Reduction of L-DOPA-Induced Dyskinesia by the Selective Metabotropic Glutamate Receptor 5 Antagonist 3-[(2-Methyl-1,3-thiazol-4-yl)ethynyl]pyridine in the 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-Lesioned Macaque Model of Parkinson’s Disease

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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 obstacles in the treatment of Parkinson’s disease. Dysfunctional glutamatergic neurotransmitter systems have been observed in both the untreated parkinsonian and dyskinetic states and represent novel targets for treatment. Here, we assess the pharmacokinetic profile and corresponding pharmacodynamic effects on behavior of the orally active, selective metabotropic glutamate receptor type 5 (mGlu5) antagonist, 3-[(2-methyl-4-thiazolyl)ethynyl]pyridine (MTEP) (as the hydrochloride salt) in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (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 (331/110061 mg/kg p.o.). Motor activity, parkinsonian disability, and dyskinesia were assessed for a 6-h period. Plasma drug levels were assessed by liquid chromatography-tandem mass spectrometry. MTEP had no antiparkinsonian action as monotherapy. However, administration of L-DOPA in combination with MTEP (36 mg/kg) reduced peak dose LID by 96%. Moreover, although total on-time (duration for which L-DOPA exerted an antiparkinsonian effect) was not significantly reduced, MTEP (36 mg/kg) reduced the duration of on-time with disabling LID by 70% compared with that for L-DOPA alone. These effects were associated with a peak plasma concentration of 20.9 μM and an area under the curve from 0 to 24 h of 136.1 h μM (36 mg/kg). Although total on-time was not reduced, the peak antiparkinsonian benefit of L-DOPA/MTEP (36 mg/kg) was less than that 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 not come at the expense of the peak antiparkinsonian benefit of L-DOPA.
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 in L-DOPA-treated parkinsonian primates (Calon et al., 2002, 2003; Halllett et al., 2005). More recently, changes in the synaptic recruitment of α-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionic acid receptor subunits in L-DOPA-treated primates have 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 problems of poor efficacy and 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 because of 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 nonhuman primate models of PD and LID. However, preliminary results, presented in abstract form, from a preclinical study and a small phase II clinical trial have shown some positive benefit of using the mGlU5 antagonist to reduce LID in parkinsonian primates and patients with PD, without worsening parkinsonism (Berg et al., 2009; Gregoire et al., 2009). Here, we fully assess the antiparkinsonian and antidysskinetic actions of an orally active, selective mGlU5 antagonist, 3-(2-methyl-4-thiazolyl)ethyl(3H)-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 used a recently described method for synthesizing the hydrochloride salt (McIldowie et al., 2010) to allow full evaluation of the pharmacodynamics of its effects on behavior after oral administration.

Materials and Methods

Animals. Six cynomolgus monkeys (Macaca fascicularis) (four females and two 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 National Institutes of Health, European Union, and UK guidelines, and animals were subject to controlled conditions of temperature (22 ± 3°C), humidity (51 ± 1%), and light (12-h 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 minimize animal suffering. All studies were performed with local institutional animal care and use committee approval and in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health (Institute of Laboratory Animal Resources, 1996).

MPTP Administration and Development of Motor Complications. For a period of at least 3 months before commencement of MPTP administration, animals were acclimatized to experimental settings, blood sampling, handling, and transfer to and from observation cages. Animals received once-daily subcutaneous injection of MPTP hydrochloride (0.2 mg/kg final concentration of base; Sigma-Aldrich, Mississauga, ON, Canada) for 8 to 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.

The parkinsonian syndrome was allowed to stabilize for a minimum of a further 60 days before 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, 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; Roche, Shanghai, China) 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 an optimal antiparkinsonian 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.

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 approximately 9:00 AM on days of observation. MTEP hydrochloride was produced according to a previously described synthetic route (McIldowie et al., 2010) and identity and purity were confirmed by LC–mass spectrometry. 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 randomized fashion by 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 Behavior after Administration of MTEP, Alone and in Combination with L-DOPA. After administration of treatments, animals were transferred immediately to an observation cage (1.5 × 1 × 1.1 m). During periods of assessment, animals were not disturbed, and their behavior was recorded on DVD and by automated passive infrared activity monitoring. Ratings of behavior were made blind to treatment, by post hoc analysis of DVD recordings by a neurologist specializing in movement disorders. A measure of total parkinsonian disability was derived, summing assessments of 1) range of movement (0 = walking on the floor and/or climbing on the walls or roof of the cage to 3 = no movement); 2) bradykinesia (0 = normal speed and initiation of movement to 3 = marked slowing, or unable to move, with prolonged freezing episodes); 3) posture (0 = normal, upright to 2 = hunched body and neck, face down, may lose balance); and 4) alertness (0 = present,
looking around, observant or 1 = absent). Dyskinesia (representative of the maximum of either chorea or dystonia) was scored as follows: 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; and 4 = severe, continuous, disabling, replacing normal activity, present more than 70% of the observation period.

