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Preclinical to Human Translational Pharmacology of the Novel M_1 Positive Allosteric Modulator MK-7622

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Running Title: Translational pharmacology of MK-7622

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Abbreviations

ACh Acetylcholine

AChEIs Acetylcholinesterase inhibitors

AD Alzheimer's disease

CSF Cerebrospinal fluid

ECog Electrocorticogram

EEG electroencephalograpy

IACUC Institutional Animal Care and Use Committee

LC-MS/MS Liquid chromatography tandem mass spectrometry

ORD Object retrieval detour task

PAM Positive allosteric modulator

qEEG Quantitative electroencephalography

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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 M_1 muscarinic receptor activators. Herein we describe the preclinical and clinical pharmacodynamic effects of the M_1 muscarinic receptor positive allosteric modulator, MK-7622. MK-7622 attenuated the cognitive impairing effects of the muscarinic receptor antagonist scopolamine and altered 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 vs. human. In sum, these results are the first to demonstrate translation of preclinical cognition and target modulation to clinical effects in man for a selective M_1 muscarinic receptor positive allosteric modulator.

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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 non-selective 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 pro-cognitive effects of AChEIs.

The M_1 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 M_1 receptor is localized to brain regions involved in cognition, including the prefrontal cortex and hippocampus, and M_1 receptor knockout mice demonstrate deficits in a variety of cognitive tasks and measures of synaptic plasticity (Bymaster et al, 2003; Young and Thomas, 2014; Shinoe et al, 2005). Furthermore, several non-selective muscarinic agonists have shown pro-cognitive 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 M_1 receptor function for cognition, there has been a great deal of effort aimed at developing novel approaches for

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more selectively activating the M_1 receptor. Significant efforts have been put forth to identify selective agonists, (Heinrich et al. 2009; Greenlee et al. 2001), 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 sub-type selectivity is through the identification of allosteric ligands, either positive allosteric modulators or allosteric agonists, which have the potential to bind to less-conserved regions distal to the orthosteric binding site (Davie et al, 2013; Kuduk and Beshore, 2012).

Toward this end, we have identified and described several positive allosteric modulators (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 non-human primate cognition models have further supported the hypothesis that the M_1 receptor activation contributes to the pro-cognitive effects of non-selective muscarinic receptor activators (Uslaner et al, 2013; Ma et al, 2009; Puri et al, 2015; Vardigan et al, 2015; Lange et al, 2015) and is not responsible for the gastrointestinal side-effects (Vardigan et al, 2015) (although some recent data suggests 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 pro-cognitive 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. Herein, we disclose the novel PAM MK-7622 and describe its effects in both non-human 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).

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Materials and Methods

Studies in *Rhesus Macaque*

Synthesis of M_1 Positive Allosteric Modulators

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

Drug Preparation and Administration

Scopolamine hydrobromide trihydrate (10–30 μg/kg; Sigma-Aldrich, St. Louis, MO) was formulated fresh daily in saline and administered 60 min prior to testing. Scopolamine was administered via an intramuscular (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 task (ORD). Animals had *ad libitum* access to water and were fed chow (Purina High Protein Monkey Diet no. 5045), 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 and 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 which 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 pre-potent 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; Jentsch et al, 2000; Wilkinson et al, 1997). 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 their 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 min 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.

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Subjects were tested 2 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 SEM ≤ 10 on easy and difficult trials for the 3 previous sessions). Next, subjects were characterized on scopolamine to demonstrate sufficient impairment compared to 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 a 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 intramuscularly 60 min 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 followed by Dunnett post-hoc tests to examine treatment differences compared to 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 eleven adult male rhesus non-human primates (*Macaca mulatta*, 9-15 kg, 5-11 years of age) were housed in standard laboratory conditions in accordance with standards of the Merck 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 subcutaneous telemetric (D70-EEE, Data Sciences International, St. Paul, MN, USA) implantation allowing for continuous and simultaneous electroencephalograpic (EEG), electromyographic, electrooculographic and generalized locomotor activity recordings, which were

subsequently analyzed off-line. Non-human 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, USA). EEG data were artifact rejected based on eye movement artifacts, extreme values, non-physiologic 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 min 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 min 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 (Supplementary Materials S1, S2 and S3). 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 3 strength forms: 1 mg, 10 mg and 100 mg. Sixteen subjects aged 22-45 years were randomized to one of two panels (A-B). Panels A and B were administered single-ascending oral doses of MK-7622 ranging from 1-70 mg or placebo in alternating panels. Each panel consisted of 8 subjects, who received MK-7622 or placebo in up to 5 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 herein. Results from the pilot qEEG study informed the sample size for Part II, which was powered to detect a difference from placebo in sigma frequency activity.

