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ARTICLE

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A randomized, double-blind, placebo-controlled comparison study of sarcosine (*N*-methylglycine) and D-serine add-on treatment for schizophrenia

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Abstract

Recent evidence indicates that enhancing *N*-methyl-D-aspartate (NMDA) neurotransmission with the treatment of NMDA/glycine site agonists, such as D-serine, or a glycine transporter-1 (GlyT-1) antagonist, *N*-methylglycine (sarcosine), can improve symptoms of schizophrenia. To compare these two novel approaches, 60 patients with chronic schizophrenia were enrolled into a 6-wk double-blind, placebo-controlled trial of add-on treatments at the reported effective dosages (2 g/d). Clinical assessments were conducted every other week. Treatment group × treatment duration interaction analysis by multiple linear regression showed that sarcosine was superior to placebo at all four outcome measures of Positive and Negative Syndrome Scale (PANSS) total ($p=0.005$), Scale for the Assessment of Negative Symptoms (SANS) ($p=0.021$), Quality of Life (QOL) ($p=0.025$), and Global Assessment of Functioning (GAF) ($p=0.042$). However, D-serine did not differ significantly from placebo in any measure. Sarcosine treatment was better than D-serine in effect sizes for all outcome measures. Sarcosine also surpassed placebo in most of the measures of five PANSS factors and five SANS subscales. All treatments were well tolerated. These findings suggest that the GlyT-1 inhibitor is more efficacious than the NMDA/glycine site agonist in treatment for schizophrenia, including life quality and global function, at the dosages tested.

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Introduction

In addition to monoaminergic theory, glutamatergic dysfunction has been implicated in the pathophysiology of schizophrenia on the basis of the psychotomimetic action of phencyclidine (PCP) and ketamine, both of which block *N*-methyl-D-aspartate (NMDA) subtype glutamate receptor-mediated neurotransmission (Javitt, 1987; Tsai & Coyle, 2001). Consequently, enhancement of NMDA neurotransmission has been proposed as a potential treatment of schizophrenia (Deutsch *et al*. 1989). Several studies have targeted the glycine site of the NMDA receptor (NMDA/glycine site). The agents included full agonists such as D-serine (Heresco-Levy *et al*. 2005; Tsai *et al*. 1984), glycine (Heresco-Levy *et al*. 1996, 1999, 2004), D-alanine (Tsai *et al*. 2006), and the partial agonist D-cycloserine (Goff *et al*. 1999; Heresco-Levy *et al*. 2002). As add-on therapy to antipsychotics, these agonists improve negative and cognitive symptoms, but the efficacy of glycine and D-cycloserine appear inconsistent (Buchanan *et al*. 2007; Goff *et al*. 2005). Moreover, both D-serine and D-alanine also reduce positive symptoms (Heresco-Levy *et al*. 2005; Tsai *et al*. 1998, 2006).

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Another strategy to improve NMDA neurotransmission is increasing the synaptic glycine level by blocking glycine reuptake through the glycine transporter-1 (GlyT-1) (Javitt, 2008; Johnson *et al*. 2003). The GlyT-1 is vital in maintaining glycine within synapses at a sub-saturating level (Johnson *et al*. 2003), and its anatomical distribution parallels that of the NMDA receptor (Smith *et al*. 1992). *N*-methylglycine (sarcosine) is a potent and prototype endogenous Gly-T1 inhibitor, with IC_{50} at low micromolar range (Herdon *et al*. 2001; McBain *et al*. 1989). Sarcosine is present at high concentrations in humans (Glorieux *et al*. 1971). Sarcosine is also a methyl donor, and there is no other known neurotransmitter system affected by sarcosine. Supporting the role GlyT-1 plays in NMDA neurotransmission, *N*[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine (NFPS), a sarcosine analogue and a potent GlyT-1 inhibitor, enhances NMDA neurotransmission (Bergeron *et al*. 1998; Chen *et al*. 2003). In behavioural studies, the potency of a series of GlyT-1 antagonists for the inhibition of PCP-induced hyperactivity *in vivo* correlated significantly with their potency in antagonizing GlyT-1 *in vitro* (Javitt *et al*. 1999). In rodents, treatment with NFPS prevents dopaminergic dysregulation following chronic or subchronic PCP administration (Javitt *et al*. 2004), and improves MK-801-induced cognitive deficits (Karasawa *et al*. 2008). Further, Gly-T1 heterozygous knockout mice are more resistant to PCP-induced disruption of prepulse inhibition and possess better working memory (Tsai *et al*. 2004*a*).

