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Title Page:

Reduction of L-DOPA-induced dyskinesia by the selective metabotropic glutamate receptor 5 (mGlu5) antagonist MTEP in the MPTP-lesioned macaque model of Parkinson's disease

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Running title page: Actions of mGlu5 blockade in the MPTP-lesioned macaque

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Words in Discussion: 1494

Abbreviations:

PD, *Parkinson's disease*; L-DOPA, *L-3,4-dihydroxyphenylalanine*; LID, *L-DOPA-induced-dyskinesia*; mGlu, *metabotropic glutamate receptor*, MPTP, *1-methyl-4-phenyl, 1,2,3,6-tetrahydropyridine*; MTEP, *3-((2-methyl-4-thiazolyl)ethynyl)pyridine*; MPEP, *3-(2-Methyl-6-(phenylethynyl))pyridine*; mGlu5, *metabotropic glutamate receptor type 5*; ANOVA, *Analysis of variance;* LC-MS/MS*, Liquid Chromatography-Tandem Mass Spectrometry; cf., Compare [Lat.]; UPDRS, Unified Parkinson's Disease Rating Scale* .

Recommended section assignment: Behavioural Pharmacology

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Abstract

Long-term motor complications of dopamine-replacement, such as L-DOPA-induced dyskinesia (LID) and reduced quality of L-DOPA-action remain an obstacle in the treatment of Parkinson's disease (PD). Dysfunctional glutamatergic neurotransmitter systems have been observed in both the untreated parkinsonian and dyskinetic state, and represent novel targets for treatment. Here, we assess the pharmacokinetic profile and corresponding pharmacodynamic effects on behaviour of the orally-active, selective mGlu5 antagonist, 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP, as the hydrochloride salt) in the MPTP-lesioned macaque. Six parkinsonian, MPTP-lesioned cynomolgus monkeys, with established LID, were administered acute challenges with MTEP (4.5 – 36 mg/kg, *p.o.*), or vehicle, either alone or in combination with L-DOPA (33 ± 1 mg/kg, *p.o.*). Motor activity, parkinsonian disability and dyskinesia were assessed for a 6 h period. Plasma drug levels were assessed by LC-MS/MS. MTEP had no anti-parkinsonian action as monotherapy. However, administration of L-DOPA in combination with MTEP (36 mg/kg) reduced peak dose LID by 96%. Moreover, though total on-time (duration for which L-DOPA exerted an anti-parkinsonian effect) was not significantly reduced, MTEP (36 mg/kg) reduced the duration of on-time with disabling LID by 70% compared to L-DOPA alone. These effects were associated with a peak plasma concentration of 20.9 μM and an $AUC_{0\text{-}24}$ of 136.1 h.μM (36 mg/kg). While total on-time was not reduced, the peak anti-parkinsonian benefit of L-DOPA/ MTEP (36 mg/kg) was less than with L-DOPA alone. Selective mGlu5 inhibitors may have significant potential to ameliorate dyskinesia but care should be taken to ensure that such effects do come at the expense of the peak anti-parkinsonian benefit of L-DOPA.

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Introduction

Effective treatment of the motor complications of dopamine-replacement therapy in Parkinson's disease (PD) remains a significant unmet clinical need. Such problems include L-DOPA-induced dyskinesia (LID) and become increasingly common following long-term treatment with L-DOPA in PD (Fabbrini et al., 2007; Poewe, 2009). LID can be troublesome and impact significantly on quality of life in PD, the severity of which is often in direct proportion to the number of years since first diagnosis (Pechevis et al., 2005). Current pharmacological approaches to treatment may reduce dyskinesia in only a subset of patients, e.g. amantadine, or, by lowering L-DOPA dose, can lessen the problem but simultaneously reduce anti-parkinsonian benefit (Goetz et al., 2005; Pahwa et al., 2006).

Abnormal glutamate signalling within the basal ganglia is evident in L-DOPA-induced motor-complicated states (Calabresi et al., 2000). Enhanced levels of striatal N-methyl-D-aspartate (NMDA)-type glutamate receptor have been observed in both PD patients with LID as well as L-DOPA-treated parkinsonian primates (Calon et al., 2002; Calon et al., 2003; Hallett et al., 2005). More recently, changes in the synaptic recruitment of α-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) receptor subunits in L-DOPA treated primates has been observed (Silverdale et al., 2010). Indeed, the potential of various ionotropic glutamate receptor antagonists as treatments for motor complications in PD has been explored in several animal models of PD (Marin et al., 1996; Blanchet et al., 1999; Nash et al., 2004; Bibbiani et al., 2005; Silverdale et al., 2005). Unfortunately, interventions targeting such receptors have, to date, been beset by

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problems of poor efficacy, tolerability and side-effects in clinical studies (Fox and Lang, 2008; Nutt et al., 2008).

An alternative approach to modulating glutamate transmission in PD and LID may lie in modulation of metabotropic glutamate (mGlu) receptors (Gubellini et al., 2004; Ossowska et al., 2007; Fox et al., 2008). mGlu receptors are broadly classified into three groups, I, II and III, based on molecular and pharmacological properties (Conn and Pin, 1997). Enriched within the basal ganglia (Testa et al., 1995; Marino et al., 2002), mGlu receptor subtype 5 (mGlu5) appears to be a promising potential target for modulation of motor function due to a wider therapeutic index and less potential to induce side-effects, particularly worsening of parkinsonism. Indeed, in rat models blockade of mGlu5 has shown benefit both in alleviating parkinsonian symptoms (Breysse et al., 2003; Ossowska et al., 2005) and in reducing LID (Mela et al., 2007; Gravius et al., 2008). To date, these actions have not been fully investigated in non-human primate models of PD and LID. However, preliminary results, presented in abstract form, from a pre-clinical study and a small Phase II clinical trial have shown some positive benefit of using the mGlu5 antagonist AFQ056 to reduce LID in parkinsonian primates and PD patients, without worsening parkinsonism (Berg et al., 2009; Gregoire et al., 2009). Here, we fully assess the anti-parkinsonian and anti-dyskinetic actions of an orally active, selective mGlu5 antagonist, 3-((2-methyl-4-thiazolyl)ethynyl)pyridine (MTEP) (Cosford et al., 2003), administered alone and in combination with L-DOPA in the 1-methyl-4-phenyl, 1,2,3,6-tetrahydropyridine- (MPTP) lesioned primate. Given the poor solubility of MTEP, we have employed a recently described method of synthesising the hydrochloride salt

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(McIldowie et al., 2009) to allow full evaluation of pharmacodynamics of effects on behaviour following oral administration.

Methods

Animals

Six cynomolgus monkeys (*Macaca fascicularis)* (4 females and 2 males, 5.2 ± 0.8 kg, 7.5 ± 0.7 years, at the time of the study) were obtained from Shared Animal Health, Beijing, PRC. Female animals were group housed, male animals were housed individually. All housing exceeded NIH, EU and UK guidelines and animals were subject to controlled conditions of temperature (22 ± 3^{o}C), humidity (51% ± 1%) and light (12 hour light-dark cycle, lights on at 7.00 am). Primate diet and water were available *ad libitum* and supplemented daily with fresh fruit. Housing was enriched with both auditory and tactile stimuli. All efforts were made to reduce to a minimum the number of animals necessary for statistically valid analyses and to minimise animal suffering. All studies were performed with local IACUC approval and in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the United States National Institutes of Health (Institute of Laboratory Animal Resources (U.S.). Committee on Care and Use of Laboratory Animals, 1996).

MPTP administration and development of motor complications

For a period of at least 3 months prior to commencement of MPTP administration, animals were acclimatised to experimental settings, blood sampling, handling and

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transfer to and from observation cages. Animals received once daily subcutaneous injection of MPTP hydrochloride (0.2 mg/kg final concentration of base, Sigma, Canada) for 8-30 days, until the first appearance of parkinsonism (MPTP hydrochloride was dissolved in 0.9% sterile saline and administered at a dose volume of 0.2 ml/kg). A parkinsonian syndrome was then allowed to develop over at least a 90 day period, during which time additional MPTP administrations were given as necessary, until animals reached moderate to marked levels of disability. Because individual macaques have varying sensitivity to MPTP, the total dose of MPTP required to produce stable, marked to moderate parkinsonism was different in each animal , the cumulative dose of MPTP across the group was 11.3 ± 1.5 mg.

