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Effects of a novel phosphodiesterase 10A inhibitor in non-human primates: a therapeutic approach for schizophrenia with improved side effect profile

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Abstract

Schizophrenia symptoms are associated with alterations in basal ganglia-cortical networks that include the cyclic nucleotides (cAMP/cGMP) signaling pathways. Phosphodiesterase 10A (PDE10A) inhibitors have been considered as therapeutic agents for schizophrenia because the regulation of cAMP and cGMP in the striatum by PDE10A plays an important role in the signaling mechanisms of the striatal-cortical network, and thereby in cognitive function. In the present study we assessed in non-human primates (NHPs) the effects of a novel PDE10A inhibitor (FRM-6308) that has demonstrated high potency and selectivity for human recombinant PDE10A in vitro. The behavioral effects of FRM-6308 in a dose range were determined in rhesus monkeys using a standardized motor disability scale for primates, motor tasks, and the “drug effects on the nervous system” (DENS) scale. The neuronal metabolic effects of FRM-6308 were determined with [(18)F]-fluorodeoxyglucose PET imaging. Results showed that FRM-6308 did not have any specific effects on the motor system at s.c. doses up to 0.32 mg/kg in NHPs, which induced a significant increase in the FDG-SUV in striatum (F 16.069, $p < 0.05$) and cortical (F 15.181, $p < 0.05$) regions. Higher doses induced sedation and occasional involuntary movements with clear development of tolerance after repeated exposures. These findings suggest that FRM-6308 has the adequate pharmacological profile to advance testing in clinical trials and demonstrate antipsychotic efficacy of PDE10A inhibition for the treatment of schizophrenia patients.

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Keywords

PDE10A inhibitor; Phosphodiesterase; Schizophrenia; Striatum; FDG PET imaging

Introduction

Schizophrenia is a multifaceted psychosis of chronic evolution that typically develops in adolescents and young adults inflicting lifelong disability (van Os and Kapur, 2009). Symptomatic treatment with antipsychotics acting on dopamine D₂ receptors can ameliorate positive symptoms such as hallucinations and delusions, but are ineffective against negative and cognitive symptoms. These poorly controlled symptoms can significantly affect the day to day functioning of patients; in fact, the severity of cognitive deficits has predictive value for functional outcome (Green, 1996; Simpson et al., 2010). The pathophysiology of these symptoms remains unclear, but several neuropathological studies have shown abnormalities in cAMP signaling pathways including the phosphorylation of the cAMP response element binding protein (CREB) (Muly, 2002; Xie and Rothstein, 1995). CREB participates in mechanisms of corticostriatal synaptic plasticity (Calabresi et al., 2000; Threlfell et al., 2009), and regulates the transcription of genes that are involved in cognitive functions including memory and learning (Carlezon et al., 2005; Hardingham et al., 2001; Lonze and Ginty, 2002). In addition, cGMP that is known to play a role in the short-term changes in excitability in striatal neurons may be involved in the pathophysiology of cognitive symptoms (Padovan-Neto et al., 2015). On the other hand, imaging studies in schizophrenia patients have shown abnormalities in the basal ganglia (BG)-prefrontal cortex (PFC) network (Altamura et al., 2013; Buchsbaum et al., 2007; Lehrer et al., 2005; Tregellas, 2014), to which the striatum is a major contributor. Taken together these data indicate that the regulation of cyclic AMP/GMP and CREB-associated signals in striatal neurons may have a high impact on the cognitive symptoms of schizophrenia patients (Menniti et al., 2007; Schmidt et al., 2008; Siuciak et al., 2006). Striatal cAMP and cGMP are regulated by hydrolysis, which is largely catalyzed by the phosphodiesterase 10A (PDE10A). This isoenzyme is expressed principally in striatal neurons (Grauer et al., 2009; Smith et al., 2013), and its inhibition has significant effects on cAMP and cGMP levels and their associated signals in striatal cells (Nishi et al., 2008; Schmidt et al., 2008). Therefore, selective PDE10A inhibitors that specifically control striatal signaling mechanisms may offer a strategy for the overall treatment of positive, cognitive, and negative symptoms of schizophrenia.

