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Dose-related target occupancy and effects on circuitry, behavior, and neuroplasticity of the glycine transporter-1 inhibitor, PF-03463275, in healthy and schizophrenia subjects

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Abstract

Background—Glycine transporter-1 (GlyT1) inhibitors may ameliorate cognitive impairments associated with schizophrenia (CIAS). The dose-related occupancy and target engagement of the GlyT1 inhibitor, PF-03463275 were studied to inform optimal dose selection for a clinical trial for CIAS.

Methods—In substudy #1, the effects of PF-03463275 (10, 20, and 40 mg BID) on occupancy of GlyT1 were tested using PET and ¹⁸F-MK-6577, and visual long-term potentiation (LTP) in schizophrenia patients (SZs) and healthy subjects (HSs). Furthermore, the capacity of PF-03463275 to attenuate ketamine-induced disruption of working memory-related activation of a “working memory” circuit was tested only in HSs using fMRI. Subsequently, the effects of PF-03463275 (60 mg BID) on occupancy of GlyT1 and LTP were examined only in SZs (substudy #2).

Results—PF-03463275, 10 mg, 20 mg, 40 mg, and 60 mg BID produced ~44%, 61%, 76%, and 83% GlyT1 occupancy respectively in SZs with higher ligand binding to GlyT1 in subcortical versus cortical regions. PF-03463275 did not attenuate any ketamine-induced effects but did improve working memory accuracy in HSs. PF-03463275 increased LTP only in SZs with peak effects at 40 mg BID (~75% GlyT1 occupancy) and with a profile suggestive of an inverted ‘U’ dose response. PF-03463275 was well-tolerated.

Conclusions—The dose-related GlyT1 occupancy of PF-03463275 is linear. While PF-03463275 did not show evidence of facilitating NMDA-R function in the ketamine assay, it enhanced neuroplasticity in SZs. Together, these findings provide support for a clinical trial to test the ability of PF-03463275 to enhance CR towards addressing CIAS. [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01911676) (NCT01911676)

Introduction

Glycine transporter-1 (GlyT1) inhibitors are among the most promising medications for the treatment of schizophrenia (1). These drugs may attenuate the impact of *N*-methyl-D-aspartate receptor (NMDA-R) functional deficits associated with schizophrenia (2). In animals, GlyT1 inhibitor enhances NMDA-R function and NMDA-R-related neuroplasticity (3, 4). GlyT1 inhibitors reduce NMDA-R antagonist-induced behavioral effects in rodents (5), working memory deficits in non-human primates (6), and ketamine-induced psychotomimetic effects in humans (7).

Though the GlyT1 inhibitors sarcosine (8, 9) and bitopertin (10) showed evidence suggestive of efficacy in clinical trials, definitive trials of bitopertin failed to replicate this effect (11). These negative results did not preclude the possibility that GlyT1 inhibitors could play a role in the treatment of schizophrenia. First, it was not clear whether the failed trials achieved optimal occupancy of GlyT1. In part, this was because it is challenging to identify the optimal degree of occupancy for a drug class that may have a non-linear (“inverted-U”) dose-response curve (12). Second, it might be possible that GlyT1 inhibitors could enhance neuroplasticity rather than reduce symptoms directly (13). Studies conducted to date were designed to detect effects of GlyT1 inhibition on symptoms, effects that could emerge from normalizing the schizophrenia-related dysregulation of circuits. Yet the neurobiology of schizophrenia is complex and it is not clear that GlyT1 inhibition could accomplish this broad objective, even with normalization of NMDA-R signaling, without accompanying therapies to guide functional change.

Recent studies in schizophrenia have focused on a biomarker associated with an NMDA-R-dependent form of neuroplasticity, long-term potentiation (LTP) (14). GlyT1 inhibitors enhance LTP in animals (3) and might promote neuroplastic capacity that could facilitate other forms of therapy e.g., by enhancing neuroplasticity, GlyT1 inhibitors might augment the effectiveness of cognitive remediation (CR).

