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Antiherpes Virus–Specific Treatment and Cognition in Schizophrenia: A Test-of-Concept Randomized Double-Blind Placebo-Controlled Trial

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Objective: **To test our hypothesis that valacyclovir, an antiherpes virus–specific medication, added to antipsychotics (APs) would improve cognitive performance and psychopathology among schizophrenia subjects exposed to neurotropic herpes simplex virus, type 1 (HSV1).** ***Methods:*** **Using a double-blind placebo-controlled design, we randomized 24 HSV1-seropositive schizophrenia subjects to receive either valacyclovir ($n = 12$) or placebo ($n = 12$) for 18 weeks in addition to stable doses of APs. Valacyclovir dose was stabilized at 1.5 g twice daily orally. At each visit, subjects were evaluated for severity of psychopathology and side effects using standardized scales and a study-specific semistructured checklist. A computerized neurocognitive battery validated on both schizophrenia and healthy subjects was administered at baseline and follow-up. Intent-to-treat analysis, using linear regression models that included all randomized subjects, were used to examine differential changes in cognition and psychopathology scores over 18 weeks between valacyclovir and placebo, accounting for placebo response.** ***Results:*** **Valacyclovir group improved in verbal memory, working memory, and visual object learning compared with placebo group. The effect sizes (Cohen's *d*) were 0.79 for working memory, 1.14 for immediate verbal memory, and 0.97 for the visual object learning. Psychotic symptom severity did not improve.** ***Conclusions:*** **Supplemental valacyclovir may alleviate impairments in cognitive domains that are often observed in schizophrenia but not psychotic symptoms in those exposed to HSV1. If replicated, this approach could provide a novel strategy to treat cognitive impairments in a subgroup of schizophrenia subjects who can be reliably identified using a blood test.**

Key words: psychosis/cognitive impairments/therapeutics/valacyclovir/herpes simplex virus/neurotropic viruses

Introduction

Cognitive impairments are considered a core deficit[1] and a separate domain[2] of schizophrenia. Cognitive deficits contribute significantly to its poor long-term outcome[3] resulting in increased family burden and societal costs.[4] Moreover, cognitive deficits respond minimally to antipsychotics (APs).[5] Therefore, systematic research to discover potentially treatable factors associated with cognitive impairments and designing interventions to target such factors could provide a novel strategy.

A number of lines of evidence suggest that it is worthwhile to explore antiherpes agents for amelioration of cognitive impairments in schizophrenia. First, associations between exposure to neurotropic herpes simplex virus, type 1 (HSV1) and cognitive impairments, especially for the working memory and verbal memory are noted repeatedly.[6–9] Second, reductions in the prefrontal cortex (PFC) gray matter volume were replicated in HSV1-exposed schizophrenia subjects compared with schizophrenia subjects not exposed to HSV1 and healthy controls.[9–11] Third, HSV1-exposed schizophrenia subjects were noted to have longitudinal decline in executive functions along with progressive gray matter loss compared with HSV1 seronegative schizophrenia subjects.[12] Cross-sectional and longitudinal imaging studies report correlation of gray matter loss with cognitive impairments, suggesting that gray matter loss may be functionally significant.[9,12] These observations were made on schizophrenia subjects without a history or clinical evidence of encephalitis suggesting that HSV1 exposure may be a risk factor for cognitive impairments even when subjects do not develop encephalitis. Postencephalitic phase is commonly characterized by cognitive impairments along with neurological sequelae.[13] Further, these observations were made on schizophrenia subjects

who were previously not treated with antiherpes medications. Because effective antiherpes medications are available, we conducted a proof-of-concept trial of valacyclovir add-on treatment on HSV1-seropositive schizophrenia subjects using a randomized double-blind placebo-controlled design.

HSV1 is an enveloped double-stranded DNA virus[14] that commonly infects human beings through the orofacial, ocular, and the nasal mucosae. Following infection, the viruses enter the sensory and autonomic nerve termini and are transported through retrograde axonal transport to the trigeminal ganglia.[15] Within the ganglionic neuronal soma, HSV1 DNA enters nucleosomes of the host cells and establishes latency with lifelong periodic reactivation cycles.[15] In human beings, latent HSV1 infection is maintained primarily in the trigeminal ganglia.[14] During reactivation cycles, HSV1 propagates from the ganglionic neurons through retrograde axonal transport to other brain regions and through anterograde transport to peripheral lesions where they are shed in body fluids. HSV1-specific antibodies are detectable in the serum throughout an infected individual's life because HSV1 would be in reactivation phase in a minority of the neuronal population at any given time although a majority harbor latent viruses.[16] Neuronal death during reactivation and dysfunction during latency secondary to modulation of apoptosis, oxidative stress, and membrane alterations are reported.[17] Apart from recurrent cold sores, no serious symptoms or signs are evident during an individual's lifetime. Hence, HSV1 infection was considered benign in the absence of encephalitis. However, a growing body of data on structural brain changes and cognitive impairments even in the absence of encephalitis may have important implications for psychiatric disorders such as schizophrenia.

