Preclinical to Human Translational Pharmacology of the Novel M₁ Positive Allosteric Modulator MK-7622[§]

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ABSTRACT

The current standard of care for treating Alzheimer's disease is acetylcholinesterase inhibitors, which nonselectively increase cholinergic signaling by indirectly enhancing activity of nicotinic and muscarinic receptors. These drugs improve cognitive function in patients, but also produce unwanted side effects that limit their efficacy. In an effort to selectively improve cognition and avoid the cholinergic side effects associated with the standard of care, various efforts have been aimed at developing selective M₁ muscarinic receptor activators. In this work, we describe the preclinical and clinical pharmacodynamic effects of the M₁ muscarinic receptor-positive allosteric modulator, MK-7622. MK-7622 attenuated the cognitive-impairing effects of the

muscarinic receptor antagonist scopolamine and altered quantitative electroencephalography (qEEG) in both rhesus macaque and human. For both scopolamine reversal and qEEG, the effective exposures were similar between species. However, across species the minimum effective exposures to attenuate the scopolamine impairment were lower than for qEEG. Additionally, there were differences in the spectral power changes produced by MK-7622 in rhesus versus human. In sum, these results are the first to demonstrate translation of preclinical cognition and target modulation to clinical effects in humans for a selective M₁ muscarinic receptor-positive allosteric modulator.

Introduction

Alzheimer's disease (AD) is marked by accumulation of plaques, tangles, and cholinergic neuronal cell death, manifesting in cognitive dysfunction. The long-standing hypothesis that restoring cholinergic function would improve cognitive deficits associated with AD was postulated more than 40 years ago (Davies and Maloney, 1976; Bartus et al., 1982; Whitehouse et al., 1982) and is supported by the moderate beneficial effects of acetylcholinesterase inhibitors (AChEIs), such as tacrine and donepezil (Davis et al., 1992; Farlow et al., 1992; Knapp et al., 1994; Rogers and Friedhoff, 1996; Rogers et al., 1998). However, these therapies also carry unwanted side effects, including nausea, vomiting, diarrhea, and other gastrointestinal effects, which have been postulated to result from the nonselective activation of the muscarinic and nicotinic receptor systems. These side effects reduce the clinical utility and constrain dosing

regimens of AChEIs, potentially limiting efficacy that might otherwise be possible. For these reasons, there have been sustained and significant efforts aimed at selectively targeting the receptors that are involved in the procognitive effects of AChEIs.

The M₁ muscarinic receptor has been demonstrated to play an important role in cognition, and for this reason has garnered attention as a potential target for a novel therapeutic. The M₁ receptor is localized to brain regions involved in cognition, including the prefrontal cortex and hippocampus, and M₁ receptor knockout mice demonstrate deficits in a variety of cognitive tasks and measures of synaptic plasticity (Bymaster et al., 2003; Shinoo et al., 2005; Young and Thomas, 2014). Furthermore, several nonselective muscarinic agonists have shown procognitive effects in humans; the most extensively studied of these, xanomeline, demonstrated efficacy in a large Phase 2 AD study (Bodick et al., 1997). However, despite its beneficial effects on cognition, xanomeline was so poorly tolerated that over half of the patients on the high-dose arm withdrew from the clinical study due to poor tolerability and adverse events.

As a result of the body of preclinical and clinical data suggesting the importance of M₁ receptor function for cognition,

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ABBREVIATIONS: ACh, acetylcholine; AChEI, acetylcholinesterase inhibitor; AD, Alzheimer's disease; ANOVA, analysis of variance; AUC, area under the curve; CSF, cerebrospinal fluid; ECoG, electrocorticogram; EEG, electroencephalography; IACUC, Institutional Animal Care and Use Committee; LC-MS/MS, liquid chromatography tandem mass spectrometry; ORD, object retrieval detour; PAM, positive allosteric modulator; P-gp, P-glycoprotein; P.O., oral; qEEG, quantitative electroencephalography.

there has been a great deal of effort aimed at developing novel approaches for more selectively activating the M_1 receptor. Significant efforts have been put forth to identify selective agonists (Greenlee et al., 2001; Heinrich et al., 2009); however, due to the high homology of the orthosteric ligand binding site, developing selective orthosteric or bitopic agonists has remained a challenge within the field. An alternative strategy to achieve receptor subtype selectivity is through the identification of allosteric ligands, either positive allosteric modulators (PAMs) or allosteric agonists, which have the potential to bind to less-conserved regions distal to the orthosteric binding site (Kuduk and Beshore, 2012; Davie et al., 2013).

Toward this end, we have identified and described several PAMs, including BQCA and PQCA, which are highly selective for M_1 over the other muscarinic receptor subtypes and have suitable properties for preclinical evaluation (Ma et al., 2009; Kuduk et al., 2011). Characterization of these compounds in rodent and nonhuman primate cognition models has further supported the hypothesis that the M_1 receptor activation contributes to the procognitive effects of nonselective muscarinic receptor activators (Ma et al., 2009; Uslaner et al., 2013; Lange et al., 2015; Puri et al., 2015; Vardigan et al., 2015) and is not responsible for the gastrointestinal side effects (Vardigan et al., 2015) (although some recent data suggest that M_1 activation could contribute to gastrointestinal effects [Alt et al., 2016; Davoren et al., 2017]). These findings suggested that a selective M_1 receptor activator could have procognitive effects in humans with a greater therapeutic window than the current standard of care.