Part II was a double-blind, randomized, placebo-controlled, 4-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 0h and 2, 4, 8 and 12 hours post-dose while the subject was awake with their 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 4 panels (A - D) consisting of eight subjects each (N = 6 active; 2 placebo) were

enrolled. In each panel, eight subjects received daily single oral doses of MK-7622 or matching placebo in a 3:1 ratio, respectively, for a total of 10 consecutive days. The oral doses in Panels A, B, C and D were respectively 10 mg, 20 mg, 30 mg 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 mg, 10 mg, and 70 mg and donepezil at 10 mg (active control) in reversing the cognitive deficits associated with a subcutaneous injection of 0.5 mg scopolamine in healthy males. Thirty-two men aged 18-45 years were enrolled and 30 completed (2 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, in order 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.

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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 a ultra high throughput screening campaign utilizing a functional readout in CHO cells stably expressing human M_1 in the presence of an EC_{15} of acetylcholine, 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, non-carboxylic acid-containing PAMs were realized through removal of the keto and acid-functionality of these earlier leads (Figure 1). The non-carboxylic 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 positive allosteric modulator of the M_1 receptor with exquisite selectivity over the $M_{2\text{-}5}$ receptor subtypes (>1,000-fold selective, data not shown) with 21 nM functional activity (EC_{50}) in the presence of an EC_{10} of acetylcholine (ACh) but an EC_{50} of ~1700 nM in the absence of ACh, consistent with it being a highly cooperative positive allosteric modulator of ACh (see below). MK-7622 has good physicochemical properties (HPLC LogD = 2.6), moderate pH-dependent solubility (pH 2, 191000 nM; pH 7, 17000 nM), and as a crystalline fumarate salt, possesses good solubility (2.3 mg/mL) in simulated gastric fluid). MK-7622 exhibits similar extent of binding to proteins in rhesus and human

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plasma (95.8% and 95.1% bound, respectively). In LLC-PK1 cells, MK-7622 demonstrated good passive permeability (Papp = 31-35·10^{-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 1000 nM tested concentration. No significant pharmacological activities were observed in vitro with MK-7622 in a CEREP panel of 140 targets with only two findings below 5000 nM (5-LO IC_{50} = 4400 nM, PDE_4 IC_{50} = 2100 nM).

The allosteric properties of MK-7622 were examined in CHO cells overexpressing the human M_1 receptor (Figure 2). Positive allosteric modulation of the ACh response was examined over a range of concentrations of ACh (0.012 to 1000 nM) and MK-7622 (0.022 to 22000 nM). The results of two independent experiments were averaged and analyzed using global curve fitting to an operational model of receptor allostery (Zhang and Kavana, 2015). In the analysis, some parameters were constrained (kai=0; bb=1; KA=42666) 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 (Supplementary Materials S4). In this analysis, MK-7622 possesses 946 nM affinity for the unbound receptor (K_B) and is highly cooperative with receptor endogenous ligand ACh, α of 339, while exhibiting a τ_B of 1.067.

Effects of MK-7622 in Rhesus Monkeys on the Object Retrieval Detour Task

Figure 3 shows the effects of scopolamine, MK-7622 and donepezil on performance of the object retrieval detour 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 SD of 10.3 nM.

