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The Pharmacokinetics of Subcutaneous Methylnaltrexone Bromide in Rhesus Macaques (*Macaca mulatta*)

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Opioids are an integral component of pain management for nonhuman primates. These potent analgesics also adverse gastrointestinal (GI) effects that include constipation, bloating, and delayed gastric emptying. Methylnaltrexone bromide (MNTX) is a selective, peripherally acting μ- and κ-opioid receptor antagonist that can be used to mitigate the GI effects associated with opioid administration. Unlike naltrexone, a similar drug in this class, MNTX possesses an N-methyl-quaternary amine group that prevents it from crossing the blood brain barrier. This blockage allows inhibition of peripheral GI opioid receptors without affecting opioid-mediated analgesia in the central nervous system. We conducted a pharmacokinetic analysis of MNTX in serum and CSF of 6 healthy juvenile male rhesus macaques after subcutaneous administration of a 0.15-mg/kg dose. We hypothesized that the macaques would demonstrate a T_{max} of 0.5 h, similar to that of humans, and that no MNTX would be detected in the CSF. This treatment resulted in a peak serum concentration of 114 ± 44 ng/mL at 0.25 ± 0.00 h; peak CSF at concentrations were 0.34 ± 0.07 ng/mL at the T_{max}. These data show that subcutaneous administration of MNTX to rhesus macaques may block peripheral adverse effects of opioids without interfering with their central analgesic effects.

Abbreviations and Acronyms: BBB, blood-brain-barrier; MNTX, methylnaltrexone bromide; CSF, cerebrospinal fluid, T_{max}, time to peak drug concentration

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Synthetic opioids are potent analgesics used in human and veterinary medicine for pain management.[9,11,13,14,32] At the California National Primate Research Center (CNPRC), opioids are an essential component of multimodal analgesia for rhesus macaques (*Macaca mulatta*) undergoing major surgeries, or for those with conditions associated with moderate to severe pain. Opioids therefore are an important tool for providing adequate veterinary care.[3,4,22]

Opioids can be divided into 2 subtypes: endogenous and exogenous. Endogenous opioids include enkephalins, endorphins, endomorphins, and dysnorphins. Exogenous opioids that are commonly used in human and veterinary medicine include, but are not limited to morphine, buprenorphine, methadone, oxycodone, hydromorphone, and fentanyl. Exogenous opioids can be further classified by their pharmacological properties and the opioid receptors that they affect. The 5 classes of opioid receptors are mu (μ), delta (δ), kappa (κ), nociception receptor (NOR), and zeta (ζ). Activation of μ and κ receptors provide analgesia, while activation of the subclass μ2 receptor causes increase gastrointestinal tract motility, resulting in ileus and constipation.[11,25] Opioid receptors are found throughout the body on various tissue types in the central nervous system (CNS) and the peripheral nervous system (PNS). The former are responsible for central analgesia, while the latter are associated with the gastrointestinal adverse effects of opioid use.[25,35] Mu-opioid receptors are widely distributed through the body in the CNS as well as in cardiac and GI tissues.[11,13,19,30]

Opioids are often associated with gastrointestinal (GI) side effects, including nausea, vomiting, constipation, bloating, and delayed gastric emptying.[13,18-20,25,30] These adverse effects can be exacerbated by GI surgery (intestinal resection and anastomosis or gastrostomy).[13,18] Nonhuman primates (NHP) are phylogenetically similar to humans and thus have the similar opioid mechanism of actions and adverse effects.[12] At our institution we have observed pruritis, delayed gastric emptying and poor appetite develop in rhesus macaques that have received long-term multiple week administration of a μ-opioid agonist. Opioid induced side effects in both humans and NHP are more commonly associated with full μ-opioids agonists, such as morphine and fentanyl, than partial opioid agonists such as buprenorphine.[12] Our macaques receive full μ-opioid agonists during and after soft tissue, orthopedic, or neurologic surgical procedures. The longer an opioid is administered, the more likely it is to induce an adverse side effect.[1,18,25] Universal opioid receptor antagonists, such as naltrexone or naloxone, are used to treat opioid overdose and narcotic dependence. These agents cross the blood brain barrier (BBB) and block opioid receptors in the CNS, reversing both the central analgesic effects of opioid agonists and peripheral any side-effects. These agents therefore cannot be used to treat opioid-associated GI side effects in individuals being treated for pain.

