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Sulforaphane Effects on Cognition and Symptoms in First and Early Episode Schizophrenia: A Randomized Double-Blind Trial

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Objective: **Cognitive symptoms are associated with significant dysfunction in schizophrenia. Oxidative stress and inflammation involving histone deacetylase (HDAC) have been implicated in the pathophysiology of schizophrenia. Sulforaphane has antioxidant properties and is an HDAC inhibitor. The objective of this study was to determine the efficacy of sulforaphane on cognition dysfunction for patients with schizophrenia.** ***Methods:*** **This double-blind randomized 22-week trial of patients with first-episode schizophrenia was conducted in four psychiatric institutions in China. Patients were randomized to three groups (two doses of sulforaphane vs. placebo) and symptomatic and cognitive assessments were completed at multiple times. The primary outcome measure was change in the MATRICS Composite score. The secondary outcomes were change in MATRICS Domain scores, PANSS Total Scores and change in side-effects.** ***Results:*** **A total of 172 patients were randomized and 151 patients had at least one follow up evaluation. There were no significant effects of sulforaphane, on the primary outcome, MATRICS overall composite score. However, on secondary outcomes, sulforaphane did significantly improve performance scores on MATRICS battery Domains of spatial working memory ($F = 5.68$, $P = 0.004$), reasoning-problem solving ($F = 2.82$, $P = 0.063$), and verbal learning ($F = 3.56$, $P = 0.031$). There were no effects on PANSS symptom scores. Sulforaphane was well tolerated.** ***Conclusion:*** **Although the primary outcome was not significant, improvement in three domains of the MATRICS battery, suggests a positive cognitive effect on some cognitive functions, which warrants further clinical trials to further assess whether sulforaphane may be a useful adjunct for treating some types of cognitive deficits in schizophrenia.**

Key words: schizophrenia/cognitive symptoms/psychiatric symptoms/sulforaphane

Introduction

Schizophrenia is characterized by both symptomatic expressions, such as hallucinations and delusions, and multiple cognitive deficits. These cognitive defects are found throughout the illness, both in the prodromal state before the onset of the full-blown disease and persisting even after symptomatic remission.[1–3] First or second generation antipsychotics have not shown definitive efficacy

in reducing cognitive deficits associated with the disorder. It is important to find adjunctive treatments which will improve cognitive deficits in schizophrenia and may further ameliorate primary symptoms.

Sulforaphane (SFN: 1-isothiocyanato-4-methylsulfinylbutane) (SF) is an organosulfur isothiocyanate derived from a glucosinolate precursor (glucoraphanin) found primarily in the cruciferous vegetable broccoli, which is an indirect antioxidant, modulator of the inflammatory response, and HDAC inhibitor.[4–7] Its antioxidant effects operate through nuclear factor erythroid 2-related factor2 (Nrf2); SFN binds with Keap1 (Kelch-like ECH-associated protein 1), which releases Nrf2, a transcription factor, that then moves from the cytoplasm to the nucleus and binds to the antioxidant response element on DNA, and upregulates the gene expression of more than 100 antioxidative and Phase 2 detoxifying enzymes; it also inhibits the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) pro-inflammatory cascade.[7–12]

The now classic Nrf2-Keap1 mechanism is most well-known for its upregulation of antioxidant and detoxification pathways. Early work focused primarily on this ability to induce antioxidant and cytoprotective pathways including those involved in the synthesis of glutathione (GSH), drug metabolism and transport, metabolic (energetics) enzymes, heme and iron metabolism, and a variety of transcription factors.[10, 11, 13] The role of SF in inducing and enhancing GSH levels in the body via the Keap1/Nrf2 pathway is a major mechanism by which SF confers its many benefits to human health. Glutathione is the body's most prevalent endogenous antioxidant, and it continuously toggles back and forth between its reduced (GSH) and oxidized (GSSG-Glutathione disulfide) state, the former being critically poised for its antioxidant and detoxification roles, the latter being a result of its performing those functions. SF induces the production of GSH and promotes the synthesis of enzymes that recycle GSSG back to its active, GSH state.[14] SF inhibit NF-κB.[8, 9, 11, 15–24] Among other applications, in animal studies, sulforaphane has been found to suppress the proinflammatory activation of macrophages in liver and adipose tissue, thus having implications that cross over into type 2 diabetes and insulin resistance.[25]

These may be involved in some of the underlying pathophysiology of schizophrenia. The higher expression of inflammatory cytokines and oxidative stress related biomarkers have been reported in subjects with prodromal symptoms, first-episode schizophrenia and chronic schizophrenia,[26–28] and elevated inflammatory cytokine levels are associated with the deficit syndrome subtype and negative symptoms.[29] Both first episode and chronic schizophrenics have low levels of GSH determined by 7T-MRS scans in the dorsal anterior cingulate (dACC) and other brain areas compared to controls,[30,31–33] and these are correlated with plasma levels (GSH does not pass the blood brain barrier, but CNS and plasma levels are often parallel).[30] Plasma, serum, RBC levels and CSF levels of GSH are decreased in both acute and chronic schizophrenia, including decreased RBC levels in those prodromal that later convert to schizophrenia.[34] Plasma GSH and dorsal anterior cingulate levels correlated with composite performance score on tests of cognitive functions.[30] Parvalbumin containing GABAergic interneurons and their perineuronal nets are reduced in the prefrontal postmortem brains,[35,36] thalamic reticular nucleus,[37,38] inferior colliculus[39] of schizophrenics and other areas,[40–42] in postmortem brains of schizophrenics in comparison to controls. The parvalbumin containing GABA neurons, are particularly vulnerable to oxidative stress[32, 38] and decrease levels parvalbumin containing GABA neurons in postmortem schizophrenic have been linked to the decrease in cognitive performance such as working memory, seen in schizophrenia.[40–42] Decrease in CNS and plasma levels of GSH and brain levels of glutamate in schizophrenia were related to decrease performance in cognitive tests.[30] Oral sulforaphane increased GSH levels in the brain's hippocampus as monitored by 7T-MRS scans in normal individuals.[43] These studies support the suggestion that the anti-oxidative and anti-inflammatory effects of sulforaphane may be relevant to the mechanisms of sulforaphane's effect in psychiatric illness and cognitive deficits in schizophrenia.