Effects of MK-7622 in Rhesus Macaque on qEEG

Continuous electrocorticograms (ECoG, EEG) were recorded continuously in independent, multi-day, cross-over designed, vehicle controlled studies in rhesus macaques (N=4-8/study). These studies were conducted in the animals' active phase (Zeitbeger 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 4-8 Hz, alpha 8-12 Hz, sigma 12-16 Hz, beta 19-30 Hz, gamma 35-100Hz) using custom developed Matlab (MathWorks, Natick, MA) scripts utilizing the Signal Processing Toolbox's short-time fast Fourier function. Continuous ECoG segments were binned in time (0-2 h, 2-4 h, 4-8 h, 8-12 h) 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 (Figure 4). Theta, alpha, and sigma showed reductions in power, which was most robust during the first 8h following drug treatment. In contrast, delta power appeared to be most heavily impacted at the 8 and 12 hour timepoints, 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 oral 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 oral doses (1-70 mg), the median time to reach maximum concentration (T_{max}) for MK-7622 was ~2-4 hours post-dose. The concentration-time profiles for MK-7622 appeared to be biphasic post-maximum concentration (C_{max}), with the inflection between alpha and beta phases occurring between 8-12 hours post-dose (Figure 6). The harmonic mean terminal half-life ($t_{½}$) values for MK-7622 were 25 and 26 hours following 40 mg 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 – 1600 nM and the area-under-the-curve over 24 hours ($AUC_{0\text{-}24h}$) ranged between 300 – 22000 nM•h across doses (1-70 mg). These data suggest that $AUC_{0\text{-}24h}$ 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 oral doses of 40 mg, the mean steady state $AUC_{0\text{-}24h}$ on Day 10 was 23550 nM•h. The mean accumulation ratio for $AUC_{0\text{-}24h}$ (Day 10/ Day 1) for MK-7622 ranged from 1.87-2.16

across doses (10-40 mg). MK-7622 steady state $AUC_{0\text{-}24h}$ 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.

The mean MK-7622 concentration in the CSF samples collected 8 hours post dose on day 9 after QD oral 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 0.4-0.6 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 monkey.

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 were lower than pre-dose baseline at all time points (1, 2, 3, 4, 6 hours); performance on other tasks were lower than pre-dose baselines at a number of time points post dose. The pre-specified 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 1-4 hour analysis, which had been utilized 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 Human

The effects of MK-7622 on qEEG in Part II of Study 001 are shown in Figure 7. Sigma frequency activity increased compared with placebo in a dose dependent fashion, with statistically significant increases observed for the 40 mg and 70 mg doses. Increases in the spectral power of beta frequency activity were observed with 40 mg 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.

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Discussion

This is the first description of the pharmacokinetic and pharmacodynamic effects of an M_1 PAM in humans, and also the first description of the translatability of M_1 PAM effects from preclinical species to humans. MK-7622 is a selective and potent M_1 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 pharmacodynamics 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 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 psychological and physiological 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, physiological and psychological state has shown to have dramatic effects on ACh release in the hippocampus and PFC in rodents (eg. Mark et al, 1996). This is particularly relevant because MK-7622 is a positive allosteric modulator 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 telenzepine 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 an M_1 PAM are heavily state/tone dependent. Specifically, they report that the M_1 receptor agonist VU0364572 and the M_1 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 psychological and physiological states during testing. Studies specifically designed to vary psychological and/or physiological state and characterize the effects of MK-7622 on qEEG or other pharmacodynamics 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 vs. 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 human, 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 an M_1 PAM on these two pharmacodynamics 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), an 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 EC_{50} value of MK-7622 in the presence of ACh but is well below the EC_{50} value of MK-7622 in the absence of ACh (~1700 nM). Nevertheless, because the aforementioned EC_{50} values were generated using an M1 over-expressing cell line *in vitro* system and potency can be influenced by various factors, including receptor number, the agonist and PAM EC_{50} 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 herein 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 in order to inform on dose selection for a Phase 2 clinical study in AD patients (Voss et al, 2016). While 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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Authorship Contributions

Participated in research design: All authors.

Conducted experiments: All authors.

Performed data analysis: Uslaner, Kuduk, Wittmann, Lange, Fox, Pajkovic, Beshore.

Wrote or contributed to the writing of the manuscript: All authors.

Footnotes

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Conflicts of interest

All authors except SW are current or former employees of, and own or owned stock/stock options, in Merck & Co., Inc., Kenilworth, NJ, USA. SW has received research funding from Merck & Co., Inc.