This was allowed to stabilise for a minimum of a further 60 day prior to commencing induction of L-DOPA-induced motor complications. LID, including both choreiform and dystonic dyskinesia and reduced duration of action of L-DOPA, “wearing-off”, were evoked by chronic L-DOPA treatment (Madopar®, Roche, L-DOPA: benserazide, ratio 4:1, L-DOPA dose 20 mg/kg and administered at a dose volume of 1 ml/kg, *p.o.* dissolved in water) for at least 90 days. At this time, dose-finding observations were conducted (data not shown) to individually titrate the L-DOPA dose (range 25 – 40 mg/kg, mean L-DOPA dose, 33 ± 1 mg/kg) for each animal to allow optimal anti-parkinsonian benefit lasting up to 4 h but which was compromised by disabling dyskinesia. The responses to these doses of L-DOPA were assessed to ensure stability and reproducibility within each animal on successive L-DOPA administrations.

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Treatments

The effects of six different treatment combinations were assessed in an acute challenge study design. Thus, animals were administered L-DOPA, or vehicle, in combination with MTEP hydrochloride (vehicle, 4.5, 18 or 36 mg/kg final concentration of base) at ~9 a.m. on days of observation. MTEP hydrochloride was produced according to a previously described synthetic route (McIldowie et al., 2009) and identity and purity confirmed by LC-MS. MTEP and L-DOPA were dissolved in water and were administered in the animal's home cage via a nasogastric tube in a volume of 1 ml/kg. All animals received all treatments once, in a randomised fashion using a complete Latin Square design using EDGAR 1.0 software (http://www.edgarweb.org.uk). A minimum of 72 h was allowed between treatments in the same animal.

Assessment of behaviour after administration of MTEP, alone and in combination with L-DOPA

Following administration of treatments, animals were transferred immediately to an observation cage (1.5 x 1 x 1.1 m). During periods of assessment, animals were not disturbed and their behaviour was recorded on DVD and by automated passive infra-red activity monitoring. Ratings of behaviour were made, blinded to treatment, by *post hoc* analysis of DVD-recordings by a neurologist specialising in movement disorders. A measure of total parkinsonian disability was derived summating assessments of a) *range of movement* (0 = walking on the floor and/ or climbing on the walls or roof of the cage to 3 = no movement), b) *bradykinesia* (0 = normal speed and initiation of movement to 3 = marked slowing, or unable to move, with prolonged freezing episodes), c) *posture* (0 =

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normal, upright, to 2 = hunched body and neck, face down, may lose balance) and d) *alertness* (0 = present, looking around, observant or 1 = absent). Dyskinesia (representative of the maximum of either chorea or dystonia) was scored from 0 = absent, 1 = mild, fleeting, not interfering with normal activity, present less than 30% of the observation period, 2 = moderate, not interfering with normal activity, present more than 30% of the observation period, 3 = marked, at times disabling, i.e. interfering with normal activity, present less than 70% of the observation period, 4 = severe, continuous, disabling, replacing normal activity, present more than 70% of the observation period (Visanji et al., 2009). Parkinsonian disability and dyskinesia, respectively, were assessed for 5 minutes every 10 minutes, the score given was the one most representative of each 5 minute observation period.

Scores were cumulated for each hour across the entire 6 h of observations for time-course analyses and during the first two hours of assessment, the peak-effect period. The duration of anti-parkinsonian action, i.e. on-time, was defined as the number of minutes for which bradykinesia was absent i.e. score equal to zero. In addition, the duration of on-time associated with dyskinesia of varying severity was defined as follows; "*good*" quality on-time represents the number of minutes for which bradykinesia was zero whilst dyskinesia was either absent or of mild or moderate severity. Meanwhile, "*bad*" quality on-time represents the number of minutes for which bradykinesia was zero whilst dyskinesia was either marked or severe.

Assessment of plasma MTEP levels following oral administration

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In a series of studies conducted independently from behavioural observations, the plasma levels of drug associated with a single oral administration of MTEP (4.5 – 36 mg/kg) were assessed. All animals received all treatments once, in a non-randomised, ascending dose fashion. A minimum of 7 days was allowed between treatments in the same animal. Samples of venous blood, approximately 1.0 ml, were taken from the cephalic vein of each animal. Blood was removed at each of the following time-points: pre-dose and at 10, 20, 30 min and 1, 2, 3, 6, 8 and 24 h post-drug. Each blood sample was transferred into K^+-EDTA coated tubes (BD, Canada), gently inverted and centrifuged at $1500g_{av}$ for 10 min at 4^oC. Plasma layers were frozen and stored at $–80^oC$ prior to LC-MS/MS analysis.

To a 30 µl sample of plasma was added 30 µl of methanol / water (1:1 v:v), 30 µl of internal standard (100 ng/ml, propranolol) and 150 µl acetonitrile. Samples were briefly mixed and vortexed at $500g_{av}$ for 2 min. After centrifugation at $30000g_{av}$ for 5 min, 5 µl of supernatant was injected onto LC-MS/MS. Separations were carried out at 25°C on a Boston Crest ODS C-18 (2.1 mm × 50 mm x 5 µm) with a Phenomenex Security Guard C-18 guard column (4 mm × 2 mm). A Shimadzu 20 AD series HPLC consisting of a degasser, binary pump, autosampler, and thermostated column compartment was used. The mobile phase consisted of 1 mM ammonium acetate and 0.025% formic acid in water for pump B, with 1 mM ammonium acetate and 0.025% formic acid in acetonitrile for pump A. The mobile phase A was maintained at 2% for 6 sec and then increased quickly to 90% over a further 6 sec and held constant for 66 sec, followed by re-equilibration to starting conditions for 78 sec. The flow rate was 0.4 ml/min and each run lasted a total of 156 sec. The mass spectrometer utilized for this work was an API 4000

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triple quadrupole equipped with an atmospheric pressure chemical ionization source. The nebulizer current was 5 mA and the source temperature was 400^{o}C. The collision energy was set at 35 V. The collision gas was nitrogen. The instrument was operated using multiple reaction monitoring (MRM) following the transitions from the protonated molecular ion to a diagnostic fragment ion for MTEP (201.0 → 159.8) and the internal standard propranol (260.3 → 116.1).

Statistical analysis and data presentation

Categorical, discontinuous scores for parkinsonian disability use a descriptive scale to label the Y-axis as follows: 0 = none, 2.25 = mild, 4.5 = moderate, 6.75 = marked, 9 = severe. Graphs of dyskinesia also use a descriptive scale to label the Y-axis as follows: 0 = none, 1 = mild, 2 = moderate, 3 = marked, 4 = severe. Since data presented represent several assessments, the values that correspond to the Y-axis description were the cumulated totals (of descriptive scores) multiplied by the number of time-points that were cumulated. Time course and 0-2 h data are graphed as median scores with individual values (peak-effect only). Time course data for disability and LID scores were ranked by animal across each of the six treatments and subjected to statistical analysis using a parametric non-matched 2-way ANOVA followed by multiple Bonferroni *post-hoc* tests. Cumulated peak-effect data (0-2 h) were analysed non-parametrically using a Friedman test followed by a Dunn's multiple comparison *post-hoc* test.

Continuous data derived from activity (time course and peak-effect totals (0-2 h)) and calculations of on-time and quality of on-time in terms of presence or absence of non-

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disabling ("good") or disabling ("bad") dyskinesia are plotted as mean ± s.e.m. Statistical analyses for time course data were carried out using a parametric repeated measures two-way ANOVA followed by a Bonferroni *post-hoc* test. Peak-effect data (0-2 h) were analysed using a one-way analysis of variance (RM-ANOVA) followed by a Tukey's *post-hoc* multiple comparison test.

For all analysis, significance was set at $P \leq 0.05$. Analyses were performed using GraphPad Prism® v.5.2.

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Results

Orally administered MTEP was well tolerated at all doses assessed, whether given alone (36 mg/kg) or in combination (4.5 – 36 mg/kg) with L-DOPA. Thus, no abnormal behaviour or sedation was observed.

Activity

Over the 6 h period of observation, there was a significant effect of time, treatment and the interaction between the two, on activity in MPTP-lesioned macaques ($F_{time\ 5,\ 150}$ = 35.4, $F_{treatment\ 5,\ 150}$ = 6.1, $F_{interaction\ 25,\ 150}$ = 5.6, $P<0.001$, 2-way-ANOVA, Figure 1A). *Post-hoc* Bonferroni analysis revealed that animals treated with L-DOPA alone (average dose 33 ± 1 mg/kg) expressed a significant increase in activity compared to vehicle-treated animals, this increase in activity lasted for up to 4 h following drug administration (Figure 1A).

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During the period of peak-effect (0-2 h), there was also a significant effect of treatment on activity ($F_{6, 30}$ = 10.1, P<0.001, Figure 2A). L-DOPA alone increased activity by 203% compared to vehicle-treated animals. *Post-hoc* Tukey analysis showed that co-administration of MTEP dose dependently reduced peak-effect activity (P<0.05 at 18 and 36 mg/kg, *p.o.*, compared to L-DOPA alone) (by 35% at 18 mg/kg and 55% at 36 mg/kg; Figure 2A).