Valacyclovir is the L-valyl ester prodrug of acyclovir which is an acyclic guanine nucleoside analog lacking a 3′-hydroxyl on the side chain.[18] Valacyclovir has been approved by the Food and Drug Administration for the treatment of herpes infections. Valacyclovir is rapidly converted to acyclovir through enzymatic hydrolysis in the intestine and liver.[18] Bioavailability of acyclovir following oral administration of valacyclovir is considerably more than that observed with oral administration of acyclovir itself.[19] Clinically useful antiviral activity of acyclovir is limited to herpes viruses. The highest activity of acyclovir is against HSV1, whereas it is half as active against HSV2, a tenth as active against varicella-zoster and Epstein-Barr viruses and least active against cytomegalovirus.[19]

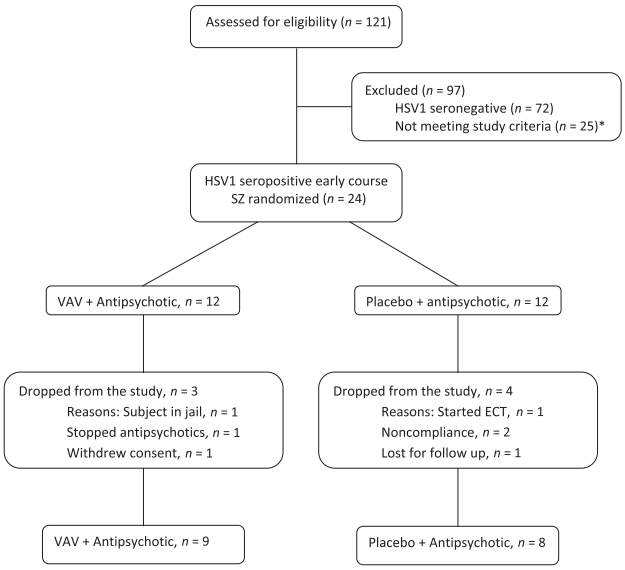
We hypothesized that HSV1-exposed schizophrenia subjects who received valacyclovir in addition to antipsychotics (VAV + APs) would demonstrate better cognitive performance, especially in the working memory and verbal memory domains, and decreased severity of psychopathology at the end of 18 weeks compared with those who received placebo added to antipsychotics (PL + APs).

Methods

Subjects

The study was conducted at the Western Psychiatric Institute and Clinic, Pittsburgh, and the Wayne State University, Detroit. Subjects were required to test positive for HSV1-specific IgG antibodies, be on stable doses of APs for $\geq$1 month and score $\geq$4 on at least one item of the Positive and Negative Syndrome Scale (PANSS)[20]; the latter criterion was included to ensure that subjects were not asymptomatic at baseline. Subjects who fulfilled *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) criteria for substance abuse in the last month or dependence during 6 months prior to recruitment were excluded. Other exclusion criteria were history of medical/neurological illnesses (eg, epilepsy, head injury with loss of consciousness, encephalitis/meningitis), history of immune disorders, being on immunosuppressants or regular antiherpes treatment, and mental retardation per DSM-IV. Of the 121 schizophrenia subjects screened, 24 HSV1-seropositive subjects with DSM-IV schizophrenia/schizoaffective disorder of both sexes between the ages 18 and 50 years were randomized (figure 1). The Structured Clinical Interview for DSM IV (SCID)[21] was administered to these randomized subjects at baseline by experienced clinical evaluators. A consensus DSM-IV diagnosis of schizophrenia/schizoaffective disorder was assigned in a meeting of 2 or more senior diagnosticians after pooling the SCID data, clinical observations from the professionals who provided direct care and a review of medical records. Throughout the study, AP dose remained unchanged, APs were not switched and no new medications were added. After fully explaining the study procedures, informed consents were obtained from all subjects. The University of Pittsburgh Institutional Review Board (IRB) and the Wayne State University Human Investigations Committee approved the study.

We collected demographic data including socioeconomic status (SES) using the Hollingshead scale.[22] The PANSS was used to rate the presence and severity of psychopathology. The side effects were evaluated using the Abnormal Involuntary Movements Scale, Barnes Akathisia Scale, and a detailed side effects checklist that was specifically developed for this study and approved by the IRB. These scales were administered by a physician (K.M.P.) or an experienced clinical evaluator at baseline and during follow-ups (weeks 2, 4, 6, 8, 12, 16, and 18). During these follow-ups, substance use and adherence to all medications were monitored. The former was clarified through history and urine drug screen when necessary and the latter through patient report and pill count.



*Substance use disorder, $n = 3$; Duration of illness longer than 10 years, $n = 8$; Refusal to participate after undergoing screening, $n = 7$; No psychiatric diagnosis, $n = 1$; Seizure disorder, $n = 1$; Not on psychiatric treatment at the time of assessment, $n = 1$; Unsure of participation, $n = 1$; Lost contact following screening, $n = 3$; Withdrew consent, $n = 1$; In and out of hospital but the study ended, $n = 1$. Total number exceeds 25 because some subjects met more than one criteria.

Fig. 1. Subject flow accounting for all participants in the study.