Scores were cumulated for each hour across the entire 6 h of observations for time course analyses and during the first 2 h of assessment, the peak effect period. The duration of antiparkinsonian 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 while dyskinesia was either absent or of mild or moderate severity; and “bad” quality on-time represents the number of minutes for which bradykinesia was zero while dyskinesia was either marked or severe.

Assessment of Plasma MTEP Levels after Oral Administration. In a series of studies conducted independently from behavioral 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 nonrandomized, 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: predose and at 10, 20, and 30 min and 1, 2, 3, 6, 8, and 24 h postdrug. Each blood sample was transferred into K+-EDTA-coated tubes (BD-Canada, Mississauga, ON, Canada), gently inverted, and centrifuged at 1500g for 10 min at 4°C. Plasma layers were frozen and stored at −80°C before 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 of acetonitrile. Samples were briefly mixed and vortexed at 500g for 2 min. After centrifugation at 3000g for 5 min, 5 μl of supernatant was injected for LC-MS/MS. Separations were carried out at 25°C on a Boston Crest ODS C18 column (2.1 mm × 50 mm × 5 μm) with a Phenomenex Security Guard C18 cartridge (4 × 2 mm). A Shimadzu 20 AD series high-performance liquid chromatography system 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. Mobile phase A was maintained at 2% for 6 s and then increased quickly to 90% over a further 6 s and held constant for 66 s, followed by re-equilibration to starting conditions for 78 s. The flow rate was 0.4 ml/min, and each run lasted a total of 156 s. An API 4000 triple quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization source was used for this work. The nebulizer current was 5 μA, and the source temperature was 400°C. The collision energy was set at 35 V. The collision gas was nitrogen. The instrument was operated using a parametric two-way analysis of variance (ANOVA) followed by multiple Bonferroni post hoc tests. Cumulated peak effect data (0–2 h) were analyzed nonparametrically using Friedman’s test followed by a Dunn multiple comparison 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 the presence or absence of nondisabling (good) or disabling (bad) dyskinesia were plotted as means ± S.E.M. Statistical analyses for time course data were performed using a parametric two-way repeated-measures ANOVA followed by a Bonferroni post hoc test. Peak effect data (0–2 h) were analyzed using a one-way repeated-measures (RM) ANOVA followed by a Tukey post hoc multiple comparison test.

For all analysis, significance was set at P ≤ 0.05. Analyses were performed using GraphPad Prism (version 5.2).

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 behavior 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, and F_{interaction,25,150} = 5.6, P < 0.001, two-way ANOVA) (Fig. 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 with vehicle-treated animals, this increase in activity lasted for up to 4 h after drug administration (Fig. 1A).

During the period of peak effect (0–2 h), there was also a significant effect of treatment on activity (F_{t,30} = 10.1, P < 0.001) (Fig. 2A). L-DOPA alone increased activity by 203% compared with that for vehicle-treated animals. Post hoc Tukey analysis showed that coadministration of MTEP dose-dependently reduced peak effect activity (P < 0.05 at 18 and 36 mg/kg p.o. compared with L-DOPA alone) by 35% at 18 mg/kg and 55% at 36 mg/kg (Fig. 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 for time alone on parkinsonian disability in MPTP-lesioned macaques (F_{time,5,150} = 0, F_{treatment,5,150} = 25.150 = 8.5, and F_{interaction,25,150} = 2.0, P < 0.01, two-way ANOVA) (Fig. 1B). Post hoc Bonferroni analysis revealed that animals treated with L-DOPA alone expressed a significant decrease in parkinsonian disability compared with that in vehicle-treated animals; this benefit was maximal in the first 2 h and lasted from 0 to 3 h after drug administration (Fig. 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 with that for vehicle treatment in the period 0 to 1 h or 1 to 3 h when L-DOPA was combined with higher doses of MTEP (18 and 36 mg/kg, respectively; two-way ANOVA) (Fig. 1B).