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TABLE 1

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

Dose	C_{max} (nM)	T_{max} (h)[a]	$AUC_{0\text{-}24h}$ (nM•h)	AUC_{last}[b] (nM•h)
1 mg	28.3 ± 4.73	2.0 (1.0, 2.0)	330 ± 74	500 ± 110
2 mg	54.6 ± 5.93	2.0 (1.0, 4.0)	640 ± 110	950 ± 210
5 mg	150 ± 25.3	2.0 (2.0, 2.0)	1820 ± 390	2870 ± 0720
10 mg	256 ± 65.1	2.5 (2.0, 3.0)	2970 ± 620	4460 ± 880
20 mg	536 ± 98.6	3.0 (2.0, 3.0)	6410 ± 1310	10100 ± 2170
40 mg	818 ± 118	2.0 (2.0, 4.0)	10600 ± 1440	18300 ± 1430
70 mg	1600 ± 204	4.0 (2.0, 6.0)	22000 ± 2860	40600 ± 8220

[a] Median (Min, Max)

[b] Last sampling time was 48h across 1-20 mg panels, and 72 h for 40 and 70 mg panels

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TABLE 2

Mean (SD) 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)	$AUC_{0\text{-}24h}$ (nM.h)	Accumulation Ratio
10 mg	1		2 (1, 3)	262 ± 29.4	3230 ± 380	
20 mg	1		2.5 (2, 4)	449 ± 85.8	5710 ± 1000	
30 mg	1		3.5 (2, 6)	642 ± 109	8030 ± 1370	
40 mg	1		3.0 (2.0, 6.0)	904 ± 96.0	11850 ± 1610	
10 mg	10	25.9 ± 6.4	2.0 (1.0, 4.0)	434 ± 98.4	6340 ± 1530	1.96 ± 0.39
20 mg	10	34.2 ± 11.0	3.5 (1.0, 4.0)	809 ± 315	12390 ± 5300	2.16 ± 0.81
30 mg	10	27.6 ± 6.8	2.5 (1.0, 4.0)	953 ± 118	14850 ± 2380	1.87 ± 0.25
40 mg	10	30.0 ± 5.2	2.5 (2.0, 4.0)	1550 ± 270	23550 ± 4160	1.98 ± 0.20

[a]Harmonic mean with pseudo SD

[b]Median (Min, Max)

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TABLE 3

Individual and mean CSF concentration for MK-7622 8 hours post dose 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	17.37	598.85	29.94	0.58
Subjects	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 (SD: 0.06)

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

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TABLE 4

Summary of results for CogState tests: time weighted average (TWA) over 1-4 hours (lower score = better performance)

		Baseline		TWA_{1-4h}		Treatment Comparison		
Treatment	**N**[a]	**Mean**	**SD**	**LSMean**	**95% CI**	**Label**	**LSMean**	***P*-value or (90% CI[b])**
Detection Test (Log_{10} msec)								
A Scop	30	2.48	0.11	2.581	(2.556, 2.607)		.	
B Scop+MK 1mg	30	2.49	0.11	2.565	(2.539, 2.591)	B-A	-0.016	***P*=0.047**
C Scop+MK 10mg	30	2.47	0.10	2.557	(2.531, 2.582)	C-A	-0.025	***P*=0.006**
D Scop+MK 70mg	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} msec)								
A Scop	30	2.70	0.10	2.749	(2.726, 2.772)		.	
B Scop+MK 1mg	30	2.69	0.11	2.738	(2.715, 2.761)	B-A	-0.011	(-0.025, 0.003)
C Scop+MK 10mg	30	2.68	0.09	2.749	(2.727, 2.772)	C-A	0.000	(-0.014, 0.014)
D Scop+MK 70mg	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 1mg	30	11.80	21.80	21.415	(12.876, 29.954)	B-A	-1.079	(-5.084, 2.927)
C Scop+MK 10mg	30	9.87	13.77	19.276	(10.728, 27.824)	C-A	-3.218	(-7.261, 0.826)
D Scop+MK 70mg	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 1mg	30	36.40	13.40	57.213	(50.571, 63.855)	B-A	3.883	(-0.708, 8.474)
C Scop+MK 10mg	30	37.03	12.56	52.699	(46.061, 59.336)	C-A	-0.632	(-5.216, 3.953)

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D Scop+MK 70mg	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)

Scop: Scopolamine 0.5 mg; MK: MK-7622; Donep: Donepezil 10 mg; LSMean: Least squares mean; $TWA_{1\text{-}4h}$: Time weighted average over 1-4 hours.

[a] Actual sample sizes are provided, including two subjects that were replaced.

[b] Significant differences are bolded: comparisons either with a *P*-value <0.05 (calculated for scopolamine+MK-7622 vs. scopolamine alone in the Detection Test) or with 90% CI s that did not overlap zero.

