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Phosphodiesterase 10A inhibitor MP-10 effects in primates: Comparison with risperidone and mechanistic implications

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Abstract

Phosphodiesterase 10A (PDE10A) is highly expressed in striatal medium spiny neurons of both the direct and indirect output pathways. Similar to dopamine D₂ receptor antagonists acting on indirect pathway neurons, PDE10A inhibitors have shown behavioral effects in rodent models that predict antipsychotic efficacy. These findings have supported the clinical investigation of PDE10A inhibitors as a new treatment for schizophrenia. However, PDE10A inhibitors and D₂ antagonists differ in effects on direct pathway and other neurons of the basal ganglia, indicating that these two drug classes may have divergent antipsychotic efficacy and side effect profile. In the present study, we compare the behavioral effects of the selective PDE10A inhibitor MP-10 to those of the clinical standard D₂ antagonist risperidone in rhesus monkeys using a standardized motor disability scale for parkinsonian primates and a newly designed “Drug Effects on Nervous System” scale to assess non-motor effects. Behavioral effects of MP-10 correlated with its plasma levels and its regulation of metabolic activity in striatal and cortical regions as measured by FDG-PET imaging. While MP-10 and risperidone broadly impacted similar behavioral domains in the primate, their effects had a different underlying basis. MP-10-treated animals retained the ability to respond but did not engage tasks, whereas risperidone-treated animals retained the motivation to respond but were unable to perform the intended actions. These findings are discussed in light of what is currently known about the modulation of striatal circuitry by these two classes of compounds, and provide insight into interpreting emerging clinical data with PDE10A inhibitors for the treatment of psychotic symptoms.

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Keywords

PDE10A inhibitors; Phosphodiesterase; Schizophrenia; Striatum; D2 receptors; Medium spiny neuron

INTRODUCTION

Phosphodiesterase 10A (PDE10A) is one member of the super family of enzymes that regulate signaling in the brain through metabolic inactivation of cAMP and cGMP (Menniti et al., 2006). PDE10A is unique in that it is expressed highly only in striatal medium spiny neurons (MSNs) (Coskran et al., 2006; Fujishige et al., 1999; Loughney et al., 1999; Seeger et al., 2003; Soderling et al., 1999). Pharmacological inhibition of the enzyme increases cAMP and cGMP in MSNs (Grauer et al., 2009; Schmidt et al., 2008; Smith et al., 2013) regulating downstream signaling molecules and gene expression (Kleiman et al., 2011; Nishi et al., 2008; Strick et al., 2010) that translate into changes in the MSN responsiveness to excitatory input (Threlfell et al., 2009). The MSNs are the gateway of the basal ganglia circuitry, integrating cortical and thalamic glutamatergic inputs with midbrain dopaminergic signals (Albin et al., 1989; Graybiel, 2005). Dysfunction within this circuitry is implicated in a number of neurologic and psychiatric conditions (DeLong and Wichmann, 2007). In particular, a subpopulation of MSNs highly express dopamine D₂ receptors and blockade of these receptors is the putative mechanism for the antipsychotic efficacy of drugs currently used to treat schizophrenia (Kapur and Mamo, 2003). The localization of PDE10A to the MSNs raised interest in the enzyme as a therapeutic target in disorders associated with basal ganglia dysfunction, and a number of laboratories have demonstrated that PDE10A inhibitors produce behavioral effects in rodents predictive of antipsychotic activity similar to D₂ antagonists (Grauer et al., 2009; Roberds et al., 2011; Sano et al., 2008; Schmidt et al., 2008; Siuciak et al., 2008; Smith et al., 2013). These data have prompted a number of drug discovery efforts to develop PDE10A inhibitors to treat schizophrenia (Chappie et al., 2009; Chappie et al., 2012; Cutshall et al., 2012; Kehler et al., 2007; Menniti et al., 2007; Piccart et al., 2011).

The similarity of effects of PDE10A inhibitors and D₂ antagonists is accounted for by the fact that both types of agents increase the activity of MSNs that express D₂ receptors (Menniti et al., 2007; Schmidt et al., 2008; Strick et al., 2010; Threlfell et al., 2009). However, MSNs conform two subpopulations according to their expression of dopamine D₁ or D₂ receptors (Kitai and Surmeier, 1993; West et al., 2003). D₁ receptors are expressed in MSNs that give rise to the direct striatal output pathway projecting to the basal ganglia output nuclei, the internal pallidal segment and the substantia nigra pars reticulata. D₂ receptors are expressed in MSNs that give rise to the indirect pathway, a polysynaptic route linking the striatum with the output nuclei via the intercalated external pallidal segment and the subthalamic nucleus (Albin et al., 1989; Gerfen, 1992; Surmeier et al., 1996). Significantly, PDE10A is expressed in both subsets of MSNs (Seeger et al., 2003), and there is electrophysiological and biochemical evidence indicating that inhibition of the enzyme impacts the function of both MSN subtypes (Strick et al., 2010; Threlfell et al., 2009). Since the basal ganglia output reflects the integrated signaling of both the direct and indirect striatal pathways (Albin et al., 1989; Cohen and Frank, 2009; Haber, 2003), D₂ antagonists and PDE10A inhibitors may be expected to differ in some behavioral effects. In contrast to D₂ antagonists, PDE10A inhibitors fail to reverse deficits in a number of animal models of prepulse inhibition (Schmidt et al., 2008; Weber et al., 2009) and produce a distinct pattern of catalepsy in rats (Schmidt et al., 2008). PDE10A inhibitors also are reported to uniquely improve cognitive performance (Grauer et al., 2009; Rodefer et al., 2005; Sano et al., 2008; Smith et al., 2013) and are active in a rodent model of the negative symptoms of

schizophrenia (Langen et al., 2012). These differences are hypothesized to arise from the balanced activation of the direct and indirect output pathways by PDE10A inhibitors (Menniti et al., 2007; Schmidt et al., 2008). Thus, PDE10A inhibitors may result in distinct therapeutic and side effect profile compared with D₂ antagonists.