Study Design

We used a randomized double-blind placebo-controlled design. The randomization scheme developed by generating random numbers was provided to the pharmacy for dispensing the medications. This scheme was provided to the psychiatric emergency room in a sealed envelope that was to be opened if a participant reported to the emergency room with serious side effects. None of the subjects reported to the emergency room; therefore, the blind was not broken until the end of the study. The starting dose of valacyclovir was 1 g orally twice daily for 2 weeks and then increased to 1.5 g orally twice daily until the end of the study because oral administration of valacyclovir 3 g per day would provide steady state cerebrospinal fluid (CSF) concentrations of 0.72–1.66 μg/ml.[23] This CSF concentration reaches or exceeds the in vitro 50% minimum inhibitory concentrations (IC_{50}) for HSV1 (0.02–0.9 μg/ml).[19]

Serological Assays

The IgG and IgM antibodies to HSV1 (gG-1 protein) and HSV2 (gG-2 protein) were assayed using the Focus Diagnostics HerpeSelect I ELISA kits. Recombinant gG-1/gG-2 antigen coated on polystyrene microwells was used. Diluted serum samples and controls were incubated in the well to allow specific antibody in the samples to react with antigen. After removing nonspecific reactants, enzyme substrate and chromogen were added, and the color was allowed to develop that was quantified by a spectrophotometric optical density (OD) reading. Sample OD was compared with reference cutoff OD to determine the results. The sensitivity and specificity of this method compared with Western blot is 96% and 95.2%, respectively.

Neuropsychological Evaluations

The Penn Computerized Neurocognitive Battery (CNB)[24] was administered at baseline and at 18 weeks. We selected the following domains for this study: working memory (letter *n*-back), verbal memory (immediate and delayed), face memory (immediate and delayed), visual object learning test (immediate and delayed), spatial processing, and emotion processing. Working memory holds certain information online while a response selection is made and is related to frontal lobe functioning. Immediate components of verbal, face, and visual memories tap the recognition memory that is known to be regulated by hippocampal and prefrontal network. We scored all subjects on accuracy and speed of processing on these tests except for the working memory for which only accuracy was scored. Speed of processing was measured as median response time on 5 of 6 cognitive domains, namely the immediate and delayed components of verbal memory, visual object learning, face memory, and spatial processing and emotion recognition. Thus, there were 17 neuropsychological outcome measures. The raw scores (accuracy and processing speed) were normed with age-matched CNB data from 564 community controls to derive *z* scores for statistical analyses. The rationale for selecting these domains is based on the neurocognitive data on HSV1-exposed schizophrenia subjects available at the time of study initiation in 2006 that indicated significant verbal memory and working memory impairments,[7] along with prefrontal gray matter reduction in HSV1-exposed schizophrenia subjects.[10,11] More details about the tests and administration of CNB are provided in previous publications.[25,26]

Statistical Approach

We first compared the study groups on demographic, clinical, and cognitive variables. Independent variables were the treatment types (VAV + AP and PL + AP). Based on the existing neurocognitive and imaging studies, we selected working memory and verbal memory as the main outcome variables. All other outcomes were examined post hoc. We used Bonferroni's corrections on these post hoc comparisons but not for the 2 main outcomes. We computed neurocognitive/PANSS change scores by subtracting the baseline scores from the follow-up scores. These change scores were used within linear regression models to compare the 2 groups adjusting for age and sex for all outcomes. Because of dropouts, we conducted both a completer analysis and an intent-to-treat (ITT) analysis using multiple imputations (MI) for the missing data. We preferred

ITT because with the ITT, the estimates are less biased and the estimates of drug effect are more likely to reflect how the drug may be used in the target population when clinically deployed.[27] We chose MI over last observation carried forward or expectation maximization because MI is a more robust method incorporating the MAR (missing at random) missingness mechanism and replaces missing values by a set of $m > 1$ plausible values to generate m plausible complete data sets.[28] Neither the baseline PANSS score nor the SES was associated with the outcomes; therefore, we did not include them as covariates in order to reduce the degrees of freedom. For MI, we generated 40 imputations by specifying the covariates so that the structure of missingness is appropriately represented for imputation and used the Rubin's method for estimating SDs and computing P values.[29] With approximately 30% missing data, 40 imputations provides an efficiency of 99.25% (efficiency of estimate = $(1 + \gamma/m)^{-1}$, where γ is the fraction of missing data and m is the number of imputations).[28]

The psychopathology (total PANSS) scores were analyzed similarly, using linear regression models within the ITT MI for missing data and a completers-only analysis without the imputed data.

Results

Subject Flow

The subject flow is shown in figure 1. Briefly, of the 121 subjects screened for eligibility, 97 were excluded because 72 subjects tested negative for HSV1, and 25 for not meeting other study criteria. Thus, 24 subjects were randomized to receive either VAV + AP (n = 12) or PL + AP (n = 12) on whom demographic, clinical, and neuropsychological data were obtained. Seven subjects dropped out of the study (PL + AP = 4; VAV + AP = 3). Thus, 17 subjects competed all study procedures for the entire length of the study (PL + AP = 8; VAV + AP = 9). Subjects who dropped out from the VAV + AP group were younger (19.81 ± 2.03 y) compared with completers (32.78 ± 8.64 y) (t = 2.51, df = 10, P = .03) and all were males. Within the PL + AP group, subjects who dropped out (27.21 ± 10.77 y; 3 males and 1 female) were not different from completers (29.41 ± 10.31 y; t = 0.41, df = 10, P = .35). Subjects who dropped out did not differ from completers within each group with respect to the duration of illness (PL + AP: dropouts 6.87 ± 1.53 y, completers 4.95 ± 3.53 y and VAV + AP: dropouts 2.51 ± 2.21 y, completers 3.68 ± 2.98 y), the baseline PANSS total score (PL + AP: dropouts 88.25 ± 11.98 y, completers 80.88 ± 14.71 y and VAV + AP: dropouts 76.50 ± 2.12 y, completers 69.80 ± 13.56 y), and mean SES (PL + AP: dropouts 37.50 ± 11.26 y, completers 30.00 ± 8.63 y and VAV + AP: dropouts 26.00 ± 5.66 y, completers 26.22 ± 6.51 y) (all $P > .17$).

