, MO) for generation of calibration curves and quality control samples in blank monkey serum. An analytical standard for the metabolite 8-hydroxymirtazapine was purchased from Cayman Chemical (Ann Arbor, MI). Mirtazapine calibration curve samples between 0.1 and 250 ng/mL (11 nonzero concentrations), 8-hydroxy mirtazapine calibration curve samples between 1 and 100 ng/mL (6 nonzero concentrations), quality control (4 each at 1, 5, 25, and 100 ng/mL), and unknown (experimental) serum samples were prepared by protein precipitation with acetonitrile. For extraction, 50 µL of standard, quality control, or unknown serum sample was added to 1.5-mL polypropylene tubes containing 5 µL of 50 ng/mL trazadone followed by 50 µL of acetonitrile. Samples were then vortex-mixed for 5 min and centrifuged for 10 min at 18,800 *g*; 100 µL of supernatant was then transferred to autosampler vials with glass inserts. The assay was evaluated for accuracy and precision of calibration curves, inter- and intraday coefficient of variation, and stability for 24 h in the autosampler.

Pharmacokinetic analysis. Serum drug concentrations were plotted on semilogarithmic graphs for visual assessment. Noncompartmental analysis of the serum mirtazapine concentration time data was performed to estimate pharmacokinetic parameters using commercially available software (Phoenix WinNonlin, v8.3.3, Certara, Princeton, NJ). Prediction of serum concentrations after administration of the 0.5-mg/kg dose was performed by nonparametric superposition in Phoenix WinNonlin.

Statistical analysis. Pharmacokinetic parameters are reported as mean with standard deviation with the exceptions of elimination rate constants and half-lives, which are reported as harmonic mean with pseudo standard deviation, and sex-based T_{max} calculations, which are reported as geometric mean with geometric standard deviation. Statistical significance of the sex-based comparison was analyzed using a Mann-Whitney *U* test. Graphing and statistical analysis of results were performed in GraphPad Prism version 9.5.1 (GraphPad Software, Boston, MA).

Results

Mirazapine assay performance. The accuracy and precision of the calibration curve were both within 10%, and the accuracy and precision of quality control samples were within 10% and 5%, respectively. The calibration curve was linear between 0.1 and 250 ng/mL (r = 0.999), and lower limit of quantitation (signal-to-noise ratio >10) and limit of detection (signal-to-noise ratio >3) were 0.1 ng/mL and 0.05 ng/mL, respectively. Samples were stable in the autosampler, with calculated concentrations of calibrators and quality control samples within 5% of initial value upon reinjection after 24 h.

Mirtazapine pharmacokinetics. Individual and group pharmacokinetic parameters for the 6 macaques are shown in Table 2. Graphical representations of individual and group serum concentration data are shown in Figure 1A and 1B, respectively. The mean C_{max} of a single 0.5-mg/kg dose of TDMTZ was 1.2 ± 0.3 ng/mL, while the mean T_{max} was 16 ± 10 h after dosing.

Table 2. Individual and group pharmacokinetic parameters for a single transdermal dose of 0.5 mg/kg mirtazapine in rhesus macaques (n = 6; 3 male, 3 female)

ID (sex)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{0\text{-}T}$ (ng × h/mL)	AUC_{INF} (ng × h/mL)	AUC_{INF} (% extrapolated)
1 (F)	1.0	24	29	40	50	22
2 (F)	1.5	24	31	50	64	22
3 (F)	1.0	12	38	39	53	27
4 (M)	1.2	24	37	43	56	24
5 (M)	1.0	6	46	34	51	34
6 (M)	1.6	3	25	55	64	14
All females	1.2 ± 0.3	19 ± 25[a]	33 ± 4[b]	43 ± 6	56 ± 7	23 ± 3
All males	1.3 ± 0.3	8 ± 3[a]	34 ± 11[b]	44 ± 11	57 ± 7	24 ± 10
All animals	1.2 ± 0.3	16 ± 10	33 ± 7[b]	43 ± 8	56 ± 6	24 ± 7

All values for grouped animals are reported as mean ± one standard deviation, with the following exceptions:
[a]T_{max} values of sex-grouped animals are reported as geometric mean ± one geometric standard deviation.
[b]Half-life values for grouped animals are reported as harmonic mean ± one pseudo standard deviation.