Until now, selective M_1 receptor activation has not been evaluated to determine whether the preclinical findings in rodent and rhesus translate to human subjects. In this study, we disclose the novel PAM MK-7622 and describe its effects in both nonhuman primate and human with respect to its ability to attenuate the cognitive-disrupting effects of scopolamine and to alter cortical activity as measured by quantitative electroencephalography (qEEG).

Materials and Methods

Studies in Rhesus Macaque

Synthesis of M_1 PAMs. Experimental details and procedures for the preparation of 2-(2-fluorophenyl)-5-(4-methoxybenzyl)-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one (Compound 1) [WO 2011049731] and 3-((1S,2S)-2-hydroxycyclohexyl)-6-((6-methylpyridin-3-yl)methyl)benzo[h]quinazolin-4(3H)-one (MK-7622) [patent US9708273B2] have been described.

Drug Preparation and Administration. Scopolamine hydrobromide trihydrate (10–30 μ g/kg; Sigma-Aldrich, St. Louis, MO) was formulated fresh daily in saline and administered 60 minutes prior to testing. Scopolamine was administered via an i.m. injection at a volume of 0.1 ml/kg. MK-7622 (0.1, 0.3, and 1.0 mg/kg) was formulated daily in 0.5% methylcellulose and administered at 2.0 ml/kg via oral (P.O.) gavage 5 hours prior to testing. The positive control donepezil (3 mg/kg) was also formulated daily in saline and administered P.O. 4 hours prior to testing with a dose volume of 2.0 ml/kg. Compounds were administered on Tuesday and Friday of each week, and experimenters administering compounds and assessing behavior were kept blind to treatment conditions.

Object Retrieval Detour Task. Eight adult male rhesus monkeys (*Macaca mulatta*) were trained on the object retrieval detour (ORD) task. Animals had ad libitum access to water and were fed chow (Purina High Protein Monkey Diet 5045 [Purina, St. Louis, MO]), a multivitamin, and fruits and vegetables after cognition testing at ~15:00. Temperature and relative humidity were maintained at 21–24°C and 50%–55%,

respectively. All experimental protocols described in this study were approved by the Merck & Co., Inc. Institutional Animal Care and Use Committee (IACUC) and conducted in accordance with the *Guide for Care and Use of Laboratory Animals*. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to use alternatives to in vivo methods where possible.

The ORD task, as described in previous reports (Diamond et al., 1989; Uslaner et al., 2013), requires subjects to retrieve a food object from a clear acrylic box with a single opening. ORD test sessions consisted of a fixed arrangement of easy ($n = 8$) and difficult ($n = 11$) trials. During easy trials, the reward was positioned either 1) inside the box, with the opening directly in the line of sight of the subject, 2) slightly protruding from the box with the opening to the left or right of the subject, or 3) just inside the box opening, which was positioned either to the left or right of the subject. Easy trials were used to detect potential adverse events under drug conditions, such as drug-related motor or visuospatial impairment. During difficult trials, the reward was placed deep inside the box, opposite the box opening that was positioned either to the left or right of the subject. Difficult trial performance was used as the dependent measure for assessing cognitive function. With difficult reaches, the monkey must inhibit the prepotent response (i.e., immediately grab the treat) in favor of an attentive, inhibited/controlled response (i.e., avoid the barrier). As such, these difficult trials require attention and impulse control and are dependent on the prefrontal cortex (Diamond et al., 1989; Dias et al., 1996; Wilkinson et al., 1997; Jentsch et al., 2000). Trials were scored as correct if subjects successfully reached into the open side of the box and retrieved the reward on their first attempt. Reaches were scored incorrect if the subject contacted one of the solid sides of the box on the initial attempt to retrieve the reward. Subjects were not punished for incorrect reaches, and all subjects eventually retrieved all rewards. If a subject ceased to perform the task, the session was terminated after 3 minutes had elapsed from the most recent attempt. A newly cleaned box was presented for every trial to eliminate any potential visual cues from previous handling. Prior to each trial, a barrier was placed in front of the acrylic box to prevent the subject from observing the position of the reward prior to the commencement of each trial.

Subjects were tested two times weekly with at least 3 days between test sessions. Subjects were first tested under vehicle conditions (saline administered i.m. in the case of scopolamine and 0.5% methylcellulose administered P.O. for MK-7622) until their performance stabilized (defined as S.E.M. ≤ 10 on easy and difficult trials for the three previous sessions). Next, subjects were characterized on scopolamine to demonstrate sufficient impairment compared with their vehicle baseline. Due to individual differences in sensitivity to scopolamine, each subject's dose was titrated such that the dose produced a ~25% deficit on difficult trials and did not significantly impact easy trial performance. Once each subject's dose was identified, the dose was replicated at least once per subject. When vehicle and scopolamine baseline performance stabilized and after 1-week washout period, MK-7622 (0.1, 0.3, or 1.0 mg/kg) characterization was initiated. MK-7622 was administered orally 5 hours prior to testing, and scopolamine was administered i.m. 60 minutes prior to testing. Donepezil (3 mg/kg) was also included as a positive control. Easy and difficult trial data were analyzed with a one-way repeated-measures analysis of variance (ANOVA), followed by Dunnett post hoc tests to examine treatment differences compared with scopolamine performance. Plasma was collected from all animals after receiving the 0.3 mg/kg dose following testing in object retrieval, and MK-7622 plasma concentrations were measured using liquid chromatography tandem mass spectrometry (LC-MS/MS) method.