The potent PDE10A inhibitors papaverine, TP-10, and MP-10 exhibited efficacy for positive, cognitive and negative symptoms in rodent models of schizophrenia (Grauer et al., 2009; Schmidt et al., 2008). Furthermore, FDG-PET imaging in the normal primate demonstrated that MP-10 effects on striatal activity were accompanied by a distinctive effect on motivation, which contrasted with the risperidone actions directly affecting motor function as a dopamine D₂ receptor antagonist (Uthayathas et al., 2014). However, in Phase 2A clinical trial, MP-10 failed to reach the primary end point for a reduction of antipsychotic symptoms, and caused undesirable side effects (Targum et al., 2012). The mechanisms underpinning the disappointing clinical results of MP-10 are not clear, but

given the specificity for regulation of striatal activity and the significant effects on attention and motivation, it may be critical to develop PDE10A inhibitors with differences in their pharmacodynamic profiles. FRM-6308 (FORUM Pharmaceuticals, Inc.) is a novel, potent and highly selective PDE10A inhibitor that has also demonstrated antipsychotic efficacy in rodent models (conditioned avoidance responding, stimulant-induced locomotor activity and prepulse inhibition tests). FRM-6308 was active at doses that did not produce decreases in spontaneous locomotor activity or catalepsy (unpublished data). These data are contrasting with the effects of other inhibitors such as the class of MP-10, which had significant motor effects at the active doses (Schmidt et al., 2008). Here, we report FRM 6308 effects in a dose escalating manner in non-human primates assessing behavioral responses in the motor and non-motor domains and correlating them with changes in brain activity measured with FDG-PET imaging. Results of these FRM 6308 tests in the primate support further evaluation of selective PDE10A inhibitors for the treatment of schizophrenia.

Materials and Methods

Animals

Four monkeys (*Macaca mulatta*), one male and three females, all adults weighing between 5 and 8 kg were kept in controlled housing conditions with constant temperature, relative humidity, and a 12-h light dark cycle. Animals had free access to food, fresh fruit supplements and water. All studies were conducted in accordance with the Institute of Laboratory Animal Resources (ILAR, 1996), and approved by the Institutional Animal Care and Use Committee.

Drugs

The novel PDE10A inhibitor, FRM-6308 was synthesized at FORUM Pharmaceuticals Inc. The compound is highly selective for PDE10A. The IC_{50} (i.e., half maximal inhibitory concentration) values for FRM-6308 at the different PDE isoforms were evaluated at Scottish Biomedical Research labs (Glasgow, UK) according to previously published methods (Thompson and Appleman, 1971). The IC_{50} values were: PDE1A3 >100 μ M, PDE1B >100 μ M, PDE1C >100 μ M, PDE2A3 = 50.0 μ M, PDE3CAT >100 μ M, PDE4CAT = 2.04 μ M, PDE4B2 = 1.75 μ M, PDE4D3 = 2.08 μ M, PDE5CAT >100 μ M, PDE6AB >100 μ M, PDE7A = 49.0 μ M, PDE8A = 69.0 μ M, PDE9A1 >100 μ M, PDE10A1 = 0.0114 μ M and PDE11A1 = 43.0 μ M. Overall, the data suggest that FRM-6308 is both a potent and selective PDE10A1 inhibitor, with greater than 150-fold selectivity for PDE10A1 over the other PDE isoforms evaluated. FRM-6308 was prepared fresh daily as a solution with DMSO (10%), Cremophor (10%) in distilled water to a volume of 1-2 ml for subcutaneous administration.

Plasma levels of FRM-6308

To verify that the FRM-6308 dose range chosen for this study achieved the unbound plasma concentration ($C_{p,u}$) in the targeted range, total plasma concentrations (C_p) of FRM-6308 were determined in three of the four animals used in behavioral tests at 3 representative doses (0.1, 0.32 and 1.0 mg/kg, s.c.). Catheters for blood sample collection were placed in a superficial vein of the leg prior to drug administration to chaired animals without sedation.

Blood samples were collected immediately before subcutaneous drug administration for background (time 0), and thereafter at 1, 2, 3 and 7.5 hrs. In each monkey, blood samples were taken in two repeated experiments for each FRM-6308 dose. Plasma was separated and stored at -80°C prior to its processing for FRM-6308 quantification using a previously described liquid chromatography-tandem mass spectrometry analysis (LC-MS/MS). For each time point, $C_{p,u}$ was determined as the product of C_p and the equilibrium dialysis-determined unbound fraction of FRM-6308 in nonhuman primate plasma, and expressed in nM units using FRM-6308 molecular weight (412.44 g/mol).