A pilot clinical trial (Tsai *et al*. 2004*b*) demonstrated that sarcosine adjuvant therapy improved positive and negative symptoms in patients with chronically stable schizophrenia who were receiving typical or atypical antipsychotics. More recent studies further suggest that sarcosine, superior to D-serine, both at 2 g/d, can benefit the negative symptoms of acutely ill schizophrenia patients on concurrent atypical antipsychotic therapy (Lane *et al*. 2005), and that sarcosine can be used as monotherapy in acutely symptomatic patients (Lane *et al*. 2008). These findings suggest that the GlyT-1 inhibitor may be more efficacious than the NMDA/glycine site agonist for treatment of schizophrenia at the dosages tested (Lane *et al*. 2005). On the other hand, both sarcosine and D-serine improve comprehensive symptom components in chronically symptomatic patients. To compare the efficacy of these two novel treatments, we conducted a placebo-controlled study of add-on sarcosine and D-serine, at the reported effective dosages, in chronic patients with schizophrenia, who had been stabilized with atypical antipsychotic therapy for at least 3 months. Furthermore, we investigated whether these two novel compounds can improve life quality and functioning. The design can minimize the confounding of psychotic exacerbation, whose improvement by the add-on study agent may have been obscured by the concomitant antipsychotic treatment in the previous study for patients with acute exacerbation (Lane *et al*. 2005).

Method

Subjects

Patients were recruited from the in-patient units of China Medical University, which is a major medical centre in Taiwan, between 1 January 2005 and 31 December 2006. The research protocol was approved by the Institutional Review Boards (IRB) of the institute. Ethnically Han Chinese patients were screened and evaluated by the research psychiatrists. After complete description of the study to the subjects, written informed consent was obtained in line with the IRB's guidelines. The Structured Clinical Interview for DSM-IV (APA, 1995) was conducted for the diagnosis. Patients were enrolled into this study if they: (1) were physically healthy and had all laboratory assessments (including urine/blood routine, biochemical tests, and electrocardiograph) within normal limits, (2) aged 18–60 yr, (3) satisfied DSM-IV criteria for schizophrenia (APA, 1994), (4) had no DSM-IV diagnosis of substance (including alcohol) abuse or dependence, (5) remained symptomatic but without clinically significant fluctuation and the antipsychotic doses were unchanged for at least 3 months, and (6) had a minimum baseline total score of 60 on the Positive and Negative Syndrome Scale (PANSS; Kay *et al*. 1987).

Study design

The dosing strategy for the concurrent atypical antipsychotics, based upon recent studies (Lane *et al*. 2000, 2004), is to optimize efficacy while minimizing side-effects, especially extrapyramidal side-effects (EPS). After achieving optimal clinical treatment response, patients' antipsychotic doses remained constant for at least 3 months prior to enrolment in the study and remained on the same antipsychotic regimens for the study period. All patients were treated with atypical antipsychotics, risperidone for the majority (Table 1).