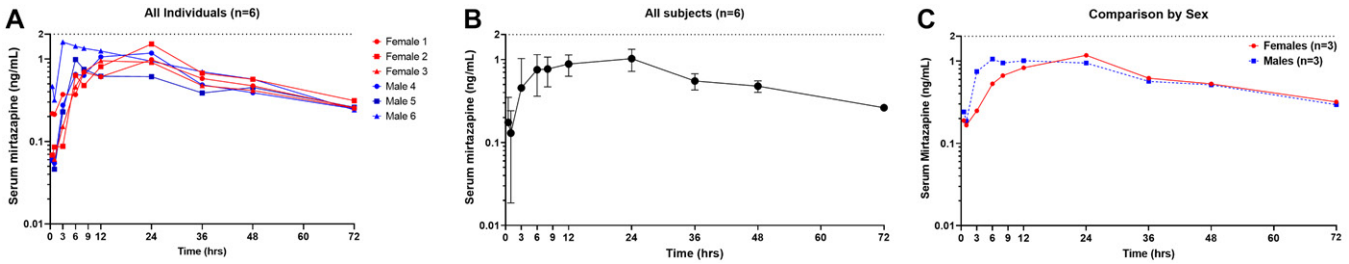

Figure 1. All rhesus macaques had a measured transdermal mirtazapine concentration of 0 ng/mL at $t = 0$ and received a single transdermal 0.5 mg/kg mirtazapine dose. (A) Individual serum mirtazapine concentrations. (B) Mean serum mirtazapine concentrations at each time point. (C) Sex-based comparison of mean serum mirtazapine concentrations.

Harmonic mean drug half-life was 33 ± 7 h. The AUC during the 72-h evaluation period was 43 ± 8 ng × h/mL; the extrapolated AUC from zero to infinity was 56 ± 6 ng × h/mL. The 8-hydroxy mirtazapine metabolite was not identified in any of the samples analyzed.

We further determined pharmacokinetic estimates for both female and male macaques (Figure 1C; Table 2). Mean C_{max} values for females and males were 1.2 ± 0.3 ng/mL (range: 1.0 to 1.5 ng/mL) and 1.3 ± 0.3 ng/mL (range: 1.0 to 1.6 ng/mL), respectively. Average T_{max} values for females and males were 19 ± 2 h (range: 12 to 24 h) and 8 ± 3 h (range: 3 to 24 h), respectively. Half-life values were similar for both sexes, with females and males averaging 33 ± 4 h (range: 30 to 38 h) and 34 ± 11 h (range: 25 to 46 h), respectively. AUC from 0 to 72 h was similar between sexes, with female and male values of 43 ± 6 ng × h/mL (range 39 to 50 ng × h/mL) and 44 ± 11 ng × h/mL (range: 34 to 55 ng × h/mL), respectively. Time point values were not statistically different ($P > 0.05$) between sexes.

Predicted serum mirtazapine concentrations over a 7-d course of daily transdermal administration of 0.5 mg/kg are shown in Figure 2. Accumulation of serum mirtazapine would be expected to occur with daily dosing, and steady-state concentrations are predicted to be approximately 2-fold higher than measured after a single dose. Given the estimated half-life of approximately 33 h, steady-state concentrations would be predicted to be attained by the fourth day of daily dosing (approximately 3 half-lives).