Drug Experiments

All experiments were performed in the morning after overnight fast and in the same environmental conditions. All monkeys were trained to sit in a primate chair and transported to a testing room for behavioral assessment before and after drug treatment. After collections of base line data, the animals received subcutaneous FRM-6308 injections for post injection assessment at fixed intervals (see below). FRM-6308 was tested at escalating doses (0.032, 0.1, 0.32 and 1 mg/kg) and compared to the vehicle (0 mg/kg) injection to determine any behavioral effect on the normal primate due to PDE10A inhibition. The FRM-6308 doses were chosen to produce plasma drug exposures across a broad range centered on levels for predicted efficacy in preclinical models of antipsychotic activity (unpublished data). Each dose was tested three times, and scores were averaged to yield a mean from three data points for each treatment in each monkey for statistical analysis.

Behavioral Assessment

a. Parkinsonian motor disability—To determine whether FRM-6308 had motor side effects, monkeys were evaluated by a blinded investigator trained in scoring motor behavior in primates. A standardized “motor disability scale for parkinsonian primates” (Papa and Chase, 1996) was used to assess induction of parkinsonian signs in normal animals because parkinsonism is a common side effect of antipsychotic agents. Animals were scored at regular intervals before and after drug administration. In addition, animals were videotaped for deferred scoring by another blinded investigator. Baseline scores were taken immediately before drug injection (time 0) and again starting 30 min after injection and thereafter every 20 min until the animal returned to normal or up to 170 min. Animals received a score between 0 and 3 (0 = normal, 1 = mild, 2 = moderate, 3 = severe) for each item (maximum score: 27 points).

b. Klüver Board test—Animals were tested for fine motor skills that included finger dexterity and hand movement speed using the Klüver board test. Prior to the tests, monkeys were trained to pick food pellets from small, spherical wells on a modified Klüver Board (Papa et al., 2004). The well diameter is so small that the animal must take the pellet out of the well with one finger before retrieving it. Monkeys were permitted to use either hand to perform the task, but they could only access the board with one hand. The time required to retrieve and move 3 pellets to mouth (action time) was recorded. Action time as well as the number of retrievals (index of success) were measured. Animals were given 60 seconds to perform this task.

c. Perch test—The “Perch test” was used to evaluate stability and balance in primates. The animal is placed in a tall play cage that is equipped with perches on a single central rod from the floor to the ceiling of the cage. This test consists of climbing the perches to reach the ceiling of the cage where there is a food reward. The climbing ability was scored at different time points after drug administration (Papa et al., 2004) assessing 4 items: 1) body swinging: none to severe (0 to 3), 2) tilting: none to severe (0 to 3), 3) number of lapses, and 4) number of falls. Animals were also filmed for deferred scoring.

d. Other Effects on the Nervous System (DENS) scale—To assess other potential neurological side effects of FRM-6308, animals were also assessed with the recently developed “Drug Effects on the Nervous System” (DENS) scale. This scale is used to rate the common side effects of psychoneurotropic drugs that can readily be assessed in primates, ranging from sedation/excitation to frequent motor and autonomic symptoms. The scale is composed of three categories, namely, cortical, extrapyramidal motor and autonomic functions (see detailed description of items and scoring in (Uthayathas et al., 2013).

¹⁸F-FDG PET imaging

In the present study, we performed ¹⁸F-fluoro-2-deoxy-D-glucose (¹⁸F-FDG) PET scans to determine the effects of FRM-6308 on brain activity following its systemic administration at two doses. In vivo ¹⁸F-FDG PET imaging is an excellent tool to examine the regional activation in the brain by measuring changes in glucose metabolism induced by the experimental drug (Buchsbaum et al., 2009; Murphy and Mackay, 2011). The experimental procedure for FDG-PET imaging in the monkeys has been described elsewhere (Uthayathas et al., 2014). Briefly, animals were chaired and given 10 min to acclimatize. Subsequently, FRM-6308 (0.1 or 0.32 mg/kg or vehicle) was administered subcutaneously (time 0). After 60 min, ¹⁸F-FDG was injected via an intravenous catheter into a superficial vein of the leg. After a 30 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 100 min from time 0, a 15 min transmission scan was obtained for attenuation correction, and at 120 min the FDG-PET scan began and continued for a total duration of 15 min (see 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 cut-off 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 interests (ROI) were manually drawn on the average image of each hemisphere (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 (OPFC) prefrontal cortex, and cingulate cortex (CC). 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 measure activity. FDG uptake was expressed as Standard Uptake Values (SUV), accounting for body mass and minor variations in 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), as well as 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 EVP 6308. Data were collected with a minimum interval of one week between each scan.