All patients were then randomly assigned under double-blind conditions to receive a 6-wk trial of placebo, or the only reported effective dosages of

Table 1. Demographics, illness and treatment characteristics of the patients assigned to placebo, D-serine, or sarcosine plus their chronically stable atypical antipsychotic treatments

	Study groups			
	Sarcosine ($n=20$)	D-serine ($n=20$)	Placebo ($n=20$)	p value
Demographics				
No. (%), female	8 (40)	8 (40)	11 (55)	0.55[a]
Age (yr), mean (S.D.)	30.4 (10.6)	30.7 (9.6)	31.5 (7.9)	0.70[b]
Body weight (kg), mean (S.D.)	66.6 (11.7)	63.0 (11.4)	67.3 (13.5)	0.53[b]
Age at onset of psychosis (yr), mean (S.D.)	22.4 (7.3)	20.0 (5.8)	21.6 (5.9)	0.44[b]
No. of hospitalizations, mean (S.D.)	2.9 (3.2)	2.9 (2.9)	2.8 (2.0)	0.87[b]
Schizophrenia subtype no. (%)				0.67[c]
Paranoid	15 (75)	13 (65)	11 (55)	
Disorganized	2 (10)	4 (20)	3 (15)	
Undifferentiated	3 (15)	3 (15)	6 (30)	
Risperidone dose (mg), mean (S.D.)	4.1 (1.5, $n=16$)	4.2 (1.5, $n=17$)	3.9 (1.8, $n=17$)	0.87[b]
Olanzapine dose (mg)	–	10, 20	20	
Quetiapine dose (mg)	400, 400, 500, 600	400	600, 600	

[a] χ^2 test.
[b] Kruskal–Wallis test.
[c] Fisher's exact test.

D-serine or sarcosine (2 g/d) (Lane *et al*. 2005, 2008; Tsai *et al*. 1998, 2004*b*). Patients were randomized in blocks of six subjects, without stratification, through a computer-generated randomization table to receive placebo or active drug in a 1:1:1 ratio. Study medication was provided in coded containers with a supply of identically appearing capsules of placebo or either of the active compounds. To ensure concealment of the randomization assignment, the research pharmacist implemented random allocation and masked treatment assignment was communicated by telephone to research staff. Patients, caregivers, and investigators (except for the investigational pharmacist) were all masked to the assignment. The doses of both amino acids were equivalent to those used in earlier studies (Lane *et al*. 2005, 2008; Tsai *et al*. 1998, 2004*b*). Patient's compliance and safety were closely monitored by the research psychiatrists and the in-patient nursing staff.

The sample size was similar to that of an earlier trial which has effect sizes between 0.5–0.9 and power of 0.4–0.8 for sarcosine treatment (Lane *et al*. 2005).

Measures

The outcome measures were psychopathology changes measured by PANSS (Kay *et al*. 1987) and Scales for the Assessment of Negative symptoms (SANS; Andreasen, 1983) total scores, Quality of Life (QOL) scale (10 items for in-patient use) (Heinrichs *et al*. 1984; Lane *et al*. 2008), and Global Assessment of Function (GAF) (Axis V in DSM-IV) (APA, 1994). A secondary analysis aimed to explore whether the positive results (if any) from the PANSS or SANS were due to a general effect on all components or to an effect on a specific component(s). Treatment response was defined as a $\geqslant 20\%$ reduction of the PANSS total score.

Originally, the PANSS contained three subscales: positive, negative, and general psychopathology (Kay *et al*. 1987). However, further factor analyses revealed five components: positive, negative, cognitive, depression, and excitement (Lindenmayer *et al*. 1994). In the present study, we thus applied the five-factor analysis for PANSS. For the assessment of negative symptoms, we *a priori* chose SANS rather than PANSS negative to avoid multiple comparisons because SANS is more comprehensive, consisted of five subscales: blunted affect, alogia, apathy, anhedonia/asociality, and attention (Andreasen, 1983). Of the original 21 items on the QOL scale (Heinrichs *et al*. 1984), 10 (social activity, social initiatives, social withdrawal, sense of purpose, motivation, curiosity, anhedonia, aimless inactivity, capacity for empathy, emotional interaction) were selected for the in-patient setting (Lane *et al*. 2008). The GAF (Axis V in DSM-IV)

included clinical symptoms in the anchors (APA, 1994), but the raters ignored the symptom components and focused on the global functioning when using GAF.