Quantitative Electroencephalography (qEEG). Eight to 11 adult male rhesus nonhuman primates (*Macaca mulatta*, 9–15 kg, 5–11 years of age) were housed in standard laboratory conditions in accordance with standards of the Merck & Co., Inc. IACUC and the United States Department of Agriculture. Temperature, humidity, and lighting (12:12 light:dark) were controlled and monitored continuously. Animals had ad libitum access to water and were fed a calorie-controlled diet of laboratory chow supplemented with fruits and vegetables. All animals underwent s.c. telemetric (D70-EEE; Data Sciences International, St. Paul, MN)

implantation allowing for continuous and simultaneous electroencephalographic (EEG), electromyographic, electrooculographic, and generalized locomotor activity recordings, which were subsequently analyzed offline. Nonhuman primates were free from prior drug exposure for a minimum of 2 weeks prior to study start. In independent 4-day crossover-designed studies, three doses of MK-7622 were tested at 1, 3, and 10 mg/kg. All surgical and experimental procedures were IACUC approved.

qEEG power spectrum was analyzed using custom-developed and compiled code using Matlab (R2014a; Mathworks, Natick, MA). EEG data were artifact rejected based on eye movement artifacts, extreme values, nonphysiologic waveforms, and signal dropout. Subsequently, power spectral estimates were calculated using a short-time fast Fourier function transform in 3-second epochs and averaged over a 30-minute bin over the course of 24 hours for each animal. Spectral frequencies were banded into delta (0.5–4 Hz), theta (4–7 Hz), alpha (8–12 Hz), sigma (12–16 Hz), beta (19–30 Hz), and gamma (35–100 Hz) power bands. Within-subject comparisons of compound and vehicle treatments were averaged, and a linear mixed effects model (nlme, cran.us.r-project.org; R Foundation for Statistical Computing, Vienna, Austria) with repeated-measures ANOVA was performed along the time series to calculate 30-minute binned statistics using the R statistical programming environment within each spectral frequency band.

Plasma and cerebrospinal fluid (CSF) were collected from a satellite group of rhesus macaque receiving 3 and 10 mg/kg MK-7622 at 1, 2, 4, 6, and 24 hours following administration, and MK-7622 concentrations were measured using LC-MS/MS.

Studies in Humans

Three studies were performed to assess MK-7622 plasma pharmacokinetics (Studies 001 and 002), CSF concentrations (Study 002), and pharmacodynamics, as assessed by qEEG (Study 001) and reversal of scopolamine-induced cognitive deficits (Study 003).

Methods

Key aspects of the methods are summarized below. Further details of the methods and statistical analyses are provided in the study protocols (Supplemental Materials 1–3). The studies were performed according to good clinical practice guidelines and were approved by the relevant institutional review boards. All subjects were healthy males who provided written informed consent.

Study 001: Single-Dose Plasma Pharmacokinetics and qEEG Effects. Part I was a double-blind, randomized, placebo-controlled, single-ascending dose study that evaluated the pharmacokinetics of MK-7622. In all the clinical studies, MK-7622 was administered as the active pharmaceutical ingredient in dry-filled capsules in three strength forms: 1, 10, and 100 mg. Sixteen subjects aged 22–45 years were randomized to one of two panels (A and B). Panels A and B were administered single-ascending P.O. doses of MK-7622 ranging from 1 to 70 mg or placebo in alternating panels. Each panel consisted of eight subjects, who received MK-7622 or placebo in up to five treatment periods. In each period, six subjects were randomized to receive MK-7622, and two subjects received

placebo. A different pair of subjects received placebo in each period. Part I also included an assessment of food effect and a pilot assessment of qEEG effects, neither of which is reported in this work. Results from the pilot qEEG study informed the sample size for Part II, which was powered to detect a difference from placebo in σ frequency activity.

Part II was a double-blind, randomized, placebo-controlled, four-period crossover study to assess the central nervous system effects of MK-7622 as measured by awake qEEG. Twenty-eight subjects aged 23–45 years were randomized to MK-7622 (10, 40, and 70 mg) and placebo in a balanced crossover design. A 27-lead electrode array was used for EEG recording with placement per the international 10–20 system. The EEG data were analyzed by digital processing techniques for power spectral density in different frequency bands by treatment. The following frequency bands were evaluated: delta = 0.5–4.25 Hz, theta = 4.25–8 Hz, alpha = 8–12 Hz, sigma = 12–15 Hz, beta = 15–24 Hz, gamma = 24–32 Hz. EEG was recorded at 0 and 2, 4, 8, and 12 hours postdose while the subject was awake with his eyes closed.

Study 002: Multiple Dose Plasma Pharmacokinetics and CSF Concentrations. This was a double-blind, randomized, placebo-controlled, rising multiple-dose study. Thirty-two subjects aged 19–45 years, divided into four panels (A–D) consisting of eight subjects each ($N = 6$ active; 2 placebo), were enrolled. In each panel, eight subjects received daily single P.O. doses of MK-7622 or matching placebo in a 3:1 ratio, respectively, for a total of 10 consecutive days. The P.O. doses in panels A, B, C, and D were, respectively, 10, 20, 30, and 40 mg MK-7622 or placebo. An assessment of MK-7622 CSF concentration was obtained at the 30 mg dose level (panel C).