An important quality of opioids is their ability to cross the BBB to reach target receptors in the CNS, and thereby exert their central analgesic effects. Opioids can cross the BBB by various transport mechanisms. Opioid receptor agonists can diffuse or

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be actively transported, via carrier-mediated influx, across the BBB. Passive diffusion of a drug across the BBB depends on its molecular weight, volume of distribution, and lipophilicity.[9,10,37] Efflux transporters, specifically P-glycoproteins (p-gp), are heavily involved in the efflux of certain drugs from the brain. For instance, morphine crosses the BBB via active mediated influx, but is also associated with MRP-mediated and p-gp–mediated efflux mechanisms. Hydrocodone, codeine, and sufentanil can passively diffuse through the BBB with no effect of efflux transporters.[24,37] Therefore, each opioid has a variable degree of brain entry. Opioid antagonists like naltrexone can pass through BBB and reverse the central analgesic effect of opioid agonists, which does not occur with methylnaltrexone.

Methylnaltrexone bromide (MNTX), like naltrexone, is a selective μ- and κ-opioid receptor antagonist.[6,17,37,39] However, unlike naltrexone, MNTX does not cross the BBB due to its large, polar structure N-methyl-quaternary amine, which is attached to the main naltrexone molecule.[1,6,16,24,27,33,39] Consequently, MNTX can reduce the GI side-effects of opioids without affecting their central analgesia.[1,2,7,8] MNTX has therefore been classified as a peripheral μ-opioid receptor antagonist (PAMORA).[6] MNTX was approved by the US Food and Drug Administration as a subcutaneous injection for humans in 2008. In 2016 it was re-formulated and approved as an oral tablet for human use. Both formulations are used to treat opioid-induced constipation in humans.[1,2,5,19,30]

The pharmacokinetics and pharmacodynamics of MNTX have not been studied in rhesus macaques. Such studies would provide important information relevant to using MNTX to refine pain management in NHPs. The goal of this study was to determine the pharmacokinetics of MNTX, administered subcutaneously at 0.15 mg/kg, in rhesus macaques, and to assess whether MNTX crosses the BBB. We hypothesized that at this dose, MNTX would have a T_{max} of 0.5 h, as it does in humans,[1] and that there would be no drug would be detected in the cerebrospinal fluid (CSF) due to the structural inability of MNTX to cross the BBB.

Materials and Methods

Animals. Six juvenile male indoor-housed rhesus macaques (age, 3.0 ± 0.7 y; weight, 5.2 ± 1.0 kg) were included in this pharmacokinetic and biodistribution study. All macaques were housed and maintained in an AAALAC-accredited facility, in accordance with the Animal Welfare Act and Regulations, Public Health Service Policy and the Guide for the Care and Use of Laboratory Animals.[3,4,22] The current study was approved by the University of California, Davis IACUC. The macaques were bred in house in a colony that is free SPF the specific pathogens Macacine herpesvirus 1, simian retrovirus type D, SIV, and simian T-lymphotropic leukemia virus. They were fed a commercial NHP diet (LabDiet Monkey Diet 5047, Purina St. Louis, MO) twice daily, with supplemental produce, forage, and fruit provided twice weekly, and water provided ad libitum through an automatic watering system. Toys and coconuts were provided for environmental enrichment. Trained personnel provided high-value treats drug administration and blood collection as positive reinforcement and for acclimation training. The macroenvironment was maintained at a constant temperature (23 °C ± 3 °C) and relative humidity (30% to 70%). Macaques were maintained on a 12:12-h light:dark cycle (lights on, 0700:1900). During the study, 2 of the 6 macaques were housed individually and 4 were pair-housed in stainless steel cages. The two individual housed macaques were in the process of being paired. Pair macaques were separated briefly during dosing and blood collection to minimize injury and accurately check for potential adverse reactions.

Before inclusion in the study, the clinical history of each macaque was reviewed and a physical exam, hemogram, and serum biochemistry analysis were performed. Criteria for enrollment in the study included a body condition score of at least 2 on a 5-point scale for ease of cerebrospinal fluid collection, and absence of any clinically relevant abnormalities on physical exam, hemogram and serum biochemistry.

Study Design. This study was designed to determine the pharmacokinetics and CSF 202 WILLIAMS ST, Seymour, TN, United States, 37865distribution of subcutaneous MNTX at 0.15 mg/kg, in 2 phases (Figure 1).