The selective inhibitor MP-10 (PF-02545920) (Verhoest et al., 2009) has advanced to clinical testing in patients diagnosed with schizophrenia and suffering an acute exacerbation of symptoms. MP-10 did not meet the primary endpoint for a reduction in psychotic symptoms in a recent Phase 2A trial (ClinicalTrials.gov identifier: NCT01175135), although the compound was reported to cause sedation and side effects consistent with target engagement (DeMartinis, 2012). Further analyses of the physiological and behavioral effects of PDE10A inhibition will be needed to understand this emerging clinical data as it relates to the therapeutic potential of PDE10A inhibitors and the neurobiology of psychosis. Here we report the first detailed analysis in primates of the effects of PDE10A inhibition on behavior and regional brain activity as measured with FDG-PET imaging. We also compare the motor effects of MP-10 with those of the clinical standard D₂ antagonist risperidone in rhesus monkeys.

Materials and Methods

Animals

One male and three female adult Rhesus monkeys (*Macaca mulatta*) weighing between 5 and 8 kg were used for these studies. Animals were kept in controlled housing conditions with constant temperature and relative humidity on a 12-h light/dark cycle. Animals had free access to food, fresh fruit, and water with the exception of test days when they were fasted overnight. All studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (ILAR, 1996), and approved by the Institutional Animal Care and Use Committee.

Drugs

The PDE10A inhibitor MP-10 (2-[4-(1-methyl-4-pyridin-4-yl)-1*H*-pyrazol-3-yl]-phoxymethyl]-quinoline 3; PF-02545920) was synthesized at Pfizer, Inc. (Verhoest et al., 2009). MP-10 was dissolved in 5% DMSO and 5% cremophor in distilled water, with the pH adjusted to approximately physiologic levels. The corresponding vehicle was used as control. Risperidone (Sigma-Aldrich, St. Louis, MO) was dissolved in distilled water. MP-10 and risperidone were administered subcutaneously at escalating doses. Doses of MP-10 (0.021, 0.067, 0.21, 0.67, 1.33 mg/kg) were selected to produce unbound plasma drug concentrations ($C_{p,u}$) across a broad range, but centered on efficacious $C_{p,u}$ in preclinical rodent models predictive of clinical antipsychotic activity. Doses of risperidone (0.01, 0.032, 0.1 mg/kg) were selected to produce $C_{p,u}$ of risperidone and its active metabolite 9-hydroxyrisperidone that result in D₂ receptor occupancy in the range for antipsychotic efficacy (Uthayathas et al., 2013). Partial data of risperidone tests have been reported recently to validate the newly designed “Drug Effects on Nervous System” scale (Uthayathas et al., 2013), and are included here for detailed comparison of effects between risperidone and MP-10.

Systemic exposures of MP-10

Plasma levels—To verify that the MP-10 dose range chosen for this study achieved the $C_{p,u}$ in the targeted range, total plasma MP-10 concentrations (C_p) were determined in three of the four test animals at two representative doses (0.21 and 0.67 mg/kg). Catheters for blood sample collection were placed in a superficial vein of the leg prior to drug administration. Blood samples were collected immediately before subcutaneous drug

administration for background (time 0), and thereafter at 1, 2 and 3 hrs. In each monkey, blood samples were taken in two repeated experiments for each MP-10 dose. Plasma was separated and stored at -80°C prior to its processing and MP-10 quantification using a previously described liquid chromatography-tandem mass spectrometry method (Schmidt et al., 2008). For each time point, $C_{p,u}$ was determined as the product of C_p and the equilibrium dialysis-determined unbound fraction of MP-10 in nonhuman primate plasma ($f_{u,p}$, 0.00204; unpublished Pfizer internal data) and expressed in nM units using MP-10 molecular weight (395.5 g/mol):

$$(C_p/\text{MW}) \times f_{u,p} \times 1000$$

MP-10 is metabolized to an *N*-demethylated derivative that has similar PDE10A inhibitory potency ($\text{IC}_{50} \sim 0.2 \text{ nM}$). The systemic exposure of the metabolite was not determined in the present study. However, in a separate study in non-human primates, a single IV bolus (0.03 mg/kg) of MP-10 was administered, and both MP-10 and the metabolite were quantified in plasma over time. The systemic AUC_{0-8} -based ratio for the *N*-demethylated metabolite-to-MP-10 was found to be 0.25. It is reasonable to extrapolate this ratio to an estimate for the present study since first-pass metabolism is bypassed after both subcutaneous (present study) and intravenous dosing. This ratio is very similar to that reported for human (0.2; DeMartinis, 2012). It is also noted that, whereas the MP-10 brain-to-plasma ratio in both mouse and rat is ~ 0.9 , this ratio for the metabolite in rat is 0.2, indicating that the metabolite has less brain penetrability than the parent. Thus, the facts that the systemic exposure of the metabolite is only $\sim 25\%$ of MP-10 and that it has 5-fold less brain penetrance than MP-10 suggest that the metabolite contributes only a minor fraction of the PDE10A inhibitory activity after subcutaneous administration of MP-10. Nonetheless, the metabolite is accounted for in the method used to estimate PDE10A target occupancy, as indicated in the next section.