Statistical analysis

Scores of motor disability and CNS drug effects were graded within wide ranges; thus, data composed continuous variables. Two-factor analysis of variance (ANOVAs) for repeated measures followed by post hoc tests were used to compare behavioral data. Significance was taken at $p < 0.05$. All results are expressed as mean values $\pm$ S.E.M.

Results

3.1 Pharmacokinetics of FRM-6308

After subcutaneous administration, the plasma concentrations of FRM-6308 reached its peak between 1 and 3 h post-injection depending upon doses (Table 1), and declined slowly thereafter. At 1 h after doses of 0.1, 0.32 or 1.0 mg/kg, mean concentrations (C_p) of FRM-6308 were 31.5, 81.8 and 140 ng/mL, respectively, indicating a strong although not completely linear dose- C_p relationship at this time point across a 3-fold dose range. The unbound plasma concentrations were at and above the in vitro IC₅₀ (4-5 ng/mL) and the active concentrations in tests of conditioned avoidance responding (CAR; ~10 ng/mL), which would be in the target occupancy range. The mean plasma concentrations of FRM-6308 declined at 3 hr for 0.1 mg/kg or later for 0.32 and 1 mg/kg (Table 1).

3.2. Behavioral effects of FRM-6308

Motor Effects—Administration of FRM-6308 0.032, 0.1 or 0.32 mg/kg had no clear motor effects, as shown by invariable motor disability scores (Fig. 2). Although monkeys had a tendency to relax, their mobility was normal, and they did not exhibit other side effects or changes in social interaction. In contrast, 1 mg/kg of FRM-6308 changed the pattern of mobility in all 4 monkeys. A pronounced calmness was accompanied by reduction of the typical animal activity, which translated into score increases in the motor disability scale for 250 min after drug injection. Changes in subscales captured a more detailed characterization of the movement reduction. Posture and mobility were as much affected as the movements of each hand or leg (Fig. 2A-D). This pattern of changes that do not include typical motor effects of psychotropic drugs (bradykinesia or slowness of intended movements, rigidity and tremor, etc.) is indicative of primary sedative effects causing generalized low activity, and driving the global score increase in the primate motor disability scale (Fig. 2E). Motor disability scores (MDS) reached a maximum between 90 and 180 min after injection of FRM-6308 1 mg/kg, which corresponds to the peak plasma level in the monkey (Table 1).

Therefore, FRM-6308 induced mild dose-dependent sedation, but has no specific extrapyramidal effects as measured by MDS.

The two parameters measured in the Klüver Board test of coordinated hand movement were action time and index of success, and both were unchanged after injection of FRM-6308 at doses of 0.1 and 0.32 mg/kg (Fig. 2F). As expected, monkeys did not perform the task with the highest FRM-6308 dose (1 mg/kg) that had sedative effects. This task requires motivation/intention to move in addition to adequate movement coordination and speed. Normal scores in the Klüver Board test in absence of sedative effects (0.1-0.32 mg/kg FRM-6308) indicate spared fine motor performance. In the “Perch test”, postural stability and balance in continued climbing were unaffected by FRM-6308 injections. Compared to baseline, scores remained unchanged at the peak effect of FRM-6308 at the two doses selected for this test (0.1 and 0.32 mg/kg, s.c.).

Other behavioral effects—FRM-6308 induced dose-dependent effects on the nervous system of normal monkeys that were clearly demonstrated by several items in the DENS scale. Typical changes in brain functions that are commonly seen after administration of psychotropic drugs can be readily assessed with the DENS scale. FRM-6308 induced changes in total DENS scores only at the highest dose of 1 mg/kg in keeping with effects observed in the motor disability scale (Fig. 2). These total score changes derived predominantly from impaired *Attentiveness* and *Reactivity* (Figure 3A-B), which are the parameters most sensitive to sedative drug effects. Instead, eye movement that was mildly affected by FRM-6308 1 mg/kg (Fig. 3C) can be related to either extrapyramidal motor control or reduced alertness or sleepiness.