Side-effect assessments included the Simpson–Angus Rating Scale for EPS (Simpson & Angus, 1970), Abnormal Involuntary Movement Scale (AIMS) for dyskinesia (Guy, 1976), and Barnes Akathisia Scale (BAS; Barnes, 1989). Systemic side-effects of treatments were evaluated by means of routine physical and neurological examinations, laboratory tests, and reviewed by applying the Udvalg for Kliniske Undersogelser (UKU) Side-effects Rating Scale (Lingjaerde *et al*. 1987).

Clinical ratings were performed by the research psychiatrists who were trained and experienced in the rating scales. Inter-rater reliability was analysed with the ANOVA test. Only raters reaching the intra-class correlation coefficients of $\geqslant 0.90$ during pre-study training were allowed to rate the study patients. To maintain high inter-rater reliability and to prevent rater drift, raters met at least once a month for training and reliability re-testing. To minimize inter-rater variability, each individual patient was assessed by the same research psychiatrist throughout the trial. Assessments were completed at baseline and at the end of weeks 2, 4, and 6.

Statistical analysis

The demographic and clinical characteristics of the patients, antipsychotic doses, response rate, and side-effects among groups were compared by Kruskal–Wallis tests (or ANOVA tests if the distribution was normal) for continuous variables and by χ^2 tests (or Fisher's exact tests) for categorical variables.

Since the interception random effect in the mixed-effects model was not sufficient to model the correlation structure within the subject and might lead to overestimation of treatment effects, we applied an autoregressive structure of random errors in multiple linear regression with the generalized estimating equation (GEE) method (Zeger *et al*. 1988) for the treatment (sarcosine, D-serine, or placebo) by time (0, 2, 4, 6 wk) interaction analysis, which simultaneously compared the three treatment groups using a single analysis and controlled for baseline psychopathology. Subjects with at least one post-treatment measurement were included. The results of GEE models were analysed by the SAS/STAT (SAS Institute Inc, USA) PROC GENMOD procedure with AR (autoregressive) (*1*) working correlation structure using the marginal model. Since there were three comparison groups, the placebo group was initially selected to be compared with the other two groups in a single analysis. In the next step, for direct comparison of the two active treatment groups, the sarcosine group was selected to be compared with the other two groups. Because ANOVA and multiple linear regression can be applied only if the distribution of the response values is normal, we examined the distribution pattern using the Kolmogorov D package in SAS/INSIGHT v. 8.2. Before treatment × time interaction analysis, linear change over time was checked for all outcomes. Unlike ANOVA, the GEE model did not obtain a statistical value or a p value among all groups.

We also applied mixed-effects models (Lange & Ryan, 1989) (with intercept as the random effect) for all normally distributed outcomes, with main effects for treatment (sarcosine, D-serine, or placebo), time (0, 2, 4, 6 weeks), and the treatment × time interaction. Significance of treatment effects over time was assessed by the significance of the treatment × time interaction while controlling for the main effects. The requirement for the mixed-effects model is the same as that for the GEE model, as shown above.

All hypothesis tests were two-sided and conducted at $\alpha = 0.05$ significance level. The p values of the four outcome measures were corrected by Bonferroni correction of multiple comparisons. After the significant findings in PANSS total or SANS total were confirmed by the stringent GEE analysis, secondary analysis on PANSS factors or SANS subscales were conducted.

To compare across the treatments, Cohen's d effect sizes (Rosnow & Rosenthal, 1996) between endpoint and baseline were calculated.

Results

Sixty schizophrenia patients were enrolled and 51 patients completed the double-blind, placebo-controlled study. Three patients (one on sarcosine, two on placebo) dropped out after the week-2 assessment, and another six (four D-serine, two placebo) dropped out after the week-4 assessment due to non-adherence to protocol (delayed return of day pass); not due to symptom change (Fig. 1).