Estimated PDE10A target occupancy (TO)—TO of PDE10A after MP-10 administration was not directly determined. However, Plisson et al., (2011) determined the MP-10 unbound plasma IC_{50} for PDE10A TO in non-human primate using PET imaging of [^{11}C]MP-10 after single IV boli of nonradiolabelled MP-10 over the dose range of 0.2 to 1.5 mg/kg (Plisson et al., 2011). This empirical IC_{50} takes into account the contribution of both MP-10 and the metabolite to TO. Thus, we use an IC_{50} of 1.0 nM as reported by Plisson et al. (2011) to estimate PDE10A TO from the unbound plasma MP-10 concentrations ($C_{p,u}$) determined in the present study using the equation (where $C_{p,u}$ and IC_{50} are in nM units):

$$(\text{MP-10 } C_{p,u}) / (\text{MP-10 } C_{p,u} + \text{IC}_{50}) \times 100$$

[^{18}F] Fluorodeoxyglucose -PET imaging

To verify that MP-10 had a pharmacodynamic effect in brain tissue in the tested dose range, the effect of the compound on brain [^{18}F] Fluorodeoxyglucose (FDG) uptake was determined by positron emission tomography (PET) imaging, using a previously described protocol (Capuron et al., 2007; Henry et al., 2010). Briefly, animals were chaired and given 20 min to acclimatize. Subsequently, MP-10 (0.21, 1.33 mg/kg or vehicle) was administered subcutaneously (time 0). After 30 min, [^{18}F] FDG (5.0 – 6.00 mCi) was injected via an intravenous catheter into a superficial vein of the leg. After a 20 min period of FDG uptake, animals were anesthetized and placed in the PET scanner. Whole brain, 3D imaging data were collected with a Siemens Focus 220 microPET scanner (Yerkes Imaging Center). Starting at 60 min from time 0, a 15 min transmission scan was obtained for attenuation

correction, and at 75 min the FDG-PET scan began and continued for a total duration of 15 min (see Supplementary Fig. 1A for the complete timeline of the experiment). All images were reconstructed using the manufacturer supplied software with measured attenuation correction, zoom factor 8, and Shepp – Logan reconstruction filter cutoff at 1 cycle/cm. The reconstructed resolution is 1.7 mm in all directions. The acquired data were corrected for random background events, scatter, attenuation, and dead-time. All PET images from the same animal were superimposed using IDL software (ITT Visual Information solutions, Boulder, CO), and averaged. Regions of interest (ROI) were manually drawn on the average image of each hemisphere (Supplemental Fig. 1B). Based on the functional topography of corticostriatal projections in nonhuman primates (Parent and Hazrati, 1995), the striatum was divided as: putamen/associative (PA, putamen rostral to the anterior commissure), putamen/motor (PM, dorsolateral sector of the postcommissural portion of the putamen), caudate nucleus (CA), and nucleus accumbens (AC). The cortical ROI were delineated as: dorsal (DPFC), medial (MPFC), ventral (VPFC) and orbital prefrontal cortex (OPFC), cingulate cortex (CC) and ventral posterior thalamus (VPT). The cerebellum, ventrolateral thalamus, external pallidum, internal pallidum, subthalamic nucleus and substantia nigra pars reticulata were also studied. The manually drawn ROIs on the average image were then superimposed onto the individual images to obtain activity. FDG uptake was expressed as Standard Uptake Values (SUV), accounting for body mass and injected dose of tracer. SUV allows precise assessment of activity changes in response to drug treatments (Boellaard et al., 2004). Because the number of animals studied was small (n=4), SUVs for striatal regions (PA, PM, CA, and AC) and for cortical regions (CC, DPFC, MPFC, and OPFC) were pooled for statistical analyses. Each of the 4 animals received vehicle and each of the two doses of MP-10. Data were collected with a minimum interval of one week between each scan.

Behavioral Assessment

All tests were performed under the same environmental conditions in the morning after overnight fast by an investigator who was blinded to the treatment. All tests were repeated three times, and data were averaged to yield a mean of three data points for each treatment in each monkey for statistical analysis.

Parkinsonian motor effects

1. Standardized scale: Animals were directly observed and scored using a standardized motor disability scale for parkinsonian primates (Cao et al., 2007; Papa and Chase, 1996). The scale consists of two parts, parkinsonian motor disability (Part I) and drug-induced adverse effects (Part II; effects mostly related to dopaminergic drugs). Only part I was used here to assess the drug induction of parkinsonism. Experiments were also filmed and a subset of them was randomly chosen for deferred scoring to confirm the results of direct scoring. Scores were taken just before drug injection (time 0) and afterwards, starting at 30 min and continuing every 20 min up to 170 min. Each item of the scale is scored according to standardized criteria within a range from 0 to 3.

2. Klüver Board test: The Klüver board test measures finger dexterity and the ability to perform fast hand movements. Prior to the tests, monkeys were trained to remove roughly spherical food pellets from wells on a modified Klüver Board (Lawrence and Kuypers, 1968; Papa et al., 2004; Xerri et al., 1999) in which the well diameters were such that the animal must use one finger to obtain the pellet. Monkeys were permitted to use either hand to perform this retrieval task, but they could only access the board with one hand at a time. The time (action time) required to retrieve and move 3 pellets to mouth was recorded by stopwatch. The action time and the index of success (the percentage of successful retrievals) were recorded. Animals were allowed 60 seconds to perform this task. *3-Perch test (MP-10 only).* This behavioral test to evaluate stability and balance (Papa et al., 2004) was carried

out in tall play cages equipped with perches placed on a rod extending from floor to ceiling. The monkey had to climb the perches to reach the ceiling of the cage in order to obtain a small food reward. At different time points after drug administration, 4 items were scored: body swinging: none to severe (0 to 3); tilting: none to severe (0 to 3); number of lapses; number of falls. Animals were also filmed for deferred scoring.

3. Other effects. Drug Effects on Nervous System (DENS) scale: The newly developed DENS scale (Uthayathas et al., 2013) was used to assess other motor and non-motor drug effects on cortical-, extrapyramidal motor- and autonomic-mediated functions. The assessments and scoring are in Table 1.

Statistical analysis

Total scores of motor effects and other drug effects were graded within wide ranges, and scale items analyzed separately included non-integer values; thus, data composed continuous variables. Two-factor analysis of variance (ANOVAs) for repeated measures followed by the Tukey post hoc test was used to compare data in behavioral tests. PET data were subjected to factorial ANOVA for repeated measures for treatment and factor regions for cortical or striatal subregions. Significance was taken at $p < 0.05$. All results are expressed as mean $\pm$ S.E.M.