A further mechanism by which sulforaphane may modify the course of schizophrenia is through a GABAergic mechanism.[44, 45] A defect in GABAergic markers in postmortem brain studies and decrease in synaptic spines is one of the most consistently replicated abnormalities and might explain some of the decrease in grey matter and associated volumetric abnormalities in the brain which have been correlated with cognitive impairment.[46–48] This decrease in GABAergic function in schizophrenia may be caused by the epigenetic silencing of *glutamic acid decarboxylase*$_{67}$ (GAD$_{67}$) expression due to both increase *DNA methyltransferase* (DNMT) and lower levels of Histone deacetylase2 (HDAC2) activity in dorsolateral prefrontal cortex (DLPFC) observed in postmortem of patients with schizohrenia.[49] Indeed, a recent Positron Emission Tomography (PET) study showed lower levels of HDAC, in the DLPFC of subjects with schizophrenia compared to controls, and the levels of HDAC activity correlated positively with several cognitive domains measured on the MATRICS battery.[50] Several compounds with HDAC inhibitory activity have been shown to increase levels of histone acetylation and decrease methylation effects through DNMT,[4,5] with a consequent increase of GAD$_{67.}$ The HDAC inhibitory activity of sulforaphane has been demonstrated in multiple studies and can lead to an increase in acetylated histones and a decreased the expression of several methylation enzymes DNMT1 and DNMT3a and DNMT3b.[5] A study in humans showed that after ingestion of 68 g

of broccoli sprouts there was a significant (>50%) reduction in HDAC activity in blood PMCs and an increase in acetylated histones H3 and H4[51] and a study in prostate cancer patients[52] suggested that 200 µmoles/day of sulforaphane could increase histone acetylation in some patients. These studies suggest that sulforaphane effects on histone acetylation might reduce cognitive deficits seen in some patients with schizophrenia.

Studies in mice have shown that SF can ameliorate some of the cognitive deficits, pre-pulse inhibition (PPI) deficits, and brain histological changes induced by phencyclidine (PCP), a drug which has been used as an animal model of schizophrenia.[53, 54] A clinical trial in patients with autism spectrum disorder has shown SFN to be effective in reducing some of the social and behavioral deficits associated with this disorder.[55,56] At the time we initiated our trial, the only published study of SF in schizophrenia was one small (seven patient) open-label study of SFN in Japanese patients with schizophrenia, which showed improvement on a visual recognition learning task from the CogState Battery,[57] but no improvement on PANSS scores.

This background provided the rationale for our current Phase 2A study, a double-blind placebo-controlled study of sulforaphane in Chinese patients' early episode schizophrenia which can more fully evaluate the cognitive effects of sulforaphane in schizophrenia. We chose to study these early stage patients because their symptomatic and cognitive deficits may be more amenable to changes with pharmacological intervention. The primary outcome measure was cognitive improvement measured by Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Consensus Cognitive Battery (MCCB)[58] Overall Composite Score. Secondary outcomes included changes in MCCB Domain scores, Positive and Negative Symptom Scale[59] (PANSS) Total score, PANSS factor scores, and changes in side-effects scores.

METHODS

Study Design

Participants with a diagnosis of first-episode schizophrenia or recent symptom exacerbation during early episode were enrolled in both inpatient and outpatient settings. During a 2-week run-in phase prior to sulforaphane randomization, patients were either initiated on risperidone or their basic antipsychotic medication was switched to risperidone and titrated to 4–6 mg/day; this dose remained constant during the study unless serious emergent clinical changes warranted a change in dose. Then patients were randomly assigned to 1–3 groups: one of two doses of sulforaphane—(A) 6 tablets/day, designated the higher dose (HD), (B) 4 sulforaphane tablets/day plus 2 placebo tablets, designated the lower dose (LD), or (C) placebo group (6 placebo tablets), for a period of 22 weeks. Sulforaphane was delivered as Avmacol (Nutramax Laboratories, Inc., Edgewood, MD, USA), or a similar looking placebo tablet produced and supplied by the same manufacturer. Avmacol tablets contain both glucoraphanin and active myrosinase enzyme and are formulated to support sulforaphane production from ≥ 30 µmol of glucoraphanin per tablet as certified by the manufacturer (see supplement for quality control data of lots used in this study and SF conversion rates). Measured glucoraphanin content was 34 and 39 µmol/tablet (15 and 17 mg/tablet) for production lots RD0416-02 and RD1215-04, respectively, averaging 36.5 µmol/tablet. The lower dose, 4 tablets, and the higher dose, 6 tablets, were estimated to yield about 146 and 219 µmol/participant/day of glucoraphanin. Based on bioavailability study data from studies performed at John Hopkins University,[60] we estimated delivery of approximately 66 and 99 µmol of sulforaphane daily in the lower and higher dose group. Participants were randomly assigned 1:1:1 to two doses of sulforaphane or placebo through a computer-generated random number in blocks of four by research technician unconnected to clinical administration of medication. Treating clinicians, research staff, and patients were blinded to medication assignment.

The study was conducted at four medical centers (the Second Xiangya Hospital of Central South University, the first affiliated hospital of Zhengzhou University, Huiai hospital of Guangzhou, the second affiliated hospital of Xinxiang Medical University) between November 2016 and June 2019. 172 patients met enrollment criteria and were randomized into three study groups. The Institutional Review Boards of the Second Xiangya Hospital of Central South University and the other sites approved this trial protocol. All participants provided written informed consent.

Inclusion and Exclusion Criteria

Inclusion criteria were subjects who were aged 18–50 years, were experiencing a first or early psychotic episode of schizophrenia and met diagnostic criteria of DSM-V. Subjects PANSS score was 75 or greater on initial enrollment. The diagnosis of schizophrenia was determined by clinical interview and history by two different chief psychiatrists, or with an associate chief physician, using DSM-V criteria. A formal SCID interview was not performed. The following participants were excluded from this study, people who: (a) pregnant or lactating, (b) had a history of substance dependence or abuse or other diagnosable mental disorders, (c) had a history of traumatic brain injury, seizures or other known neurological or organic diseases of the central nervous system, (d) had current suicidal or homicidal thoughts, (e) had clinically significant abnormal renal, liver function or other metabolic results.

Assessments

Basic background demographic information, medical and psychiatric history and family history of mental illnesses were collected at baseline. Routine lab chemistries were obtained at baseline and weeks 4 and 22 of study drug treatment.