Phase 1. During the pharmacokinetic phase, macaques were injected with 0.15 mg/kg MNTX subcutaneously (n = 6), in the dorsal interscapular region cage-side. The squeeze-back mechanism of the cage modular was used to orient and secure the dorsal interscapular region to the front of the cage for ease of access in dosing. Prior to dosing, the drug was stored at room temperature (20° to 25 °C) and protected from light. Animal weights were obtained a week before and the day of injection to ensure accurate dosing. The interscapular region was shaved with clippers a day before MNTX administration. The dose and route of administration were based on human clinical trials.[1] MNTX was administered by using a 1-mL tuberculin syringe with a 22-gauge needle; volume was rounded to the nearest hundredth of a milliliter. Shaved injection sites were assessed for adverse reactions for 15 min after dosing and at subsequent blood collection time points. Attitude, mentation, appetite, hydration status, and fecal output and consistency were monitored daily. Blood was collected from the cephalic vein by trained personnel using a cage-side 'arm pull' technique and a syringe. Serial 3-mL blood samples were obtained at time points 0, 0.25, 0.5, 1, 3, 6, 12, 24, 30, 36, 48, and 72 h. These collection time points were selected based on previous MNTX pharmacokinetic literature, including in human patients, and on the ability to determine an area under the curve (AUC) for pharmacokinetic analysis.[1,15]

Samples were transferred into 3.5-mL serum separator tube with no anticoagulant. The samples were centrifuged 15 min after collection at 1,500 × g for 15 min at 10 °C min. Serum was collected and stored at −80 °C in 1.8-mL Nalgene CryoTube vials (Thermo Fischer Scientific 4000, Roskilde, Denmark) until submitted for analysis.

Phase 2. Phase 2 was contingent on the pharmacokinetic data analysis (T_{max}) obtained from phase 1 and used the same 6 macaques. After a washout period of one month, the 6 rhesus macaques received MNTX at the same dose and route as in phase 1. Fifteen minutes before the serum T_{max} of 0.25 h, as determined in phase 1, all macaques were sedated with ketamine (5 to 10 mg/kg) and dexmedetomidine (0.0075 to 0.015 mg/kg) intramuscularly, as no drug-drug interactions have currently been identified between ketamine and MNTX.[1]

Macaques were shaved from the last cervical vertebral body to the cranial aspect of the occipital condyles of the skull. They were then placed in ventral recumbency and aseptically prepped with betadine and 70% isopropyl alcohol. Sterile technique was used to obtain 1 mL of CSF from the cisterna magna with a 3-mL syringe and 22-gauge needle (Figure 2). CSF was placed in 1.8-mL Nalgene CryoTube vials (Thermo Fischer Scientific 4000 Roskilde, Denmark) and stored at −80 °C. A single dose of the nonsteroidal anti-inflammatory drug ketoprofen (5 mg/kg) was administered intramuscularly for analgesia