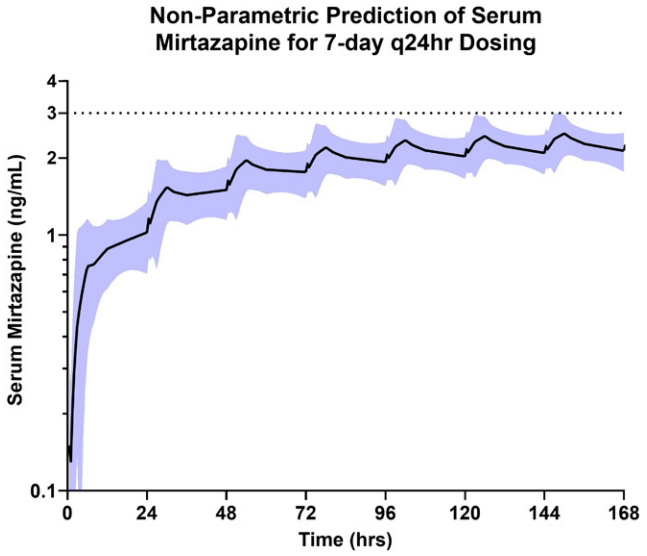

Figure 2. Nonparametric prediction of cumulative mirtazapine concentrations with serial daily transdermal dosing with 0.5 mg/kg. The black line represents mean serum concentration, while purple shading represents the 95% confidence interval of the prediction.

Discussion

The data. Table 2 describes the pharmacokinetics of a single dose of TDMTZ, given at a dose that was previously shown to ameliorate hyporexia in previous studies in both macaques[24] and cats. These data imply that despite published information indicating clinical efficacy in promoting food intake and weight gain in rhesus at 0.5 mg/kg, this dose of TDMTZ reaches a C_{max} that is over an order of magnitude lower than that found in cats. Average half-life was several hours longer in rhesus than cats. However, T_{max} occurred at a similar time in both species.

Comparative pharmacokinetics. The pharmacokinetics of TDMTZ in rhesus differed from those reported for domestic cats, which are the FDA-approved species for the TDMTZ ointment we examined. Most notably, in a single-dose study[7] of 8 cats that used the same dose of 0.5 mg/kg that we used in macaques, the mean C_{max} was reported as 21.5 ng/mL, nearly 18-fold higher than our rhesus of mean C_{max} of 1.2 ng/mL. The mean half-life in this same cohort of cats was reported to be 26.8 h, while our mean half-life in rhesus was 33 h. The mean T_{max} was similar in both species, with peak serum concentrations achieved in cats and rhesus at 15.9 h and 16 h after dosing, respectively.

The differences in blood concentrations between rhesus and cats warrant further investigation; several factors may have contributed to the nearly 20-fold difference in maximum serum concentration between these species. One obvious difference is the relative size and anatomy of the pinnae; cats have proportionally larger and thinner pinnae that are highly vascular, while rhesus have thicker cartilage and relatively smaller pinnae as a proportion of body size. This difference in shape and differences in pinnal thickness, surface area, and vasculature between species may directly influence differences in the rate and extent of absorption. However, the similarity in T_{max} between species seems to indicate the rate of absorption may be less affected than the extent of absorption.

Another consideration is that our monkeys were dosed while sedated with ketamine, while cats in the TDMTZ study[7] were not sedated for dosing. Ketamine is generally considered to maintain blood pressure during sedation in veterinary species[6] and humans,[23] although one study[18] using ketamine in rhesus found that hypotension developed over longer sedation times, particularly in infants. Although we cannot rule out a decrease in pinnal blood pressure secondary to sedation, our animals received a single dose of ketamine and all recovered from sedation less than one hour after dosing. In addition, sedation seems unlikely to account for the magnitude of difference in C_{max} between rhesus and cats. Transient hypothermia due to sedation could also affect absorption, but our anecdotal observations in rhesus suggest that peripheral temperature would not decrease significantly during a brief sedation with a relatively low dose of ketamine.