The primary cognitive instrument was the MCCB adopted and standardized in China,[61] and was performed by research assistants who were certified in MCCB (details in supplement). We used additional exploratory tests based on previous research in China, using tests which showed a large effect size ($d \geq 0.8$) between patients with schizophrenia and controls,[62] including the following: Color Trails I, Color Trail II, Paced Auditory Serial Addition Test (PASAT), Grooved Pegboard Tests (GPT) (dominant and nondominant hand), Category Fluency (animal and action naming), Wisconsin Card Sort Test (WSC), Stroop test (word and color word). Furthermore, a Global Deficit Score (GDS) (range 0–5, see supplement for details) was calculated according to rules described in our previous publication (22). Cognitive assessments were done at baseline and weeks 10 and 22 of study drug.

Psychopathology was evaluated by the PANSS scale and Calgary Depression Scale, and side effects by the SAFTEE,[63] Abnormal Involuntary Movement Scale—AIMS[64], Barnes Akathisia Scale—BAS,[65] and Simpson-Angus Scale—SAS,[66] at enrollment, baseline (week 2), and weeks 4, 10 and 22 of study drug administration.

Statistical Analysis

The primary analysis was an intent to treat analysis of all patients who had at least one post baseline value on SF or placebo for that variable. We used statistical programs SAS 9.4 and SPSS 25. Statistical significance was set at $P < .05$, and trend level significance was $P < .08$. The analysis of symptom and cognitive variables used mixed model analysis using SAS mixed procedure to handle missing data, from drop-outs or other causes. If variables deviated markedly from the normal distribution, transformations (log, square root) were attempted before analysis to achieve a better approximation to normal distribution; where distributions were still very skewed we developed syntax for mixed model with nonnormal distributions using proc glim mix and appropriate transformations. The main analysis was a mixed model analysis of difference scores from baseline with baseline scores rating at end of run in as covariate. Additional analyses used mixed model original values at the indicated time points without covariate, and completer analysis at each time point. Effect size for the overall mixed model treatment effect was computed for variables with statistically significant treatment effects or strong trends, using additionally developed SAS syntax based on the suggestions of Tippley and Longnecker (http://www.scsug.org/wp-content/uploads/2016/11/Ad-Hoc-Method-for-Computing-Effect-Size-for-Mixed-Models_PROCEEDINGS-UPDATE-1.pdf). Corrected significance levels across scales or subscales for a specific variable was assessed by Benjamini–Hochberg (BH) protected significance level (at $\alpha = .05$).[67,68] Effect size output used η^2 which we translated into Cohen's d (through Psychometrica, www.psychometrica.de/effect). Effect size at individual time points was analyzed by computation in an excel program for treatment and control groups with Cohen's d and Hedges correction. Ratings on the PANSS scale used PANSS Total score and PANSS 5-factor scores derived from factor analysis of the PANSS scale based on 5-factor score derived from studies with Chinese and international studies of patients with schizophrenia.[69–71]

Differences in side effects between sulforaphane treatment groups and placebo were nonnormal in distribution and analyzed by Kruskal Wallis analysis of variance, using scores for the difference from baseline at each time point for the following variables: (a) each item of SAFTEE scale, (b) total score on AIMS, BARNES, and Simpson-Angus scale. The results are based on completer analyses at each time point. Additionally, the total score of all items on the SAFTEE scale was analyzed in mixed model analysis. Differences in changes in routine laboratory values in the three treatment groups were assessed by analysis of variance.

Results

Background Characteristics

The flow of patients is shown in figure 1 with more detailed reasons for drop-outs at each stage in the web-supplement. Background characteristics of 151 participants who completed at least one evaluation after beginning treatment with sulforaphane or placebo are shown in table 1. Subjects were first or early episode schizophrenia who had been ill for an average of 15 months (range 0.04–3 years) and 86% were antipsychotic drug naïve at study entry. About 73.5% were inpatients and 26.5% were outpatients at study entry. There were no significant differences in age, sex, education, inpatient/outpatient status, antipsychotic drug treatment, or baseline PANSS or cognitive scores between treatment groups. Almost all the patients showed acute psychiatric symptoms with only three patients having PANSS Total scores below 73 at baseline evaluation just before study drug initiation.

Medication Ingestion Compliance

Medication ingestion, as determined by pill counts in bottles returned, was high and there were no differences between the assigned drug groups (see web supplement for details).

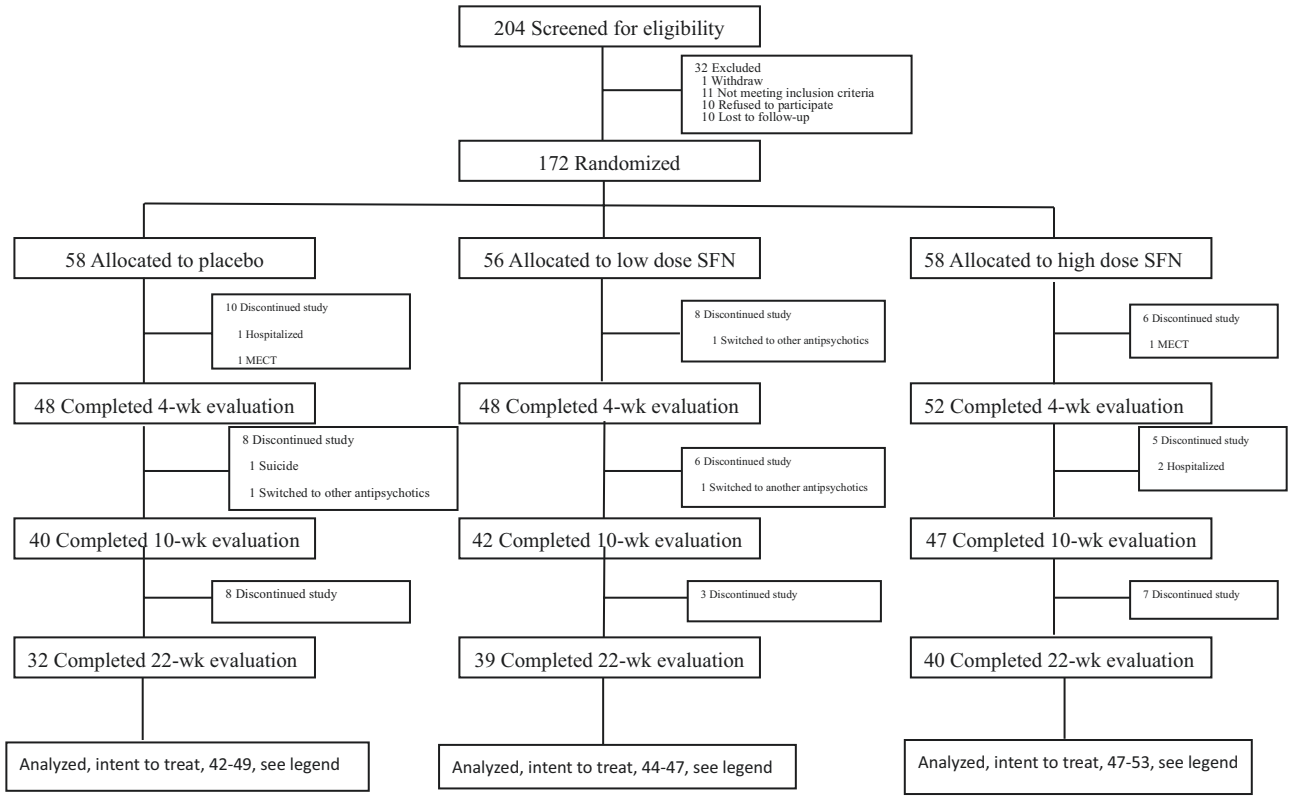

Fig. 1.