This study was conducted to inform the design of a clinical trial evaluating the capacity of the GLYT1 inhibitor PF-03463275 to enhance CR for treating the cognitive impairments associated with schizophrenia (CIAS). The first aim of the study was to establish the dose occupancy relationship of PF-03463275 using Positron Emission Tomography (PET) and the GYT1 specific radiotracer ^{18}F -MK-6577 (15) in both schizophrenia patients (SZs) and healthy subjects (HSs). The second aim of the study was to demonstrate target engagement by testing the dose-related ability of PF-03463275 to attenuate the ketamine-associated reduction of activation in a pre-defined working memory (WM) network during the encoding and early maintenance (EEM) phase of working memory in HSs, as measured by blood oxygenated level-dependent (BOLD) signal, the contrast agent in functional magnetic resonance imaging (fMRI). As a third aim we tested effects of PF-03463275 on NMDA-mediated neuroplasticity using an electroencephalographic (EEG) assay of long-term potentiation (LTP).

METHODS AND MATERIALS (See supplement for details)

General Study Design

Healthy subjects (HSs) and schizophrenia subjects (SZS) completed 3 treatment phases each lasting 1 week in duration, separated by at least 1 week washout (fig. S1A, table S1A). Subjects received placebo and only 2 of 3 possible doses of (10, 20 or 40 mg BID) PF-03463275 under double-blind, randomized conditions in a crossover design (substudy #1). On the 6th day of each treatment phase when PF-03463275 was at steady state, all SZS and a sub-set of HSs underwent a PET scan followed by assessment of LTP. In HSs only, a ketamine-fMRI study was conducted on the last day of each treatment phase.

The results of substudy #1 led to substudy #2 that was conducted to determine receptor occupancy and LTP at a higher dose of PF-03463275 (fig. S1B, and table S1B). For this, only SZS received either PF-03463275 placebo or 60mg BID in random order for 7 days with PET and LTP tested on Day 6.

Regulatory Approvals

The study had Institutional Review Board and U.S. Food and Drug Administration (IND #118880) approvals and was registered on clinicaltrials.gov (Text S1).

Subjects

SZS and HSs were recruited according to specific criteria (Text S2a and b) after a comprehensive screening process (Text S3). In order to reduce known variability in PF-03463275 plasma levels, only subjects genotyped as P450 2D6 extensive metabolizers were included (Text S4) and in substudy #1 the only permissible antipsychotics were risperidone and aripiprazole.

Drugs (Text S5)

PF-04958242—For substudy #1, subjects were randomly assigned to one of three treatment groups (A: placebo, 10 mg, and 20 mg BID; B: placebo, 10 mg, and 40 mg BID; C: placebo, 20 mg, and 40 mg BID) (Text S6). Thus, each subject received only two of the possible three doses of PF-03463275. Within each group, subjects were randomized to one of the six possible medication orders so that there was at least one and no more than two subjects assigned to every possible order. For substudy #2 (fig. S1B) SZS participated in two treatment periods where they received PF-03463275 60 mg BID or placebo in random order for 7 days.

Ketamine—The ketamine dose (0.23 mg/kg bolus over 1 minute followed by 0.58 mg/kg/hour constant infusion for ~45 minutes) was selected as it been shown to safely produce an array of transient schizophrenia-relevant effects including working memory deficits and psychotomimetic effects (14, 16-20), and to reduce working-memory related activation as measured by fMRI (21, 22).

PET study of GlyT1 Occupancy by PF-03463275 (Text S7)—A high quality 3-D MPRAGE MRI was obtained for coregistration. In substudy #1, the dose-related occupancy of GlyT1 by PF-03463275 (10, 20, and 40 mg BID vs. placebo) was evaluated in HSs and SZs using ¹⁸F-MK-6577 on the High Resolution Research Tomograph (HRRT). Distribution volume (V_T) was estimated using the one-tissue and two-tissue compartment models (1TC and 2TC) and multilinear analysis 1 (MA1) for each scan. Transporter occupancy (Occ) was derived from a graphical occupancy plot and related to PF-03463275 dose (or PF-03463275 plasma concentration) to determine the drug dose (or drug concentration) that achieves 50% of the maximum occupancy (ID_{50} and IC_{50}) (Text S7). In substudy #2 the occupancy of GlyT1 by PF-03463275 (60 mg BID) was studied only in SZs. ID_{50} and IC_{50} values derived from only HSs, only SZs, and all subjects were compared.