Although our study design did not quantify appetite, intake, or elimination, no abnormal increases or decreases in these parameters were noted by husbandry or project staff during or after mirtazapine administration. Our animals were fed their normal ration throughout the study. The most common adverse effect of TDMTZ in cats is erythema at the application site,[25] while in humans the most common adverse physiologic effects of oral mirtazapine are somnolence and xerostomia.[32] Less common but more clinically significant side effects reported in humans and cats include vomiting and nausea, particularly in the context of overdose and resultant serotonin toxicity.[11,19] No adverse effects were observed in our study. Clinicians should be aware of, and monitor for, these common side effects observed in other species, but our experimental and clinical use of this drug in rhesus has not been associated with adverse effects.

Dosing considerations in rhesus. Despite the marked difference in C_{max} between rhesus and cats, several common traits of these species may inform clinicians in dosing rhesus with TDMTZ. One commonality is that both rhesus and domestic cats are fastidious groomers and may remove topical drugs before full absorption. One study showed that the use of an Elizabethan collar to restrict grooming extended TDMTZ's half-life in cats.[7] However, the use of similar protective devices is not realistic for most clinical treatments in NHP species due to their ability to manipulate and remove devices. Clinicians could consider applying TDMTZ before surgery, chemical restraint, or chair restraint whenever possible to extend the time for absorption, as the animal will not be able to groom its ears during these periods. Allogrooming, another important species-specific behavior shared by rhesus and cats, may also interfere with absorption of transdermal therapeutics. Manufacturer instructions for Miratаz indicate that cats receiving treatment should be isolated from both humans and animals for 2 h after administration;[25] temporary separation of paired or socially housed rhesus after TDMTZ administration may be warranted to ensure delivery and absorption in the proper recipient, especially when separation is combined with application during restraint or sedation periods during which the treated animal cannot groom.

The potential for sex-related pharmacokinetic variation merits consideration, given that human biologic males and females also have statistically significant pharmacokinetic differences in mirtazapine and related antidepressants.[5,21,37] Although we did not detect a significant difference in TDMTZ serum level between male and female rhesus, possibly due to a lack of study power at $n = 3$ per sex, one study[24] reported sex differences in the number of hyporexic days after TDMTZ administration in macaques. Male 6 in our study reached T_{max} more quickly than the other subjects, which likely contributed to overall variability in our sex-based analysis. Oral mirtazapine's peak concentration has been reported to be up to 50% higher in human females as compared with males,[36] and a similar sex difference has been reported for $T_{1/2}$ after oral dosing in humans.[5] These differences are thought to be related to variation in several metabolic and hormonal pathways involved in mirtazapine metabolism and clearance, including sex differences in cytochrome P450 isozymes, estrogen concentration, and sex differences in liver size and blood flow.[5] Currently dose adjustments based on sex have not been recommended for humans,[16] but given the higher degree of sexual dimorphism found in rhesus, additional research on this topic could be clinically useful. In our animals, C_{max} was nearly identical between sexes, and the marked difference in concentration as compared with cats occurred in both sexes. Our mean T_{max} was also not significantly different between sexes, although males showed greater variability (female range, 12 to 24 h; male range, 3 to 24 h). Half-life in our rhesus also followed a pattern of wider variability in males (female range, 30 to 38 h; male range, 25 to 46 h) despite similar mean values. To our knowledge, sex differences have not been reported in other nonhuman species receiving TDMTZ.

Hypoproteinemia is a common concurrent finding in macaques with inappetence due to inadequate intake and losses to trauma, diarrhea, renal, or hepatic disease or due to experimental manipulations. Serum chemistry, performed as part of preproject screening, indicated animals in our study had total serum protein levels between 6.5 and 7.3 g/dL and albumin ranged between 4.1 and 4.5 g/dL. We measured total mirtazapine and did not quantify free compared with bound drug. As mirtazapine is moderately protein bound in the bloodstream (approximately 85% in humans after oral dosing[32]), varying normal and abnormal protein levels could affect pharmacological parameters and efficacy, although such variation would likely be clinically insignificant except in highly compromised animals. Variations in BCS and body composition must also be considered when adjusting dose for potentially ill and/or hyporexic animals, although the risk of overdose in mirtazapine is considered low given its relatively wide margin of safety in humans.[26] Animals in our study were assigned BCS by CNPRC veterinary staff during preproject screening using an established and validated method;[8,35] all animals ranged between 2.0 and 3.0 out of a possible 5.0 at the time of screening.