Demographic and Clinical Data

The mean age of all subjects in the study was 29.11 ± 8.78 years; the mean age of subjects in each study group were not different (t = 0.24, df = 22, P = .82). The mean duration of illness of all randomized subjects was 4.37 ± 3.19 years; the groups did not statistically differ with respect to the duration of illness (t = 1.74, df = 22, P = .095). The PANSS scores were higher among PL + AP compared with VAV + AP group (t = 2.31, df = 22, P = .031) (table 1). All subjects were on atypical APs. Four subjects (VAV + AP = 3 and PL + AP = 1) were on anticholinergic medication (benztropine). We maintained compliance for greater than 75% of the treatment duration.

Six subjects on placebo and 5 on VAV reported the following side effects: drooling, muscle tightness, mild tremors, akathisia, bloating of the stomach, feeling tired, elbow pain, increased sexual drive, insomnia, and leg cramps in PL + AP group; constipation, stomach pain, motion sickness, occasional muscle twitch, tremor, and upset stomach in VAV+AP group (see online supplementary table). All side effects were mild; none of the subjects required discontinuation of treatment.

Neurocognitive Performance

Both the ITT MI and completer analysis gave the same qualitative results with rather small numerical differences.

The total working memory accuracy scores (0-, 1-, and 2-back) showed a trend toward improvement in both the ITT MI and completer analyses. However, 2-back accuracy scores improved significantly over 18 weeks in VAV + AP group but not in PL + AP group. We did not note such improvements in 0- and 1- back accuracy. The effect sizes were approximately 0.79 for total scores and 0.72 for 2-back scores.

The processing speed of immediate verbal memory improved significantly with valacyclovir (Cohen's d = 1.21). The completer analysis showed a trend (P = .087) for improvement with a comparable effect size (d = 1.06). The delayed verbal memory (accuracy and processing speed) did not improve significantly (table 2 and figure 2).

Among the other cognitive domains (visual object learning, face memory, spatial processing, and emotion recognition) that were examined post hoc, the accuracy of delayed visual object learning showed significant improvements (P = .026; d = 0.91). The completer analysis also showed improvement (P = .051; d = 1.06). However, this difference did not reach the Bonferroni corrected significance ($P < .05/14 = .0036$). The other 3 domains did not improve (table 3).

Psychopathology Scores

Although there was an overall reduction in PANSS scores over 18 weeks across the entire sample, we did not observe significant effect of VAV over placebo

Table 1. Demographic and Clinical Characteristics

Variable	VAV + AP	PL + AP	Statistic	Significance
Age			0.24[a]	.82
In years	29.54 ± 9.44	28.67 ± 8.47		
Range	18.89–44.12 y	20.19–50.38 y		
Sex				
Male	6	7	0.17[b]	.68
Female	6	5		
SES				
Average parental SES	25.50 ± 9.23	25.75 ± 8.95	1.84[a]	.08
Subject SES	26.12 ± 6.10	32.50 ± 9.78	0.07[a]	.95
Duration of illness (in years)	3.49 ± 2.82	5.59 ± 3.08	1.74[a]	.095
PANSS at baseline[c]				
Total	70.92 ± 12.55	83.33 ± 13.79	2.31[a]	**.031**
Positive	15.58 ± 3.70	19.67 ± 4.74	2.35[a]	**.028**
Negative	21.58 ± 4.44	22.83 ± 3.04	0.81[a]	.430
General	33.75 ± 7.52	40.83 ± 8.12	2.22[a]	**.037**

Note: VAV, valacyclovir; PL, placebo; AP, antipsychotics; SES, socioeconomic status; PANSS, Positive and Negative Syndrome Scale. Statistically significant differences are bolded.
[a]*t* statistic.
[b]χ^2 test.
[c]PANSS raw scores here are different from that in table 3 because the scores in this table include all 24 subjects who entered into the study.

with either ITT MI (positive symptoms, $P = .90$; negative symptoms, $P = .21$; and general symptoms, $P = .74$) or completers-only analyses (table 3).

Discussion

This pilot study was designed to test our hypothesis that HSV1 exposure is a treatable factor associated with cognitive impairments in schizophrenia. The VAV + AP group showed significant improvements in verbal memory, working memory, and visual object learning tasks providing preliminary support to our hypothesis. Valacyclovir was well tolerated without significant adverse effects or clinically identifiable drug interactions over 18 weeks while on atypical APs. Our results suggest that valacyclovir supplemented to APs was a safe and

Table 2. Changes in Test Scores of Hypothesized Neuropsychological Outcomes Within VAV + AP and PL + AP Groups From Baseline to 18 Weeks Using Both the ITT MI Analyses and Completers-only Analyses. Age and Gender Were the Covariates in All Analyses