The demographic characteristics, illness course, diagnostic subtype, stable antipsychotic medication, and clinical severity at baseline of the patients were similar in the three treatment groups (Tables 1 and 2). The doses of co-administered risperidone treatment were similar to earlier studies without or with add-on D-serine or sarcosine treatment (Lane *et al*. 2000, 2004, 2005; Tsai *et al*. 2004*b*). The clinical severity of the subjects was also close to that of previous clinical trials

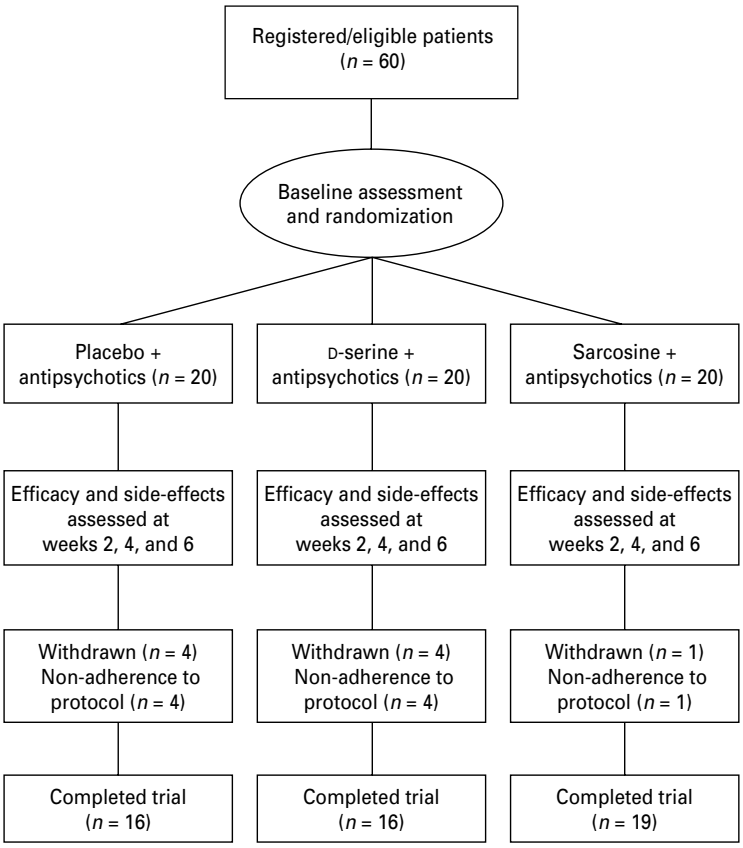

Fig. 1. Progress of 60 patients during the trial. There were nine dropouts, all due to non-adherence to protocol (see Results section).

of D-serine and sarcosine (Lane *et al*. 2006; Tsai *et al*. 1998, 2004*b*).

Clinical outcomes

Clinical changes in outcomes are presented in Table 2. To determine the efficacy of the sarcosine and D-serine treatments, we chose placebo group as the reference group to be compared with the other two groups (Table 2). The multiple linear regression with the GEE method (Zeger *et al*. 1988) was for the treatment (sarcosine, D-serine, or placebo) by time (0, 2, 4, 6 wk) interaction analysis, which simultaneously compared the three treatment groups using a single analysis and controlled for baseline psychopathology. The results were similar to, but more stringent than the findings from the mixed-effects model. Sarcosine treatment was effective for all outcome measures, including PANSS total ($p=0.0052$), SANS total ($p=0.021$), QOL scale ($p=0.025$), GAF ($p=0.042$) (Table 2), but D-serine treatment did not improve any measure (Table 2).

The sarcosine group was numerically superior to the D-serine group in all outcome domains but the differences did not reach statistical significance after Bonferroni correction (results not shown). Due to the non-normal distributions of PANSS factors and SANS subscales, Mann–Whitney tests were applied to compare sarcosine with placebo in these subcomponents and significance was assessed by comparing endpoint data while controlling for baseline data. Sarcosine was better than placebo in PANSS positive ($z=-2.11$, $p=0.040$), PANSS negative ($z=-2.58$, $p=0.010$), PANSS cognitive ($z=-1.97$, $p=0.050$), PANSS depression ($z=-3.01$, $p=0.002$), SANS affect ($z=-2.30$, $p=0.023$), SANS alogia ($z=-2.36$, $p=0.021$), SANS apathy ($z=-2.65$, $p=0.010$), and SANS anhedonia ($z=-3.31$, $p=0.001$), but not in PANSS excitement ($z=-0.87$, $p=0.40$) and SANS attention ($z=-1.32$, $p=0.21$). In contrast, D-serine treatment did not differ significantly from placebo in any of the secondary measures.