Given our findings, we recommend that TDMTZ can be used to promote intake and weight gain in rhesus macaques when dosed at 0.5 mg/kg for at least 4 consecutive days to achieve a steady state concentration. Because the label recommends a 14-d course of treatment for cats,[25] we suggest a 7- to 14-d course of treatment to promote the positive long-term effects in rhesus macaques, as previously recommended by others.[24] This recommendation might be appropriate for cynomolgus macaques and other common Old World monkey species, but pharmacokinetic (and pharmacodynamic in the case of other species) validation should be performed in cynomolgus and other monkey species.

Study limitations. A limiting factor in the interpretation of our data is the low sample size, with 6 total animals and 3 of each sex. We feel our data are useful despite this limitation because a minimum of 3 animals per time point is broadly considered acceptable for pharmacokinetic analysis in animals.[40] Our reported drug levels were consistently different than those reported for cats.[7] However, clinical efficacy in rhesus was previously demonstrated at our dosage of 0.5 mg/kg.[24] Furthermore, the estimates of half-life in our study must be interpreted with caution because our sampling schedule ended at 72 h after dosing, which is less than 3 estimated half-lives; therefore, our estimates may not be accurate and could affect the predicted rate of accumulation and time needed to reach steady state. However, the clinical need for dosing over multiple days to achieve a pharmacodynamic effect supports our predictions in this study.

We cannot exclude small variations in our results related to veterinary intervention during the experimental period. One rhesus received a single dose of ketoprofen, which may use the same hepatic metabolic pathways as TDMTZ; however, the specific clearance processes of these 2 drugs have not been fully elucidated in rhesus. Two rhesus also received a single prophylactic saline treatment; this could also affect serum concentration measurements. However, animals also had free

access to drinking water throughout the study, and we did not control for water intake or elimination. Overall, animals remained clinically hydrated throughout our study. In clinical situations, veterinary intervention that includes fluid therapy and analgesia will often be necessary for animals that receive TDMTZ as part of a clinical treatment regimen.

Finally, because our study included 2 animals that had screened negative for MHV-1 and 4 that were considered as MHV-1 exposed ("conventional" rhesus), we cannot exclude possible differences in metabolism related to subclinical MHV-1 infection. None of the animals had a history of clinical MHV-1 signs before or during our study.

Future directions. Given the large C_{max} differences between rhesus and cats and the high therapeutic margin of mirtazapine reported in most species, testing higher TDMTZ doses in rhesus may increase the benefit to animals receiving this treatment. Related studies of increased dosage and resultant pharmacodynamic effect, particularly in association with serial dosing, could also promote positive clinical outcomes and welfare of rhesus that receive TDMTZ. Further delineation of pharmacodynamic efficacy at varying doses, particularly quantification of weight gain and increases in food intake, would also guide clinical use of the drug.

Investigation of various routes of administration (for example, pairing an initial oral or intravenous dose with serial topical administration to hasten the onset of the pharmacodynamic effect) could provide flexibility and better clinical results for prescribers. Pharmacokinetics after dosing at different body sites (for example, flank, limb, or shoulder) could also provide more options for clinical use and/or promote higher or more consistent absorption. Additional investigation of the sex differences we described in pharmacokinetic parameters could determine the clinical significance of sex-dependent dosing.

Finally, given mirtazapine's labeled human use as a therapy for MDD, the potential for improved mental welfare in NHPs treated with TDMTZ should not be ignored. Mirtazapine's antidepressive effects may be useful for reducing distress that macaques may experience in association with hospitalization or experimental procedures and improve sleep quality: a health concern and research confounder often underestimated in the NHP research environment.[4]

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