fMRI study of PF-03463275 effects on working memory circuits (Text S8)—In substudy #1, the dose-related capacity of PF-03463275 at steady state levels to attenuate ketamine-induced disruption of a predefined cortical “working memory” circuit was tested only in HSs with fMRI using a previously developed procedure (21). On the 7th day of each treatment phase, subjects received saline followed by ketamine in a fixed sequence, in order to avoid potential carryover effects. For the first scan, subjects fixated on a cross projected on a screen. Approximately 75 seconds into the scan, they received a saline bolus for one minute and then a constant saline infusion for the rest of the saline portion of the scan. During saline infusion, they completed 8 runs of the spatial working memory (SWM) task and a resting run. They then received a ketamine bolus and ketamine constant infusion prior to repeating the SWM task. Before and after each scanning session, they were assessed with the Positive and Negative Symptom Scale (PANSS)(23), the Clinician-Administered Dissociative States Scale (CADSS)(24), and the Hopkins Verbal Learning Test (HVLTL)(25), a list learning task. Blood was sampled periodically to assay plasma ketamine, norketamine, and PF-03463275 levels, and vital signs were continuously recorded.

PF-03463275 effects on neuroplasticity (Text S9)—LTP was tested in an experimental procedure during EEG recording. The experiment was based on a conventional two-stimulus visual oddball task (fig. S2). Two blocks of standard trials (Pre 1, Pre 2) were first administered to establish a baseline, followed by a block of high-frequency photic stimulation used to potentiate the visual-evoked potential (VEP) in a manner similar to tetanic stimulation in classical LTP (14, 26-28). Post-tetanus blocks were administered following the identical format as baseline and evaluated as tests of change from pre-tetanus VEP amplitude. Further details on the task and stimulus parameters are provided in supplement (text S9). In substudy #1, LTP was compared across PF-03463275 dose (placebo, 10 mg, 20 mg and 40 mg BID) both in HSs and SZs, while in substudy #2, LTP was compared across PF-03463275 dose (placebo and 60 mg BID) only in SZs (fig. S1b).

PF-03463275 and ketamine levels were assayed using methods described in text S10.

Medication Adherence (Text S11)—At each visit adherence was monitored with weekly pill counts. Only in substudy #2, adherence was visually confirmed using Cellphone Assisted Remote Observation of Medication Adherence (CAROMA) (29).

Safety Assessments (Text S12)—Physical and neurological examinations, laboratory tests, vital signs, EKG, and adverse events were collected during the study treatment periods.

Statistical Analyses—Details of the statistical approach to analysis of the PET, fMRI and LTP is presented in the Supplement.

PET data (Text S7)—The primary endpoint was volume of distribution (V_T) and non-displaceable distribution volume (V_{ND}). Differences in V_T (V_{ND}) between groups were analyzed using a 2-tailed t test. The F test was used to evaluate the goodness of fits by the 1TC and 2TC models, the two occupancy models with one (ID_{50} or IC_{50}) or two parameters (I_{max} and ID_{50} or IC_{50}). To test whether the ID_{50} or IC_{50} differs between groups, the occupancy model was separately fitted to each group data set and globally fitted to all the

data sets at once, sharing ID_{50} or IC_{50} . The residual sums of squares were compared using the F test. A P value of less than 0.05 was considered statistically significant.

fMRI data (Text S8)—The primary endpoint was working memory-related activation measured by fMRI. Additional endpoints of interest were SWM accuracy and reaction time, HVLIT immediate and delayed recall, and PANSS positive, negative and cognitive subscales scores. Dose was the originally specified dependent variable but because of variability within each dose (Table S7), PF-03463275 plasma level was also evaluated. Models for each endpoint contained infusion (ketamine, saline) and period (1,2,3) as factors. A non-parametric approach for repeated measures was used to analyze PANSS scores since there were floor effects for these data. The model for SWM performance contained an additional factor for trial type (2-item, 4-item, control) and the model for task-related activation contained factors for trial type and region of interest (ROI). We initially analyzed a 5-ROI model based on our piloting with glycine that contained anatomically pre-defined working memory areas. Additional analyses were conducted using a 3-ROI model based on our published ketamine work (30). For HVLIT immediate recall, trial (1, 2, 3) was included as a factor.