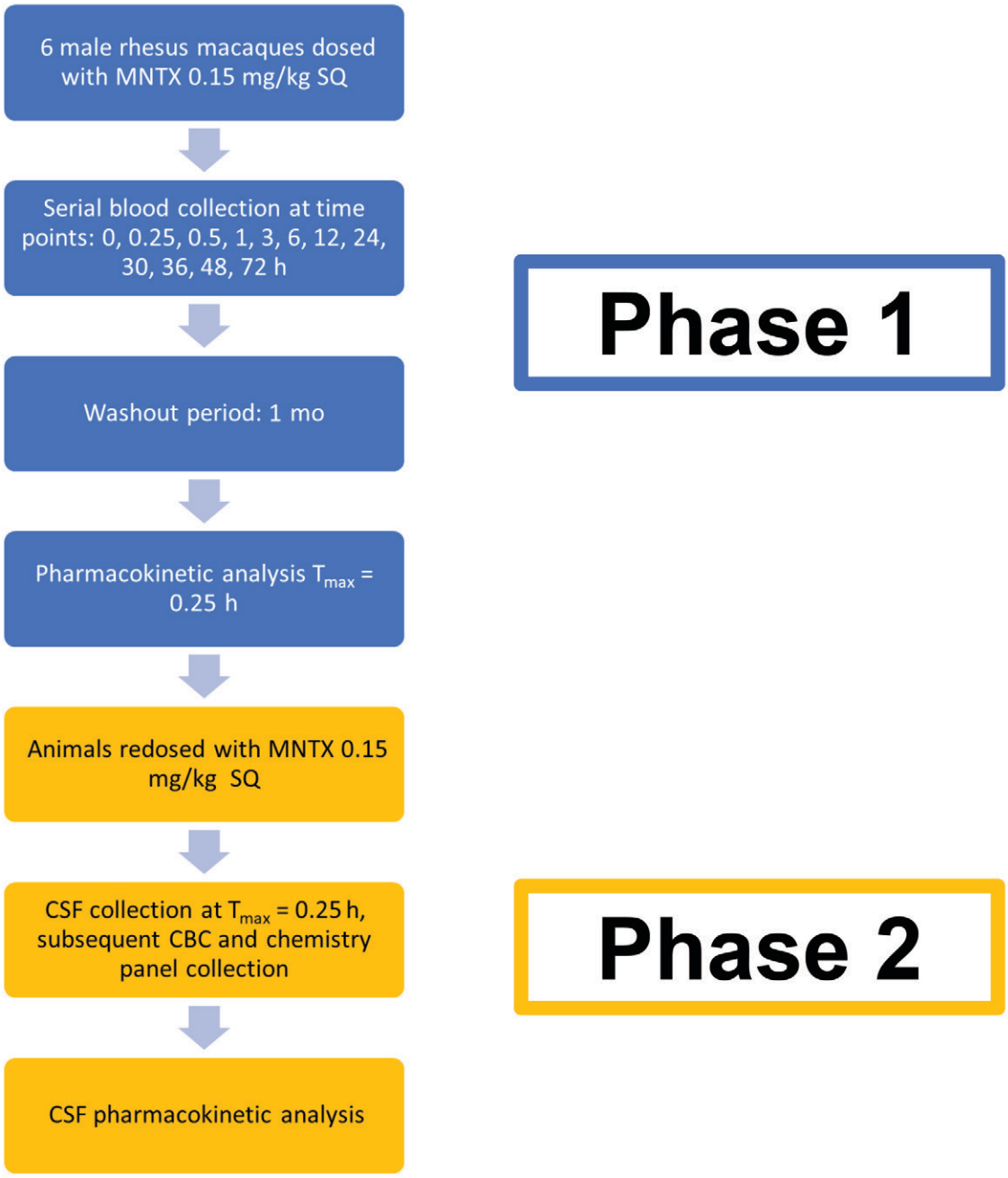

Figure 1. Study outline with phase 1 and phase 2.

after CSF collection. Blood was collected for a hemogram, biochemistry panel, and methylnaltrexone measurement in conjunction with the CSF. CSF cytology was performed to verify that no blood contamination occurred at the time of collection. After completion of sample collection, atipamezole was administered intramuscularly at the same volume of dexmedetomidine as a reversal agent. One macaque had blood contamination of the CSF, as indicated by hemolysis; thus, phase 2 was repeated in this macaque after a 1-mo washout period.

Determination of methylnaltrexone concentrations in serum samples. Serum calibrators were prepared by dilution of the methylnaltrexone working standard solutions (Sigma Aldrich, St. Louis, MO) with drug free rhesus macaque serum to concentrations ranging from 0.05 to 400 ng/mL. Calibration curves and negative control samples were prepared fresh for each quantitative assay. In addition, quality control samples (drug-free serum fortified with analyte at 3 concentrations within the standard curve) were included with each sample set as an additional check of accuracy.

Prior to analysis, 0.2 mL of serum was diluted with 2 mL 0.1M pH 7 ammonium formate buffer and 0.2 mL water containing the internal standard, propantheline (Sigma Aldrich, St. Louis, MO) at 50 ng/mL. All samples were vortexed gently to mix, and subjected to solid phase extraction using Isolute CBA

Columns, 3 mL 200 mg Biotage (Charlotte, NC). The columns were conditioned with 2.5 mL of methanol and 2.5 mL of 0.1M pH 7 ammonium formate buffer and the samples loaded onto the columns. The columns were subsequently rinsed with 3 mL water and 3 mL methanol, prior to elution with 1.7 mL of 1% formic acid in methanol. Samples were dried under nitrogen, dissolved in 120 µL of 5% acetonitrile (ACN) in water, with 0.2% formic acid and 30 µL injected into a liquid chromatography tandem mass spectrometry (LC-MS/MS) system.

The concentration of methylnaltrexone was measured in serum by LC-MS/MS using positive electrospray ionization (ESI(+)). Quantitative analysis was performed on a TSQ Altis triple quadrupole mass spectrometer coupled with a Vanquish liquid chromatography system (Thermo Scientific, San Jose, CA). The spray voltage was 3500V, the vaporizer temperature was 350 °C, and the sheath and auxiliary gas were 50 and 10 respectively (arbitrary units). Product masses and collision energies of each analyte were optimized by infusing the standards into the TSQ Altis. Chromatography employed an ACE 3, C18, 10 cm × 2.1 mm, 3-µm column (Mac-Mod Analytical, Chadds Ford, PA) and a linear gradient of ACN in water with a constant 0.2% formic acid at a flow rate of 0.4 mL/min. The initial ACN concentration was held at 5% for 0.40 min, ramped to 80% over 4.6 min and ramped to 90% over 0.2 min, before re-equilibrating for 3.2 min at initial conditions.