Parkinsonian disability and on-time

Over the 6 h period of observation, there was a significant effect of treatment and interaction between time and treatment but not time alone, on parkinsonian disability in MPTP-lesioned macaques ($F_{time\ 5, 150}$ = 0, $F_{treatment\ 5, 150}$ = 8.5, $F_{interaction\ 25, 150}$ = 2.0, P<0.01, 2-way-ANOVA, Figure 1B). *Post-hoc* Bonferroni analysis revealed that animals treated with L-DOPA alone expressed a significant decrease in parkinsonian disability, compared to vehicle-treated animals, this benefit was maximal in the first 2 h, and lasted from 0-3 h following drug administration (Figure 1B). Examination of the time course of parkinsonian disability showed that, in contrast to L-DOPA alone or L-DOPA in combination with MTEP (4.5 mg/kg), disability was not significantly reduced, compared to vehicle treatment, in the period 0-1 h, or 1-3 h, when L-DOPA was combined with higher doses of MTEP (18 and 36 mg/kg, respectively) (2-way ANOVA, Figure 1B).

During the peak-effect period, vehicle-treated MPTP-lesioned animals expressed parkinsonian disability of moderate–marked levels (Figure 2B). During this period there was a significant effect of treatment on disability (Friedman Statistic (FS) = 21.6,

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P<0.001, Figure 2B). *Post-hoc* Dunn's analysis showed that treatment with L-DOPA alone produced significant anti-parkinsonian benefit, compared to vehicle-treated animals, reducing disability to mild or absent levels (P<0.01, Figure 2B). Examination of duration of anti-parkinsonian benefit or on-time (the number of minutes for which bradykinesia was absent) revealed there to be a significant effect of treatment across the whole 6 h of observation ($F_{5, 25}$ = 11.4, P<0.001, Figure 3A). In animals that received L-DOPA alone, on-time was 233 ± 39 min (Figure 3A). Combination with the higher doses of MTEP (18 – 36 mg/kg), but not the lower dose of MTEP (4.5 mg/kg) caused a modest reduction in the anti-parkinsonian efficacy of L-DOPA such that during the peak-effect period parkinsonian disability was increased from absent / mild to mild / moderate levels compared to animals treated with L-DOPA alone (0-2 h parkinsonian disability; L-DOPA / vehicle *cf.* L-DOPA / MTEP (36 mg/kg), 9.5 *cf.* 24.5, out of a maximum score possible of 108). Indeed, *post-hoc* Dunn's analysis revealed that levels of disability in animals receiving L-DOPA in combination with higher doses of MTEP (18 and 36 mg/kg) were not significantly different to those seen in vehicle-treated animals (P>0.05, Figure 2B).

Across the whole 6 h of observation there was a trend, with increasing doses of MTEP, towards a reduction in total on-time (L-DOPA / vehicle *cf.* L-DOPA / MTEP (36 mg/kg), 233 ± 39 min *cf.* 190 ± 28 min, 19% reduction, Figure 3A). However, at no time was on-time in animals treated with L-DOPA in combination with MTEP different to that seen in animals treated with L-DOPA alone and remained significantly different to that of animals treated with vehicle (all P<0.01, Tukey's Multiple Comparison test).

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Dyskinesia and quality of on-time

As with parkinsonian disability, over the 6 h period of observation, there was a significant effect of treatment and an interaction between time and treatment on dyskinesia in MPTP-lesioned macaques ($F_{time\ 5,\ 150}$ = 0, $F_{treatment\ 5,\ 150}$ = 19.4, $F_{interaction\ 25,\ 150}$ = 4.1, P<0.001, 2-way-ANOVA, Figure 1C). During the peak-effect period there was also a significant effect of treatment on levels of dyskinesia (FS = 25.7, P<0.001, Figure 2C). *Post-hoc* Dunn's analysis showed that treatment with L-DOPA alone elicited significant levels of dyskinesia (reaching disabling, marked or severe levels) compared to vehicle-treated animals, that was significant for up to 4 h following administration of drug (FS = 25.7, P<0.001, Figure 2C). Treatment with MTEP caused a dose-dependent reduction in levels of dyskinesia evoked by L-DOPA which, during the peak-effect period with the highest dose assessed (36 mg/kg), were virtually eliminated compared to those seen with L-DOPA alone (LID score; L-DOPA / vehicle *cf.* L-DOPA / MTEP (36 mg/kg), 24 *cf.* 1, median values out of a maximum score of 48). Indeed, MTEP (36 mg/kg), in combination with L-DOPA, reduced dyskinesia such that levels were not significantly different to those seen following treatment with vehicle alone (moderate – marked dyskinesia reduced to mild or absent levels, P>0.05, Figure 2C).

There was a significant effect of treatment on on-time associated with disabling (marked or severe) dyskinesia (i.e. "bad" on-time) ($F_{5,\ 25}$ = 15.4, P<0.001, Figure 3B). Treatment with L-DOPA alone elicited 122 ± 24 min of on-time associated with disabling dyskinesia (51% of total on-time). L-DOPA in combination with MTEP (36 mg/kg) produced significantly less "bad" on-time, compared to L-DOPA alone (70 % reduction)

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(P<0.01, LID score; L-DOPA / vehicle *cf.* L-DOPA / MTEP (36 mg/kg), 122 ± 24 min *cf.* 37 ± 21 min, Figure 3B). There was also a significant effect of treatment on the total on-time during which dyskinesia was absent or non-disabling ("good" on-time, $F_{5, 25}$ = 3.2, P<0.05, Figure 3C). Treatment with L-DOPA alone elicited 111 ± 23 min of "good" on-time (49% of total on-time), although this was not significantly different to that seen in animals treated with vehicle alone. However, *post-hoc* Tukey's analysis further revealed that combination of L-DOPA with MTEP (36 mg/kg) caused an increase (of 37 %) in "good" on-time compared to L-DOPA alone (LID score; L-DOPA / vehicle *cf.* L-DOPA / MTEP (36 mg/kg), 122 ± 24 min *cf.* 37 ± 21 min, Figure 3B), such that the duration of "good" on-time was significantly greater than seen in vehicle-treated animals (P<0.05).

Pharmacokinetic profile

In MPTP-lesioned cynomolgus macaques, MTEP was readily detectable in plasma following oral administration of the drug in its hydrochloride form. On all days of analysis, MTEP was undetectable in the pre-treatment sample. MTEP concentrations were measurable as early as 10 min post-drug administration and were still above lowest quantifiable limits at 24 h post-administration (Figures 4, B and C). Times to peak levels of drug (t_{max}) were between 1.3 and 3.3 h after dosing (4.5 and 36 mg/kg, respectively) and were associated with plasma concentrations ranging from 2.2 to 20.9 μM and an $AUC_{0\text{-}24}$ of 10.6 to 136.1 h.μM (4.5 and 36 mg/kg, respectively).

The elimination phase half-life, $t_{1/2}$, for MTEP in the MPTP-macaque was approximately 3 h.

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Discussion

The current study characterises the behavioural response to selective blockade of mGlu5 in the MPTP-lesioned macaque, the foremost animal model of parkinsonism and motor complications in PD. We have synthesised the hydrochloride salt of MTEP to permit full investigation of dose-response and pharmacodynamics following oral administration. We find that the mGlu5 antagonist MTEP reduces peak-dose L-DOPA-induced motor activity, a measure of total movement, in MPTP-lesioned macaques with established motor complications. Assessment of dyskinesia and parkinsonian disability scores revealed the majority of this MTEP-evoked decrease in activity can be attributed to a large and highly significant reduction in L-DOPA-induced dyskinesia (LID). In this respect, we broadly support the findings of a recent report using MTEP (though as free base), where qualitatively similar effects on dyskinesia, though of lesser magnitude, were also observed (Morin et al., 2010). We also show that while MTEP might compromise peak anti-parkinsonian benefit, overall it improved quality of anti-parkinsonian action, by reducing the proportion of total anti-parkinsonian action (on-time) that was associated with disabling LID. The effects described here thus continue to support the concept of mGlu5 blockade as a strategy for treatment of motor complications in PD,. However, we suggest the exercise of caution in translation to PD patients, and inclusion of clinical measures of quality of peak anti-parkinsonian benefit, not just on-time and dyskinesia, in subsequent patient studies, particularly at Phase III.

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MTEP is a prototypical antagonist of mGlu5 (Cosford et al., 2003) that is orally active and of considerably greater selectivity for mGlu5 (Lea and Faden, 2006), than other mGlu5 agents e.g. MPEP (Breysse et al., 2003; Levandis et al., 2008) and SIB-1830 (Hill et al., 2001) that have been employed in some previous studies to assess potential in PD.