During the peak effect period, vehicle-treated MPTP-le-
sioned animals expressed parkinsonian disability of moderate to marked levels (Fig. 2B). During this period there was a significant effect of treatment on disability [Friedman statistic (FS) = 21.6, \( P < 0.001 \)] (Fig. 2B). Post hoc Dunn analysis showed that treatment with \( L\)-DOPA alone produced a significant antiparkinsonian benefit, compared with that in vehicle-treated animals, reducing disability to mild or absent levels (\( P < 0.01 \)) (Fig. 2B). Examination of duration of antiparkinsonian benefit or on-time (the number of minutes for which bradykinesia was absent) revealed that there was a significant effect of treatment across the whole 6 h of observation (\( F_{5,25} = 11.4, P < 0.001 \)) (Fig. 3A). In animals that received \( L\)-DOPA alone, on-time was 233 ± 39 min (Fig. 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 antiparkinsonian efficacy of \( L\)-DOPA such that during the peak effect period parkinsonian disability was increased from absent/mild to mild/moderate levels compared with animals treated with \( L\)-DOPA alone (0–2 h parkinsonian disability, \( L\)-DOPA/vehicle compared with \( L\)-DOPA/MTEP (36 mg/kg): 9.5 versus 24.5, of a maximum score possible of 108). Indeed, post hoc Dunn 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 from those seen in vehicle-treated animals (\( P > 0.05 \)) (Fig. 2B).

Across the whole 6 h of observation there was a trend, with increasing doses of MTEP, toward a reduction in total on-time [\( L\)-DOPA/vehicle compared with \( L\)-DOPA/MTEP (36 mg/kg): 233 ± 39 versus 190 ± 28 min, 19% reduction] (Fig. 3A). However, at no time was on-time in animals treated with \( L\)-DOPA in combination with MTEP different from that seen in animals treated with \( L\)-DOPA alone, and it remained significantly different from that of animals treated with vehicle (all \( P < 0.01 \), Tukey multiple comparison test).

**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 (\( F_{5,150} = 0, F_{5,150} = 19.4, \) and \( F_{interaction 25,150} = 4.1, P < 0.001, \) two-way-ANOVA) (Fig. 1C). During the peak effect period there was also a significant effect of treatment on levels of dyskinesia (FS = 25.7, \( P < 0.001 \)) (Fig. 2C). Post hoc Dunn analysis showed that treatment with \( L\)-DOPA alone elicited significant levels of dyskinesia (reaching disabling, marked, or severe levels) compared with that for vehicle-treated animals, which was significant for up to 4 h after administration of drug (FS = 25.7, \( P < 0.001 \)) (Fig. 2C). Treatment with MTEP caused a dose-dependent reduction in levels of dyskinesia evoked by \( L\)-DOPA that, during the peak effect period with the highest dose assessed (36 mg/kg), were virtually eliminated compared with those seen with \( L\)-DOPA alone (LID score, \( L\)-DOPA/vehicle compared with \( L\)-DOPA/MTEP (36 mg/kg): 24 versus 1, median values 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 from those seen after treatment with vehicle alone (moderate to marked dyskinesia reduced to mild or absent levels, \( P > 0.05 \)) (Fig. 2C).

There was a significant effect of treatment on on-time associated with disabling (marked or severe) dyskinesia (i.e., bad on-time) (\( F_{5,25} = 15.4, P < 0.001 \)) (Fig. 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 with that for \( L\)-DOPA alone (70% reduction) (\( P < 0.01, \) LID score, \( L\)-DOPA/vehicle compared with \( L\)-DOPA/MTEP (36 mg/kg): 122 ± 24 versus 37 ± 21 min) (Fig. 3B). There was also a significant effect of treatment on the total on-time during which dyskinesia was absent or nondisabling (good on-time, \( F_{5,25} = 3.2, P < 0.05 \)) (Fig. 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 from that seen in animals treated with vehicle alone. However, post hoc Tukey analysis further revealed that combination of \( L\)-DOPA with MTEP (36 mg/kg) caused
an increase (of 37%) in good on-time compared with L-DOPA alone (LID score, L-DOPA/vehicle compared with L-DOPA/MTEP (36 mg/kg): 122 ± 24 versus 37 ± 21 min) (Fig. 3B), such that the duration of good on-time was significantly greater than that seen in vehicle-treated animals (P < 0.05).