Study 003: Scopolamine Reversal. This was a double-blind, randomized, placebo-controlled crossover to evaluate the efficacy of MK-7622 at 1, 10, and 70 mg and donepezil at 10 mg (active control) in reversing the cognitive deficits associated with a s.c. injection of 0.5 mg scopolamine in healthy males. Thirty-two men aged 18–45 years were enrolled and 30 completed (two withdrew and were replaced to achieve the target of 30 subjects completing). Both MK-7622 and donepezil were dosed 3.5 hours before the scopolamine injection, to coincide with the approximate timing of anticipated peak effects of both drugs. The efficacy assessments for this study included the detection, identification, continuous paired associate learning, and Groton maze learning test modules from the CogState computerized cognitive test battery (Fredrickson et al., 2010). These tasks evaluate a range of cognitive domains, including executive function, psychomotor function/information processing, visual attention, and visuospatial associative learning. The CogState battery was administered 5 hours before and 1, 2, 3, 4, and 6 hours after the scopolamine injection. The performance in the CogState battery 5 hours before scopolamine injection was used as the baseline for comparison in each period.

Results

Discovery of MK-7622. The identification of a selective ligand for the M_1 receptor was initiated at Merck Research Laboratories in 2004. From an ultra high-throughput screening campaign utilizing a functional readout in Chinese hamster ovary cells stably expressing human M_1 in the

Fig. 1. Structural evolution of M_1 PAMs that led to the identification of the clinical development candidate MK-7622.

presence of an EC₁₅ of acetylcholine (ACh), a screening hit was identified, BQCA (Ma et al., 2009), and drug optimization efforts led to the identification of quinolizidinone PQCA (Kuduk et al., 2011). Modest central nervous system penetration, low confidence in human pharmacokinetic predictions, and potential liabilities associated with acyl glucuronides, all related to the carboxylic acid present in BQCA and PQCA, hampered the identification of a suitable development candidate. As such, noncarboxylic acid-containing PAMs were realized through removal of the keto and acid functionality of these earlier leads (Fig. 1). The noncarboxylic acid pyrazolone 1 addressed the liabilities of its progenitors. Ring expansion to a pyrimidinone, optimization of the southern aryl ring to a methylpyridine, and replacement of the northern fluorophenyl ring with a (*S,S*)-trans-cyclohexanol resulted in substantial potency enhancement, resolution of off-target liabilities, and appropriate drug properties that culminated in the identification of MK-7622 as a clinical development candidate.

In Vitro Assessment of MK-7622. MK-7622 is a PAM of the M₁ receptor with exquisite selectivity over the M_{2–5} receptor subtypes (>1000-fold selective, data not shown) with 21 nM functional activity (EC₅₀) in the presence of an EC₁₀ of ACh but an EC₅₀ of ~1.7 μ M in the absence of ACh, consistent with it being a highly cooperative PAM of ACh (see below). MK-7622 has good physicochemical properties (high-pressure liquid chromatography LogD = 2.6) and moderate pH-dependent solubility (pH 2, 191 μ M; pH 7, 17 μ M), and, as a crystalline fumarate salt, it possesses good solubility (2.3 mg/ml) in simulated gastric fluid. MK-7622 exhibits similar extent of binding to proteins in rhesus and human plasma (95.8% and 95.1% bound, respectively). In LLC-PK1 cells, MK-7622 demonstrated good passive permeability (Papp = 31–35 $\times 10^{-6}$ cm/s). Transport (BA/AB) ratios for MK-7622 in LLC-PK1 cells stably transfected with African

green monkey MDR1 and human MDR1 cDNAs were determined to be 2.4 and 1.8, respectively, suggesting that MK-7622 is not a substrate for human and monkey P-glycoprotein (P-gp) at 1 μ M tested concentration. No significant pharmacological activities were observed in vitro with MK-7622 in a panel of 140 targets with only two findings below 5 μ M (arachidonate 5-lipoxygenase IC₅₀ = 4.4 μ M phosphodiesterase 4 IC₅₀ = 2.1 μ M).

The allosteric properties of MK-7622 were examined in Chinese hamster ovary cells overexpressing the human M₁ receptor (Fig. 2). Positive allosteric modulation of the ACh response was examined over a range of concentrations of ACh (12 pM–1000 nM) and MK-7622 (22 pM–22 μ M). The results of two independent experiments were averaged and analyzed using global curve fitting to an operational model of receptor allosterity (Zhang and Kavana, 2015). In the analysis, some parameters were constrained (kai = 0; bb = 1; KA = 42,666), and the average maximum was defined as the average of all maximum–minimum values for the top ACh concentration of every curve in the data set (Supplemental Material 4). In this analysis, MK-7622 possesses 0.946 μ M affinity for the unbound receptor (K_B) and is highly cooperative with receptor

Fig. 2. MK-7622 positive allosteric modulation of Ca⁺⁺ flux in response to ACh in human M₁ Chinese hamster ovary cells. Ca⁺⁺ flux was measured in M₁ Chinese hamster ovary cells in response to increasing concentrations of ACh (12 pM–1 μ M) in the absence or presence of 15 increasing concentrations (22 pM–22 μ M) of MK-7622. The responses were normalized to the ACh response at 1000 nM and are presented as the average $\pm$ S.E. of two independent experiments. The values in the table represent the geometric mean and geometric S.D. for K_B, and the arithmetic mean $\pm$ range for α and τ_B global fit values from the two independent experiments.

Fig. 3. Effect of MK-7622 on a scopolamine-induced deficit in object retrieval. Scopolamine impaired performance on the difficult, but not easy trials. The impairment produced by scopolamine was dose dependently attenuated by MK-7622. Donepezil (DON) given at 3 mg/kg was equally effective as a positive control. *Indicates significantly different from scopolamine alone ($P < 0.05$).

endogenous ligand ACh, α of 339, while exhibiting a τ_B of 1.067.