Results

Systemic Exposures of MP-10

After subcutaneous administration, plasma concentrations of MP-10 peaked between 1 and 2 h post-injection (Table 2), were < 2 -fold different between 1 and 3 h, and declined steadily and slowly thereafter. At 1 h following doses of 0.211 or 0.67 mg/kg, mean C_p were 36.8 and 136 ng/mL, respectively, indicating a linear dose- C_p relationship at this time point across this 3-fold dose range; these C_p equate to $C_{p,u}$ of 0.19 and 0.71 nM, respectively (Table 2). As reported previously for MP-10, the in vitro IC_{50} for recombinant rat PDE10A is 0.18 nM. Furthermore, a MP-10 $C_{p,u}$ of 0.16 nM corresponds to the IC_{50} for suppression of conditioned avoidance responding in rats, an effect also produced by clinically relevant concentration of D_2 receptor antagonists (Olsen et al., 2008; Schmidt et al., 2008). Thus, assuming linearity, the dose range of MP-10 administered to monkeys in this study achieved exposures from 0.1- to 6-fold the $C_{p,u}$ found to be efficacious in rodent models of antipsychotic efficacy, with the mid-doses of 0.21 and 0.67 mg/kg yielding clinically tested systemic exposures (DeMartinis, 2012). The TO of PDE10A at these two doses are estimated at 16% and 41%, respectively (Table 2). MP-10 is metabolized to an *N*-demethylated derivative that has similar PDE10A inhibitory potency. However, this metabolite likely contributes little to the TO (see Methods).

These MP-10 doses may be compared with those of risperidone used here. Based on a previous study (Uthayathas et al., 2013), and accounting for both risperidone and its active metabolite 9-hydroxyrisperidone, 0.01, 0.032 and 0.1 mg/kg of risperidone were estimated to result in dopamine D_2 receptor TOs at 1 h of 36%, 79% and 95%, respectively (Uthayathas et al., 2013). Levels of D_2 occupancy above approximately 55% in patients with schizophrenia are associated with antipsychotic efficacy (Natesan et al., 2006; Olsen et al., 2008).

MP-10 increases regional brain [^{18}F] FDG uptake

The effect of MP-10 on brain [^{18}F] FDG uptake was determined by PET imaging to verify that the compound had a pharmacodynamic effect in targeted brain areas in the tested dose range. Administration of 0.211 or 1.33 mg/kg of MP-10 resulted in statistically significant

increases in the SUV of [^{18}F] FDG in both striatal ($F_{(2, 36)} = 7.9$, $p < 0.01$,) and cortical ($F_{(2, 30)} = 6$, $p < 0.02$) regions compared to vehicle treatment (Figure 1A-B). The magnitude of the increase was not different between the two doses of MP-10 despite a predicted 6-fold change in $C_{p,u}$. An ineffective dose was not identified. There were no statistically significant differences between MP-10 and vehicle treatments for any of the striatal or cortical subregions, due to the increased variability incurred from sampling these smaller areas. These results demonstrate that MP-10 directly affects striatal and cortical brain function over the dose range tested. No significant differences relative to control (vehicle) were found in other brain regions in FDG SUV following the two doses of MP-10 (see Supplemental Table 1).

Behavioral effects of MP-10

Parkinsonian motor effects—Administration of MP-10 0.021, 0.067, or 0.21 mg/kg had no effect on motor scores during the 3 hours after drug administration (Figure 2A). This is despite the fact that the 0.21 mg/kg dose caused a significant change in striatal and cortical [^{18}F] FDG uptake. Although monkeys had a tendency to relax, their mobility was normal and they did not exhibit other side effects or changes in social interaction. At the higher doses of MP-10 of 0.67 and 1.33 mg/kg, a change in behavior was noted in 3 of the 4 monkeys. Animals had a tendency to be still and calm, and this movement reduction drove the increase in global scores on the motor disability scale ($F_{(2, 63)} = 252$, $p < 0.001$; Figure 2A). The maximum increase in global motor score was similar at both doses, with scores returning to baseline sooner after 0.67 mg/kg than after 1.33 mg/kg. A more detailed characterization of the movement reduction is captured in the score changes on subscales. Posture and mobility scores increased in a dose dependent fashion ($F_{(2, 63)} = 71$ and 152, respectively, $p < 0.001$; Figures 3A & B). In contrast, impairment in hand and leg movements was mild and similar at both doses ($F_{(2, 63)} = 147$ and 122, respectively, $p < 0.001$; Figures 3C & D). The lack of mobility is also reflected in a decrease in social interaction, which was similar at both doses ($F_{(2, 63)} = 35$, $p < 0.001$; Figure 3E). Thus, reduced activity, as opposed to extrapyramidal motor effects (slowness of gait and hand/leg movements, tremor, etc.), appeared as the primary factor underlying the increase in global motor disability score caused by MP-10.

This analysis is further supported by the results of the Klüver board and Perch tests. The Klüver board test requires fine movement coordination and speed, and is a highly sensitive measure of parkinsonian motor disability. MP-10 at doses up to 0.21 mg/kg had no effect on the action time and index of success in this assessment (Figure 2C). However, at the higher doses of MP-10, monkeys appeared inattentive, lacked interest and failed to perform the task. The Perch test is a sensitive measure of impairments in stability and balance, and these motor deficits are also typically associated with parkinsonism. Monkeys did not show any compromise in the performance of this test following administration of MP-10 at 0.21 or 0.67 mg/kg. Scores taken before and 90 min after either dose of MP-10 injections remained at zero (normal). It is noteworthy that the 0.67 mg/kg dose of MP-10 was expected to induce low activity and possibly lack of incentive to the animals to perform in the Perch test. However, the Perch test was done after all other tests after the animals had been exposed repeatedly to MP-10. Thus, it is possible that the lack of effect of 0.67 mg/kg was the result of some tolerance to MP-10 having developed at the time of this test.