Sulforaphane Effects on Cognition

Table 2 shows the effects of sulforaphane treatment, with the F_{TR} showing the overall effects, and the mean difference (from baseline) at specific doses and time points shows more specific effects of the higher vs lower dose compared to placebo. Sulforaphane did not improve Overall Composite scores on the MCCB battery or Global Cognitive Deficit (GDS) scores (table 2, figure 2 and supplementary table S3 web-supplement). However, there were significant improvement on the MCCB Domain scores, Spatial Working Memory (F_{TR} = 5.68, DF = 2,129, ***P* = .004**), Verbal Learning (F_{TR} = 3.56, DF = 2,126, ***P* = .031**), and a near significant trend for Reasoning and Problem Solving (F_{TR} = 2.82, DF = 2,129, ***P* = .063**) (table 2, figure 2). The overall treatment effect on spatial working memory difference score was still significant after BH correction for multiple comparisons on 7 MCCB domain or composite scores (table 2). The effect sizes (Cohen's *d*), for these three domain scores were small to moderate (table 2). For verbal learning and problem solving the strongest improvements were for higher dose of SF, but for spatial working memory the stronger effect was from the lower SF dose. Similar effects were seen for the main analysis of difference score with bassline covariate (table 2), and the analysis of scores at each time point without covariate adjustment (figure 2), but the difference score analysis showed a somewhat stronger effect. Completer analysis showed fairly similar results. (supplementary table S4). There were no effects of sulforaphane treatment on measures from several other cognitive tests, including the Wisconsin Card Sort, Stroop tests, Grooved Pegboard and PASAT (see supplementary table S2 for full results). On the Color Trials test (supplementary table S2), the lower SF dose reduced the decrement than placebo at follow up times in the more challenging Color Trials II test. There were no statistically significant "interaction" effects of drug treatment × site, or drug treatment × time × site for any of the MCCB domains which showed improvement with sulforaphane. However, the Reasoning and Problem Solving domain showed a significant overall site effect and for this domain and for spatial working memory some sites tended to show a stronger effect at specific time points. There were no significant interactions effects of whether subjects started the study as inpatient vs outpatient status and sulforaphane treatment effects on cognitive variables (see supplement).

Sulforaphane Effects on Psychiatric Symptoms

There were no statistically significant treatment effects of sulforaphane on psychiatric symptoms, as measured by PANSS total scores or any of the 5-factors of the PANSS scale (table 3, figure 2) above that of placebo, although all groups improved with very similar substantial decreases in scores. Although there was no overall treatment effect

Table 1 Subject Characteristics

Characteristic	Placebo ($N = 50$)	Low dose Sulforaphane ($N = 48$)	High dose Sulforaphane ($N = 53$)	Statistical Tests
Age (m)	23.18 ± 6.43	24.73 ± 7.57	24.64 ± 6.39	$F = 0.819$, df = 2,148, $P = 0.443$
Sex (M/F) (*n*)	24/26	23/25	20/33	$X^2 = 1.457$, df = 2, $P = 0.483$
BMI (m)	21.59 ± 3.53	21.26 ± 3.40	21.71 ± 4.16	$F = 0.192$, DF = 2,148, $P = 0.826$
Education (yrs.) (m)	10.96 ± 2.407	10.69 ± 2.594	11.32 ± 2.840	$F = 0.742$, df = 2,148, $P = 0.478$
Months ILL (m)	13.79 ± 11.48	16.66 ± 13.08	14.37 ± 13.51	$F = 0.695$, df = 2,148, $P = 0.501$
Ethnicity (H/O) (*n*)	50/0	48/0	48/5	$X^2 = 9.562$, df = 2, $P = 0.008$
Study Site (n) (CHANGSHA/ZHENZHOUG/ XINXIANG/GUANGZHOU)	14/14/12/10	18/11/11/8	19/11/13/10	$X^2 = 1.530$, df = 6, $P = 0.957$
Cigarette Smoker (Y/N) (n)	9/41	4/44	8/45	$X^2 = 2.007$, df = 2, $P = 0.367$
Antipsychotic drug naïve before study [number (%)]	42 (84.0%)	42 (87.5%)	46(86.8%)	$X^2 = 0.284$, df = 2, $P = 0.868$
Duration of illness in drug naïve subjects before study entry (months)	11.99 ± 10.95 ($n = 42$)	15.93 ± 13.00 ($n = 42$)	13.36 ± 13.64 ($n = 46$)	$F = 1.053$, df = 2,127, $P = 0.352$
Inpatient/Outpatient at start of study	40/10	33/15	38/15	$X^2 = 1.729$, df = 2, $P = 0.412$
Length of hospital stay for inpatients during this study period (days) (m)	17.75 ± 4.73	16.03 ± 4.00	16.68 ± 4.60	$F = 1.385$, df = 2,108, $P = 0.255$
Risperidone dose (m)	4.80 ± 1.16	5.07 ± 1.28	4.79 ± 1.18	$F = 0.863$, df = 2,148, $P = 0.424$
Antidepressant (Y/N) (*n*)	0/50	2/46	1/52	$X^2 = 2.188$, df = 2, $P = 0.335$
Mood stabilizer drug (Y/N) (*n*)	0/50	0/48	0/53	NR
Benzodiazepine drug (Y/N) (*n*)	1/49	1/47	1/52	$X^2 = 0.005$, df = 2, $P = 0.997$
Baseline PANSS total scores	91.69 ± 16.71 ($n = 49$)	88.79 ± 14.28	87.60 ± 14.90	$F = 0.949$, df = 2,147, $P = 0.390$
Baseline MATRICS scores				
Overall composite (m)	35.75 ± 6.00 ($n = 36$)	39.09 ± 5.98 ($n = 38$)	37.65 ± 7.45 ($n = 45$)	$F = 2.392$, df = 2,116, $P = 0.096$
Working memory (m)	35.98 ± 13.02 ($n = 42$)	37.86 ± 13.51 ($n = 44$)	38.81 ± 11.01 ($n = 47$)	$F = 0.582$, df = 2,130, $P = 0.560$
Reasoning-problem solving (m)	36.14 ± 12.19 ($n = 42$)	38.36 ± 11.47 ($n = 44$)	39.98 ± 11.55 ($n = 47$)	$F = 1.191$, df = 2,130, $P = 0.307$