Effects of MK-7622 in Rhesus Monkeys on the ORD Task. Figure 3 shows the effects of scopolamine, MK-7622, and donepezil on performance of the ORD task. A one-way repeated-measures ANOVA revealed a main effect of treatment on difficult trials ($F_{(5, 35)} = 7.743$, $P < 0.001$). Post hoc analysis revealed that scopolamine treatment impaired performance on difficult trials relative to vehicle ($P < 0.001$), and that 0.3 and 1.0 mg/kg MK-7622 significantly attenuated the scopolamine effect ($P = 0.003$ and $P = 0.010$, respectively) as did the positive control donepezil ($P = 0.001$). The lowest dose, 0.1 mg/kg MK-7622, did not have a significant effect on the scopolamine deficit ($P = 0.254$).

A one-way repeated-measures ANOVA also revealed a main effect of treatment on easy trials ($F_{(5, 35)} = 2.503$, $P = 0.049$). However, post hoc analysis revealed that for easy trials

animals treated with scopolamine did not differ significantly from vehicle ($P = 0.997$) or any of the other treatment groups ($P = 0.143$ – 0.999).

Plasma was collected from animals on the day they received 0.3 mg/kg MK-7622 following object retrieval testing (i.e., approximately 5 hours after drug administration). Mean total plasma concentration was 39.4 nM with an S.D. of 10.3 nM.

Effects of MK-7622 in Rhesus Macaque on qEEG. Continuous electrocorticograms (ECoG, EEG) were recorded continuously in independent, multiday, crossover-designed, vehicle-controlled studies in rhesus macaques ($N = 4$ – 8 /study). These studies were conducted in the animals' active phase (Zeitgeber time +4.5). ECoG data were subsequently processed offline for power spectral analysis. Within a treatment group (vehicle + 1 mg/kg MK-7622; vehicle + 3 mg/kg MK-7622; vehicle + 10 mg/kg MK-7622), each animal's average power spectrum was calculated at 6 frequency ranges (delta 0.5–4 Hz, theta

Fig. 4. Effect size of MK-7622 1, 3, and 10 mg/kg versus vehicle in rhesus macaque by time by band averaged (dark colors indicate statistically significant).

Fig. 5. CSF and total plasma exposures of MK-7622 in rhesus macaque as a function of time and dose.

4–8 Hz, alpha 8–12 Hz, sigma 12–16 Hz, beta 19–30 Hz, gamma 35–100 Hz) using custom-developed Matlab (MathWorks) scripts utilizing the Signal Processing Toolbox's short-time fast Fourier function. Continuous ECoG segments were binned in time (0–2, 2–4, 4–8, 8–12 hours), and a Cohen's *d* effect size was calculated comparing the paired treatment groups at each time bin and frequency band.

MK-7622 showed a dose-responsive effect across delta-sigma power bands, whereas beta and gamma bands appear to have been most affected at the two lower doses (Fig. 4). Theta, alpha, and sigma showed reductions in power, which was most robust during the first 8 hours following drug treatment. In contrast, delta power appeared to be most heavily impacted at the 8- and 12-hour time points, primarily during the transition from lights on to lights off. Finally, the highest frequencies beta and gamma were both modestly increased, and only during the first 2 hours following treatment with MK-7622.

Figure 5 shows the CSF and total plasma concentrations of MK-7622 following administration of 3 and 10 mg/kg MK-7622 as a function of time. Exposures were roughly dose-proportional, and the concentration–time profile appeared to align most closely with the reductions in theta, alpha, and sigma.

In a separate study, plasma, CSF, and brain (frontal cortex) concentrations of MK-7622 were determined in rhesus monkey following 3 months of daily P.O. dosing. Using *in vitro* unbound brain concentrations of MK-7622, calculated free brain concentrations were consistent with CSF concentrations of MK-7622 (data not shown), confirming that CSF levels can

be used as a surrogate for free brain concentrations in respective *in vivo* studies.

Human Pharmacokinetics of MK-7622. Pharmacokinetic parameters for single doses of MK-7622 in Study 001 are summarized in Table 1. Following single P.O. doses (1–70 mg), the median time to reach maximum concentration (T_{max}) for MK-7622 was ~2–4 hours postdose. The concentration–time profiles for MK-7622 appeared to be biphasic postmaximum concentration (C_{max}), with the inflection between α and β phases occurring between 8 and 12 hours postdose (Fig. 6). The harmonic mean terminal half-life values for MK-7622 were 25 and 26 hours following 40 and 70 mg, respectively (sampling at lower doses was not sufficient to characterize the terminal phase). The mean C_{max} for MK-7622 ranged from 28 to 1600 nM, and the area under the curve over 24 hours ($AUC_{0-24 \text{ hours}}$) ranged between 0.3 and 22 $\mu\text{M} \times \text{h}$ across doses (1–70 mg). These data suggest that $AUC_{0-24 \text{ hours}}$ and C_{max} were dose proportional over the evaluated dose range.

The pharmacokinetic parameters for multiple doses of MK-7622 in Study 002 are summarized in Table 2. Following daily P.O. doses of 40 mg, the mean steady state $AUC_{0-24 \text{ hours}}$ on day 10 was 23.55 $\mu\text{M} \times \text{h}$. The mean accumulation ratio for $AUC_{0-24 \text{ hours}}$ (day 10/day 1) for MK-7622 ranged from 1.87 to 2.16 across doses (10–40 mg). MK-7622 steady state $AUC_{0-24 \text{ hours}}$ and C_{max} increased approximately dose proportionally over the evaluated dose range. Day 1 pharmacokinetic parameters for MK-7622 after 10, 20, and 40 mg doses of MK-7622 were consistent with pharmacokinetics from single dosing in Study 001.