DENS scores—The DENS scale was used to assess alterations in brain functions that are often seen after administration of psychotropic drugs but that are not captured by the scales used to assess parkinsonian motor disability. In keeping with the effects seen on the parkinsonian motor scale, only the two highest doses of MP-10 (0.67 and 1.33 mg/kg) had significant effects on the total DENS scores ($F_{(2, 63)} = 156$, $p < 0.001$; Figure 4A). Both

doses caused a similar magnitude of increase, with the higher dose producing a greater increase in score at early and late time points. However, there was a considerable variation in these responses with repetition of the same dose in the same animal, suggesting that tolerance may develop with repeated administration. Changes in total DENS scores derived largely from decreased attentiveness and reactivity ($F_{(5, 60)} = 91$ and 85 , respectively, $p < 0.001$; Figure 5A and B). Eye movements are affected by either sedation or parkinsonism; however, the lack of other specific parkinsonian features suggests that the MP-10 effect on eye movements may be caused by sedation ($F_{(5, 60)} = 58$, $p < 0.001$; Figure 5C). MP-10 caused non-significant increases in salivation (Figure 5D).

MP-10 had no effect on the involuntary movements component of the DENS scale in any monkey receiving doses up to 0.21 mg/kg. However, the higher MP-10 doses (0.67 and 1.33 mg/kg) induced oral dyskinesias ($F_{(5, 60)} = 25$, $p < 0.001$; Figure 5E). These involuntary movements affected the mouth, tongue and jaw, and were markedly stereotypic with smacking, chewing and biting, but with a mildly choreodystonic component. Slight dystonia of the tongue was occasionally seen. The appearance and severity of these dyskinesias was highly episodic, and consequently was variable when averaged across monkeys and over time. This likely accounts for the fact that the average scores across animals were not significantly different from vehicle control treatment at most of the time points. It also appeared that there may have been a decrease in the severity of the oral dyskinesia with repeated treatment, although this was not possible to establish quantitatively due to the episodic nature of the response. Oral dyskinesias induced by MP-10 markedly decreased by 170 min after drug injection, indicating that they were exposure-related.

Behavioral effects of risperidone

Parkinsonian motor effects—Risperidone was administered at 3 doses, 0.01 , 0.03 and 0.1 mg/kg that were estimated to result in D_2 receptor TO in the range of 36–95%. Whereas 0.01 mg/kg had no effect on motor behavior during the 3 hours after drug administration, both 0.032 and 0.1 mg/kg caused significant increases in motor disability scores ($F_{(2, 63)} = 113$, $p < 0.001$; Figure 2B). These effects were both dose- and time-dependent, with 0.1 mg/kg causing a greater effect of longer duration. In comparison to MP-10, risperidone caused roughly comparable increases in posture and social interaction scores ($F_{(2, 63)} = 115$ and 94 , respectively, $p < 0.001$; Figure 3F and J), but a notably less effect in mobility scores (i.e., less reduction in mobility, $F_{(2, 63)} = 30$, $p < 0.001$; Figure 3G). On the other hand, risperidone caused greater, dose-dependent increases in hand and leg movement scores ($F_{(2, 63)} = 130$ and 103 , respectively, $p < 0.001$; Figure 3H & I) than MP-10. Also in contrast to MP-10, these effects of risperidone were consistent in repeated tests and affected all animals similarly. Thus, the overall motor disability caused by risperidone can be attributed more to specific motor deficits than sedation. This is also reflected in the results of the Klüver board test. Whereas monkeys receiving 0.032 and 0.1 mg/kg risperidone attempted to perform the task, performance was severely compromised and action time scores were at the 60 s cut off (Figure 2D). The Perch test was not performed following risperidone administration because of risks of injuries in animals with parkinsonian motor deficits.

DENS scale—Risperidone at 0.01 mg/kg had minimal effects on behaviors scored using the DENS scale. However, at 0.032 and 0.1 mg/kg there were increases in total DENS scores that were greater and of longer duration at the higher dose ($F_{(2, 63)} = 123$, $p < 0.001$; Figure 4B). Overall, the DENS scores were lower with risperidone than with MP-10, but the pattern of changes in subscale ratings was markedly different for the two compounds. Risperidone caused less impairment in attentiveness and reactivity than MP-10 ($F_{(1, 42)} = 181$ and 173 , respectively, $p < 0.001$; Figure 5F & G), items that clearly relate to sedation. Risperidone compromised eye movement ($F_{(1, 42)} = 93$, $p < 0.001$; Figure 5H) to an extent

similar to MP-10, and this effect could represent sedation or parkinsonian impairment. Risperidone caused considerably more salivation ($F_{(1, 42)} = 36$, $p < 0.001$; Figure 5I) than MP-10, a clear parkinsonian symptom suggesting more extrapyramidal impairment.

Risperidone caused significant involuntary movements in all monkeys at 0.032 mg/kg and the intensity of these involuntary movements was further increased at 0.1 mg/kg. These were predominantly choreodystonic oral dyskinesias ($F_{(1, 42)} = 113$, $p < 0.001$; Figure 5J), more dystonic and less stereotypic than those seen with MP-10. Risperidone-induced dyskinesias affected predominantly the tongue and had clear dystonic features. On average, the intensity of involuntary movements was slightly lower with risperidone than with high doses of MP-10. Oral dyskinesias induced by risperidone were also peak-exposure effects, disappearing 170 min after drug injection.

Discussion

We examined the motor and behavioral effects of MP-10 in an old world primate, the rhesus monkey, in comparison with the D₂ antagonist risperidone, one of the most widely used antipsychotics. Macaques have been used extensively to model human motor dysfunction in Parkinson's disease because of the close resemblance of this model to human parkinsonism (Jenner, 2003). We took advantage of this similarity to carefully compare differential effects of PDE10A and D₂ antagonism on primate motor behavior. The results of our study provide valuable insight into the nature of the behavioral effects of PDE10A inhibitors, which may help interpret such effects in humans.

