Priority Communication

Biological Psychiatry

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. 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 cognitive impairments associated with schizophrenia.

METHODS: In substudy 1, the effects of PF-03463275 (10, 20, and 40 mg twice a day) on occupancy of GlyT1 were tested using positron emission tomography and ^{18}F-MK-6577, and visual long-term potentiation (LTP) in schizophrenia patients (SZs) and healthy control subjects. 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 healthy control subjects using functional magnetic resonance imaging. Subsequently, the effects of PF-03463275 (60 mg twice a day) on occupancy of GlyT1 and long-term potentiation were examined only in SZs (substudy 2).

RESULTS: PF-03463275 at 10, 20, 40, and 60 mg twice a day 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 healthy control subjects. PF-03463275 increased long-term potentiation only in SZs with peak effects at 40 mg twice a day (~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 *N*-methyl-D-aspartate receptor function in the ketamine assay, it enhanced neuroplasticity in SZs. These findings provide support for a clinical trial to test the ability of PF-03463275 to enhance cognitive remediation toward addressing cognitive impairments associated with schizophrenia.

Keywords: Cognition, Glycine transporter inhibitor, Long-term potentiation, Neuroplasticity, NMDA receptor, Positron emission tomography, Receptor occupancy, Schizophrenia

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Glycine transporter-1 (GlyT1) inhibitors are promising medications for the treatment of schizophrenia (1). These drugs may attenuate the impact of *N*-methyl-D-aspartate receptor (NMDAR) functional deficits associated with schizophrenia (2). In animals, GlyT1 inhibitors enhance NMDAR function and NMDAR-related neuroplasticity (3,4).

GlyT1 inhibitors reduce NMDAR antagonist–induced behavioral effects in rodents (5), working memory deficits in nonhuman 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 nonlinear (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 NMDAR signaling, without accompanying therapies to guide functional change.

Recent studies in schizophrenia have focused on a biomarker associated with an NMDAR-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; for example, 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. The first aim of the study was to establish the dose occupancy relationship of PF-03463275 using positron emission tomography (PET) and the GlyT1-specific radiotracer ^{18}F-MK-6577 (15) in both schizophrenia patients (SZs) and healthy control subjects (HCs). 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 predefined working memory (WM) network during the encoding and early maintenance phase of working memory in HCs, as measured by blood oxygen level–dependent 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 assay of LTP.

METHODS AND MATERIALS

Details regarding the methods and materials used in this study can be found in the Supplement.

General Study Design

HCs and SZs completed three treatment phases each lasting 1 week in duration, separated by at least 1 week of washout (Supplemental Figure S1A and Supplemental Table S1A). Subjects received placebo and only two of three possible doses of PF-03463275 (10, 20, or 40 mg twice a day) under double-blind, randomized conditions in a crossover design (substudy 1). On day 6 of each treatment phase, when PF-03463275 was at a steady state, all SZs and a subset of HCs underwent a PET scan followed by assessment of LTP. In HCs 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 (Supplemental Figure S1B and Supplemental Table S1B). For this, only SZs received either PF-03463275 placebo or 60 mg twice a day 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 (investigational new drug no. 118880) approvals and was registered on clinicaltrials.gov (Supplemental Text S1).

Subjects

SZs and HCs were recruited according to specific criteria (Supplemental Text S2A, B) after a comprehensive screening process (Supplemental Text S3). To reduce known variability in PF-03463275 plasma levels, only subjects genotyped as P450 2D6 extensive metabolizers were included (Supplemental Text S4), and in substudy 1 the only permissible antipsychotics were risperidone and aripiprazole.

Drugs

Study drug preparations can be found in Supplemental Text S5.

PF-03463275. For substudy 1, subjects were randomly assigned to one of three treatment groups (A: placebo, 10 mg, and 20 mg twice a day; B: placebo, 10 mg, and 40 mg twice a day; C: placebo, 20 mg, and 40 mg twice a day) (Supplemental 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 (Supplemental Figure S1B), SZs participated in two treatment periods where they received PF-03463275 60 mg twice a day 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 because it has been shown to safely produce an array of transient schizophrenia-relevant effects including WM deficits and psychotomimetic effects (14,16–20) and to reduce WM-related activation as measured by fMRI (21,22).

PET Study of GlyT1 Occupancy by PF-03463275

A high-quality three-dimensional magnetization prepared rapid acquisition gradient-echo MRI was obtained for coregistration. In substudy 1, the dose-related occupancy of GlyT1 by PF-03463275 (10, 20, and 40 mg twice a day vs. placebo) was evaluated in HCs and SZs using ^{18}F-MK-6577 on the high-resolution research tomograph (Supplemental Text S7). Distribution volume (V_T) was estimated using the one- and two-tissue compartment models and multilinear analysis 1 for each scan. Transporter occupancy 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}) (Supplemental Text S7). In substudy 2, the occupancy of GlyT1 by PF-03463275 (60 mg twice a day) was studied only in SZs. ID_{50} and IC_{50} values derived from only HCs, only SZs, and all subjects were compared.

fMRI Study of PF-03463275 Effects on WM Circuits

In substudy 1, the dose-related capacity of PF-03463275 at steady state levels to attenuate ketamine-induced disruption of a predefined cortical WM circuit was tested only in HCs with fMRI using a previously developed procedure (21) (Supplemental Text S8). On the day 7 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 1 minute and then a constant saline infusion for the rest of the saline portion of the scan. During saline infusion, they completed eight runs of the spatial WM (SWM) task and a resting run. They then received a ketamine bolus and ketamine constant infusion before repeating the SWM task. Before and after each scanning session, they were assessed with the Positive and Negative Syndrome Scale (PANSS) (23), the