TABLE 1

Mean (S.D.) pharmacokinetic parameter values for MK-7622 following administration of single oral doses in healthy men

Dose	C_{max} (nM)	T_{max} (h) ^a	$AUC_{0-24 \text{ h}}$ ($\mu\text{M} \times \text{h}$)	AUC_{last} ^b ($\mu\text{M} \times \text{h}$)
1 mg	28.3 ± 4.73	2.0 (1.0, 2.0)	0.33 ± 0.074	0.50 ± 0.11
2 mg	54.6 ± 5.93	2.0 (1.0, 4.0)	0.64 ± 0.11	0.95 ± 0.21
5 mg	150 ± 25.3	2.0 (2.0, 2.0)	1.82 ± 0.39	2.87 ± 0.72
10 mg	256 ± 65.1	2.5 (2.0, 3.0)	2.97 ± 0.62	4.46 ± 0.88
20 mg	536 ± 98.6	3.0 (2.0, 3.0)	6.41 ± 1.31	10.1 ± 2.17
40 mg	818 ± 118	2.0 (2.0, 4.0)	10.6 ± 1.44	18.3 ± 1.43
70 mg	1600 ± 204	4.0 (2.0, 6.0)	22.0 ± 2.86	40.6 ± 8.22

^aMedian (minimum, maximum).

^bLast sampling time was 48 hours across 1–20 mg panels, and 72 hours for 40 and 70 mg panels.

Fig. 6. Mean plasma concentration profiles for MK-7622 following administration of single oral doses of MK-7622 to healthy men (semi-log scale).

The mean MK-7622 concentration in the CSF samples collected 8 hours postdose on day 9 after every day P.O. dosing of 30 mg MK-7622 was 15.5 nM (Table 3). Individual ratios of CSF to unbound plasma concentrations of MK-7622 ranged from 0.4 to 0.6 and were consistent between subjects (Table 3). The underlying mechanism for CSF to unbound plasma ratio being less than 1 is not clear. As described above, *in vitro* experiments suggest that MK-7622 is not a substrate for human P-gp, and therefore, P-gp efflux is not a likely reason for this observation. It is therefore reasonable to assume that CSF levels are a good surrogate for unbound brain concentration, as further supported by data generated in rhesus monkeys.

Effects of MK-7622 on Scopolamine-Induced Cognitive Deficits in Human. In the scopolamine-alone arm, the performance of subjects receiving only scopolamine on tasks such as the detection task was lower than predose baseline at all time points (1, 2, 3, 4, 6 hours); performance on other tasks was lower than predose baselines at a number of time points postdose. The prespecified analysis looking at peak effect did not show a statistically significant effect of the active control donepezil and was therefore considered uninformative. Instead, a time-weighted average over 1- to 4-hour analysis, which had been used in previous studies conducted by Merck & Co., Inc. (data not shown), was retrospectively applied and indicated a positive effect of both donepezil and MK-7622 on reversal of scopolamine impairment as measured by the detection task (Table 4). The effects of MK-7622 were statistically significant at all dose levels and appeared to show a dose-dependent trend. Donepezil also had a statistically

significant effect on identification, but not on continuous paired associate learning or the Groton maze learning task (Table 4). MK-7622 did not have a statistically significant effect on identification, continuous paired associate learning, or the Groton maze learning task (Table 4). The absence of statistical improvement of donepezil on continuous paired associate learning and the Groton maze learning task may be due to a smaller scopolamine impairment effect seen in the current study compared with previous studies conducted by Merck & Co., Inc. (data not shown) and/or due to insufficient sample size.

Effects of MK-7622 on qEEG in Humans. The effects of MK-7622 on qEEG in Part II of Study 001 are shown in Fig. 7. The sigma frequency activity increased compared with placebo in a dose-dependent fashion, with statistically significant increases observed for the 40 and 70 mg doses. Increases in the spectral power of beta frequency activity were observed with 40 and 70 mg. There was also a statistically significant increase in spectral power in the delta and theta frequency bands for the 70 mg dose at 2 hours. There were no statistically significant effects on alpha and gamma over all dose levels and time points. Overall, there were no statistically significant effects at 10 mg.

Discussion

This is the first description of the pharmacokinetic and pharmacodynamic effects of a M₁ PAM in humans, and also the first description of the translatability of M₁ PAM effects from preclinical species to humans. MK-7622 is a selective and potent M₁ PAM with appropriate drug-like properties for evaluation in both rhesus macaque and humans. In both species, similar exposures of MK-7622 generated pharmacodynamic effects, as measured by the ability to attenuate the effects of scopolamine on cognition. Likewise, similar drug concentrations in both species produced electrophysiological effects, as measured by qEEG. The overall effects of MK-7622 on cognition, as measured by performance in the scopolamine model as well as effects in qEEG, translated across species. However, there were also some important differences between species and between pharmacodynamic measures, which are discussed in greater detail below.