With the analysis of treatment group × treatment duration interaction using the mixed-effects model,

Table 2. Primary outcome measures for the 6-wk add-on sarcosine or D-serine treatment

Scale[a]	Treatment[a]	Baseline[a]	Week 2[a]	Week 4[a]	Endpoint[a]	Difference in score- changing rate *vs.* placebo, mean (S.E.)[b]	*Z* (*p* value)[b]	*t* (*p* value)[c]
PANSS total	Placebo	88.7 (17.4)	88.1 (21.1)	84.6 (21.7)	85.2 (23.6)			
	D-serine	88.4 (14.8)	83.1 (14.3)	78.8 (14.9)	75.4 (15.4)	−1.55 (0.65)	−2.40 (0.065)	−3.52 **(0.0024)**
	Sarcosine	85.3 (11.5)	77.2 (12.9)	73.1 (15.2)	70.9 (14.6)	−1.67 (0.52)	−3.23 **(0.0052)**	−3.64 **(0.0016)**
SANS total	Placebo	55.8 (15.1)	54.2 (16.2)	52.4 (15.8)	52.7 (17.3)			
	D-serine	54.7 (19.6)	52.9 (21.1)	49.5 (19.5)	48.1 (19.5)	−0.58 (0.39)	−1.49 (0.55)	−1.96 (0.21)
	Sarcosine	51.6 (15.5)	46.9 (16.3)	42.5 (17.2)	42.0 (16.0)	−1.04 (0.37)	−2.80 **(0.021)**	−3.44 **(0.0028)**
QOL	Placebo	19.2 (7.0)	20.3 (6.7)	19.8 (6.8)	20.7 (7.1)			
	D-serine	18.8 (12.1)	19.0 (10.5)	21.5 (9.7)	23.1 (10.6)	0.53 (0.31)	1.70 (0. 36)	2.83 **(0.021)**
	Sarcosine	21.2 (8.2)	23.8 (7.7)	26.2 (8.2)	26.8 (8.1)	0.67 (0.24)	2.77 **(0.025)**	3.31 **(0.0048)**
GAF	Placebo	37.0 (10.0)	37.3 (12.0)	39.8 (11.4)	39.0 (12.7)			
	D-serine	42.9 (8.5)	45.1 (9.1)	46.5 (9.2)	48.2 (8.7)	0.50 (0.28)	1.75 (0.31)	2.31 (0.088)
	Sarcosine	41.3 (8.5)	44.6 (7.5)	46.5 (8.7)	47.8 (8.6)	0.66 (0.26)	2.56 **(0.042)**	3.13 **(0.0084)**

PANSS, Positive and Negative Syndrome Rating Scale; SANS, Scales for the Assessment of Negative symptoms; GAF, Global Assessment of Function.

Value at each visit, mean (S.D.) of raw data.

[a] Clinical severity at baseline was similar in three treatment groups by ANOVA test (PANSS total, $F=0.320$, $p=0.73$; SANS total, $F=0.327$, $p=0.72$; QOL, $F=0.389$, $p=0.68$; GAF, $F=2.298$, $p=0.11$; all d.f. = 2).

[b] See also Statistical Analyses and Clinical Outcome sections. Treatment group × treatment duration (week) interaction effects between D-serine *vs.* placebo and between sarcosine *vs.* placebo using a single multiple linear regression analysis with the generalized estimating equation (GEE) method controlling for baseline psychopathology. Y = baseline + treatment + time + treatment × time (in week, as a continuous variable) + constant.