MP-10 and risperidone were compared over a range of clinically relevant doses. For risperidone, antipsychotic efficacy is observed at $> 55\%$ D₂ receptor TO (Olsen et al., 2008). For the three risperidone doses used in this study maximal D₂ receptor TO spanned 35–95% (Uthayathas et al., 2013). For MP-10, the range of doses afforded C_{p,u} bracketing the IC₅₀ for rat recombinant PDE10A *in vitro* (0.18 nM) and the IC₅₀ for suppression of conditioned avoidance responding in rats (0.16 nM) (Schmidt et al., 2008). In fact, mean MP-10 C_{p,u} 1–3 h after 0.21 and 0.67 mg/kg were approximately 0.19 nM and 0.89 nM, respectively, which were similar to those examined in the MP-10 Phase 2A study (DeMartinis, 2012). The ratio of MP-10 total brain-to-total plasma concentrations in rat (Schmidt et al, 2008) and mouse (Verhoest et al, 2009) are ~0.9 and there is no evidence that it is a substrate for blood-brain barrier efflux transporters (Verhoest et al, 2009). These data suggest that MP-10 readily crosses the blood-brain barrier. Consistent with this, we also observed that in the monkey, 0.21 and 1.33 mg/kg MP-10 caused significant, homogeneously distributed elevations in FDG uptake in striatal and cortical regions measured by PET. These data confirm that MP-10 accessed the brain to impact neural activity. Occupancy of PDE10A by MP-10 was not determined in this study. However, Plisson et al (2011) measured in baboons the MP-10 total plasma concentrations required to achieve 50% PDE10A occupancy by PET using [¹¹C]MP-10; the total plasma IC₅₀ was estimated at 126 to 198 ng/ml. These total plasma MP-10 concentrations are similar to those observed in the present study. However, further work is clearly warranted to better determine the relationship between behavioral effects and levels of inhibition of PDE10A.

The motor and behavioral effects of MP-10 and risperidone were scored using two scales, a *Motor Disability* scale for parkinsonian primates (Cao et al., 2007; Papa and Chase, 1996) and the recently developed *DENS* scale (Uthayathas et al., 2013). The former scale was used since risperidone is known to cause parkinsonian symptoms in primates including humans. However, the effects of MP-10 were not well described in terms of parkinsonian motor impairment alone, and the DENS scale was used to further characterize the effects of this novel pharmacological agent. While risperidone and MP-10 produced similar total scores on

both scales, and both disrupted performance in the Klüver Board test, the behavioral underpinnings of these effects were different.

The major effect of MP-10 in the primate was to reduce spontaneous activity and responsiveness, which was captured in the Attentiveness and Reactivity subscales of the DENS scale, as well as on the mobility measure of the motor disability scale. These MP-10 effects imply more the motivational aspects of motor behavior and the impact of generalized sedative effects. Consistent with this interpretation, MP-10 caused relatively mild impairment of hand and leg coordination and little salivation, measures reflecting parkinsonian motor effects. Furthermore, in the Perch test, a measure of stability and balance sensitive to parkinsonian motor impairment, animals receiving MP-10 performed the task and evidenced no impairment. In contrast, risperidone caused notable motor impairment associated with parkinsonism, particularly at the high dose, but had relatively milder effects on Attentiveness, Reactivity and Mobility. The difference between MP-10 and risperidone is strikingly illustrated in the nature of the disruption these two agents caused on the Klüver board test. Whereas risperidone-treated animals attempted the task but failed due to lack of fine motor control, MP-10-treated animals simply did not attempt the task. Both MP-10 and risperidone produced involuntary movements in the form of oral dyskinesias, albeit of subtly different presentation. For MP-10, these movements included stereotypic smacking, chewing and biting, with occasional dystonia of the tongue. Oral dyskinesias produced by risperidone were of a lower intensity, predominantly affected the tongue, and were more dystonic and less stereotypic than those seen with MP-10.

A notable difference between MP-10 and risperidone was in the dose responsiveness of effects. Risperidone effects were clearly dose responsive on each motor disability and DENS scale item. Furthermore, effects were of longer duration at the higher dose, all monkeys were equally impacted, and there was little variability in individual responses across replicate experimental sessions. In sharp contrast, the effects of MP-10 were more all-or-none in nature. There were no notable behavioral effects of MP-10 at 0.21 mg/kg, whereas essentially maximal effects were observed at 0.67 and 1.33 mg/kg on most of the rated behaviors. This pattern of response is particularly clear for the hand and leg movement subscales, where the magnitudes of effects were not at a ceiling. The lack of behavioral effect at 0.21 mg/kg is noteworthy since this dose caused an elevation of FDG uptake measured by PET that was equivalent to that after 1.33 mg/kg. More generally, there was variability in response to MP-10 between monkeys and for individual monkeys across replicate experimental sessions. In rhesus, as in other species, MP-10 exposure was proportional to dose and the variability in exposure was low between subjects and for individuals on replicate dosing. On the other hand, the duration of MP-10 effects consistently waned more rapidly after 0.67 mg/kg than 1.33 mg/kg, indicating that the effects were directly related to drug exposure. These data indicate that the unusual dose responsiveness and variability of response to MP-10 is not related to variability in systemic drug exposure. Thus, whereas the behavioral effects of risperidone are graded with increasing exposure, those caused by MP-10 occur more in an all-or-none fashion, and it appears that the tipping point for this effect may be quite sensitive given the variability in response to MP-10 and lack of dose responsiveness. The differences in dose-response characteristic between MP-10 and risperidone likely represent a mechanistic difference.

Overall, while MP-10 and risperidone broadly impacted similar behavioral domains in the primate, the nature of the behavioral disruption had a different underlying basis. Simply put, MP-10-treated animals retained the ability to respond but did not engage tasks, whereas risperidone-treated animals retained the motivation to respond but were unable to perform the intended actions. These findings can be interpreted in light of what is currently known about the modulation of striatal circuitry by these two classes of compounds.

PDE10A and D₂ receptors are both highly expressed by MSN of the indirect pathway and both PDE10A inhibitors and D₂ antagonists increase the indirect pathway output (Nishi et al., 2011; Threlfell et al., 2009). This anatomical and functional overlap is hypothesized to account for their similarity in rodent models predictive of antipsychotic activity (Menniti et al., 2007; Schmidt et al., 2008). However, the input signals to the indirect pathway MSNs driving cyclic nucleotide signaling regulated by PDE10A are not well characterized. A key area for further study is identification of these input signals to fully interpret the effects of PDE10A inhibition on indirect pathway activity.