	Intent to Treat (MI) Analyses						Completer Analyses					
Cognitive Measure	Mean (SD) VAV + AP	Mean (SD) PL + AP	β	*F* (*df*)[a]	*P*[b]	*d*[c]	Mean (SD) VAV + AP	Mean (SD) PL + AP	β	*F* (*df*)	*P*	*d*[c]
Hypothesized neuropsychological outcome variables												
Immediate verbal memory (processing speed)	−0.11 (0.09)	−0.01 (0.08)	−.097	4.49 (1, 169)	**.036**	**1.21**	−0.09 (0.09)	−0.02 (0.08)	−1.88	**3.53 (1, 11)**	**.087**	**1.06**
Immediate verbal memory (accuracy)	1.03 (2.98)	−0.38 (3.64)	1.23	0.45 (1, 162)	.50	0.38	0.99 (2.75)	0.01 (4.03)	.59	0.34 (1, 11)	.57	0.33
Working memory (total)	3.76 (4.90)	0.25 (4.90)	3.86	2.71 (1, 127)	**.10**	**0.79**	2.50 (4.16)	−1.06 (5.85)	1.80	3.24 (1, 11)	**.099**	**0.79**
Working memory (2-back)	2.13 (2.68)	0.13 (2.97)	2.25	4.42 (1, 190)	**.037**	**0.72**	1.56 (2.41)	−0.86 (3.33)	2.41	4.23 (1, 11)	**.064**	**0.82**

Note: VAV, valacyclovir; PL, placebo; AP, antipsychotics; ITT, intent-to-treat; MI, multiple imputations. Statistically significant differences and associated effect size estimates are bolded.
[a]Degrees of freedom for the ITT MI analysis are based on variance inflation factors during MI, and thus differ across analysis even though all MI analyses included all 24 subjects.
[b]*P* values are combined using the Rubin's method.
[c]Effect size estimates using Cohen's *d*.

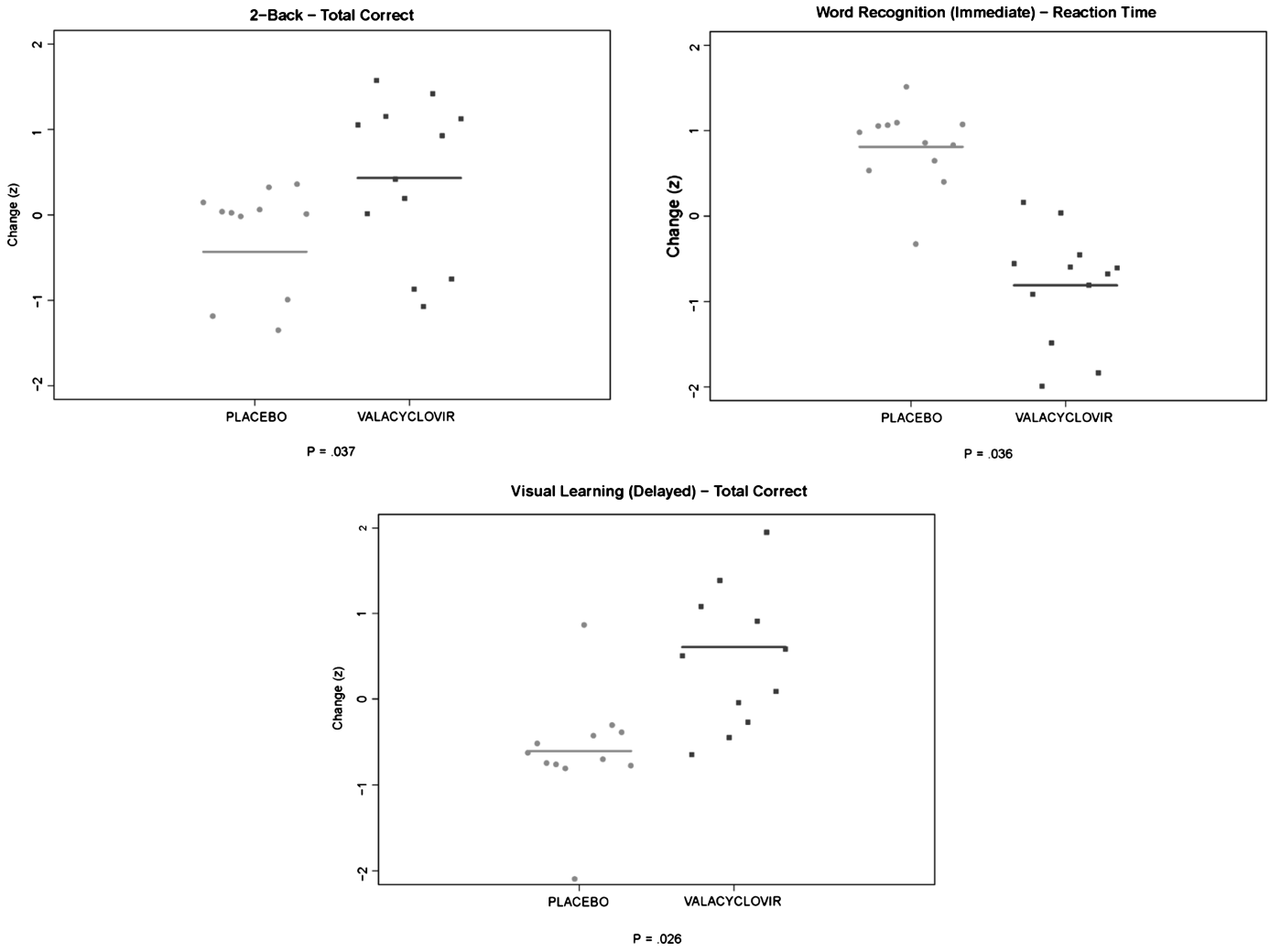


Fig. 2. Changes in neuropsychological scores over 18 weeks in the intent-to-treat multiple imputations analysis.

well-tolerated treatment that may alleviate cognitive impairments in HSV1-seropositive schizophrenia subjects. The improved cognition occurred without concurrent changes in psychotic symptom severity suggesting that valacyclovir may more selectively affect cognitive impairments than psychotic symptoms. However, these preliminary observations require confirmation and replication in adequately powered samples.