Background characteristics of subjects included in Analysis-Subjects who completed at least one post-baseline measure on one variable. Sex: M = Male, F = Female. Ethnicity: H = Chinese Han, O = Other. Y = Yes, N = No. (m) Mean ± S.D. (*n*) = number of subjects $F = F$ values from univariate analysis of variance. X^2 = Chi-Square value. NR = Calculation no relevant. 84% of the subjects were in the age range 17–30 and only 4 were above 40 years of age.

Table 2 Effects of Sulforaphane MATRICS Battery and Other Cognitive Scale Scores

Scale	Treatment	Baseline Score (Mean ± s.e.m.)	Adjusted Estimated Difference from Baseline At Specified Time Point (Mean ± s.e.m.). *'s indicate significant difference from placebo		Overall Analysis (difference scores) F_{TR} Overall Treatment Effect Effect Size Cohen's d for scores which had statistical significant treatment effect
			10 Weeks	22 Weeks	
MATRICS battery					
Overall composite	Sulforaphane-HD (n = 45)	37.65 ± 0.98	3.09 ± 0.62	3.87 ± 0.72	F_{TR} = 0.37, DF = 2,115, P = 0.693
	Sulforaphane-LD (n = 38)	39.09 ± 1.07	2.61 ± 0.68	3.01 ± 0.77	
	Placebo (N = 36)	35.75 ± 1.10	2.20 ± 0.71	3.44 ± 0.82	
Speed of processing	Sulforaphane-HD (n = 47)	35.85 ± 1.24	2.84 ± 0.93	4.95 ± 1.01	F_{TR} = 0.27, DF = 2,127, P = 0.767
	Sulforaphane-LD (n = 43)	36.16 ± 1.30	2.52 ± 0.98	3.49 ± 1.04	
	Placebo (N = 41)	32.46 ± 1.33	2.23 ± 1.03	4.20 ± 1.11	
Attention vigilance	Sulforaphane-HD (n = 47)	38.60 ± 1.83	1.13 ± 1.18	2.56 ± 1.43	F_{TR} = 1.35, DF = 2,124, P = 0.263
	Sulforaphane-LD (n = 43)	39.07 ± 1.92	2.15 ± 1.25	5.38 ± 1.47	
	Placebo (N = 38)	37.76 ± 2.04	2.84 ± 1.34	6.28 ± 1.64	
Spatial working Memory	Sulforaphane-HD (n = 47)	38.81 ± 1.83	3.95 ± 1.29 LH	4.84 ± 1.34	F_{TR} = 5.68, DF = 2,129, **P = 0.004**[B]
	Sulforaphane-LD (n = 44)	37.86 ± 1.89	7.88 ± 1.35 ** LH (d = 0.51)	7.99 ± 1.35**(d=0.37)	d =0.35
	Placebo (N = 42)	35.98 ± 1.93	2.09 ± 1.39	2.16 ± 1.46	
Verbal Learning	Sulforaphane-HD (n = 47)	34.17 ± 1.60	2.85 ± 1.17 * LH (d = 0.35)	1.21 ± 1.38	F_{TR} = 3.56, DF = 2,126, **P = 0.031**
	Sulforaphane-LD (n = 44)	35.36 ± 1.66	−1.94 ± 1.23 LH	−1.22 ± 1.39	d=0.26
	Placebo (N = 42)	33.26 ± 1.70	−0.93 ± 1.28	−1.46 ± 1.52	
Visual Learning	Sulforaphane-HD (n = 47)	39.77 ± 13.20	5.32 ± 1.62	4.15 ± 1.88	F_{TR} = 0.70, DF = 2,169, P = 0.498
	Sulforaphane-LD (n = 43)	41.12 ± 12.26	3.93 ± 1.74	1.44 ± 1.92	
	Placebo (N = 40)	37.80 ± 9.13	4.29 ± 1.82	0.68 ± 2.07	
Reasoning-Problem Solving	Sulforaphane-HD (n = 47)	39.98 ± 1.71	5.94 ± 1.22*(d = 0.24)	8.29 ± 1.40 ǂ LH	F_{TR} = 2.82, DF = 2,129, **P = 0.063**
	Sulforaphane-LD (n = 44)	38.36 ± 1.77	4.39 ± 1.28	4.11 ± 1.40 LH	d = 0.26
	Placebo (n = 42)	36.14 ± 1.81	2.22 ± 1.32	4.54 ± 1.53	
Social Cognition	Sulforaphane-HD (n = 45)	36.933 ± 1.80	2.40 ± 1.83	1.33 ± 1.76	F_{TR} = 0.59, DF = 2,118, P = 0.558
	Sulforaphane-LD (n = 39)	40.77 ± 1.94	0.20 ± 1.20	−1.47 ± 1.86	
	Placebo (N = 38)	37.37 ± 1.20	−0.13 ± 2.03	0.14 ± 1.93	
Optimal Neuropsychological Tests (NP) Component Combined Total Scores, And Global Deficit Scores(GDS) Derived From NP Component Scores					
NP component test total score	Sulforaphane-HD (n = 43)	34.89 ± 1.17	3.86 ± 0.73	5.86 ± 0.76	F_{TR} = 1.22, DF = 2,116, P = 0.298
	Sulforaphane-LD (n = 40)	35.89 ± 1.16	2.39 ± 0.76	4.60 ± 0.78	
	Placebo (N = 37)	32.13 ± 1.21	3.62 ± 0.81	6.16 ± 0.83	
NP GDS Score	Sulforaphane-HD (n = 43)	1.71 ± 0.16	0.56 ± 0.09	−0.73 ± 0.10	F_{TR} = 1.45, DF = 2,116, P = 0.240
	Sulforaphane-LD (n = 30)	1.53 ± 0.16	−0.34 ± 0.09	−0.51 ± 0.11	
	Placebo (n = 37)	2.07 ± 0.17	−0.42 ± 0.10	−0.71 ± 0.11	