PDE10A inhibitors and D₂ antagonists diverge significantly in modulation of other components of the striatal circuitry. PDE10A, but not D₂ receptor, is highly expressed in MSNs of the direct striatal output pathway (Coskran et al., 2006; Schmidt et al., 2008; Seeger et al., 2003; Xie et al., 2006), and gene expression studies indicate that PDE10A inhibitors also activate these neurons (Strick et al., 2010). Thus, the ability of PDE10A inhibitors to activate both direct and indirect pathway MSNs constitutes a major difference with D₂ antagonists. Recently, Cui et al. (Cui et al., 2013) reported that direct and indirect striatal output pathways are both activated during action initiation, highlighting the notion that these two information streams function in counterpoint rather than competitively (Graybiel, 2005). In this context, it is interesting that in macaques the co-activation of direct and indirect pathways by PDE10A inhibition resulted in reduced task engagement. On the other hand, D₂ receptors, but not PDE10A, are expressed by striatal aspiny cholinergic interneurons (Kawaguchi et al., 1995; Kreitzer, 2009; Xie et al., 2006). These interneurons play a critical role in synchronizing the activity of MSNs to coordinate their output (Tepper et al., 2004). D₂ receptors are also expressed in substantia nigra, where they are involved in the gating of dopamine neuron activity (Smith and Kieval, 2000). It is tempting to speculate that disruption of gating functions by D₂ inhibition contributes to the impairment of movement execution. Perhaps the fact that PDE10A is functionally expressed only in striatal medium spiny neurons but not interneurons or dopamine neurons (Xie et al., 2006), accounts for the relative lack of effect of MP-10 on fine motor control.

In summary, our findings in primates provide a valuable comparison of clinically relevant motor behavioral side effects and dyskinesias caused by PDE10A inhibition and D₂ antagonism. More significantly, we reveal that PDE10A inhibition has a unique behavioral effect in primates, namely, to suppress the intent to move without impairing the ability to carry out such movement. Striatal circuitry is critically involved in all aspects of motor behavior including the attentional and motivational components, and further study is warranted to discern whether and how PDE10A inhibition may impact these cognitive aspects of striatal function. Such mechanistic studies will undoubtedly provide unique insight into how the basal ganglia regulate motivated action selection and execution, and further inform the therapeutic potential of compounds such as MP-10.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- PDE10A regulates striatal outputs with some similarity to dopamine D₂ receptors.
- We compared effects of the inhibitor, MP-10, and risperidone in rhesus monkeys.
- MP-10 effects correlated with PK data and striatal activity as measured by FDG-PET.
- MP-10 reduced the reactivity and task engagement without movement impairment.
- Contrarily, risperidone affected the ability to move causing clear parkinsonism.

Figure 1. Standard uptake values (SUV) of FDG in the monkey brain following MP-10 administration

^{18}F -FDG PET images of striatal (**A**) and frontal cortical (**B**) regions correspond to pre-commissural coronal planes of the same monkey following the administration of vehicle (top left), 0.21 mg/kg MP-10 (top middle) and 1.33 mg/kg of MP-10 (top right). Increased metabolic activity is seen throughout both brain areas following administration of MP-10. Scale bar = 10 mm. In A and B, the left graphs show FDG SUV pooling striatal or cortical subregions together. The right graphs show FDG SUV of different striatal ($F_{(2, 36)} = 7.9$) or cortical ($F_{(2, 30)} = 6$) ROIs. * $p < 0.01$; # $p < 0.05$ for differences between control and MP-10 doses. Analysis of separate subregions did not show significant differences. Values are Mean $\pm$ SEM; $n = 4$. PA: Putamen/associative. PM: putamen/motor. CA: caudate nucleus. AC: nucleus accumbens. DPFC, MPFC and OPFC: dorsal, medial and orbital prefrontal cortex, respectively. CC: cingulate cortex.

Figure 2. Parkinsonian motor effects of MP-10 compared to risperidone

Total motor disability scores produced by MP-10 doses from 0 to 1.33 mg/kg, s.c., (A) or risperidone doses from 0 to 0.1 mg/kg, s.c. (B). Scores are the “total” values obtained after addition of individual item scores taken with the standardized motor disability scale for primates (total scores produced by risperidone have been reported in Uthayathas *et al.* 2013). The baseline score was obtained just before drug injection (time 0). After MP-10 or risperidone injection scoring starts at 30 min and continues thereafter every 20 min. Values are the average of total scores for individual intervals from all monkeys (n=4). Two way ANOVAs for analysis of MP-10 and risperidone effects ($F_{(2, 63)} = 252$ and 113, respectively, $p < 0.001$) were followed by post hoc Tukey test; * $p < 0.01$, # $p < 0.05$ vs. same time point in the control test vehicle injection. Changes in motor dexterity produced by MP-10 (C) or risperidone (D) doses (same s.c. doses as in A and B). The action time in the Klüver Board test was recorded before (Baseline: Open bars) and 90 min after drug injection (90 min: Closed bars) in all monkeys. No significant differences were found with MP-10 doses up to 0.21 mg/kg or risperidone 0.01 mg/kg. Monkeys did not perform the task (up to the maximal time of 60 min) in tests of MP-10 0.67 mg/kg and 1.33 mg/kg due to sedation. Risperidone doses of 0.032 and 0.1 mg/kg impeded the performance of the tests without clear signs of sedation. *in C and D: lack of performance after time limit. Data points are mean (n = 4) $\pm$ SEM.