An important observation is the safety and tolerability of valacyclovir by schizophrenia subjects while on psychotropic medications. The study participants tolerated valacyclovir without serious adverse effects. Dropout rate was similar between the VAV + AP and PL + AP groups. Valacyclovir is metabolized through the aldehyde dehydrogenase and aldehyde oxidase and does not induce hepatic microsomal enzymes.[30] The currently prescribed psychotropic medications are mainly metabolized through the cytochrome P450 system. Therefore, the metabolism of psychotropic medications may not have been affected by the adjuvant valacyclovir. Overall, this study suggests a favorable safety profile for supplementing APs with valacyclovir.

Prior neurocognitive studies on HSV1-exposed schizophrenia subjects observed impairments mainly in the working memory, verbal memory, and executive functions.[6–8] PFC plays a key role in the regulation of these domains. Imaging studies that examined the entire brain also observed gray matter reductions in the PFC of schizophrenia subjects exposed to HSV1.[9–11] Therefore, we predicted that the cognitive improvements would be observed primarily in those domains where PFC plays a key role in their regulation. Consistent with our prediction, verbal memory and working memory improved in this study. Certain aspects of visual object learning tap prefrontal resources although other regions may be involved in the network that regulates this domain.[31] Because HSV1 propagates widely in the brain and has been isolated from frontal, occipital, and temporal regions,[15] it is likely that HSV1 could affect multiple cognitive domains. Improvement in delayed memory may

Table 3. Post Hoc Analyses of Other Neuropsychological Test Scores and Psychopathology Scores Within VAV + AP and PL + AP Groups From Baseline to 18 Weeks Using Both the ITT MI Analyses and Completers-only Analyses. Age and Gender Were the Covariates in All Analyses

	Intent to Treat (MI) Analyses						Completer Analyses					
Cognitive Measure	Mean (SD) VAV + AP	Mean (SD) PL + AP	β	F (df)[a]	P[b]	d[c]	Mean (SD) VAV + AP	Mean (SD) PL + AP	β	F (df)	P	d[c]
Accuracy of performance on cognitive measures												
Delayed verbal memory	−0.03 (4.44)	0.48 (3.88)	−.81	0.14 (1, 150)	.71	0.17	0.13 (4.40)	0.51 (3.82)	−.20	0.04 (1, 11)	.84	0.11
Immediate visual memory	−0.08 (2.16)	0.21 (1.78)	−.38	0.05 (1, 68)	.82	0.12	0.01 (2.0)	0.37 (1.85)	−.37	0.14 (1, 11)	.72	0.21
Delayed visual memory	2.44 (2.62)	−0.49 (3.11)	2.92	5.06 (1, 153)	**.026**	0.91	1.57 (1.68)	−0.99 (3.58)	2.19	4.78 (1, 11)	.051	1.02
Immediate face memory	0.42 (5.70)	1.57 (3.60)	−1.11	0.13 (1, 70)	.72	0.25	0.17 (5.59)	1.10 (3.55)	−.92	0.12 (1, 11)	.74	0.20
Delayed face memory	1.51 (6.06)	2.30 (2.89)	−.92	0.10 (1, 140)	.76	0.78	1.27 (6.44)	1.89 (2.05)	−.61	0.05 (1, 11)	.82	0.13
Spatial processing	1.63 (3.84)	1.39 (2.75)	.13	0.03 (1, 110)	.85	0.12	1.40 (4.02)	0.90 (2.88)	.49	0.08 (1, 11)	.78	0.14
Emotion recognition	−0.49 (3.16)	−0.18 (5.31)	.17	0.02 (1, 115)	.89	0.05	−1.18 (1.29)	−1.59 (6.01)	.41	0.05 (1, 11)	.83	0.09
Speed of processing of cognitive measures												
Delayed verbal memory	−83.46 (197.09)	−12.18 (217.33)	−71.60	0.33 (1, 147)	.57	0.34	−75.88 (181.44)	−25.17 (229.81)	−.46	0.21 (1, 11)	.65	0.28
Immediate visual memory	−85.76 (436.36)	−308.27 (391.74)	241.47	1.15 (1, 151)	.29	0.36	−56.70 (433.53)	−267.45 (413.70)	1.02	1.05 (1, 11)	.33	0.57
Delayed visual memory	−0.06 (0.22)	−0.12 (0.17)	.07	0.38 (1, 177)	.54	0.18	−0.06 (0.24)	−0.12 (0.17)	.51	0.26 (1, 11)	.62	0.30
Immediate face memory	−357.86 (318.69)	−148.27 (357.09)	−204.95	1.28 (1, 192)	.25	0.60	−328.59 (290.01)	−107.92 (384.96)	−220.67	1.28 (1, 11)	.28	0.64
Delayed face memory	−0.05 (245.16)	−63.65 (299.56)	63.54	0.12 (1, 104)	.73	0.28	32.02 (230.49)	−33.14 (321.52)	65.16	0.15 (1, 11)	.70	0.23
Spatial processing	−0.23 (0.36)	−0.05 (0.18)	−0.17	1.09 (1, 121)	.30	0.64	−0.22 (0.37)	−0.06 (0.14)	−.17	1.21 (1, 11)	.29	0.64
Emotion recognition	−332.10 (353.59)	−87.27 (464.64)	−231.47	0.22 (1, 132)	.64	0.29	−354.43 (311.55)	−186.37 (519.24)	−168.06	0.05 (1, 11)	.49	0.39
Psychopathology scores[d]												
PANSS total	−7.88 (15.01)	−14.33 (18.42)	5.62	0.53 (1, 322)	.47	0.38	−8.19 (10.65)	−13.35 (19.06)	5.16	0.31 (1, 10)	.59	0.32
PANSS positive symptoms	−2.91 (4.73)	−3.35 (4.84)	.34	0.02 (1, 165)	.90	0.09	−2.95 (4.55)	−3.54 (5.23)	0.59	0.04 (1, 10)	.84	0.12
PANSS negative symptoms	−2.15 (3.21)	−4.22 (3.21)	1.92	1.56 (1, 282)	.21	0.66	−2.30 (2.88)	−4.28 (3.45)	1.99	1.12 (1, 10)	.31	0.62
PANSS general symptoms	−3.05 (8.60)	−5.60 (10.18)	2.17	0.11 (1, 80)	.74	0.31	−2.95 (5.68)	−5.54 (11.27)	2.58	0.23 (1, 10)	.64	0.28