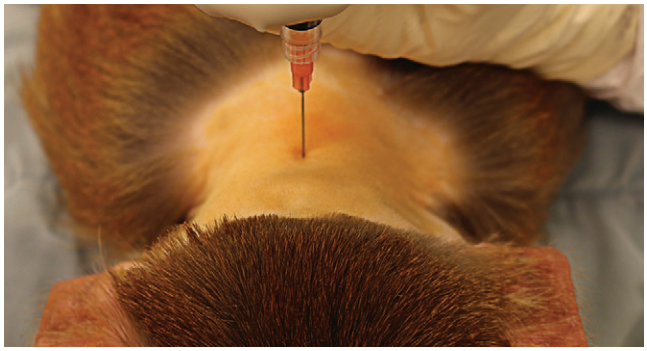

Figure 2. CSF collection process. The area of the head between the last cervical vertebral body and the cranial aspect of the occipital condyles was shaved and aseptically prepped. 1 mL of cerebral spinal fluid was obtained from the cisterna magna using a 22-gauge needle.

Detection and quantification were conducted using selective reaction monitoring (SRM) of initial precursor ion for methylnaltrexone (mass to charge ratio [m/z] 356.2) and the internal standard propantheline (m/z 368.2). The response for the product ions for methylnaltrexone (m/z 227.0, 284.1, 300.1, 302.2) and the internal standard (m/z 181.0, 326.1) were plotted and peaks at the proper retention time were integrated using Quanbrowser software (Thermo Scientific). Quanbrowser software was used to generate calibration curves and to quantify analytes in all samples by linear regression analysis. A weighting factor of 1/X was used for all calibration curves. The instrument response for methylnaltrexone was linear and gave correlation coefficients of 0.99 or better. Accuracy was reported as percent nominal concentration; precision was reported as percent relative standard deviation. Accuracy was 99% for 0.15 ng/mL, 92% for 1 ng/mL and 104% for 20 ng/mL. Precision was 10% for 0.15 ng/mL, 11% for 1 ng/mL and 6% for 20 ng/mL. The technique was optimized to provide a limit of quantitation of 0.05 ng/mL and a limit of detection of approximately 0.025 ng/mL for methylnaltrexone.

Pharmacokinetic Analysis. Pharmacokinetic parameters for methylnaltrexone were calculated using commercially available pharmacokinetic software (Phoenix Winnonlin v8.2, Certara, Princeton, NJ) and noncompartmental analysis. The maximum concentration (C_{max}) and time to maximum concentration (T_{max}) were determined by analysis of the concentration time data. The slope of the terminal portion of the curve, lambda z (λz) was used to calculate half-life ($t_{1/2}$λz) using the Equation 0.693/λz. The area under curve (AUC) from time 0 to infinity (AUC0→∞) was obtained by using the linear up log down trapezoidal rule and dividing the last plasma concentration by the terminal slope extrapolated to infinity.

Statistical analysis. MNTX serum concentrations at the various time points (0, 0.25, 0.5, 1, 3, 6, 12, 24, 30, 36, 48, 72 h after administration) were compared using ordinary least square linear regression with indicator variables for the different timepoints and robust standard errors. Any time point that was below the limit of detection value was assigned a value of 0.025 ng/mL for purposes of statistical analysis. (Figure 3).