Figure 3. Itemized motor disability scores

Scores of *Posture*, *Mobility*, *Hand Movement*, *Leg Movement*, and *Social Interaction* produced by MP-10 doses from 0 to 1.33 mg/kg, s.c., (A–E) or risperidone doses from 0 to 0.1 mg/kg, s.c., (F–J). The individual items are selected from the standardized motor disability scale for primates based on most significant compromise. The baseline score was obtained just before drug injection (time 0). After MP-10 or risperidone injection scoring starts at 30 min and continues thereafter every 20 min. Values are the average of scores for individual intervals from all monkeys. Two way ANOVAs for analysis of MP-10 effects (posture: $F_{(2, 63)} = 71$, mobility: $F_{(2, 63)} = 152$, hand movement: $F_{(2, 63)} = 147$, leg movement: $F_{(2, 63)} = 122$, and social interaction: $F_{(2, 63)} = 35$, for each item $p < 0.001$), and risperidone effects (posture: $F_{(2, 63)} = 115$, mobility: $F_{(2, 63)} = 30$, hand movement: $F_{(2, 63)} = 130$, leg movement: $F_{(2, 63)} = 103$, and social interaction: $F_{(2, 63)} = 94$, for each item $p < 0.001$) were followed by post hoc Tukey test; * $p < 0.01$, # $p < 0.05$ vs. same time point in the control test, vehicle injection. Data points are mean ($n = 4$) $\pm$ SEM.

Figure 4. Other neurologic effects of MP-10 compared to risperidone

Total DENS scores produced by MP-10 doses from 0 to 1.33 mg/kg, s.c., (A) or risperidone doses from 0 to 0.1 mg/kg, s.c. (B). Scores are the “total” values obtained after addition of individual item scores taken with the DENS scale for primates (total scores produced by risperidone have been reported in Uthayathas *et al.* 2013). The baseline score was obtained just before drug injection (time 0). After MP-10 or risperidone injection scoring starts at 30 min and continues thereafter every 20 min. Values are the average of total scores for individual intervals from all monkeys (n=4). Two way ANOVAs for analysis of MP-10 and risperidone effects ($F_{(2, 63)} = 156$ and 123, respectively, $p < 0.001$) were followed by post hoc Tukey test; * $p < 0.01$, # $p < 0.05$ vs. same time point in the control test vehicle injection. Data points are mean $\pm$ SEM.

Figure 5. Itemized DENS scores

Scores of *Attentiveness*, *Reactivity*, *Eye Movement*, *Salivation*, and *Involuntary Movements (Dyskinesias)* produced by MP-10 doses from 0 to 1.33 mg/kg, s.c., (A–E) or risperidone doses from 0 to 0.1 mg/kg, s.c., (F–J). The individual items are selected from the DENS scale for primates based on most significant compromise (Uthayathas *et al.* 2013). The baseline score was obtained just before drug injection (time 0). After MP-10 or risperidone injection scoring starts at 30 min and continues thereafter every 20 min. Values are the average of scores for individual intervals from all monkeys. Two way ANOVAs for analysis of MP-10 effects (attentiveness: $F_{(5, 60)} = 91$, reactivity: $F_{(5, 60)} = 85$, eye movement: $F_{(5, 60)} = 58$, dyskinesias: $F_{(5, 60)} = 25$, for each item $p < 0.001$), and risperidone effects (attentiveness: $F_{(1, 42)} = 181$, reactivity: $F_{(1, 42)} = 173$, eye movement: $F_{(1, 42)} = 93$, salivation: $F_{(1, 42)} = 36$, dyskinesias: $F_{(1, 42)} = 113$, for each item $p < 0.001$) were followed by post hoc Tukey test; * $p < 0.01$, # $p < 0.05$ vs. same time point in the control test, vehicle injection. Involuntary movements were mostly oral dyskinesias with choreodystonic movements of the tongue. Eye movements may indicate extrapyramidal compromise or sedation. Data points are mean ($n = 4$) $\pm$ SEM.

Table 1

“Drug Effects on the Nervous System” (DENS) scale.

Cortical function	Attentiveness	Attentive/Active = 0 Less attentive/Less active = 1 Not attentive/Inactive = 2
	Reactivity	Threatening or trying to reach the examiner = 0 Only looking at the examiner = 1 Lack of response = 2 Sleepy appearance, drooping eyelids, somnolence = 3
Extrapyramidal motor function	Eye movement	Frequent blinking/Normal eye movement = 0 Less frequent blinking/ Reduced or slow eye movement = 1 Infrequent blinking/Fixed eyes = 2
	Involuntary movements	Absent = 0 Mild = 1 Moderate = 2 Severe = 3
Autonomic function	Salivation	Normal salivation = 0 Increased salivation with some dribbling = 1 Drooling = 2
	Upper GI function	Normal appetite and interested in treats and rewards = 0 Decreased interest in treats and rewards = 1 No interest in any rewards, the animal is nauseated or vomiting = 2
	Lower GI function	No defecation or formed stool = 0 A large amount of stool or softer stool = 1 Diarrhea = 2
	Urinary function	One or two urine spots on cage pan = 0 Frequent urination = 1 Very frequent urination = 2

The DENS scale was designed to evaluate common effect of drugs on the nervous system in primates following acute administration (see detailed description of scoring in each item in Uthayathas *et al.* 2013).

Table 2

Plasma concentration of MP-10 and estimated target occupancy of PDE10A in monkeys

Dose (mg/kg, s.c.)	Time (h)		
	1	2	3
0.21	0.19 ± 0.014 (16 ± 1)	0.23 ± 0.029 (19 ± 3)	0.21 ± 0.015 (17 ± 1)
0.67	0.71 ± 0.051 (41 ± 5)	0.89 ± 0.024 (47 ± 2)	0.73 ± 0.033 (42 ± 3)

Three monkeys were administered single doses of MP-10 (0.21 or 0.67 mg/kg, s.c.), and plasma samples were obtained at 1, 2, or 3 h post-injection for determination of the total plasma MP-10 concentration (C_p). Each dose was repeated twice. The unbound plasma MP-10 concentration ($C_{p,u}$: see calculation in Methods) mean ± S.E.M for each dose at each time (n= 6 determinations) are presented. Projected PDE10A target occupancy (mean ± S.E.M.) at each $C_{p,u}$ was calculated as described in Methods and is presented in parentheses.