The effects of MK-7622 on specific spectral bands as measured by qEEG were substantially different in the rhesus macaque and human studies. MK-7622 in rhesus macaque dose-dependently decreased power in theta, alpha, and sigma

TABLE 2

Mean (S.D.) pharmacokinetic parameters for MK-7622 following multiple dosing for 10 days in healthy men

Dose	Day	$t_{1/2}$ (h) ^a	T_{max} (h) ^b	C_{max} (nM)	AUC _{0-24 h} (μM × h)	Accumulation Ratio
10 mg	1		2 (1, 3)	262 ± 29.4	3.23 ± 0.38	
20 mg	1		2.5 (2, 4)	449 ± 85.8	5.71 ± 1.00	
30 mg	1		3.5 (2, 6)	642 ± 109	8.03 ± 1.37	
40 mg	1		3.0 (2.0, 6.0)	904 ± 96.0	11.85 ± 1.61	
10 mg	10	25.9 ± 6.4	2.0 (1.0, 4.0)	434 ± 98.4	6.34 ± 1.53	1.96 ± 0.39
20 mg	10	34.2 ± 11.0	3.5 (1.0, 4.0)	809 ± 315	12.39 ± 5.3	2.16 ± 0.81
30 mg	10	27.6 ± 6.8	2.5 (1.0, 4.0)	953 ± 118	14.85 ± 2.38	1.87 ± 0.25
40 mg	10	30.0 ± 5.2	2.5 (2.0, 4.0)	1550 ± 270	23.55 ± 4.16	1.98 ± 0.20

^a $t_{1/2}$, half-life.

^aHarmonic mean with pseudo S.D.

^bMedian (minimum, maximum).

TABLE 3

Individual and mean CSF concentration for MK-7622 8 hours postdose on day 9 following administration of multiple oral doses of 30 mg MK-7622 in healthy men

	CSF Concentration (nM)	Plasma Concentration (nM)	Unbound Plasma Concentration (nM)*	Ratio of CSF to Unbound Plasma Concentration
Individual Subjects	17.37	598.85	29.94	0.58
	16.87	681.4	34.07	0.5
	15.42	564.16	28.21	0.55
	14.42	588.01	29.4	0.49
	12.84	511.59	25.58	0.5
	16.7	820.08	41.00	0.41
Mean	15.52 (90% CI: 14.36, 16.77)			0.5 (S.D.: 0.06)

CI, confidence interval.

*Unbound fraction of MK-7622 in human plasma is 0.05.

and only modestly increased beta and gamma immediately after dosing and only at the two lower doses. In humans, MK-7622 exhibited its most pronounced effects on sigma and beta, demonstrating a dose-dependent increase in power. The underlying cause of these differences is unlikely due to species, per se, but rather a difference in psychologic and physiologic state of the subjects during testing. Namely, we hypothesize that ACh tone substantially differs between rhesus macaque being treated in their home cage versus humans receiving compound in a new, unfamiliar setting, having certain expectations regarding drug effects, and being asked to close and open their eyes while qEEG is being recorded. Cognitive state, anxiety levels, and other environmental differences may account for substantial differences in ACh tone. Toward this end, physiologic and psychologic state has shown to have dramatic effects on ACh release in the hippocampus and prefrontal cortex in rodents (e.g., Mark et al., 1996). This is particularly relevant because MK-7622 is

a PAM with high cooperativity properties (α), meaning the pharmacodynamic effects of MK-7622 are heavily ACh tone dependent. Furthermore, context-induced changes in ACh release and M_1 receptor activation have been shown to translate to changes in electrophysiology. For example, in an elegant study in which behavior, ACh release, and electrophysiology were measured simultaneously, Howe et al. (2017) showed that cues that were attended to and signaled reward presentation evoked a behavioral response, ACh release, and an increase in various spectral frequency bands measured from the prefrontal cortex. These same authors also showed that infusion of the M_1 -preferring antagonist telonezepine had effects on cue- but not basal-evoked gamma, highlighting the importance of M_1 receptor activation in this context-mediated effect. A recent study by Lebois et al. (2016) further suggests that the electrophysiological effects of a M_1 PAM are heavily state/tone dependent. Specifically, they report that the M_1 receptor agonist VU0364572 and the M_1

TABLE 4

Summary of results for CogState tests in healthy men: time-weighted average over 1–4 h (lower score = better performance)

Treatment	N ^a	Baseline		TWA _{1–4 h}		Treatment Comparison		
		Mean	S.D.	LSMean	95% CI	Label	LSMean	P value or (90% CI) ^b
Detection test (Log ₁₀ ms)								
A Scop	30	2.48	0.11	2.581	(2.556, 2.607)	.	.	.
B Scop + MK 1 mg	30	2.49	0.11	2.565	(2.539, 2.591)	B-A	−0.016	P = 0.047
C Scop + MK 10 mg	30	2.47	0.10	2.557	(2.531, 2.582)	C-A	−0.025	P = 0.006
D Scop + MK 70 mg	31	2.47	0.09	2.543	(2.517, 2.568)	D-A	−0.038	P < 0.001
E Scop + Donep	31	2.46	0.08	2.554	(2.528, 2.579)	E-A	−0.028	(−0.044, −0.011)
Identification (Log ₁₀ ms)								
A Scop	30	2.70	0.10	2.749	(2.726, 2.772)	.	.	.
B Scop + MK 1 mg	30	2.69	0.11	2.738	(2.715, 2.761)	B-A	−0.011	(−0.025, 0.003)
C Scop + MK 10 mg	30	2.68	0.09	2.749	(2.727, 2.772)	C-A	0.000	(−0.014, 0.014)
D Scop + MK 70 mg	31	2.68	0.09	2.744	(2.721, 2.767)	D-A	−0.005	(−0.019, 0.009)
E Scop + Donep	31	2.68	0.08	2.730	(2.708, 2.753)	E-A	−0.019	(−0.033, −0.005)
Continuous paired associate learning								
A Scop	30	21.47	40.17	22.493	(13.933, 31.053)	.	.	.
B Scop + MK 1 mg	30	11.80	21.80	21.415	(12.876, 29.954)	B-A	−1.079	(−5.084, 2.927)
C Scop + MK 10 mg	30	9.87	13.77	19.276	(10.728, 27.824)	C-A	−3.218	(−7.261, 0.826)
D Scop + MK 70 mg	31	13.65	36.01	22.516	(14.016, 31.017)	D-A	0.023	(−3.943, 3.989)
E Scop + Donep	31	11.19	25.71	22.918	(14.411, 31.425)	E-A	0.424	(−3.587, 4.436)
Groton maze learning test								
A Scop	30	38.17	16.66	53.330	(46.694, 59.967)	.	.	.
B Scop + MK 1 mg	30	36.40	13.40	57.213	(50.571, 63.855)	B-A	3.883	(−0.708, 8.474)
C Scop + MK 10 mg	30	37.03	12.56	52.699	(46.061, 59.336)	C-A	−0.632	(−5.216, 3.953)
D Scop + MK 70 mg	31	38.94	17.30	52.625	(46.043, 59.206)	D-A	−0.706	(−5.276, 3.864)
E Scop + Donep	31	39.48	17.61	50.288	(43.704, 56.872)	E-A	−3.042	(−7.614, 1.529)