LTP data (Text S9)—LTP was defined by change in N100 (negative deflection ~ 100ms post-stimulus, aka C1) VEP amplitude from pre-tetanus baseline (average of Pre 1 and Pre 2) to the test block beginning 18-minutes post-tetanus (Post 18; fig. S2). A single LTP value was computed for statistical analysis based on the difference in Post18 Visual Evoked Potential (VEP) amplitude from predicted values obtained by regressing Post18 on individual pre-tetanus baselines. The residual gain score (31) was standardized across samples and conditions to produce an index of change that accounts for individual differences in VEP amplitude while estimating absolute gain for each test occasion relative to all others. Substudy #1 was analyzed using linear mixed models fit to assess the main effects Condition (baseline, placebo, 10mg, 20mg, 40mg), Group (SZS vs. HC), and Condition by Group interactions. A similar approach was used to analyze data in substudy #2 but only tested the within-group (SZS) effect of Condition (baseline, placebo, 60mg). The best-fitting variance-covariance structure was selected based on Schwartz-Bayesian Information Criterion. Bonferroni correction was applied to pairwise contrasts of simple effects. Significant fixed effects of group and interactions were followed by analyses of condition within each group. Period effects were tested but were dropped from final analysis as non-significant. Linear and quadratic relationships between LTP and drug plasma concentration were examined using regression curve fitting.

RESULTS

Complete data was obtained in 24 HSs and 9 SZs in substudy #1, and 10 SZs in substudy #2 (Fig. S3; Table S3). The major reason for ineligibility was genetic nonmatch (Table S2). The subjects were predominantly males (Table S3).

PET occupancy results

Arterial blood samples were not available for 10 scans, and 1 scan was aborted due to subject anxiety (Table S4). The data from these scans (20% of the total) were excluded from the analysis.

Plasma analysis—The parent fractions for the placebo scans ($n = 13$) were $32\% \pm 7\%$ and $21\% \pm 5\%$ at 15 and 60 min post-injection, respectively (Fig. S4). The plasma free (unbound protein) fraction (f_p) of ^{18}F -MK-6577 in the plasma for the placebo scans ($n = 13$) and the 40 mg blocking scans ($n = 10$) was $6.3\% \pm 1.4\%$ and $5.7\% \pm 1.2\%$, respectively.

^{18}F -MK-6577 in human brain—Averaged uptake images at the placebo and 40 mg blocking scans were determined (Fig. 1). Time activity curves (TACs) (Fig. S4) for representative brain regions revealed highest uptake of ^{18}F -MK-6577 in the pons, midbrain, and cerebellum white matter; intermediate uptake was observed in the thalamus, centrum semiovale, and cerebellum gray matter, and the lowest uptake was in the cerebral cortex (Fig. S5). Substantial reductions were seen in ^{18}F -MK-6577 activity levels with exposure to PF-03463275.

The TACs were fitted with the MA1 model (Fig. S5) which produced stable kinetic parameters. Regional distribution volumes (V_T) averaged over all subjects at different doses of PF-03463275 (Table S5) revealed a clear dose-dependent reduction in V_T was observed in all brain regions (Figure 2). Occupancy values for all subjects are presented (Table S6). Using the average nondisplaceable volume of distribution (V_{ND}) values across all subjects, baseline binding potential (BP_{ND}) were calculated and ranged from 1.24 ± 0.54 (caudate) to 4.82 ± 0.94 (pons) in HSs. Thus, more than half of the signal in the cortical regions can be attributed to specific binding (i.e., $BP_{ND} > 1$), so occupancy of specific sites can be measured well.

The ID_{50} and IC_{50} values were determined from the occupancy vs. PF-03463275 dose or plasma concentration relationships, assuming 100% occupancy could be reached (the model allowing $< 100\%$ maximum occupancy was not statistically better). Figure 3A illustrates the relationship between the dose (mg/kg) and transporter occupancy. The estimated ID_{50} was 0.26 ± 0.03 mg/kg for all subjects. ID_{50} values were separately estimated by group (HSs and SZs, Fig. 3B–C). ID_{50} from HSs (0.38 ± 0.06 mg/kg) was significantly higher ($P = 0.0004$) than that from the SZS group (0.18 ± 0.02 mg/kg). Thus, based on the SZs ID_{50} value, for a 70-kg patient, doses of 10, 20, 40, and 60 mg BID PF-03463275 produce GlyT1 occupancies of 44, 61, 76, and 83%, respectively. The relationship between plasma PF-03463275 concentration and transporter occupancy is illustrated in figure 3D. Estimated IC_{50} value was 12.3 ± 1.0 ng/mL for all subjects. As in ID_{50} value, IC_{50} from HSs (13.6 ± 1.5 ng/mL, Fig. 3E) was higher than that from SZs (10.8 ± 1.3 ng/mL, Figure 3F), however this difference was not significantly different ($P = 0.19$). This suggests that occupancy differences between HC and SZS groups are due to group differences in plasma PF-03463275 concentrations, i.e., bioavailability differences.