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Legends for Figures

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

Fig. 2. MK-7622 positive allosteric modulation of Ca^{++} flux in response to acetylcholine (ACh) in human M_1 CHO cells. Ca^{++} flux was measured in M_1 CHO cells in response to increasing concentrations of acetylcholine (0.012 to 1000 nM) in the absence or presence of 15 increasing concentrations (0.022 to 22000 nM) of MK-7622. The responses were normalized to the ACh response at 1000 nM, and are presented as the average ± SE of two independent experiments. The values in the table represent the geometric mean and geometric SD for K_B, and the arithmetic mean ± range for α and τ_B global fit values from the 2 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 ($p<0.05$).

Fig. 4. Effect size of MK-7622 1, 3, and 10 mg vs. 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.

Fig. 6. Mean Plasma Concentration Profiles for MK-7622 Following Administration of Single Oral Doses of MK-7622 to Healthy Male Subjects (Semi-log Scale)

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

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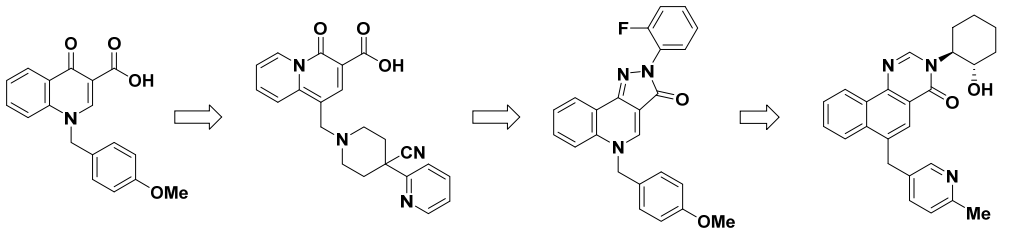

BQCA: M_1 IP = 660 nM **PQCA:** M_1 IP = 136 nM **1**: M_1 IP = 384 nM **MK-7622**: M_1 IP = 21nM

Fig. 1

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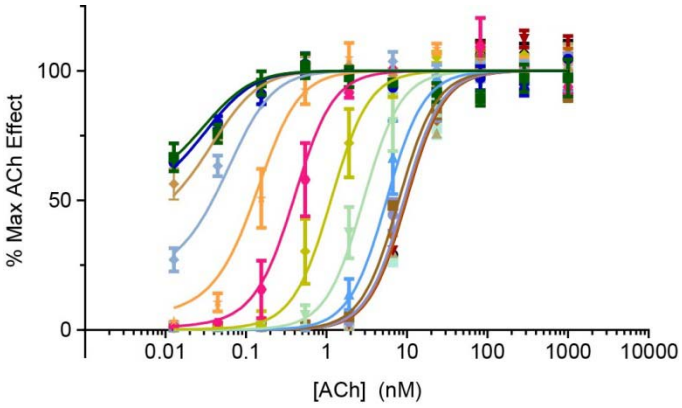

K_B (nM)	α	τ_B
948.1	338.6	1.067
±162.7*	±59.2*	±0.076*

*Standard error of parameters.

Fig. 2

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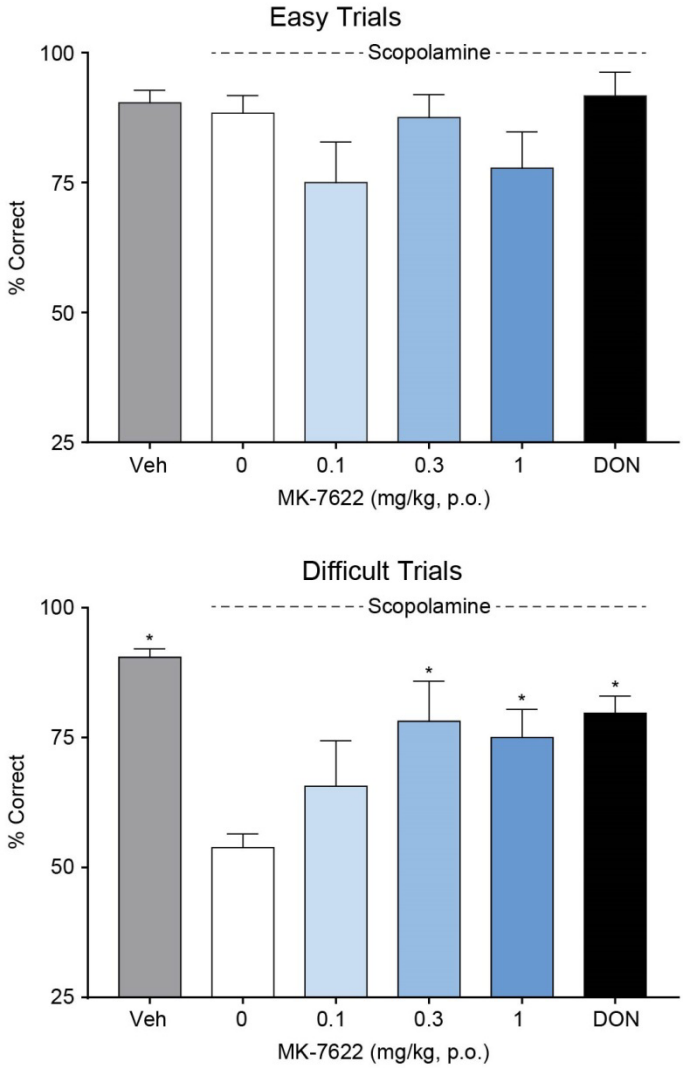