FRM-6308 at 1 mg/kg also induced mild oral dyskinesias (Fig. 3D). These dyskinesias were choreodystonic movements of the mouth and tongue with significant stereotypy and smacking. Although these characteristics match the common phenotype of tardive dyskinesias that are typically induced by neuroleptics, the involuntary movements observed with the higher dose of FRM-6308 cannot be classified as such since they were elicited by acute exposure to the drug. Moreover, repeated treatments with the same dose (1 mg/kg) had a tendency to reduce the appearance of oral dyskinesia. Also these oral dyskinesias were inconsistently expressed across monkeys (there was no effect in two monkeys and mild scores in the other two monkeys).

3.3 Functional effects of FRM-6308 in brain regions

The effect of FRM-6308 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 0.1 mg/kg FRM-6308 produced a significant increase in the FDG-SUV in both the striatal (Fig. 4A-D; $F_{15,181}$, $p < 0.05$) and cortical (Fig. 4E-H; $F_{16,069}$, $p < 0.05$) regions. No significant differences in FDG SUV following either of the two doses of FRM-6308 relative to control (vehicle) were found in other brain regions (globus pallidus, subthalamic nucleus, substantia nigra pars reticulata, thalamus and cerebellum). The increased activity seen with the lower dose (0.1 mg/kg) in striatal regions was not further augmented with the higher dose of 0.32 mg/kg (no significant difference)

although there was approximately a 3-fold change in plasma levels between these doses (Fig. 4D, H). These findings could indicate a ceiling effect on the cell metabolism reached with the lower dose, or a limitation of FDG-PET imaging to detect further metabolic differences. A critical point is that FRM-6308 doses that increased the striatal and cortical glucose uptake were not shown to produce side effects of any type. Also the increased activity seen with the lower dose (0.1 mg/kg) in cortical regions was not further augmented with the higher dose of 0.32 mg/kg with the exception of the dorsal prefrontal cortex (DPFC) where activity was slightly reduced. FDG-SUV in separate striatal regions (caudate nucleus, putamen associative and putamen motor areas) yielded some non-significant differences due to increased measurement variability in areas of small size, (Fig. 4D). The cortical effect is likely to be a product of network activity since the expression of PDE10A in the cortex is very low (Plisson et al., 2011). Thus, higher doses of FRM-6308 may be expected to produce extended and more varied network and cortical effects.

Discussion

FRM-6308 tests in normal nonhuman primates showed very low incidence of side effects that were only produced by high doses of the inhibitor, and primarily involved sedation. The escalating doses tested in this study were in the range of expected efficacy according to data obtained in rodent models of schizophrenia. Plasma levels of FRM6308 at the tested doses confirmed increasing exposures that could be expected to produce a dose-response curve. FRM-6308 administration also produced significant changes of metabolic activity in the brain (^{18}F FDG-PET) demonstrating brain penetrance even in the absence of sedative or other behavioral effects at the doses used in imaging tests. These changes corresponded to increased activity in the striatum and discrete cortical areas (mostly dorsal and orbital prefrontal cortex and cingulate) that are coherent with the drug inhibitory effect on striatal PDE10A and its impact on connected areas. Therefore, results demonstrated that doses of FRM-6308 producing significant exposures and correlated activity changes in target brain regions do not elicit the side effects commonly observed in primates with other antipsychotic agents. These findings suggest that FRM-6308 may have an advantageous profile as a new PDE10A inhibitor for clinical trials in schizophrenia.

The PK profile of FRM6308 may contribute to its improved pharmacodynamic profile. FRM-6308 at 0.1-1 mg/kg s.c. was rapidly absorbed, reached C_{max} rapidly between 1-3 hr, and its half-life was calculated between 4 and 6 hrs. These PK properties together with high selectivity for PDE10A and absence of sedative effects at doses up to 0.32 mg/kg given s.c. indicate that FRM-6308 is likely a PDE10A inhibitor with increased potency and selectivity than the previously reported agents. The selectivity for PDE10A results in more limited actions to the striatum and less unwanted effects produced by inhibiting the PDEs that are more widely distributed in the brain. On the other hand, the reduced toxicity together with efficacy at low doses (high potency) may increase the therapeutic index of FRM-6308. The comparison of active doses of MP-10 and FRM-6308 in similar tests of NHP shows that FRM-6308 has lower sedative effects and is less dyskinesigenic than MP-10 (Uthayathas et al., 2014). Overall, the pharmacological profile of FRM-6308 may be an advantage to demonstrate efficacy in clinical trials.