Note: VAV, valacyclovir; PL, placebo; AP, antipsychotics; ITT, intent-to-treat; MI, multiple imputations; PANSS, Positive and Negative Syndrome Scale. Statistically significant differences are bolded.

[a]Degrees of freedom for the ITT MI analysis are based on variance inflation factors during MI, and thus differ across analysis even though all MI analyses included all 24 subjects.

[b]P values are combined using the Rubin's method.

[c]Effect size estimates using Cohen's d.

[d]PANSS scores here are different from that in table 1 because the scores in this table are the estimated mean change scores for all 24 subjects who entered into the study for the ITT MI analyses and, for those who completed the study in the completer-only analyses.

support such possibility because delayed memory tasks may involve both hippocampus and PFC, HSV1 may affect hippocampus, in addition.[32] Further studies using functional and diffusion tensor imaging could better inform the changes in neural systems following valacyclovir therapy.

The activity of valacyclovir is specific for herpes family of viruses[18] and selectively affects herpes-infected cells. Within the herpes family, HSV1 appears to be most sensitive to valacyclovir.[19] Acyclovir distributes well into most body tissues and easily crosses the cell membranes. Herpes-infected cells contain HSV1-thymidine kinase (HSV1-tk) that phosphorylates acyclovir to acyclovir monophosphate; the latter cannot diffuse out of the cell. This creates a concentration gradient for acyclovir to diffuse into the herpes-infected cell; thus selectively increasing the uptake of acyclovir by the infected cells compared with noninfected cells.[33] Cellular enzymes convert acyclovir monophosphate to acyclovir triphosphate such that herpes-infected cells contain 40–100 times higher acyclovir than in an uninfected cell. Acyclovir triphosphate is nearly 3000-fold more selective for the viral DNA polymerase compared with the host DNA polymerase.[33] Acyclovir triphosphate competitively inhibits viral DNA polymerase and, to a much smaller extent, host DNA polymerase. Besides, acyclovir triphosphate terminates viral DNA chain elongation. The terminated DNA chain, the acyclovir triphosphate, and the enzyme form a complex that irreversibly inactivates viral DNA polymerase.

Valacyclovir is active during viral replication when the HSV1-tk is active. During latency, inactive viral DNA remains in nucleosomes, and abundant expression of latency-associated transcripts suppress viral replication making HSV1-tk unavailable. HSV1-tk is essential for the conversion of acyclovir to acyclovir monophosphate and subsequent inhibition of viral DNA transcription. Thus, valacyclovir may be ineffective during latency.[18] Because HSV1 goes through reactivation and latency cycles where a minority of neuronal population at any given time would be in reactivation,[16] treatment with valacyclovir for 18 weeks was anticipated to provide adequate time to capture windows of replication cycles and also to target the actively replicating viruses. It is not clear if longer exposure to valacyclovir would have been more effective. If so, it is unclear how long a person should be treated with valacyclovir. These issues need careful examination in future studies. Besides, an extensive literature search did not yield any published data on direct effect of valacyclovir on neurotransmitters or neuronal functions. Therefore, the observed neurocognitive improvements are likely to be related to reduction of viral load in host cells containing reactivated HSV1. Such viral load reduction may lead to alterations in immune mediators such as interleukin-6 (IL-6) and IL-1β that are known to be elevated following HSV1 exposure.[18] Direct measurement of peripheral viral load or antibody titers is unreliable. Peripheral measures of viral load depend on reactivation followed by anterograde axonal transport—but reactivation is also accompanied by retrograde axonal transport to other brain regions that cannot be assayed using available techniques. Antibody titers are highly vulnerable to factors unrelated to the viral load such as stress and host immune response variations.