To date, in primates, no information is available regarding brain mGlu5 receptor occupancy by MTEP following oral, or any other route of administration. In rodents, central mGlu5 receptor occupancy data are only available after parenteral administration of MTEP, with no assessment of related plasma levels (Cosford et al., 2003; Busse et al., 2004), and thus extrapolation between our plasma levels and receptor occupancy cannot be made directly. Moreover, because this was the first time that behavioural effects of the hydrochloride salt of MTEP were investigated, and the effects we observe appear, for similar doses, to be greater than those reported with MTEP, as free base (Morin et al., 2010), we have defined plasma levels associated with efficacy. The pharmacokinetic analyses conducted here demonstrated, after oral administration of MTEP hydrochloride, at doses that reduced dyskinesia (18 and 36 mg/kg) $AUC_{0\text{-}24h}$ of 65.1 and 136.1 h.μM respectively and peak plasma levels (C_{max}) of 9.5 ± 2.2 and 20.9 ± 4.0 μM respectively.

The neural mechanisms underlying dyskinesia, particularly that seen at times of peak L-DOPA effect, involve a combination of pre- and postsynaptic changes in the nigrostriatal dopaminergic and related systems, resulting in over-activity of the GABAergic 'direct' striatal output pathways (Crossman, 1990; Bezard et al., 2001; Brotchie et al., 2005; Cenci, 2007). Glutamatergic terminals, uptake sites and receptors within these regions

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can potentially influence activity of the direct pathway and thus drive this over-activity. Indeed, abnormal functioning of basal ganglia glutamatergic systems has been suggested as an underlying feature of the pathological motor disturbances of LID (Chase and Oh, 2000). Whilst NMDA and AMPA receptor antagonists either alone (Papa and Chase, 1996; Konitsiotis et al., 2000; Silverdale et al., 2005) or in combination (Bibbiani et al., 2005) may have some benefit in reducing motor complications, including LID, the limited tolerability may render them difficult to employ in many PD patients (Fox et al., 2008). A more subtle means of modulating excess glutamatergic function and increasing potential therapeutic index may instead reside with metabotropic glutamate receptors. Certainly, within the striatum, mGlu5 receptors are more abundant than in any other basal ganglia region (Testa et al., 1995) and, by virtue of their presence on projection neurons, are able to modulate activity of the D1 dopamine receptor-predominant direct pathway. Thus, by normalising activity of the direct striatal output pathway, mGlu5 antagonism may reduce motor complications.

The involvement of mGlu5 in dyskinesia is further supported by findings that specific mGlu5 binding is increased in the posterior putamen and pallidum of MPTP-lesioned macaques expressing LID, compared to control animals (Samadi et al., 2008). Furthermore, reduction of LID by treatment with an NMDA antagonist was associated with a normalisation of mGlu5 binding in these same areas (Samadi et al., 2008). At the molecular level, mGlu5 antagonism might exert anti-dyskinetic actions through reduction in L-DOPA-induced phosphorylation of extracellular signal-regulated kinase 1 and 2 (ERK1/2), and mitogen-and-stress activated kinase 1 (MSK-1). MTEP attenuates the L-

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DOPA-induced phosphorylation of ERK1/2 and MSK-1 in the striatum of 6-OHDA-lesioned rats (Rylander et al., 2009). Thus, dopamine D1 receptor-mediated activation of ERK1/2 and MSK-1 may be fundamental to the development of LID (Westin et al., 2007).

No robust anti-parkinsonian actions of MTEP (36 mg/kg, *p.o.*) given alone were seen in this study. This has not been previously investigated in non-human primates. In contrast studies in rodents have described anti-parkinsonian actions of mGlu5 antagonists, though not consistently. MPEP, an mGlu5 antagonist, albeit one less selective than MTEP (Lea and Faden, 2006), exhibited robust anti-parkinsonian effects in hemi-parkinsonian 6-OHDA-lesioned rats, though these were only seen following chronic, not acute, administration of drug (Breysse et al., 2002). MTEP also reversed haloperidol-induced catalepsy in rats after acute administration (Ossowska et al., 2005; Dekundy et al., 2006). On the other hand, in 6-OHDA-lesioned rats, there was no effect of acute MTEP alone on rotational behaviour (Dekundy et al., 2006) or on rotarod performance, either acutely or after 18 days of once-daily treatment (Mela et al., 2007). The lack of anti-parkinsonian efficacy observed here may reflect a lesser role of mGlu5 in the generation of parkinsonian symptoms in the primate, compared to rodent, or a need for repeated treatment to make anti-parkinsonian benefits apparent. The above discussion raises the issue of acute versus chronic therapy, and the impact of such on the actions of MTEP. The data presented here reflect acute actions only. It is clear that such acute administration can provide dramatic anti-dyskinetic actions. Unlike the anti-parkinsonian actions of MPEP in 6-OHDA-lesioned rats, chronic administration is not required to

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reveal these actions. The requirement for chronic dosing in order for some actions of MPEP to be revealed may reflect differences in the pharmacology of MPEP and MTEP. Indeed in the 6-OHDA rat, even chronic dosing is not associated with anti-parkinsonian actions. However, while we show that actions of MTEP can be obtained acutely we do not address whether chromic treatment results in tolerance. This is a different issue that would be important to further development of an mGlu5 agent.

In assessing the behavioural impact of mGlu5 antagonism on anti-parkinsonian actions of L-DOPA, in addition to assessing parkinsonism and dyskinesia, we employ measures of duration and quality of on-time to better model clinical measures of quality of a treatment's benefit. In this fashion, we seek to gain useful preclinical equivalents of proportion of time for which dyskinesia is present (Unified Parkinson's Disease Rating Scale (UPDRS) part IV, item 32, or Movement Disorder's Society-sponsored revision of (MDS)-UPDRS item 4.1) (Fahn et al., 1987; Goetz et al., 2008), and diary measures of on-time which incorporate the impact of troublesome dyskinesia, such as proportion of on-time without troublesome dyskinesia (Encarnacion and Hauser, 2008). These, unlike the conventional measures of the impact of dyskinesia employed in the majority of non-human primate studies, have been successfully employed in Phase III to provide a link through to successful clinical use (Rascol et al., 2005). In using these measures we note that, when L-DOPA was combined with doses of MTEP that also reduced LID, at no dose did MTEP significantly decrease total on-time and, indeed, increased "good" on-time, i.e., that associated with an absence of dyskinesia or dyskinesia of a non-disabling nature. Similar measures might thus represent useful endpoints in Phase II and III clinical

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studies. However, the data for peak anti-parkinsonian benefit do provide some indication of a mild reduction in maximal anti-parkinsonian benefit of L-DOPA when it is combined with doses of MTEP with anti-dyskinetic efficacy. Measures of on-time do not seem to be sensitive to this decreased maximal anti-parkinsonian benefit, but it should not be ignored, as in practice PD patients suffering from motor complications may find a maximal anti-parkinsonian benefit even with mild, non-disabling dyskinesia preferable to an absence of dyskinesia but with increased parkinsonian disability (Hung et al., 2007). Such discussion indicates the value of quality of life measures employed as early as possible in clinical development of mGlu5 antagonists to address this issue.

In conclusion, in parkinsonian, MPTP-lesioned macaques with motor complications, selective mGlu5 blockade reduces L-DOPA-induced peak-dose dyskinesia. Selective inhibition of mGlu5 may represent a promising approach to the pharmacological treatment of motor complications in PD, though careful attention should be paid to whether such benefits can be achieved while maintaining a maximal degree of peak anti-parkinsonian benefit.

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References

Berg D, Godau J, Trenkwalder C, Eggert K, Csoti I, Storch A, Gasparini F, Hariry S, Johns DG and Gomez-Mancilla B (2009) A randomized, controlled trial evaluating AFQ056 in reducing levodopa-induced dyskinesia in patients with Parkinson's disease. *Mov Disord Society Conference (Paris)*:LB-05.

Bezard E, Brotchie JM and Gross CE (2001) Pathophysiology of levodopa-induced dyskinesia: potential for new therapies. *Nat Rev Neurosci* **2**:577-588.

Bibbiani F, Oh JD, Kielaite A, Collins MA, Smith C and Chase TN (2005) Combined blockade of AMPA and NMDA glutamate receptors reduces levodopa-induced motor complications in animal models of PD. *Exp Neurol* **196**:422-429.