**Pharmacokinetic Profile.** In MPTP-lesioned cynomolgus macaques, MTEP was readily detectable in plasma after oral administration of the drug in its hydrochloride form. On all days of analysis, MTEP was undetectable in the pretreatment sample. MTEP concentrations were measurable as early as 10 min after drug administration and were still above the lowest quantifiable limits at 24 h after administration (Figs. 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 29.9 μM and an AUC_{0–24 h} 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.

**Discussion**

The current study characterizes the behavioral response to selective blockade of mGlu5 in the MPTP-lesioned macaque, the foremost animal model of parkinsonism and motor complications in PD. We synthesized the hydrochloride salt of MTEP to permit full investigation of dose response and pharmacodynamics after 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 that the majority of this MTEP-evoked decrease in activity can be attributed to a large and highly significant reduction in LID. In this respect, we broadly support the findings of a recent report using MTEP (but as free base), in which qualitatively similar effects on dyskinesia, although of lesser magnitude, were also observed (Morin et al., 2010). We also show that although MTEP might compromise peak antiparkinsonian benefit, overall it improved the quality of antiparkinsonian action by reducing the proportion of total antiparkinsonian 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 that caution be used in translation to patients with PD and that clinical measures of quality of peak antiparkinsonian benefit, not just on-time and dyskinesia, be included in subsequent patient studies, particularly at phase III. MTEP is a prototypical antagonist of mGlu5 (Cosford primates. MPTP-lesioned cynomolgus monkeys received MTEP (4.5–36 mg/kg p.o.) or its vehicle (veh) 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 of the 6-h observation period. Data are means ± S.E.M. (activity only) or median with individual values. n = 6 for all treatment groups. *, P < 0.05; **, P < 0.01; ***, P < 0.001, compared with vehicle/vehicle-treated animals. ##, P < 0.05; ###, P < 0.01, L-DOPA compared with L-DOPA/vehicle-treated animals. RM ANOVA followed by a Tukey post hoc multiple comparison test (activity only) or a Friedman test with a Dunn multiple comparison post hoc test.

**Fig. 2.** Effect of MTEP in combination with L-DOPA on peak effect activity, parkinsonian disability, and dyskinesia totals in MPTP-lesioned primate. **A** Activity. Activity levels (activity) were assessed every 5 min and cumulated into a 2-h period for the first 2 h of the 6-h observation period. Data are means ± S.E.M. (activity only) or median with individual values. n = 6 for all treatment groups. *, P < 0.05; **, P < 0.01; ***, P < 0.001, compared with vehicle/vehicle-treated animals. **B** Parkinsonian disability. Parkinsonian disability (parkinsonian disability) was assessed every 5 min and cumulated into a 2-h period for the first 2 h of the 6-h observation period. Data are means ± S.E.M. (activity only) or median with individual values. n = 6 for all treatment groups. *, P < 0.05; **, P < 0.01; ***, P < 0.001, compared with vehicle/vehicle-treated animals. **C** Dyskinesia. Dyskinesia (dyskinesia) was assessed every 5 min and cumulated into a 2-h period for the first 2 h of the 6-h observation period. Data are means ± S.E.M. (activity only) or median with individual values. n = 6 for all treatment groups. *, P < 0.05; **, P < 0.01; ***, P < 0.001, compared with vehicle/vehicle-treated animals.
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 used in some previous studies to assess potential in PD.

To date, in primates, no information is available regarding brain mGlu5 receptor occupancy by MTEP after oral or any other route of administration. In rodents, central mGlu5 receptor occupancy data are available only 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 behavioral effects of the hydrochloride salt of MTEP were investigated and the effects we observe seem, 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), $\text{AUC}_{0-24\ h}$ of 65.1 and 136.1 $\mu$M, respectively, and peak plasma levels ($C_{\text{max}}$) of 9.5 ± 2.2 and 20.9 ± 4.0 $\mu$M, 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; Brodchie et al., 2005; Cenci, 2007). Glutamatergic terminals, uptake sites, and receptors within these regions can potentially influence activity of the direct pathway and thus drive this overactivity. 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). Although NMDA and $\alpha$-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid 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, limited tolerability may render them difficult to use in many patients with PD (Fox et al., 2008). A more subtle means of modulating excess glutamatergic function and increasing the 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 normalizing activity of the direct stri-