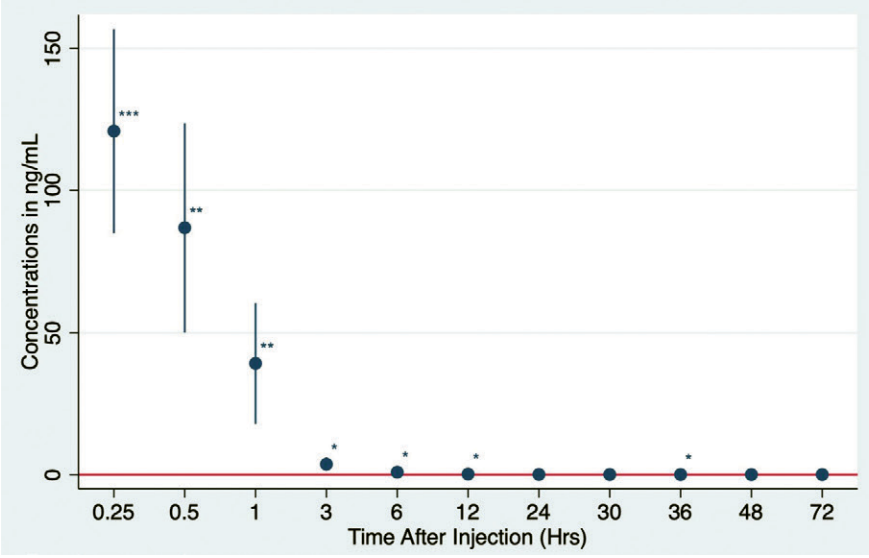

Figure 3. Least square linear regression of MNTX serum concentrations. Concentrations measured at the selected time points after administration (0.25, 0.5, 1, 3, 6, 12, 24, 30, 36, 48, 72 h) were compared with the value at time point 0 *$P < 0.1$; **$P < 0.05$; ***$P < 0.01$. The lower limit of detection was set at 0.025 ng/mL for analysis.

For phase 2, pharmacokinetic mean serum parameters were compared with the CSF mean concentrations by using a Kruskal–Wallis test with χ^2 approximation (Version 14.2, 1 Stata SE, StataCorp, College Station, TX). The same software was used to evaluate the average CSF concentrations of methylnaltrexone by using a one-sample *t* test analysis to test the one-sided hypothesis that the mean concentration in CSF exceeded the minimal detectable value (0.025 ng/mL) using the same software. Statistical significance was set as a *P* value less than 0.05.

Results

All rhesus macaques remained healthy throughout the study. No injection site reactions were noted in any animal at any time point. Physical examination and clinical laboratory tests showed no clinically relevant abnormalities before or during the study. The adverse effect that was reported in a few human patients after MNTX administration (that is, bloating) was not noted on daily observation in the macaques[1].

The pharmacokinetic parameters in rhesus macaques dosed with 0.15 mg/kg MNTX in phase 1 are shown in Table 1. The $t_{1/2}$ mean plasma concentration of MNTX was 114 ± 44 ng/mL and the T_{max} was 0.25 h. The time course of measured serum MNTX concentrations with regard to time (0, 0.25, 0.5, 1, 3, 6, 12, 24, 30, 36, 48, 72 h) is shown in Figure 4.

The mean concentration of MNTX in CSF (0.34 ± 0.07 ng/mL) significantly exceeded the minimum detectable concentration of 0.025 ng/mL ($P < 0.0001$). However, the concentration of MNTX at the T_{max} in CSF was significantly less than that measured in serum from phase 2 ($P < 0.0039$) (Figure 5). Thus, although MNTX did cross the BBB and enter the CSF the amounts detected in the CSF were significantly lower than that of serum.

Discussion

The current study investigated the pharmacokinetic profile of 0.15 mg/kg MNTX, administered subcutaneously to juvenile male rhesus macaques. This μ-opioid reversal agent has the potential to counter adverse effects commonly associated with μ-opioid receptor agonists.[11] Previous literature stated that MNTX only acts on peripheral μ-opioid receptors due to its molecular structure.[7,8,11,15] We tested whether MNTX crossed the BBB in rhesus macaques. Ultimately, we rejected our hypothesis, because low concentrations of MNTX were detected in the CSF, albeit at significantly lower concentrations than in the serum.

The structure of MNTX (the methylation of its amine group) inhibits the molecule from crossing the BBB and reversing the central analgesic effects of opioid agonists. However, few animal studies are available to show that MNTX does not cross the BBB. One study in rats found minute concentrations of MNTX in the brain at levels below the serum detection limit of MNTX and concluded that MNTX does cross the BBB albeit in limited amounts that are not statistically significant.[26] Similarly we found low amounts of MNTX in the CSF. Although the brain tissue of our macaques was not analyzed, we found low concentrations of MNTX (0.34 ± 0.07 ng/mL) in the CSF. Our hypothesis that peripheral administration of MNTX would not yield drug in the CSF was therefore disproven. Although trace amounts of MNTX crossed the BBB, CSF concentrations were significantly less than those in serum (114 ± 44). We speculate that central analgesia provided by μ-opioids agonist would probably not be significantly affected by MNTX.