Ketamine fMRI results

Ketamine significantly reduced activation (BOLD response), accuracy and increased reaction time during the spatial working memory task (Table S7). Plasma ketamine and norketamine levels increased (Figure S6) and plasma PF-03463275 increased with dose (Figure S7). Ketamine also produced significant increases in PANSS subscale scores, and impaired recall on the HVLT (Table S7). However, PF-03463275 did not attenuate any of the ketamine-induced effects reported above. These negative results were further confirmed in the analysis using plasma PF-03463275 level rather than dose as the independent variable.

Though PF-03463275 did not ameliorate ketamine-associated deficits in reaction time or accuracy on the SWM task, it did appear to improve performance regardless of whether participants were receiving ketamine or saline. In the dose analysis, post-hoc comparisons indicated that the 40 mg BID dose as compared to placebo significantly improved SWM accuracy, $t(54.5) = 2.99$, $p = 0.013$. PF-03463275 dose did not affect RT. The plasma PF-03463275 analysis confirmed that higher levels of PF-03463275 were associated with improved SWM accuracy, $F(1,39.6) = 7.54$, $p = 0.0009$. Higher levels of PF-03463275 were also associated with increased RT, $F(1,39.4) = 6.12$, $p = 0.02$.

LTP results

For the LTP task there was a small percentage of dropouts (Table S8). At baseline assessment (i.e., prior to PF-03463275 treatment), VEP amplitudes were statistically equivalent between HSs and SZs in substudy #1, both before and after high frequency photic stimulation (Table S9). Similarly, baseline VEP amplitude did not differ between the SZS samples in substudies # 1 and #2 (Table S10). Furthermore, contrary to expectation for LTP-like enhancement of VEPs following stimulation, this effect was not evident in mean values for HC or in either SZS sample at baseline (Table S11).

In substudy #1 there was a significant effect of PF-03463275 dose [$F(4, 85.48) = 4.15$, $p = .004$] on LTP interacting with Group [$F(4, 85.48) = 2.86$, $p = .028$]. Pairwise contrasts revealed significant differences between the 40mg condition and pre-drug baseline ($p = .015$), placebo ($p = .004$) and 20mg ($p = .019$) conditions. Repeating the analysis in each group separately confirmed that PF-03463275 effects were explained entirely by responses in SZS [$F(4, 25.12) = 3.20$, $p = .030$], with no detectable effect of PF-03463275 dose in HSs [$F(4, 62.18) = 0.52$, $p = .72$]. Testing of simple effects within SZS, regarded as confirmatory and conducted without correction for multiple comparisons, found that LTP was higher at 40mg dose than in all other conditions (p values .048 - .002), while the 10mg and 20mg dose levels were statistically equivalent to placebo and baseline. Substudy #2 (PF-03463275 60mg vs placebo only in SZs) detected no differences between PF-03463275 at 60 mg and placebo or baseline [$F(2, 14) = 1.41$, $p = .28$] (Fig. 4). Group average responses across conditions (Fig. 5) suggested that Pre-Post HFS VEP enhancement followed an inverted dose-response function in SZs with maximal effect at 40mg, and returning to baseline level when the dose was increased to 60mg.

Relationships between LTP and PF-03463275 estimated target occupancy (ETO) (Text S13) was evaluated based on plasma-predicted values (Fig. 6). Given the statistical equivalence in

LTP between baseline and placebo tests, only the placebo condition was included as a comparator for drug effects in this analysis. In substudy #1, across PF-03463275 doses and groups, a modest linear association was observed suggesting more effective LTP with higher ETO [$R = .21$, $B = -.58$, $F(1,84) = 3.81$, $p = .05$]. When HCs and SZs were considered separately, the strength of linear association increased in SZs [$R = .47$, $B = -.116$, $F(1,21) = 6.06$, $p = .02$], but did not show a significant relationship in HCs [$R = .12$, $B = -.35$, $F(1,61) = .92$, $p = .34$]. When all data in SZS were pooled from substudies #1 and #2 (i.e., placebo, 10, 20, 40 and 60 mg) the model was best explained by a quadratic function, though this relationship only achieved trend-level significance [$R = .36$, $B1 = -2.26$, $B2 = 2.26$, $F(2,35) = 2.63$, $p = .09$].