Fig. 3

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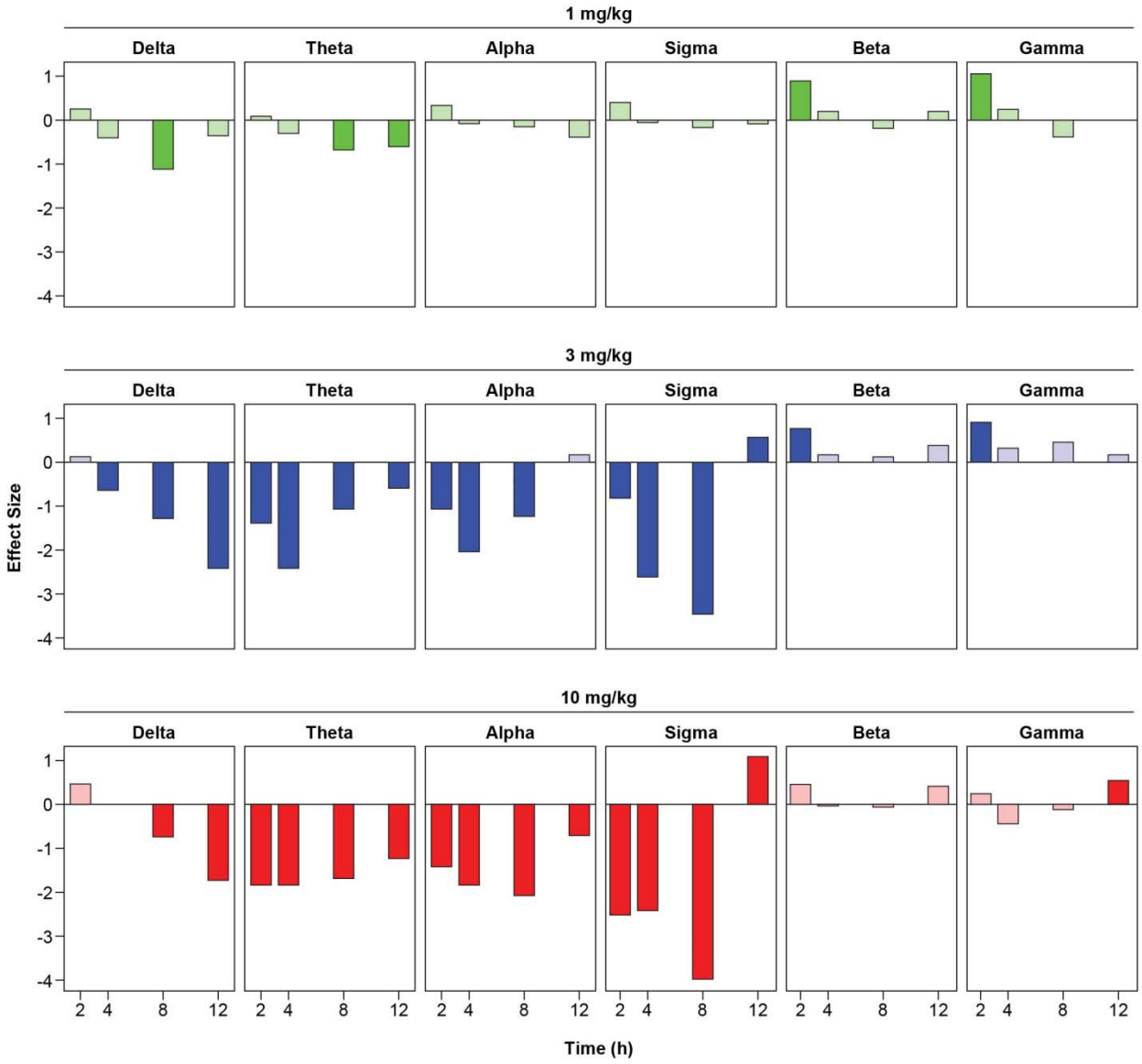