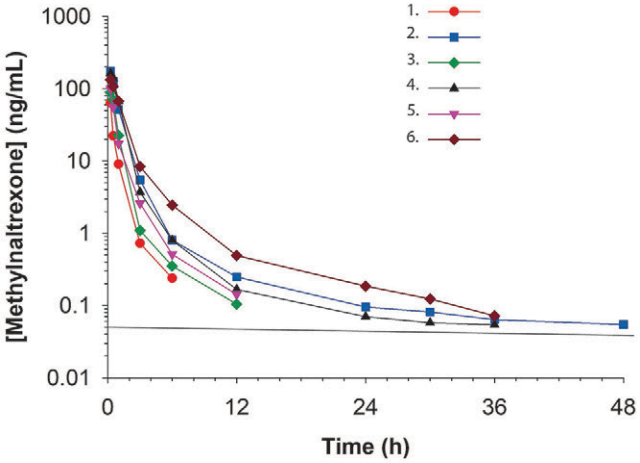

Figure 4. MNTX serum concentration for individual rhesus macaque as a function of time after administration (0, 0.25, 0.5, 1, 3, 6, 12, 24, 30, 36, 48, 72 h). The minimal detectable threshold is noted as 0.025 ng/mL.

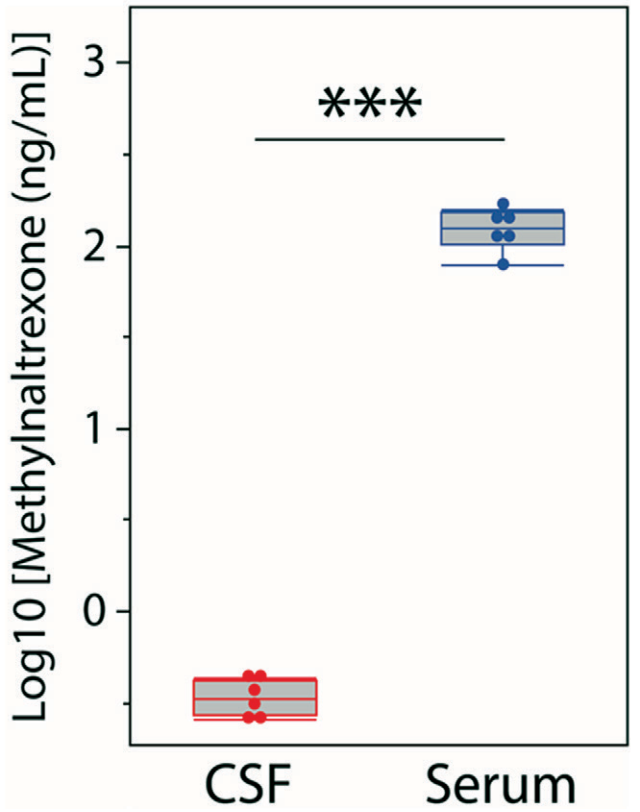

Figure 5. Statistical comparison between CSF and serum in phase 2 in a logarithmic scale. ***$P < 0.01$.

Table 1. Pharmacokinetic parameters in rhesus macaques for 0.15 mg/kg dose of MNTX in phase 1 (n = 6).

	Mean ± 1 SD
T_{max} (h)	0.25 ± 0
C_{max} (ng/mL)	114 ± 44
λ_z (1/h)	0.13 ± 030
$t_{1/2a}$ (h)	5.0 ± 8.8
$t_{1/2b}$ (h)	19 ± 17
$AUC_{0-\infty}$ (h/ng/mL)	94 ± 60

T_{max}, time to observed maximum plasma concentration; C_{max}, observed maximum plasma concentration; $AUC_{0-\infty}$, area under the plasma concentration time–curve extrapolated to infinity; AUC_{0-72}, area under the plasma concentration time curve from 0 to 72 h (3 d); λ_z, terminal slope

Two formulations of MNTX are approved for use in humans: a tablet form and an injectable formulation. Both forms are labeled for every other day (EOD) use to minimize μ-opioid induced constipation (OIC) in adults with chronic noncancer pain.[1] The FDA final phase clinical trials in humans for MNTX were performed with EOD or daily subcutaneous injectable dosing, with the EOD dosing frequency eventually selected for labeled use. This frequency may have been selected due to the strong affinity of opioid receptor antagonists to the μ-opioid receptor, which caused a slower dissociation of the drug from the receptor.[21,31] For example, naltrexone has a longer half-life in the brain (72 to 108 h) than in the plasma due to its high affinity for μ-opioid receptors.[21,28] Therefore, the plasma clearance half-life of a drug may not accurately reflect the duration of action of this drug at the CNS receptor site.[21,28] To ensure accurate dosing in NPS, we used the human FDA-approved subcutaneous formulation to test whether the pharmacokinetics of MNTX were similar in rhesus macaques and in human patients. Use of daily dosing may alter the concentration of MNTX in the CSF and thus alter the central analgesic effect of opioid administration.