CI, confidence interval; Donep, donepezil 10 mg; LSmean, least squares mean; MK, MK-7622; Scop, scopolamine 0.5 mg; TWA_{1–4 h}, time-weighted average over 1–4 hours.

^aActual sample sizes are provided, including two subjects that were replaced.

^bSignificant differences are those comparisons either with a P value < 0.05 (calculated for scopolamine + MK-7622 vs. scopolamine alone in the detection test) or with 90% CIs that did not overlap zero.

Fig. 7. Effect size of MK7622 10, 40, and 70 mg versus placebo by time by band averaged on all frontal and central leads during eyes closed in healthy men (dark colors indicate statistically significant).

receptor PAM BQCA produce quite distinct effects on hippocampal coherence, as measured by local field potentials, within CA1 and CA3 regions of the hippocampus during a behavioral task. These authors attribute the differences in the electrophysiological effects of these compounds to their differences in ability to influence the M_1 receptor as a function of ACh tone, with BQCA, like MK-7622, relying on basal levels of ACh to produce effects, whereas VU0345572, an allosteric agonist, does not. These findings suggest that rather than species differences, the distinct effects on qEEG that were observed in rhesus and human following MK-7622 treatment could be the result of different psychologic and physiologic states during testing. Studies specifically designed to vary psychologic and/or physiologic state and characterize the effects of MK-7622 on qEEG or other pharmacodynamic biomarkers are needed to further test this hypothesis.

Consistent with the notion that the effects of MK-7622 are likely to be context- and state-dependent, the drug concentrations required to affect performance in the scopolamine model versus qEEG were substantially different. In both humans and rhesus, the effects in the scopolamine model appeared to be more sensitive than qEEG; total plasma exposures of ~30 nM MK-7622 attenuated the cognitive-impairing effects of scopolamine in both rhesus and humans, whereas plasma exposures ~20-fold higher were required to impact qEEG. At first glance, this discrepancy might be surprising, but, when put in context with the above discussion regarding context and ACh tone, it might be expected that the potency of a M_1 PAM on these two pharmacodynamic measures would be heavily dependent on state. During cognitive testing, which has been shown to enhance synaptic ACh (Giovannini et al., 1998; Dalley et al., 2001), a M_1 PAM such as MK-7622 would be

expected to be much more potent relative to the less demanding cognitive state during resting qEEG recording. Furthermore, scopolamine increases extracellular ACh concentrations (Watanabe and Shimizu, 1989; Durkin et al., 1992), which could further contribute to the increased potency observed with MK-7622 in the scopolamine model. It is worth noting that the free concentrations of MK-7622 that were achieved in the brain, as reflected by total CSF levels, were likely not high enough to engender agonist activity on their own. Specifically, in all studies conducted with MK-7622 in which pharmacodynamic activity was measured, predicted CSF levels were well below 100 nM, which aligns with the EC₅₀ value of MK-7622 in the presence of ACh, but is well below the EC₅₀ value of MK-7622 in the absence of ACh (~1700 nM). Nevertheless, because the aforementioned EC₅₀ values were generated using a M1-overexpressing cell line in vitro system and potency can be influenced by various factors, including receptor number, the agonist and PAM EC₅₀ values could be different in native tissue. Studies utilizing an ex vivo native preparation to further characterize the intrinsic potency of MK-7622 would be valuable.

The studies described in this work highlight the challenges of utilizing pharmacodynamic biomarkers to aid dose selection for compounds that are dependent on receptor state. Specifically, the goal of these studies was to determine the dose–effect relationship for the pharmacodynamic effects of MK-7622 to inform on dose selection for a Phase 2 clinical study in AD patients (Voss et al., 2016). Although we were successful in establishing the dose–effect relationship for qEEG and cognition in the scopolamine model, the minimum effective doses between the pharmacodynamic biomarkers were different. Fortunately, because a 70 mg single dose of MK-7622 was well tolerated and produced pharmacodynamic effects in both the human scopolamine model and on qEEG, a dose was chosen for Phase 2 that produced (with once-daily dosing over multiple days) the equivalent exposures to a 70 mg single dose. However, additional uncertainty was recognized in translating these findings from healthy volunteers to AD patients, given that ACh levels in AD patients differ from healthy volunteers. These challenges in translation should be considered when embarking on programs for which the pharmacodynamic drug effects are heavily state-dependent.

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