Fig. 4

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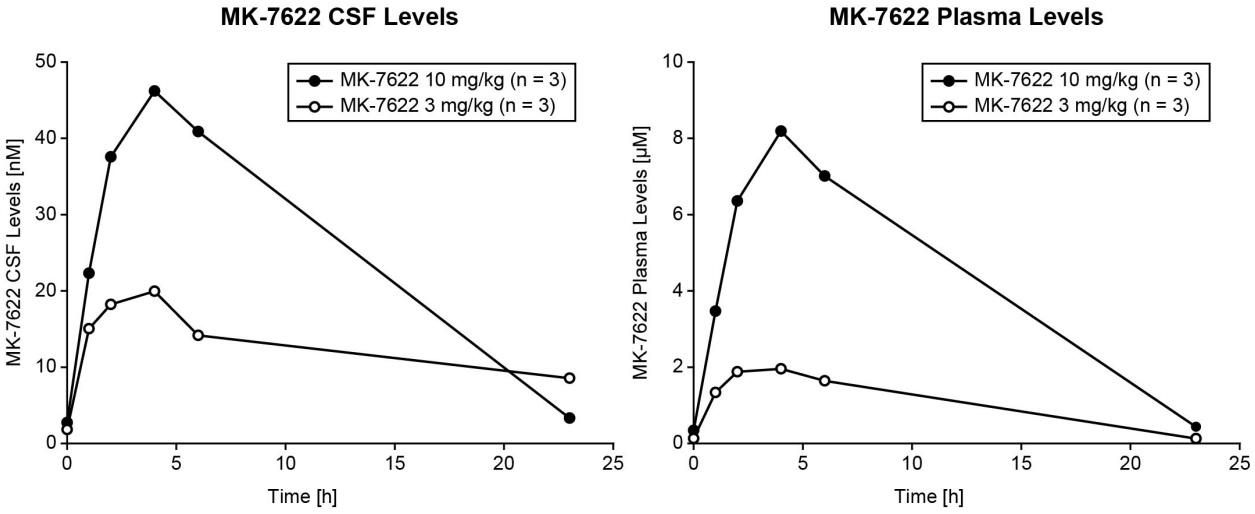

Fig. 5

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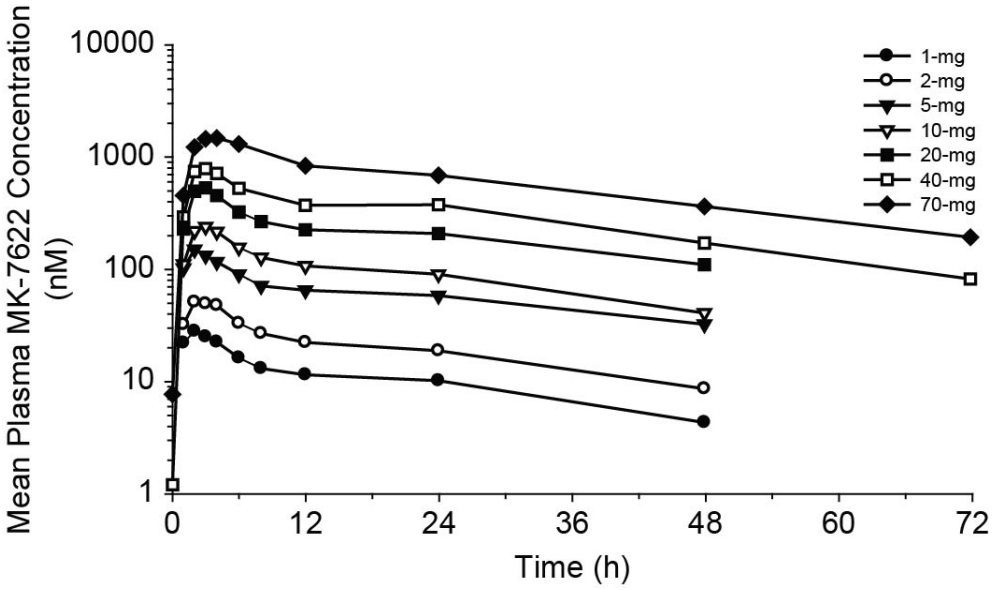