In addition to the FDA-recommended formulation of MNTX, we also tested the labeled human dose of 0.15 mg/kg. As compared with human trials, peak MNTX serum concentrations occurred sooner in rhesus macaques (rhesus T_{max}: 0.25 h, human T_{max}: 0.5 h) and reached similar concentrations (rhesus: 1143.9 ± 44 ng/mL, human: 117 ± 33 ng/mL). This indicates that the rate of absorption, which governs the T_{max}, may be higher in rhesus macaques than in humans. The earlier attainment of T_{max} in rhesus macaques may be due to a difference in one of the variables in the 2-compartmental open system model used to calculate intrinsic absorption rate of a drug compound ($Cp = Ae^{-\alpha t} + Be^{-Bt}$).[29]

In other animal studies, MNTX has been administered through an intravenous (IV) or intraperitoneal (IP) route, rather than the oral or subcutaneous FDA-approved routes labeled for humans.[7,8,15] In a pharmacokinetic study using horses, 5 horses were given 1 mg/kg MNTX IV, resulting in elimination half-lives of $T\alpha = 5.1 \pm 0.9$ min and $T\beta = 47.0 \pm 11.6$ min. That study also concluded that MNTX partially prevented GI stasis associated with concurrent administration of a higher dose (0.5 mg/kg) of morphine.[7]

As in other animal studies using MNTX,[7,8,15,30,34] we found no significant adverse effects in any of the 6 macaques used in this study. Human subjects that received MNTX EOD for 4 wk also had a low incidence of adverse reactions in clinical trials.[1] Adverse effects of MNTX reported in humans dosed with the injectable formulation include abdominal pain, nausea, diarrhea, sweating, hot flashes, tremors, and chills.[1] In the current study, serial blood collection caused mild to moderate bruising (hematoma) in 2 of the 6 animals. The bruising resolved over 3 d without treatment. One animal was given a single dose of ketoprofen (5 mg/kg IM) for a non-project related skin abrasion during the washout period after phase 1. No additional treatment or medical care was needed in any of the other animals during the study. One macaque had blood contamination in the CSF during phase 2. This contamination was evident in a higher MNTX yield in the CSF serum analysis and by marked hemolysis in the sample. This animal repeated phase 2 CSF and blood collection to maximize accuracy of data analysis.

Determining the pharmacodynamics and efficacy of MNTX in rhesus macaques was beyond the scope of this study. Additional studies are therefore necessary to evaluate the safety, efficacy and pharmacodynamic profile of MNTX in rhesus macaques. Further investigation and case-control studies of MNTX should be performed in rhesus macaques, as coadministration of MNTX with an opioid agonist could further demonstrate the utility of MNTX as a treatment for postoperative opioid-induced GI upset and constipation. Furthermore, multiple doses should be evaluated to establish safety guidelines in NHP. Sex differences were not assessed in the present study, although sex differences in response to MNTX may exist and should be considered with pharmacokinetic analysis.[36] Additional research is also needed to determine whether similar sex differences exist with rhesus macaques dosed with MNTX.

Potential limiting factors in this study include the type of syringe used for administration of MNTX. In human patients, a 1-mL syringe with a 22-gauge needle is used.[1] The rhesus macaques in this study had an average injection volume of 0.04 mL of MNTX, and all doses were rounded to the nearest hundredth in a 1-mL syringe. The use of an insulin syringe with a BD-Ultra-Fine needle may have provided more precise dosing due the small injection volume. However, the shorter less rigid insulin needle could have potentially led to intradermal administration instead of injection into the subcutaneous space, especially when administered cage-side to fully conscious macaques in phase 1 of this study. Overall, we elected to use a 1-mL syringe with a 22-gauge needle in this species due to the needle strength and length to ensure a subcutaneous injection of MNTX. Further investigation of needle size is needed to determine the best method of subcutaneous administration in various animals. This study established pharmacokinetic data for a single subcutaneous dose of MNTX at 0.15 mg/kg in rhesus macaques. Our findings confirmed that the pharmacokinetic profile of rhesus macaques differs slightly from that of humans. We also determined that MNTX's structural composition does not fully inhibit the diffusion of the molecule across the BBB, and trace concentrations can be measured in the CSF. Even though, the CSF concentrations are significantly lower than serum concentrations at the T_{max}, and may be clinically negligible, we did not test whether these low concentrations affects the level that central analgesia. Therefore, if MNTX is administered, animal pain score should be used to determine whether break-through pain occurs. MNTX is a potential adjunctive treatment that could obviate other treatments for opioid-induced adverse effects; such treatments would include laxatives, appetite stimulants, and NSAIDs. Thus, MNTX provides a potential refinement for pain management in rhesus macaques, improving welfare of animals given opioids in a research or clinical setting.

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