DISCUSSION

The PET occupancy study revealed that GlyT1 occupancy by PF-03463275 increased linearly (Fig. 3), consistent with the classic occupancy model. The highest dose tested (60 mg BID) did not saturate the binding site. The quality of the fit to the occupancy model was improved when using the plasma concentration of PF-03463275 (Figs. 3D–F) compared to the relationship based on dose (Figs. 3A–C). This suggests that the conventional direct relationship between plasma concentration and occupancy is valid, and that there is some intersubject variability in the plasma levels at the same dose within each group. Interestingly, there was a group difference in the occupancy between HSs and SZs at a given dose. However, this difference was eliminated when accounting for the plasma drug levels, i.e., SZs had higher plasma levels than the HC at the same dose. This suggests that the clearance and/or metabolism of the drug was slower in SZs. Of note, in testing the highest dose, medication adherence was monitored, perhaps contributing to less pharmacokinetic variability and a tighter dose-occupancy relationship. These results suggest that plasma drug levels rather than dose should always be used to predict occupancy, and that dosing decisions for a patient group should not be directly extrapolated from occupancy values measured in HSs.

PF-03463275 pretreatment improved working memory accuracy regardless of whether subjects received saline or ketamine, an effect that requires replication and further exploration. However, PF-03463275 did not prevent the disruptive effects of ketamine on activation of a predefined working memory circuit during the encoding and early maintenance phases. This negative finding is unlikely due to “assay sensitivity” as ketamine attenuated task-related activation, as observed in prior studies (30) (22). It is consistent with the lack of efficacy of ORG-25935, a GlyT1 inhibitor, on ketamine-induced working memory impairment in HSs (32). However, it contrasts with non-human primate (NHP) studies in which PF-03463275 and ORG-25935 reduced ketamine effects on working memory (12, 33). The reasons for the differing findings in the human and NHP studies are not clear, and might include: 1) the NHPs were over-trained on the working memory task, while the humans were not, 2) the humans were tested during ketamine infusion, while the NHPs were tested 15 minutes following ketamine injection, and 3) there may be species-related differences in the responses to these medications. Also, in contrast to the reported effects of ORG-25935 (32), PF-03463275 did not reduce the psychotomimetic effects of ketamine. In contrast to the ORG-25935 study (32) psychosis ratings in the current study

were retrospective and conducted after termination of the ketamine infusion, when symptoms were abating spontaneously; perhaps reducing the sensitivity to detect antipsychotic effects.

PF-03463275 enhanced neuroplastic capacity in SZs as estimated by a visual LTP (26). A significant increase in LTP was detected at 40mg BID relative to comparison conditions. However, increasing dose to 60mg in substudy #2 produced no appreciable change from baseline. When examined in relation to plasma-predicted occupancy, an inverted U dose-response function was suggested (Fig 6, panel B), with peak efficacy achieved at approximately 60-80% occupancy. This finding suggests that dose selection of GlyT1 inhibitors is important with optimal dosing between 60%–80% occupancy of GlyT1. Pro-cognitive effects of GLYT1 inhibitors have proved elusive(1) and the current failure of PF-03463275 to increase neuroplasticity in HSs may be consistent with this. However, the dose-dependent improvement of WM accuracy observed in the fMRI study suggests that GLYT1 inhibitors might improve some aspects of cognition. Though PF-03463275 did not affect LTP in HSs but did so in SZs, the LTP assay appears sensitive to drugs acting via the NMDA-R glycine site, and may serve as a biomarker for dose-efficacy research. Therefore, the current findings of this placebo-controlled multi-dose study, are the first to our knowledge demonstrating pharmacologic enhancement of LTP in schizophrenia and support the study of PF-03463275 for treatment of CIAS in combination with CR.

The primary aim of this study was to evaluate the hypothesis that GlyT1 inhibition by PF-03463275 was a reasonable strategy to enhance neuroplasticity in schizophrenia and to select the optimal dose to achieve this aim. Overall, this aim was achieved. This study demonstrated robust dose-related occupancy of GlyT1 by PF-03463275. While PF-03463275 failed to modify ketamine effects it produced a dose-related improvement in working memory accuracy in HSs. PF-03463275 also increased LTP in SZs at a dose that occupied ~75% of GlyT1 sites. The enhancement of LTP in SZs suggests that GlyT1 inhibition might be a strategy to promote a putatively NMDA-R-dependent form of neuroplasticity during cognitive remediation and other rehabilitative treatments. However, the current data question the utility of the reversal of ketamine effects as an assay to detect these effects of NMDA-R signaling. Finally, PF-03463275 was well-tolerated at all doses. Together, these results support testing PF-03463275 for its ability to increase neuroplasticity in SZS with the aim of facilitating CR in a clinical trial for CIAS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A: MR images. B-C: PET images summed from 40 to 90 min after injection of ^{18}F -MK-6577, averaged over B: 13 subjects at a placebo scan and C 10 subjects at steady state of 40 mg BID PF-03463275. Activity is expressed in SUV.