Blanchet PJ, Konitsiotis S, Whittemore ER, Zhou ZL, Woodward RM and Chase TN (1999) Differing effects of N-methyl-D-aspartate receptor subtype selective antagonists on dyskinesias in levodopa-treated 1-methyl-4-phenyl-tetrahydropyridine monkeys. *J Pharmacol Exp Ther* **290**:1034-1040.

Breysse N, Amalric M and Salin P (2003) Metabotropic glutamate 5 receptor blockade alleviates akinesia by normalizing activity of selective basal-ganglia structures in parkinsonian rats. *J Neurosci* **23**:8302-8309.

Breysse N, Baunez C, Spooren W, Gasparini F and Amalric M (2002) Chronic but not acute treatment with a metabotropic glutamate 5 receptor antagonist reverses the akinetic deficits in a rat model of parkinsonism. *J Neurosci* **22**:5669-5678.

Brotchie JM, Lee J and Venderova K (2005) Levodopa-induced dyskinesia in Parkinson's disease. *J Neural Transm* **112**:359-391.

Downloaded from jpet.aspetjournals.org at ASPET Journals on April 9, 2024

JPET Fast Forward. Published on March 15, 2010 as DOI: 10.1124/jpet.110.166629
This article has not been copyedited and formatted. The final version may differ from this version.

Busse CS, Brodkin J, Tattersall D, Anderson JJ, Warren N, Tehrani L, Bristow LJ, Varney MA and Cosford ND (2004) The behavioral profile of the potent and selective mGlu5 receptor antagonist 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) in rodent models of anxiety. *Neuropsychopharmacology* **29**:1971-1979.

Calabresi P, Giacomini P, Centonze D and Bernardi G (2000) Levodopa-induced dyskinesia: a pathological form of striatal synaptic plasticity? *Ann Neurol* **47**:S60-68; discussion S68-69.

Calon F, Morissette M, Ghribi O, Goulet M, Grondin R, Blanchet PJ, Bedard PJ and Di Paolo T (2002) Alteration of glutamate receptors in the striatum of dyskinetic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated monkeys following dopamine agonist treatment. *Prog Neuropsychopharmacol Biol Psychiatry* **26**:127-138.

Calon F, Rajput AH, Hornykiewicz O, Bedard PJ and Di Paolo T (2003) Levodopa-induced motor complications are associated with alterations of glutamate receptors in Parkinson's disease. *Neurobiol Dis* **14**:404-416.

Cenci MA (2007) Dopamine dysregulation of movement control in L-DOPA-induced dyskinesia. *Trends Neurosci* **30**:236-243.

Chase TN and Oh JD (2000) Striatal dopamine- and glutamate-mediated dysregulation in experimental parkinsonism. *Trends Neurosci* **23**:S86-91.

Conn PJ and Pin JP (1997) Pharmacology and functions of metabotropic glutamate receptors. *Annu Rev Pharmacol Toxicol* **37**:205-237.

Cosford ND, Tehrani L, Roppe J, Schweiger E, Smith ND, Anderson J, Bristow L, Brodkin J, Jiang X, McDonald I, Rao S, Washburn M and Varney MA (2003) 3-

Downloaded from jpet.aspetjournals.org at ASPET Journals on April 9, 2024

JPET Fast Forward. Published on March 15, 2010 as DOI: 10.1124/jpet.110.166629
This article has not been copyedited and formatted. The final version may differ from this version.

[(2-Methyl-1,3-thiazol-4-yl)ethynyl]-pyridine: a potent and highly selective metabotropic glutamate subtype 5 receptor antagonist with anxiolytic activity. *J Med Chem* **46**:204-206.

Crossman AR (1990) A hypothesis on the pathophysiological mechanisms that underlie levodopa- or dopamine agonist-induced dyskinesia in Parkinson's disease: implications for future strategies in treatment. *Mov Disord* **5**:100-108.

Dekundy A, Pietraszek M, Schaefer D, Cenci MA and Danysz W (2006) Effects of group I metabotropic glutamate receptors blockade in experimental models of Parkinson's disease. *Brain Res Bull* **69**:318-326.

Encarnacion EV and Hauser RA (2008) Levodopa-induced dyskinesias in Parkinson's disease: etiology, impact on quality of life, and treatments. *Eur Neurol* **60**:57-66.

Fabbrini G, Brotchie JM, Grandas F, Nomoto M and Goetz CG (2007) Levodopa-induced dyskinesias. *Mov Disord* **22**:1379-1389.

Fahn S, Elton RL and members Up (1987) Unified Parkinson’s disease rating scale in *Recent developments in Parkinson's disease* (Fahn S, Marsden D, Goldstein M and Calne DB eds) pp 153-163, 293-304, Macmillan Healthcare Information.

Fox SH, Brotchie JM and Lang AE (2008) Non-dopaminergic treatments in development for Parkinson's disease. *Lancet Neurol* **7**:927-938.

Fox SH and Lang AE (2008) Levodopa-related motor complications--phenomenology. *Mov Disord* **23 Suppl 3**:S509-514.

Goetz CG, Poewe W, Rascol O and Sampaio C (2005) Evidence-based medical review update: pharmacological and surgical treatments of Parkinson's disease: 2001 to 2004. *Mov Disord* **20**:523-539.

Downloaded from jpet.aspetjournals.org at ASPET Journals on April 9, 2024

JPET Fast Forward. Published on March 15, 2010 as DOI: 10.1124/jpet.110.166629
This article has not been copyedited and formatted. The final version may differ from this version.

Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, Poewe W, Sampaio C, Stern MB, Dodel R, Dubois B, Holloway R, Jankovic J, Kulisevsky J, Lang AE, Lees A, Leurgans S, LeWitt PA, Nyenhuis D, Olanow CW, Rascol O, Schrag A, Teresi JA, van Hilten JJ and LaPelle N (2008) Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Mov Disord* **23**:2129-2170.

Gravius A, Dekundy A, Nagel J, More L, Pietraszek M and Danysz W (2008) Investigation on tolerance development to subchronic blockade of mGluR5 in models of learning, anxiety, and levodopa-induced dyskinesia in rats. *J Neural Transm* **115**:1609-1619.

Gregoire L, Ouatara B, Gasparini F, Bilbe G, Johns DG, Vranesic I, Sahasramaman S, Gomez-Mancilla B and diPaolo T (2009) Antidyskinetic effect of AFQ056 a novel metabotropic glutamate receptor type 5 (mGluR5) antagonist in MPTP monkeys. . *Society for Neuroscience Abstracts*:Abs326.319.

Gubellini P, Pisani A, Centonze D, Bernardi G and Calabresi P (2004) Metabotropic glutamate receptors and striatal synaptic plasticity: implications for neurological diseases. *Prog Neurobiol* **74**:271-300.

Hallett PJ, Dunah AW, Ravenscroft P, Zhou S, Bezard E, Crossman AR, Brotchie JM and Standaert DG (2005) Alterations of striatal NMDA receptor subunits associated with the development of dyskinesia in the MPTP-lesioned primate model of Parkinson's disease. *Neuropharmacology* **48**:503-516.

Downloaded from jpet.aspetjournals.org at ASPET Journals on April 9, 2024

JPET Fast Forward. Published on March 15, 2010 as DOI: 10.1124/jpet.110.166629
This article has not been copyedited and formatted. The final version may differ from this version.

Hill MP, McGuire SG, Crossman AR and Brotchie JM (2001) The mGlu5 receptor antagonist SIB-1830 reduces L-DOPA-induced dyskinesia in the MPTP-lesioned primate model of Parkinson's Disease. *Society for Neuroscience Abstracts* **27**:Abs200.217.

Hung SW, Adeli GW, Arenovich T, Fox SH and Lang AE (2007) Patient Perception of Dyskinesias in Parkinson's disease. *Neurology* **68(suppl 1)**:64.

Institute of Laboratory Animal Resources (U.S.). Committee on Care and Use of Laboratory Animals N (1996) Guide for the care and use of laboratory animals., in, U.S. Dept. of Health and Human Services, Public Health Service, Bethesda, Md.

Konitsiotis S, Blanchet PJ, Verhagen L, Lamers E and Chase TN (2000) AMPA receptor blockade improves levodopa-induced dyskinesia in MPTP monkeys. *Neurology* **54**:1589-1595.

Lea PMt and Faden AI (2006) Metabotropic glutamate receptor subtype 5 antagonists MPEP and MTEP. *CNS Drug Rev* **12**:149-166.

Levandis G, Bazzini E, Armentero MT, Nappi G and Blandini F (2008) Systemic administration of an mGluR5 antagonist, but not unilateral subthalamic lesion, counteracts l-DOPA-induced dyskinesias in a rodent model of Parkinson's disease. *Neurobiol Dis* **29**:161-168.