Randomized study design successfully assigned schizophrenia subjects equally between the VAV + AP and PL + AP and matched subjects for age, sex, SES, duration of illness, and neuropsychological scores at baseline. However, the PANSS scores were higher among PL + AP compared with VAV + AP group. The total PANSS scores for both groups correspond to ''moderately ill'' category of the Clinical Global Impression scale[34] suggesting that the overall severity of illness was similar between groups. In addition, the differences in PANSS scores did not independently predict neurocognitive changes in this study. Therefore, our results may not have been confounded by differences in clinical severity.

Although a causal association between exposure to HSV1 and increased risk for schizophrenia is questionable,[35] its association with cognitive impairments, especially in schizophrenia appears to be robust from both neurocognitive and imaging studies.[6–12] These replicated convergent observations do not prove etiological link with cognitive impairments. However, neurocognitive,[6–9] neuroimaging,[9–11] and longitudinal studies[12] along with the results of this study raises a possibility that HSV1 exposure may be causative.[36] In addition, treating HSV1-exposed schizophrenia subjects could prevent decline in cognitive functions and gray mater loss that was noted in our longitudinal study.[12]

It is unclear why psychopathology did not improve with valacyclovir. Two previous studies conducted on chronic schizophrenia subjects did not note improved psychotic symptoms among HSV1-seropositive schizophrenia subjects with valacyclovir[37] or acyclovir.[38] APs that primarily modulate dopamine D_2 and serotonin 5-HT_2 receptors do not significantly improve cognitive performance.[5] Cognitive impairments and psychotic symptoms are thought to be mediated by distinct neural circuits.[39] Therefore, it is possible that HSV1 affects neural circuitry regulating cognition but not psychopathology. However, some of the cognitive domains examined here, and negative symptoms may be regulated by prefrontal cortical functions.[40] Such refined distinctions require systematic examination in future studies on adequately powered samples.

Usefulness of valacyclovir treatment for cognitive impairments in schizophrenia, if confirmed, can be considerable. Prevalence of HSV1 seropositivity in the United States can be up to 70% depending on age, ethnicity, and SES; higher prevalence among older individuals of Hispanic origin with a lower social status. The

seroprevalence among schizophrenia subjects in published studies ranges between 44% and 64.5% possibly because these prevalence rates are not from systematic epidemiological studies. In this study, 41% of the screened schizophrenia subjects tested positive for HSV1, possibly for the same reasons stated above. Controls do not differ in the frequency of seropositivity compared with subjects with schizophrenia.[35] The seropositivity frequency in this study, considering the subject demographics, is approximately similar to the seropositivity rates in the community.[41] For these reasons, valacyclovir therapy, if confirmed, can still help a large subgroup of schizophrenia subjects even if a prevalence of 50% is assumed. Moreover, an inexpensive, highly specific and sensitive blood test can reliably identify individuals who can be targeted for valacyclovir therapy.

There are several limitations to this study. First, the study had limited statistical power due to the relatively small sample size. However, it had adequate power to detect medium to large effect sizes. Consistent with that, the cognitive domains that showed statistically significant differences also had large effect sizes. Second, we did not directly assay the bioavailability of valacyclovir in the brain. Previous studies have reported that the dosage used in this study would provide CSF IC_{50} concentrations to inhibit HSV1 replication.[19] Third, although the mechanism of action of valacyclovir is precisely delineated, the biological basis for the improvement in cognitive performance is uncertain. It is tempting to suggest that valacyclovir through its antiherpes activity may have reduced the viral burden that in turn may have altered the immune mediators affecting the networks regulating cognitive processes. Such assumptions need to be supported by data. Fourth, we did not use the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) battery because it was being developed when this trial was initiated. However, most domains included here are reflected in the MATRICS too. Our rationale for selecting the CNB was based on its prior validation in both healthy[42] and schizophrenia subjects.[24] Further, this battery successfully detected subtle cognitive differences within the multiplex families with schizophrenia and their multigenerational relatives.[25,26] Fifth, although practice effects cannot be discounted, potential practice effects did not contribute to comparable improvement in the PL + AP group. Moreover, CNB was administered under the same conditions to all participants that may minimize such practice effects.

In summary, our test-of-concept trial suggests that valacyclovir add-on therapy may be beneficial for cognitive impairments but not psychotic symptoms. These observations require replication in adequately powered samples. The improvements were noted across multiple cognitive domains with medium to large effect size. Since effect sizes may be inflated in small sample studies, replicating a medium effect size in a larger study would still be of significance because the existing medications[5] and novel neurotransmitter modulators[43] have reported small effects. Since there are effective medications to treat psychotic symptoms and that supplementing the existing APs with valacyclovir is safe and well-tolerated, valacyclovir can be an important addition to therapeutic armamentarium for HSV1-exposed schizophrenia subjects provided these findings are replicated in a larger sample. Given the US epidemiological evidence that up to 70% of Americans are exposed to HSV1, valacyclovir add-on therapy may address cognitive impairments among a large subgroup of schizophrenia subjects. Future studies also need to examine whether valacyclovir therapy may improve cognitive performance among HSV1-exposed persons without schizophrenia.

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Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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