Analysis is from mixed model difference scores with baseline score as covariate. Values are baseline scores and model estimated difference sucres at 10 weeks and 22 weeks of treatment. HD = Higher dose of sulforaphane, LD = Lower dose sulforaphane. Different ns are due to missing values on one or more tests for some subjects. F_{TR} = overall sulforaphane treatment effect incorporating effects of both sulforaphane doses vs placebo at both time points. d = effect size. The mean difference (from baseline) in the table at 10 and 22 weeks, with significance levels from placebo indicated can be used to interpret effects of specific doses at two specific treatment time points. At specific time points mean **difference value different from placebo**: *P < 0.05, ** P < 0.01, ǂ P < 0.08. Difference in values of HD vs LD sulforaphane LH = P < 0.05. B = F values remained statistically significant with BH corrected significance levels including seven variables, MATRICS domains and overall composite scores considered in analysis (α = 0.05). Several MATRICS scores (Overall Composite, Speed of Processing, Attention-Vigilance) showed a statistically significant time effect with scores for all treatment groups increasing over time, but no difference in drug treatment effects. The Chinese MATRICS Battery does not contain the LNS test component for verbal working memory because of differences in Chinese language.

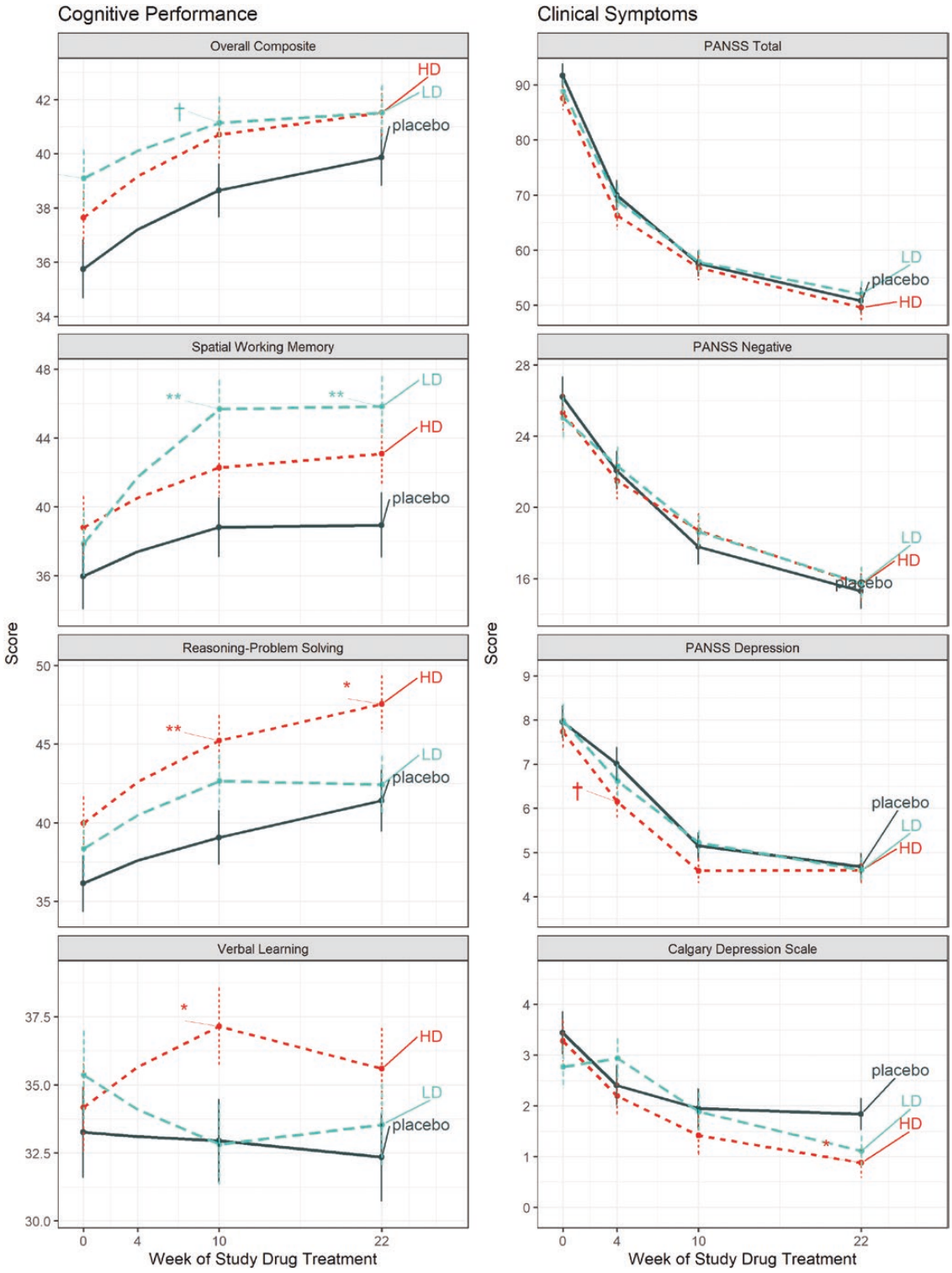

Fig. 2.

on the Calgary Depression Scale (CDS), the higher SF group showed a slightly greater decrease (P = .043) than placebo at 22 weeks of study drug treatment. For the CDS there was a sex effect (drug treatment × sex interaction, P = .002) and only females but not males showed a sulforaphane benefit vs placebo at 10 weeks and 22 weeks of study drug treatment (see additional data in web supplement).