The comprehensive examination of motor behavior in these primate tests included the itemized parkinsonian motor disability, impairment of fine finger movements (motor dexterity) and sensible changes in balance and stability. These tests showed only effects of the highest dose tested, which induced clear sedation in the DENS measurements. Thus, FRM-6308 did not have specific effects on the motor system at s.c. doses up to 0.32 mg/kg in the primate. This is an important reference for a range of exposure that may demonstrate antipsychotic efficacy in clinical trials while proving safe and free of extrapyramidal side effects. At a dose of 1 mg/kg, FRM-6308 clearly produced mild sedation that globally affected the animal behavior. Nonetheless, this effect decreased in successive tests indicating the development of tolerance. Similarly, the expression of involuntary movements only with such higher dose tended to disappear with repeated tests. Notably, the dyskinesias induced by FRM-6308 were mild oral dyskinesias with predominant stereotypic movements, contrasting with the severe abnormal movements of the neck and trunk observed with other PDE10A inhibitors. Taken together, these primate data showing only sedation and mild dyskinesias of FRM-6308 high doses suggest significant advantages in the safety profile of this PDE10A inhibitor compared to typical antipsychotic drugs.

The development of second-generation antipsychotic drugs offered the benefit of milder extrapyramidal side effects compared to the acute dystonia, akathisia, parkinsonism and tardive dyskinesia that were frequently induced by classic agents. Nonhuman primates treated with antipsychotics show motor effects that closely resemble those seen in schizophrenia patients (Casey, 1989) including the characteristic human parkinsonism (Jenner, 2003). The neuroanatomical and functional similarities of the motor system between humans and nonhuman primates provide the context for high resemblance of motor effects of drugs between these species (Emborg, 2007; Potts et al., 2014). Results of FRM-6308 tests in primates, therefore, underscore the potential for major differences between selective PDE10A inhibitors and other drug classes in their impact on the motor system of schizophrenic patients.

Part of the improved side effect profile of selective PDE10A inhibitors may rely on the restricted action on striatal neurons that express this isoenzyme. Indeed, PDE10A is expressed at high levels in medium spiny neurons of the striatum (Coskran et al., 2006; Seeger et al., 2003; Xie et al., 2006), but outside the striatum, its expression is minimally detected (Coskran et al., 2006; Seeger et al., 2003). The present FDG-PET data showed significant activity changes in cortical regions likely revealing network effects resulting from the direct action of FRM-6308 in the striatum. Similar imaging changes have been reported with other PDE10A inhibitors (Uthayathas et al., 2014). In the striatum, PDE10A is largely distributed in medium spiny neurons, which are the striatal projection units and 95% of striatal neurons. This vast distribution pattern indicates that PDE10A inhibitors influence both striatal output pathways and globally the BG circuitry (Gresack et al., 2014; Megens et al., 2014; Strick et al., 2010), which is consistent with their predicted antipsychotic efficacy (Carlsson, 2001; Goff and Coyle, 2001; O'Donnell and Grace, 1998). Moreover, the analysis of single circuit activation has recently demonstrated that both direct and indirect striatal output pathways act in coordinated motion, highlighting the notion that these two information streams function cooperatively rather than competitively (Cui et al., 2013). Clearly, the action on both striatal output pathways confers PDE10A inhibitors a unique

mechanistic profile that differs anatomically from the specific D₂ antagonistic antipsychotics acting predominantly on the indirect striatal output (Grauer et al., 2009; Schmidt et al., 2008).