Fig. 6

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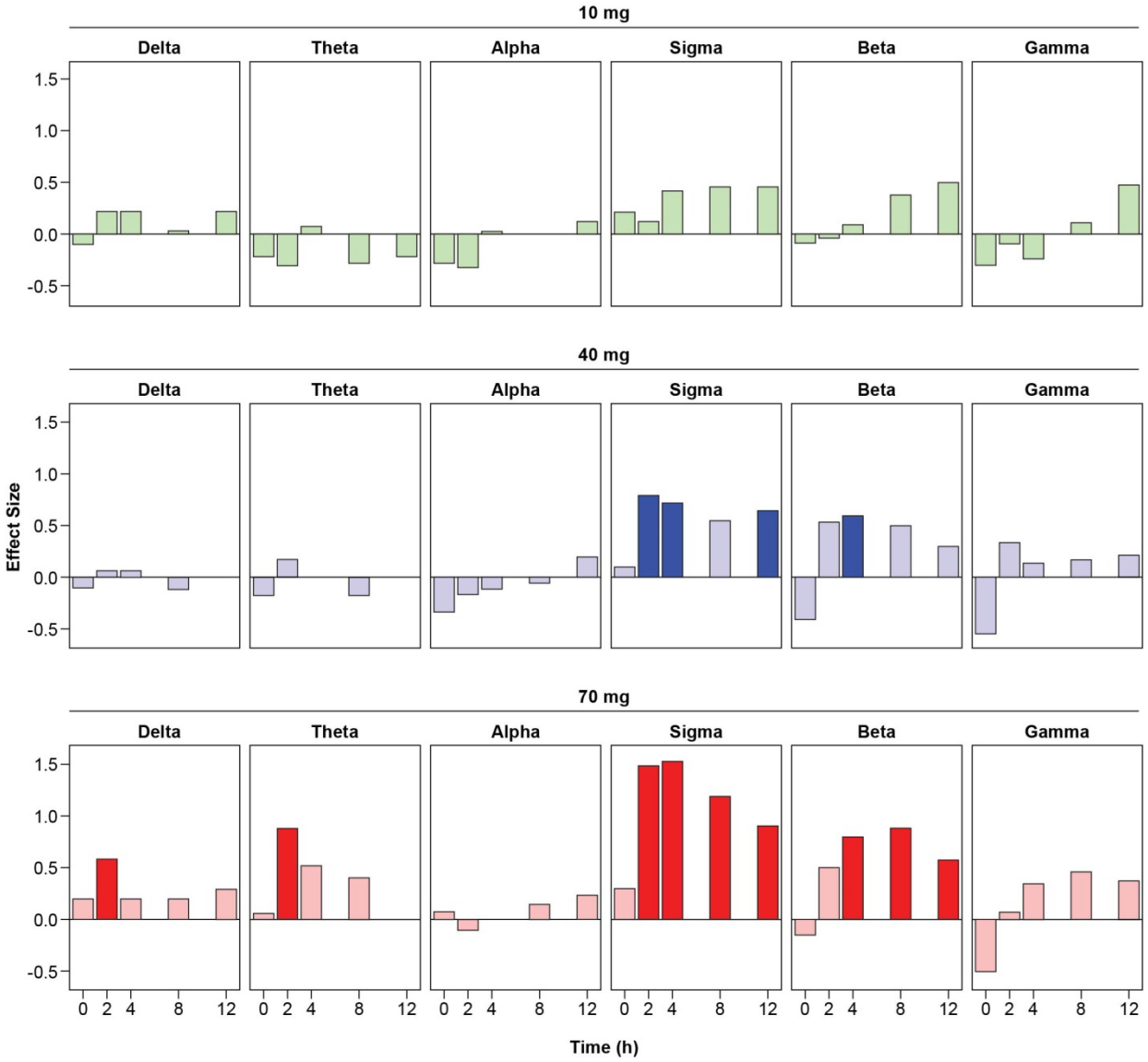

Fig. 7