Table 3 Effects of Sulforaphane on Psychopathology Scores

Scale	Treatment	Baseline Score (Mean ± s.e.m.)	Adjusted Estimated Difference from Baseline At Specified Time Point (Mean ± s.e.m.)			Overall Analysis (difference scores) F_{TR} = Overall Treatment Effect
			4 Weeks	10 Weeks	22 Weeks	
PANSS total score	Sulforaphane-HD (n = 53)	87.60 ± 2.11	−21.84 ± 2.18	−31.14 ± 2.06	−38.08 ± 2.31	F_{TR} = 0.16, DF = 2,146, P = 0.849
	Sulforaphane-LD (n = 48)	88.79 ± 2.21	−19.83 ± 2.29	−30.74 ± 2.16	−36.59 ± 2.37	
	Placebo (n = 49)	91.69 ± 2.19	−20.01 ± 2.28	−32.38 ± 2.20	−38.70 ± 2.47	
PANNS 5-Factor Scores						
Positive	Sulforaphane-HD (n = 53)	15.32 ± 0.70	−5.57 ± 0.54	−7.44 ± 0.45	−8.39 ± 0.51	F_{TR} = 0.45, DF = 2,146, P = 0.638
	Sulforaphane-LD (n = 48)	14.77 ± 0.73	−5.67 ± 0.56	−8.09 ± 0.47	−8.29 ± 0.52	
	Placebo (N = 49)	15.00 ± 0.72	−5.12 ± 0.56	−7.36 ± 0.47	−7.89 ± 0.54	
Negative	Sulforaphane-HD (n = 53)	21.47 ± 0.98	−3.81 ± 0.80	−6.54 ± 0.80	−9.34 ± 0.97	F_{TR} = 0.47, DF = 2,146, P = 0.566
	Sulforaphane-LD (n = 48)	21.15 ± 1.03	−2.90 ± 0.85	−6.57 ± 0.84	−9.49 ± 0.98	
	Placebo (N = 49)	22.04 ± 1.02	−3.64 ± 0.84	−7.92 ± 0.85	−10.48 ± 1.04	
Depression	Sulforaphane-HD (n = 53)	7.74 ± 0.36	−1.77 ± 0.34	−3.30 ± 0.27	−3.63 ± 0.28	F_{TR} = 1.87, DF = 2,146, P = 0.158
	Sulforaphane-LD (n = 48)	7.98 ± 0.38	−1.31 ± 0.36	−2.72 ± 0.28	−3.33 ± 0.28	
	Placebo (N = 49)	7.96 ± 0.38	−0.92 ± 0.36	−2.77 ± 0.29	−3.27 ± 0.30	
Cognitive	Sulforaphane-HD (n = 53)	11.43 ± 0.47	−2.64 ± 0.34	−3.59 ± 0.38	−4.33 ± 0.39	F_{TR} = 0.74, DF = 2,146, P = 0.479
	Sulforaphane-LD (n = 48)	11.77 ± 0.49	−2.47 ± 0.36	−3.16 ± 0.40	−3.80 ± 0.40	
	Placebo (N = 49)	11.33 ± 0.49	−2.80 ± 0.35	−3.57 ± 0.40	−4.66 ± 0.41	
Excitement	Sulforaphane-HD (n = 53)	9.09 ± 0.60	−1.51 ± 0.56	−2.51 ± 0.71	−2.10 ± 0.64	F_{TR} = 0.66, DF = 2,146, P = 0.522
	Sulforaphane-LD (n = 48)	9.58 ± 0.63	−1.50 ± 0.69	−2.20 ± 0.75	−1.51 ± 0.72	
	Placebo (N = 49)	9.93 ± 0.62	−1.26 ± 0.49	−1.46 ± 0.58	−1.03 ± 0.54	
Calgary Depression Scale Total Score	Sulforaphane-HD (n = 53)	3.29 ± 0.41	−1.04 ± 0.33	−1.81 ± 0.33	−2.37 ± 0.29* (d=-0.17)	F_{TR} = 2.09, DF = 2,145, P = 0.127
	Sulforaphane-LD (n = 48)	2.77 ± 0.43	−0.13 ± 0.35	−1.18 ± 0.35	−1.93 ± 0.29	
	Placebo (N = 49)	3.44 ± 0.43	−0.85 ± 0.35	−1.33 ± 0.36	−1.50 ± 0.32	

Each values of adjusted mean difference, is estimated mean difference from all subjects having at least baseline values and one post-value value derived from SAS mixed model analysis with baseline value as covariate. LD = low dose sulforaphane; HD = high dose sulforaphane. Variables which were markedly nonnormal in distribution were analyzed by modified mixed model with transforms for distribution. Statistical significance of estimated mean in sulforaphane group from placebo at specific time point, by t-test comparison in mixed model results:

*$P < 0.05$. All the symptom measures, except the excitement factor, showed a significant time effect, $P < 0.001$with subjects in all treatment groups showing improvement over time, the scores being lower (difference score more negative) at the later time points.

Relationship of Decrease in PANSS scores to Decrease in MATRICS scores

Whether the decrease in psychiatric symptoms explained the improvements in MCCB scores was explored by several analyses. First, as noted, the PANSS scored decreased equally in both the sulforaphane and placebo-treated groups whereas improvement in the Matrics scores occurred only in the sulforaphane treated patients. This may make it less likely that the decrease in PANNS scores was responsible for the improvement in Matrics scores. However, we did additional analysis to examine this question more directly. We did mediation analysis to investigate whether decreases PANSS Total score mediated the improvement in Matrics scores in the domains of spatial working memory, verbal leaning, and reasoning and problem solving. We used the criteria proposed by Kenny and associates[72–74] to determine whether PANSS decrease met the criteria as a mediator of the Matrics changes in these domains; specific explanation of the multiple criteria and procedures and relevant statistical output are found in supplementary data. According to these analyses, the PANNS Total decrease did not fulfill criteria to be a mediator of Matrics change. We also looked at correlations between increase in Matrics scores and decrease in PANSS scores; if decreases in PANSS scores substantially influenced change in Matrics scores, we would expect significant negative correlations between PANSS change and Matrics change. There were no significant negative correlations between decrease in PANSS Total, PANSS Positive, or PANSS Negative and changes in working memory, verbal learning, or reasoning and problem solving scores, except for one negative correlation between PANSS negative symptoms and increase in verbal learning ($r = -0.35$, $P < .05$) (See supplement section 10 for statistical details.)

Side Effects

There was also no difference between sulforaphane and placebo on the total scores of ratings on the AIMS, BARNES, SIMPSON-ANGUS scales (supplementary table S8), most individual items on the SAFTEE scale (supplementary table S7), or the SAFTEE total side-effect scores. There were no effects of drug treatment on changes in routine laboratory values (supplementary table S10). (See supplement section 12 for details.)