The increased levels of cAMP and cGMP as a result of PDE10A inhibition have significant effects on the cell signaling mechanisms. It has been shown that pharmacological inactivation of PDE10A or transgenic knockout of the PDE10A gene lead to accumulation of cAMP and cGMP and changes in the excitability of striatal neurons (Grauer et al., 2009; Strick et al., 2010; West and Grace, 2004). Under physiological conditions, drugs that augment cAMP or cGMP levels in medium spiny neurons (i.e.: PDE inhibitors or adenylyl cyclase activators) facilitate spontaneous and evoked cortico-striatal transmission (Colwell and Levine, 1995; Padovan-Neto et al., 2015; Threlfell et al., 2009; West and Grace, 2004). Conversely, drugs that decrease cyclic nucleotides (i.e.: cyclase inhibitors) or drugs that block protein kinase activity (PKA) have opposite effects (Colwell and Levine, 1995; Sammut et al., 2010; Yang et al., 2014). For example, forskolin, an adenylyl cyclase activator, enhances the amplitude and duration of cortically-evoked glutamatergic synaptic responses, and increases the excitatory effects of glutamate receptor agonists (Colwell and Levine, 1995). In contrast, PKA inhibition can eliminate NMDA receptor-mediated plateau depolarizations (Tseng et al., 2007). Taken together, these data indicate that activation of cAMP signaling by blocking PDE10A in striatal projection neurons facilitates corticostriatal transmission and potentiates the excitatory effects of AMPA and NMDA receptor signaling. The behavioral effects of PDE10A inhibitors have been attributed to a greater impact on indirect striatal pathway neurons (Kehler and Nielsen, 2011; Nishi et al., 2008; Nishi et al., 2011; Polito et al., 2015; Threlfell et al., 2009; Wilson et al., 2015). Simpson and colleagues reported that increased D₂ receptor activity in the striatum may alter cognition by disrupting the integration of information from cortical projections (Simpson et al., 2010). However, PDE10A inhibition effects on cAMP signaling has significant impacts on D₁- and D₂-receptor expressing striatal projection neurons. These effects have proven to influence largely motor and cognitive function including the attentional and motivational components in rodent and non-human primate studies (Smith et al., 2013). Thus, the ability of PDE10A inhibitors to modulate signaling mechanisms in both striatal pathways may be the key property for their efficacy and safety profiles. Although the present results do not address the specific effects of FRM-6308 on cognitive functions, the mechanisms of action of this class of drugs warrant further studies to determine extended efficacy against the diverse nature of schizophrenia symptoms.

Conclusion

The present results of FRM-6308 tests in normal nonhuman primates demonstrated no side effects at doses that already induced clear changes in brain activity and, at higher doses, mild sedative and occasional dyskinetic adverse effects. These data provide significant information about the improved side effect profile of selective PDE10A inhibitors in comparison with available antipsychotic agents. The specific effects on both striatal output pathways and cortical networks associated with basal ganglia outputs shown by FDG-PET imaging provide support to the potential efficacy of these inhibitors for positive, negative and cognitive symptoms of schizophrenia. It is important to note that here FRM-6308 effects

in the primate were only assessed following acute administration. However, the present results suggest that chronic FRM-6308 treatment may result in better tolerance of higher doses. Finally, future mechanistic studies of PDE10A inhibitor effects will undoubtedly provide insights into the BG-cortical regulation of motivation, attention and other aspects of cognitive function.

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Highlights

- A new PDE10A inhibitor is profiled in primates as a candidate for antipsychotic therapy.
- FRM-6308 is highly selective and potent for PDE10A, and has good PK profile.
- FRM 6308 induced only mild sedation and dyskinesia at the highest dose tested (1 mg/kg).
- A range of (s.c.) doses of FRM-6308 has shown excellent tolerability in the primate.
- Non-sedative doses of FRM 6308 increased the striatal activity as measured by FDG-PET.

Figure 1.

Experimental design of PET imaging study. A. Timeline of PET scans. Experiments began with chairing the monkey and allowing habituation to be comfortably in the chair. Subsequently, the monkey received the subcutaneous injection of FRM-6308 or vehicle (time 0). Fifty minutes post FRM-6308 injection, an intravenous catheter was placed in the external saphenous vein, and FDG was injected via the catheter. The interval between s.c. FRM-6308 injection and i.v. FDG injection was 60 min. The monkey was anesthetized at 90 min and placed in the PET scanner. A 15 min transmission scan was obtained for attenuation correction starting at 100 min. At 120 min from FRM-6308 injection, the FDG scan began and continued for a period of 15 min. B. Regions of interests (ROI) were manually drawn on the average image of each hemisphere based on the functional topography of corticostriatal projections in monkey: putamen/associative (PA), putamen/motor (PM), caudate nucleus (CA), nucleus accumbens (AC), dorsal (DPFC), medial (MPFC), ventral (VPFC) and orbital prefrontal cortex (OPFC), cingulate cortex (CC). Scale bar = 10 mm.