Figure 2.

Regional distribution volumes (V_T) of ^{18}F -MK-6577 at placebo, and at steady state of 10 mg, 20 mg, and 40 mg, and 60 mg BID PF-03463275. Bars indicated mean $\pm$ SD (Cbl WM [cerebellum white matter], MID [midbrain], CSO [centrum semiovale], Cbl GM [cerebellum gray matter], PUT [putamen], CCX [cerebral cortex]).

Figure 3.

Relationship between PF-03463275 dose and transporter occupancy from all subjects (A), healthy subjects (B), and schizophrenia subjects (C). The data from HCs and SZs are displayed in red and blue, respectively. The symbols of circles and crosses indicate the transporter occupancy estimated with and without a placebo scan, respectively. The estimated ID_{50} values with standard errors were (A) 0.26 ± 0.03 mg/kg, (B) 0.38 ± 0.06 mg/kg, and (C) 0.18 ± 0.02 mg/kg. D-F: Relationship between PF-03463275 concentration and transporter occupancy from all subjects (D), healthy subjects (E), and schizophrenia

subjects (F). The data from HCs and SZs are displayed in red and blue, respectively. The symbols of circles and crosses indicate the transporter occupancy estimated with and without a placebo scan (see Supplemental Text S8), respectively. The estimated IC_{50} values with standard errors were (D) 12.3 ± 1.02 ng/mL, (E) 13.6 ± 1.52 ng/mL, and (F) 10.8 ± 1.34 ng/mL.

Figure 4.

VEP waveforms under pre (black) and post (red) HFS conditions across drug conditions by sample and study. Waveforms depict group averaged VEPs, from which peak amplitude values at ~100ms (N100) were measured for ascertainment of LTP on a subject by subject basis. (A) HCs evidenced no change from baseline or placebo in pre-post HFS amplitude values across 10, 20, and 40mg conditions. HCs were not included in Substudy 2 or tested under 60mg condition. (B) SZs evidenced increases in post-HFS relative to pre-HFS amplitude values in 10, 20, and 40mg conditions. Both pre- and post-HFS VEP amplitudes increased with higher dosing, with VEPs in the 40mg condition evidencing the most change in amplitude and post-HFS enhancement. VEPs were relatively stable under placebo. (C) SZs in Substudy 2 showed no change from baseline in pre-post HFS amplitude values under placebo or 60mg conditions.

Figure 5.

Mean LTP scores reflect post-HFS amplitude change relative to pre-HFS predicted values standardized (z-score) across conditions. HCs (left panel) evidenced marginal post-HFS enhancement at baseline with no significant change under placebo or active drug conditions. SZs (right panel) show an opposite pattern, with LTP scores reflecting reduction in post-HFS amplitude relative to pre-HFS amplitude at baseline and placebo testing, but post-HFS enhancement under 10mg and 40mg conditions. Inclusion of the 60mg condition (Substudy 2) for sake of visual comparison suggests an inverted “U” dose-response relationship with peak enhancement LTP at 40mg.

Figure 6.

Scatterplots depict relationship of LTP to estimated target occupancy (ETO) levels. (A) Substudy 1: A statistically significant linear association ($p = 0.02$) was observed in SZs (upper panel) suggesting increases in LTP with greater occupancy at doses of 10mg to 40mg. No appreciable relationship was observed in HCs. (B) Substudies 1 and 2 SZs: Addition of 60mg dose condition reduced linear association and relationship was optimally explained by a quadratic curve reflecting reduction in LTP at ETO values above .80.

Note: 4 data points were excluded from the intent-to-treat sample due to either undetectable (1 HC and 2 SZs) or extremely high (1 SZ) PF-03463275 plasma levels. In addition, neither plasma or PET scans were available for 3 active drug test sessions (SZ $n=1$ 40mg; HC $n=1$ 10mg, $n=1$, 40m) and, thus, could not be included in this analysis.