Discussion

Although the primary hypothesis of improvement in overall cognitive function as measured by the MCCB Overall Composite scores was not confirmed, the results of this study showed that sulforaphane improved selective aspects of cognitive function, particularly Spatial Working Memory, Verbal Learning and Reasoning and Problem Solving, in patients with early episode schizophrenia. The purpose of a phase 2a study is to determine if further clinical trials are indicated; we feel that positive results on three of the seven domains of the MCCB to warrant further clinical trials in the context of our rationale which links defects in oxidative stress and HDAC2 to cognition. The strongest effect appeared to be on improvement in spatial working memory which had the largest effect size and consistent improvement at 10 weeks and 22 weeks of study drug treatment. Since the doses of sulforaphane we used were relatively low, we do not know whether much higher doses would have shown a greater or more consistent effect on our cognitive measures, and the apparent inconsistent dose effects (low dose better than high) on working memory domain are difficult to explain and confusing. On the basis of data from one review,[75] it has been suggested that the maximal biological effect at tolerated human doses may be obtained with a dose of approximately 200 μmol sulforaphane/day (for 70 kg person). In this study, the estimated of μmol SF/day delivered in our lower and higher dose groups were 66 and 99, which is about ⅓ or ½ this dose. Our doses were also lower than doses used in a sulforaphane study of the effects of air pollution in China which utilized a SF powdered drink mix and found a dose–response relationship for SF effects on an air pollutant measure benzene mercapturic acid. However, our higher dose was in the same range of does used in some double-blind clinical studies of sulforaphane in treatment of autism which showed positive effect of sulforaphane at these doses.[55,56,76] A recently published double-blind study of sulforaphane in chronic schizophrenia did not find any overall effects of the treatment on any MCCB or PANSS measures,[77] but a preliminary further report from this group did show effects of sulforaphane on improving working memory in a subset of subjects who had higher concentrations of dithiocarbamate >1 mmol/L in their urine,[78] which may be associated with higher sulforaphane levels in these subjects. This is consistent with the stronger effect on the MCCB working memory domain that we reported here.

Moreover, the effects of SF on improving cognition in this study are consistent with the positive cognitive effect SF reported in several animal studies. A study of brain injury to the right parietal lobe in rats,[79] showed that SF, improved performance on the Morris water maze task, and a delayed match to place task, in testing conducted weeks after the traumatic brain injury. In Zebra fishes,[80] sulforaphane counteracted the deficits induced by scopolamine administration in a fear condition passive avoidance task, with implications for retained memory function. PCP has been used as an animal model of many of the deficits seen in schizophrenia. A series of studies by Shirai and associates,[53,54] showed that acute or subchronic administration of SF could attenuate or reverse the effect of PCP on a measure related to memory retention expressed in exploratory preferences in mice. Treatment with a sulforaphane precursor glucoraphanin

during presumed adolescence in mice (4–8 weeks) also reduced PCP-induced cognitive deficits tested at a later time. These changes were accompanied by similar beneficial effects on histological and biochemical measures induced by PCP (a) the increase 8-oxo-dG-positive cells, and (b) the decrease in parvalbumin (PV)-positive cells in the medial prefrontal cortex and hippocampus and attenuation of the decrease in spine density.[54] Sulforaphane's antioxidant effects may work partly through a pathway involved in the with the Nrf2 and KEAP1 genes, and Shira and associates also show that SNP differences in these genes and their epistatic interaction are associated with cognitive deficits in patients with schizophrenia. Since SF has antioxidant, anti-inflammatory, and epigenetic properties, it is plausible SF could reverse known abnormalities seen patients with schizophrenia. A small open-label study with 90 mg of SFN-glucosinolate per day for 8 weeks in participants with schizophrenia patients showed no changes in psychiatric symptoms but an improvement in accuracy component of the One Card Learning Task from the CogState battery; however, only seven patients completed the trial.[57] All these studies are consistent with a hypothesized effect of SF on cognitive function in schizophrenia.

Cognitive deficits similar to those in first episode and chronic schizophrenia are also found in many high-risk subjects with prodromal symptoms, as has been previously reported by our group[81] and others.[82, 83] We are currently investigating whether SF administration in the prodromal phase may attenuate or prevent the development of cognitive deficits or the rate of conversion to schizophrenia.[84] The lack of side-effects from SF compared to placebo in this study suggests that this will be a well-tolerated intervention without major safety concerns. Other human studies have also shown a benign side-effects of SF with no major differences from placebo.[55,56,77,85]

What are potential implications of our results. There is currently no drug or other nutritional chemical compound which has been established as an effective treatment for attenuating cognitive deficits in schizophrenia. Although the effect size *d* for our positive findings cognitive effects on sulforaphane of Spatial Working Memory, Verbal Leaning and Reasoning Problem-Solving domains is modest ($d = 0.35$, 0.26, and 0.26, respectively), it is similar to the size effects of the recently introduced antipsychotics on reducing PANSS total score, which vary 0.27–0.39[86] (see web appendix to referenced article). Cognition is a different aspect of schizophrenia than symptoms, and it's possible that even a small benefit in cognition, added to that produced by standard treatment, could make a clinically important difference, if these findings are replicated in additional studies. It is not uncommon that early finding does not replicate, but we feel our finding are sufficiently positive that further studies should be undertaken.

Limitations

It is possible that the statistically positive effects on secondary outcomes can occasionally occur by chance, although the working memory effect survived a corrected BH significance test accounting for all MCCB components tested. We limited our conclusion to stressing the necessity of replication and make no claim that the evidence presented from this trial show conclusively that sulforaphane is definitely beneficial for cognition in schizophrenia. Another limitation may be that we could not fully assess compliance with medication. Although our data on pill counts suggest high compliance with medication ingestion, we do not have other more objective measures of medication compliance. Additionally, since this was only a 22-week study we do not know whether these sulforaphane effects would be persistent with continued sulforaphane treatment, but the stronger effects on verbal learning at 12 weeks compared to 22 weeks suggest that some of the effects may not persist at the same level over longer time periods. Since the doses of sulforaphane we used were relatively low we don't know whether much higher doses would have shown a greater or more consistent effect on our cognitive measures, and the apparent inconsistent dose effects on working memory domain are difficult to explain and confusing. The lack of inflammatory or oxidative stress biomarker effects of sulforaphane and their relationship to clinical response is an important deficiency in the study and we can therefore not link any biological mechanistic interpretations of sulforaphane's effects in this study or assess whether subjects baseline inflammatory state affected drug response.

Conclusions

Although there was no improvement in measures of overall cognitive function, which was the hypothesized primary outcome measure, our study found statistically significant improvement in working memory and other cognitive domains assessed by the MCCB battery in patients with early episode schizophrenia. This is the first controlled study, at the early phase two stage, and consequently too early to claim proof of concept, but we suggest that it provides enough evidence that further trials of sulforaphane should be undertaken. These results need to be replicated in additional studies before we can fully assess their potential clinical significance.

Supplementary Material

Supplementary data are available at *Schizophrenia Bulletin Open* online.

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