Figure 2.

Motor effects of FRM-6308. Motor disability produced by FRM-6308 doses from 0 to 1.0 mg/kg, s.c. is shown by the scores obtained in several individual items of the scale (standardized Motor Disability Scale for parkinsonian non-human primates): Posture (A), Mobility (B), Hand Movement (C), and Leg Movement (D). The values of hand (C) and leg (D) movements represent the addition of scores from each hand and each leg. The “total” Motor Disability Score (E) was obtained after addition of all individual item scores of the scale. The baseline score was obtained just before drug injection (time 0). After FRM-6308 injection, scoring starts at 30 min and continues thereafter every 20 min. Values are the average score at each interval for the animal group. Results of the motor dexterity test following FRM-6308 doses from 0 to 1.0 mg/kg, s.c. are shown by the action times (F) recorded before (baseline) and 90 min after drug injection as the average value for the

animal group. Monkeys did not perform the task (up to the maximal time of 60 min) in tests of FRM-6308 1.0 mg/kg s.c. due to sedation. * $p < 0.01$ versus same time point in the control vehicle injection (ANOVA for repeated measures followed by post hoc Tukey test). Data points are mean ($n = 4$ monkeys) $\pm$ SEM.

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Figure 3.

Other neurologic effects of FRM-6308. Other neurological effects produced by FRM-6308 doses from 0 to 1.0 mg/kg, s.c. (E) are shown by the scores obtained in several individual items of the DENS scale (Drug Effects on the Nervous System scale): Attentiveness (A), Reactivity (B), Eye Movements (C), and Dyskinesias (D). The “total” DENS score (E) was obtained after addition of all individual item scores of the scale. The baseline score was obtained just before drug injection (time 0). FRM-6308 0.32 mg/kg occasionally induced mild nausea (non-significant difference). After FRM-6308 injection scoring starts at 30 min and continues thereafter every 20 min. Values are the average score at each intervals for the animal group. * $p < 0.01$ versus same time point in the control vehicle injection (ANOVA for repeated measures followed by post hoc Tukey test). Data points are mean ($n = 4$ monkeys) $\pm$ SEM.

Figure 4.

Standard uptake values (SUV) of FDG in the monkey brain following FRM-6308 administration. Examples of ^{18}F -FDG PET images of striatal (A-C) and cortical (D-F) regions correspond to pre-commissural coronal planes of the same monkey following the administration of 0 (vehicle), 0.1, and 0.32 mg/kg of FRM-6308. Increased metabolic activity is seen throughout striatal and frontal cortical brain areas following administration of FRM-6308. Scale bar = 10 mm. Comparison of FDG-SUV changes following each dose of FRM-6308 is shown by the average value for the animal group in the striatal (D) or

cortical (H) ROIs. * $p < 0.01$ versus control (ANOVA for repeated measures followed by post hoc Tukey test). Data points are mean ($n = 4$ monkeys) $\pm$ SEM. 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.

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Table 1

Plasma concentration of FRM-6308 in monkeys after single drug administration

FRM 6308 dose mg/kg s c	Time (hrs)				
	0	1	2	3	7.5
0.1 mg/kg	0	31.478 (3.1252)	24.688 (3.638)	12.964 (1.3271)	3.6126 (0.454)
0.32 mg/kg	0	81.772 (18.946)	87.072 (22.46)	105.33 (13.413)	29.529 (8.1483)
1 mg/kg	0	139.9 (49.869)	183.17 (30.785)	199.33 (25.859)	41.183 (4.9827)

Plasma concentrations of FRM-6308 are given in ng/ml. Data are the averaged FRM-6308 concentrations from three animals. In each animal, the PK experiment for each dose was repeated once, and thus, individual animal data were obtained averaging results from two blood samples. Values are the average for the animal group $\pm$ SEM. There was no significant difference in FRM-6308 plasma concentrations between 1 and 2 hrs with either dose of FRM-6308