Marin C, Papa S, Engber TM, Bonastre M, Tolosa E and Chase TN (1996) MK-801 prevents levodopa-induced motor response alterations in parkinsonian rats. *Brain Res* **736**:202-205.

Downloaded from jpet.aspetjournals.org at ASPET Journals on April 9, 2024

JPET Fast Forward. Published on March 15, 2010 as DOI: 10.1124/jpet.110.166629
This article has not been copyedited and formatted. The final version may differ from this version.

Marino MJ, Awad H, Poisik O, Wittmann M and Conn PJ (2002) Localization and physiological roles of metabotropic glutamate receptors in the direct and indirect pathways of the basal ganglia. *Amino Acids* **23**:185-191.

McIldowie MJ, Gandy MN, Skelton BW, Brotchie JM, Koutsantonis GA, Spackman MA and Piggott MJ (2009) Physical and crystallographic characterisation of the mGlu5 antagonist MTEP and its monohydrochloride. *J Pharm Sci* **99**:234-245.

Mela F, Marti M, Dekundy A, Danysz W, Morari M and Cenci MA (2007) Antagonism of metabotropic glutamate receptor type 5 attenuates l-DOPA-induced dyskinesia and its molecular and neurochemical correlates in a rat model of Parkinson's disease. *J Neurochem* **101**:483-497.

Morin N, Gregoire L, Gomez-Mancilla B, Gasparini F and Di Paolo T (2010) Effect of the metabotropic glutamate receptor type 5 antagonists MPEP and MTEP in parkinsonian monkeys. *Neuropharmacology*.

Nash JE, Ravenscroft P, McGuire S, Crossman AR, Menniti FS and Brotchie JM (2004) The NR2B-selective NMDA receptor antagonist CP-101,606 exacerbates L-DOPA-induced dyskinesia and provides mild potentiation of anti-parkinsonian effects of L-DOPA in the MPTP-lesioned marmoset model of Parkinson's disease. *Exp Neurol* **188**:471-479.

Nutt JG, Gunzler SA, Kirchhoff T, Hogarth P, Weaver JL, Krams M, Jamerson B, Menniti FS and Landen JW (2008) Effects of a NR2B selective NMDA glutamate antagonist, CP-101,606, on dyskinesia and Parkinsonism. *Mov Disord* **23**:1860-1866.

Downloaded from jpet.aspetjournals.org at ASPET Journals on April 9, 2024

JPET Fast Forward. Published on March 15, 2010 as DOI: 10.1124/jpet.110.166629
This article has not been copyedited and formatted. The final version may differ from this version.

Ossowska K, Konieczny J, Wardas J, Pietraszek M, Kuter K, Wolfarth S and Pilc A (2007) An influence of ligands of metabotropic glutamate receptor subtypes on parkinsonian-like symptoms and the striatopallidal pathway in rats. *Amino Acids* **32**:179-188.

Ossowska K, Konieczny J, Wolfarth S and Pilc A (2005) MTEP, a new selective antagonist of the metabotropic glutamate receptor subtype 5 (mGluR5), produces antiparkinsonian-like effects in rats. *Neuropharmacology* **49**:447-455.

Pahwa R, Factor SA, Lyons KE, Ondo WG, Gronseth G, Bronte-Stewart H, Hallett M, Miyasaki J, Stevens J, Weiner WJ and Quality Standards Subcommittee of the American Academy of N (2006) Practice Parameter: treatment of Parkinson disease with motor fluctuations and dyskinesia (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* **66**:983-995.

Papa SM and Chase TN (1996) Levodopa-induced dyskinesias improved by a glutamate antagonist in Parkinsonian monkeys. *Ann Neurol* **39**:574-578.

Pechevis M, Clarke CE, Vieregge P, Khoshnood B, Deschaseaux-Voinet C, Berdeaux G and Ziegler M (2005) Effects of dyskinesias in Parkinson's disease on quality of life and health-related costs: a prospective European study. *Eur J Neurol* **12**:956-963.

Poewe W (2009) Treatments for Parkinson disease--past achievements and current clinical needs. *Neurology* **72**:S65-73.

Rascol O, Brooks DJ, Melamed E, Oertel W, Poewe W, Stocchi F and Tolosa E (2005) Rasagiline as an adjunct to levodopa in patients with Parkinson's disease and

Downloaded from jpet.aspetjournals.org at ASPET Journals on April 9, 2024

JPET Fast Forward. Published on March 15, 2010 as DOI: 10.1124/jpet.110.166629
This article has not been copyedited and formatted. The final version may differ from this version.

motor fluctuations (LARGO, Lasting effect in Adjunct therapy with Rasagiline Given Once daily, study): a randomised, double-blind, parallel-group trial. *Lancet* **365**:947-954.

Rylander D, Recchia A, Mela F, Dekundy A, Danysz W and Cenci MA (2009) Pharmacological modulation of glutamate transmission in a rat model of L-DOPA-induced dyskinesia: effects on motor behavior and striatal nuclear signaling. *J Pharmacol Exp Ther* **330**:227-235.

Samadi P, Gregoire L, Morissette M, Calon F, Hadj Tahar A, Dridi M, Belanger N, Meltzer LT, Bedard PJ and Di Paolo T (2008) mGluR5 metabotropic glutamate receptors and dyskinesias in MPTP monkeys. *Neurobiol Aging* **29**:1040-1051.

Silverdale MA, Kobylecki C, Hallett PJ, Li Q, Dunah AW, Ravenscroft P, Bezard E and Brotchie JM (2010) Synaptic recruitment of AMPA glutamate receptor subunits in levodopa-induced dyskinesia in the MPTP-lesioned nonhuman primate. *Synapse* **64**:177-180.

Silverdale MA, Nicholson SL, Crossman AR and Brotchie JM (2005) Topiramate reduces levodopa-induced dyskinesia in the MPTP-lesioned marmoset model of Parkinson's disease. *Mov Disord* **20**:403-409.

Testa CM, Standaert DG, Landwehrmeyer GB, Penney JB, Jr. and Young AB (1995) Differential expression of mGluR5 metabotropic glutamate receptor mRNA by rat striatal neurons. *J Comp Neurol* **354**:241-252.

Visanji NP, Fox SH, Johnston TH, Millan MJ and Brotchie JM (2009) Alpha1-adrenoceptors mediate dihydroxyphenylalanine-induced activity in 1-methyl-4-

Downloaded from jpet.aspetjournals.org at ASPET Journals on April 9, 2024

JPET Fast Forward. Published on March 15, 2010 as DOI: 10.1124/jpet.110.166629
This article has not been copyedited and formatted. The final version may differ from this version.

phenyl-1,2,3,6-tetrahydropyridine-lesioned macaques. *J Pharmacol Exp Ther* **328**:276-283.

Westin JE, Vercammen L, Strome EM, Konradi C and Cenci MA (2007) Spatiotemporal pattern of striatal ERK1/2 phosphorylation in a rat model of L-DOPA-induced dyskinesia and the role of dopamine D1 receptors. *Biol Psychiatry* **62**:800-810.

Downloaded from jpet.aspetjournals.org at ASPET Journals on April 9, 2024

JPET Fast Forward. Published on March 15, 2010 as DOI: 10.1124/jpet.110.166629
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Footnotes

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Figure Legends

Figure 1. Effect of MTEP in combination with L-DOPA on time course of activity, parkinsonian disability and dyskinesia in MPTP-lesioned primates. MPTP-lesioned cynomolgus monkeys received MTEP (4.5 – 36 mg/kg, *p.o.*) or its vehicle in combination with L-DOPA or its vehicle. Activity (**A**), parkinsonian disability (**B**) and dyskinesia (**C**) were assessed every 5 min and cumulated into 1 h periods for the duration of the 6 h time-course. Data are mean ± s.e.m. (activity only) or median values. N = 6 for all treatment groups. For accompanying significance tables * / ** / *** represents $P<0.05$, $P<0.01$ or $P<0.001$, respectively *cf.* vehicle / vehicle-treated animals, 2-way ANOVA with Bonferroni *post hoc* test.

Figure 2. Effect of MTEP in combination with L-DOPA on peak effect activity, parkinsonian disability and dyskinesia totals in MPTP-lesioned primates. MPTP-lesioned cynomolgus monkeys received MTEP (4.5 – 36 mg/kg, *p.o.*) or its vehicle in combination with L-DOPA or its vehicle. Activity (**A**), parkinsonian disability (**B**) and dyskinesia (**C**) were assessed every 5 min and cumulated into a 2 h period for the first 2 h (0-2 h) of the 6 h observation period. Data are mean ± s.e.m. (activity only) or median with individual values. N = 6 for all treatment groups. * / ** / *** represents $P<0.05$, $P<0.01$ or $P<0.001$, respectively *cf.* vehicle / vehicle-treated animals. ## / ### represents $P<0.05$ or $P<0.01$, respectively *cf.* L-DOPA / vehicle-treated animals. (RM-ANOVA) followed by a Tukey's *post-hoc* Multiple Comparison Test (activity only) or Friedman's test with Dunn's multiple comparison *post-hoc* test.