Fig. 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 (veh) 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 after drug administration. $n = 6$ for all treatment groups, $*, P < 0.05$; $**, P < 0.01$; $***, P < 0.001$, compared with vehicle/vehicle-treated animals $##, P < 0.01$ compared to l-DOPA/vehicle-treated animals. RM ANOVA followed by a Tukey post hoc multiple comparison test.
atal output pathway, mGlu5 antagonism may reduce mo-

ter complications. The involvement of mGlu5 in dyskinesia is further sup-
ported by findings that specific mGlu5 binding is increased in the posterior putamen and pallidum of MPTP-lesioned ma-
cques expressing LID compared with that in control ani-

mals (Samadi et al., 2008). Furthermore, reduction of LID by treat-
ment with an NMDA antagonist was associated with a normali-
zation of mGlu5 binding in these same areas (Samadi et al., 2008). At the molecular level, mGlu5 antagonism might exert antidyskinetic actions through a reduction in L-DOPA-induced phosphorylation of extracellu-
lar signal-regulated kinase 1 and 2 (ERK1/2) and mitogen and stress-
activated kinase 1 (MSK-1). MTEP attenuates the L-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 antiparkinsonian actions of MTEP (36 mg/kg p.o.) given alone were seen in this study. Such actions have not been previously investigated in nonhuman primates. In contrast, studies in rodents have described the antiparkinso-
nian actions of mGlu5 antagonists, although not consistently. MPEP, an mGlu5 antagonist, albeit one less selective than MTEP (Lea and Faden, 2006), exhibited robust antiparkinso-
nian effects in hemiparkinsonian 6-OHDA-lesioned rats, although these were only seen after chronic, not acute, ad-
ministration of drug (Breysse et al., 2002). MTEP also re-
versed haloperidol-induced catalepsy in rats after acute ad-
ministration (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 behavior (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 antiparkinsonian efficacy observed here may reflect a lesser role of mGlu5 in the generation of parkinsonian symp-
toms in the primates, compared with the rodent, or a need for repeated treatment to make antiparkinsonian benefits ap-
parent. The above discussion raises the issue of acute versus chronic therapy and the impact of such therapy on the ac-
tions of MTEP. The data presented here reflect acute actions only. It is clear that such acute administration can provide dramatic antidyskinetic actions. Unlike the antiparkinso-
nian actions of MPEP in 6-OHDA-lesioned rats, chronic ad-
ministration is not required to reveal these actions. The requirement for chronic dosing for some actions of MPEP to be revealed may reflect differences in the pharmacology of MPEP and MTEP. Indeed, in the 6-OHDA-lesioned rat, even chronic dosing is not associated with antiparkinsonian actions. However, although we show that the actions of MTEP can be obtained acutely, we do not address whether chronic treatment results in tolerance. This is a different issue that would be important to further development of an mGlu5 agent.

In assessing the behavioral impact of mGlu5 antagonism on the antiparkinsonian actions of L-DOPA, in addition to assessing parkinsonism and dyskinesia, we used measures of duration and quality of on-time to better model clinical mea-
sures of quality of a treatment’s benefit. In this fashion, we sought to gain useful preclinical equivalents of proportion of time for which dyskinesia is present (Unified Parkinson’s Disease Rating Scale part IV, item 32, or Movement Disorder Society-sponsored revision of Unified Parkinson’s Disease Rating Scale 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

Fig. 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 (micromolar) in plasma at times extending from 10 min to 24 h after administration was assessed via LC-MS/MS. Data are means ± S.E.M. with concentration plotted in either linear (A) or semilogarithmic (B) forms. Standard pharmacokinetic parameters (C) were calculated using GraphPad Prism (ver-
sion 5.2).
without troublesome dyskinesia (Encarnacion and Hauser, 2008). These, unlike the conventional measures of the impact of dyskinesia used in the majority of nonhuman primate studies, have been successfully applied in phase III to provide a link through to successful clinical use (Rascov 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 nondisabling nature. Similar measures might thus represent useful endpoints in phase II and III clinical studies. However, the data for peak antiparkinsonian benefit do provide some indication of a mild reduction in maximal antiparkinsonian benefit of l-DOPA when it is combined with doses of MTEP with antidyskinetic efficacy. Measures of on-time do not seem to be sensitive to this decreased maximal antiparkinsonian benefit, but it should not be ignored, as in practice patients with PD who have motor complications may find a maximal antiparkinsonian benefit even with mild, nondisabling dyskinesia preferable to an absence of dyskinesia but with increased parkinsonian disability (Hung et al., 2007). Such discussion indicates the value of use of quality-of-life measures 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, although careful attention should be paid to whether such benefits can be achieved while maintaining a maximal degree of antiparkinsonian benefit.

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References


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