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Figure 3. Effect of MTEP in combination with L-DOPA on total on-time and quality of on-time in MPTP-lesioned primates. MPTP-lesioned cynomolgus monkeys received MTEP (4.5 – 36 mg/kg, *p.o.*) or its vehicle in combination with L-DOPA or its vehicle. The total duration of on-time (time that bradykinesia was absent, **A**) and on-time with disabling (marked or severe) dyskinesia (**B**) or without disabling dyskinesia (**C**) was defined over the 6 h period following drug administration. N = 6 for all treatment groups. * / ** / *** represents $P<0.05$, $P<0.01$ or $P<0.001$, respectively *cf.* vehicle / vehicle-treated animals. (RM-ANOVA) followed by a Tukey's *post-hoc* multiple comparison test.

Figure 4. Effect of oral MTEP administration on plasma levels of drug in MPTP-lesioned primates. MPTP-lesioned cynomolgus monkeys received MTEP (4.5 – 36 mg/kg, *p.o.*). The concentration of drug (μM) in plasma at times extending from 10 min to 24 h after administration was assessed via LC-MS/MS. Data are mean ± s.e.m. with concentration plotted in either linear (**A**) or semi-logarithmic (**B**) forms. Standard pharmacokinetic parameters (**C**) were calculated using GraphPad Prism version 5.2.

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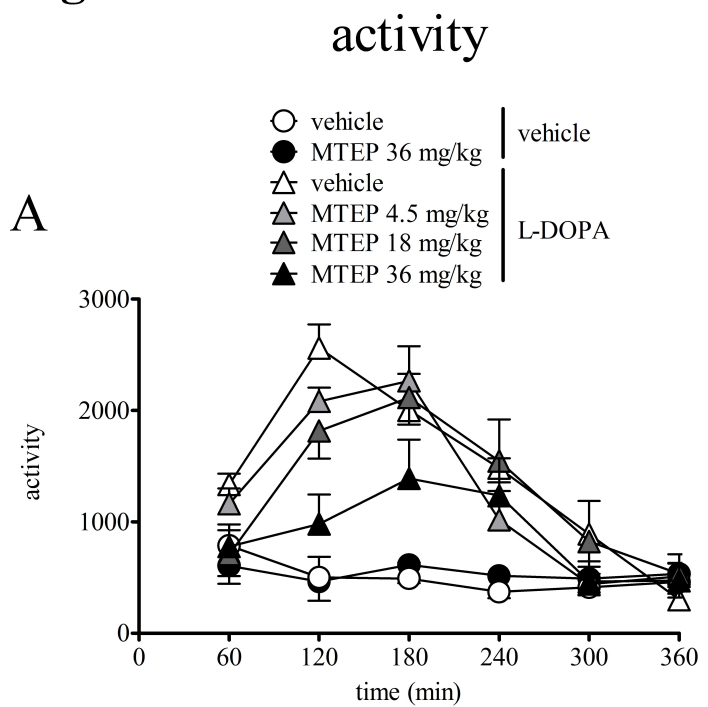
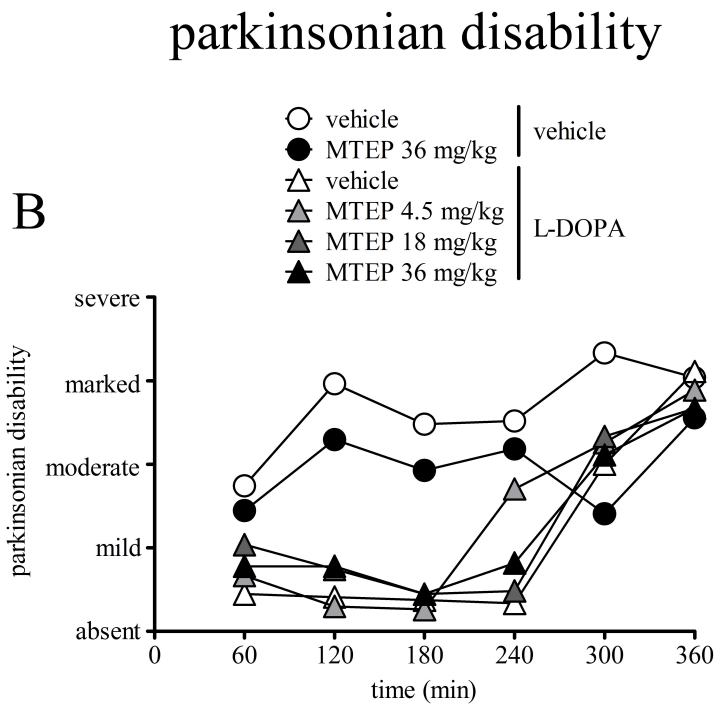
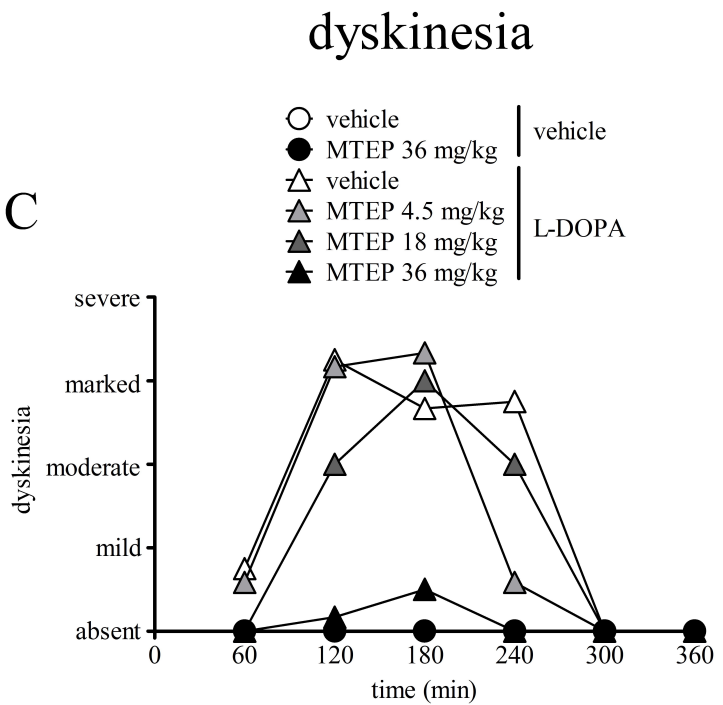
Figure 1.



		veh / veh *cf.* (min)					
	MTEP (mg/kg)	60	120	180	240	300	360
vehicle	36	ns	ns	ns	ns	ns	ns
L-DOPA	vehicle	ns	***	***	**	ns	ns
	4.5	ns	***	***	ns	ns	ns
	18	ns	***	***	**	ns	ns
	36	ns	ns	*	*	ns	ns



		veh / veh *cf.* (min)					
	MTEP (mg/kg)	60	120	180	240	300	360
vehicle	36	ns	ns	ns	ns	ns	ns
L-DOPA	vehicle	***	**	*	ns	ns	ns
	4.5	***	***	***	ns	ns	ns
	18	ns	*	*	ns	ns	ns
	36	**	ns	ns	ns	ns	ns



		veh / veh *cf.* (min)					
	MTEP (mg/kg)	60	120	180	240	300	360
vehicle	36	ns	ns	ns	ns	ns	ns
L-DOPA	vehicle	***	***	***	***	ns	ns
	4.5	***	***	***	*	ns	ns
	18	ns	**	***	***	ns	ns
	36	ns	ns	**	ns	ns	ns

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Figure 2.

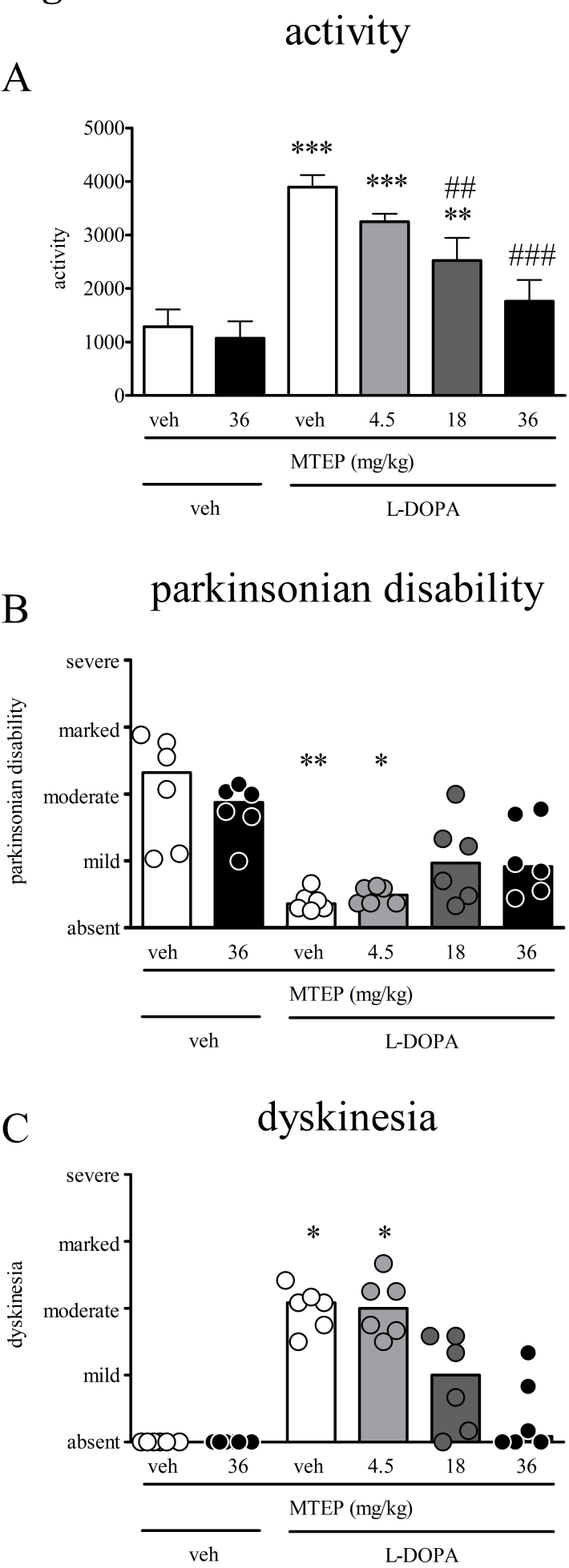

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Figure 3.

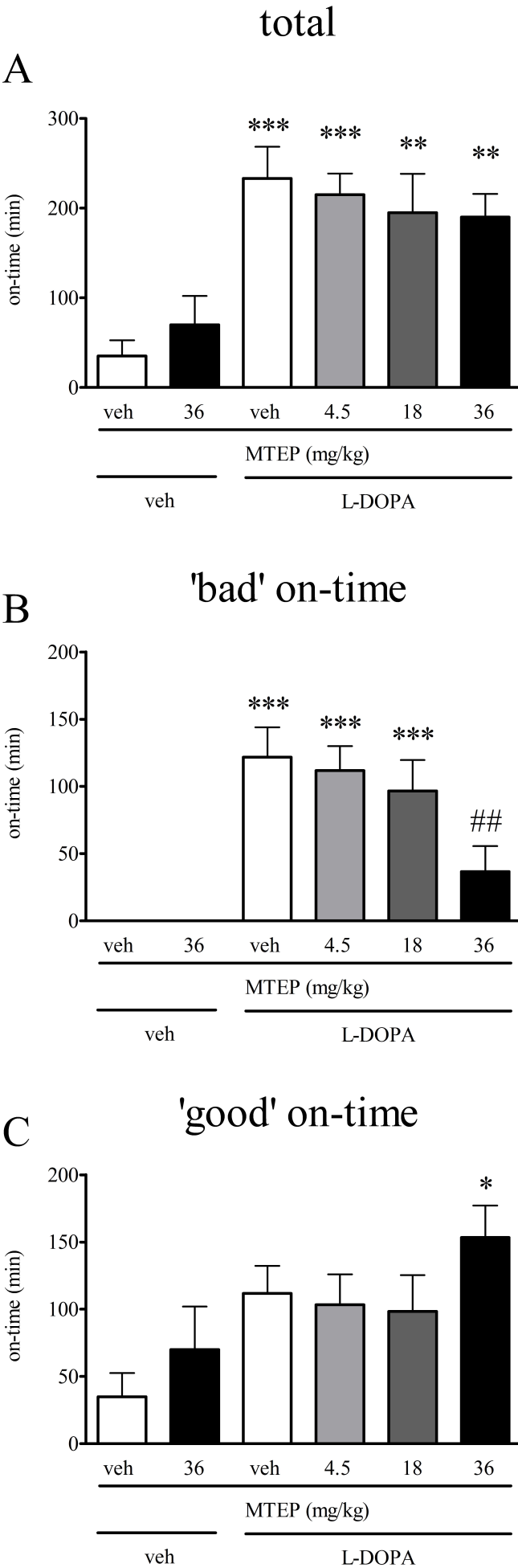
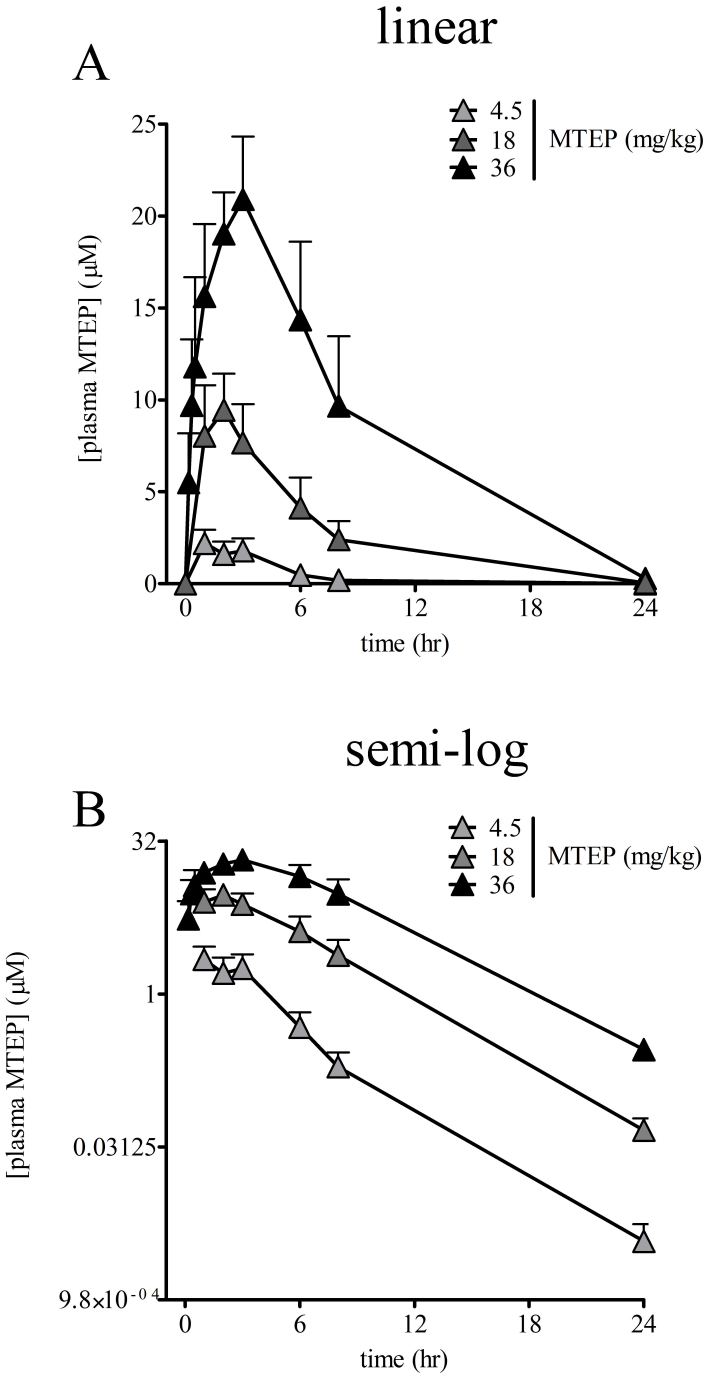


Figure 4.



C

	MTEP (mg/kg)		
	4.5	18	36
c_{max} (μM)	2.2 ± 0.8	9.5 ± 2.2	20.9 ± 4.0
t_{max} (h)	1.3 ± 0.4	2.7 ± 0.8	3.3 ± 1.2
AUC_{0-2} (h.μM)	2.6 ± 1.1	12.8 ± 3.8	18.5 ± 7.8
AUC_{0-24} (h.μM)	10.6 ± 3.8	65.1 ± 3.7	136.1 ± 57.6
k_{elim}	0.247	0.248	0.220
$t_{½}$ (h)	2.8	2.8	3.1