[c] Treatment group × treatment duration (week) interaction effects between D-serine *vs.* placebo and between sarcosine *vs.* placebo using a single mixed-effects model controlling for baseline psychopathology (with all d.f. values = 165). The difference in score-changing rate *vs.* placebo was similar to that with the GEE model (not shown).

[b,c] The *p* values of the four outcome measures were multiplied by 4 for Bonferroni correction of multiple comparisons.

sarcosine was better than placebo in the score-changing rates of all measures including PANSS total ($p=0.0016$), SANS total ($p=0.0028$), QOL scale ($p=0.0048$), and GAF ($p=0.0084$) (Table 2). D-serine was superior to placebo in PANSS total ($p=0.0024$) and QOL ($p=0.021$), but not in SANS total and GAF by the mixed-effects model.

For directly comparing the two active treatment groups, we also used the sarcosine group as the reference group, with a single analysis of the mixed-effects model. Consistent with the findings with the placebo group as the reference group, the sarcosine group was numerically superior to the D-serine group in all primary outcome domains but the differences did not reach statistical significance after Bonferroni correction (results not shown).

Analysis of intra-group effect size between endpoint and baseline showed that sarcosine treatment had the largest effect size, D-serine smaller, and placebo the smallest: PANSS total (placebo 0.17, D-serine 0.86, sarcosine 1.10), SANS total (placebo 0.19, D-serine 0.33, sarcosine 0.61), QOL scale (placebo 0.21, D-serine 0.38, sarcosine 0.69), GAF (placebo 0.17, D-serine 0.62, sarcosine 0.76) (effect sizes for secondary outcomes had similar trends, results not shown). The more comprehensive efficacy of sarcosine was not due to higher dropout rate of the D-serine group since the reasons for dropping out were protocol non-adherence, rather than changes of symptom severity that warranted discontinuation due to deterioration, or early graduation due to improvement.

At endpoint, the sarcosine group had nine responders, who had $\geqslant 20\%$ reduction of the PANSS total score; the D-serine group had seven; and the placebo group had none. Compared to the placebo group, both sarcosine (Fisher's exact test, $p=0.001$) and D-serine ($p=0.008$) groups were more likely to respond. Since no patient in the placebo group showed response, logistic regression to compare odds ratio of response rate with the other two groups was not attempted.

Table 3. Adverse events other than extrapyramidal symptoms during the study[a]

Adverse event	Study groups (no. of patients)			
	Sarcosine	D-serine	Placebo	Total
Weight gain	3	4	5	12
Insomnia	4	4	2	10
Fatigability	2	2	4	8
Sedation	2	1	3	6
Palpitations	2	3	1	6
Tension	2	1	2	5
Hypersomnia	1	1	2	4
Weight loss	2	2	0	4
Constipation	1	2	0	3
Others	1	3	3	7
Total	20	23	22	65

[a] All p values are not significant for comparisons in three study groups. Systemic side-effects of treatments were reviewed by applying the Udvalg for Kliniske Undersogelser (UKU) Side-effects Rating Scale.

Side-effects

All the three treatment groups had minimal EPS at the beginning of the study. The baseline scores of Simpson–Angus (sarcosine group 0.1 ± 0.3, D-serine group 0.1 ± 0.4, placebo group 0.2 ± 0.5), AIMS (0.1 ± 0.3, 0.0 ± 0.0, 0.0 ± 0.0) and BAS (0.1 ± 0.4, 0.0 ± 0.0, 0.0 ± 0.0) were similar in the three groups (all p values = n.s.). At endpoint of the study, the severity of EPS remained minimal and did not have significant differences among the groups (Simpson–Angus, sarcosine group: 0.1 ± 0.3, D-serine group 0.1 ± 0.3, placebo group 0.2 ± 0.4; AIMS: 0.1 ± 0.3, 0.0 ± 0.0, 0.0 ± 0.0; BAS: 0.1 ± 0.3, 0.0 ± 0.0, 0.0 ± 0.0) (all p values = n.s.).

Treatment-emergent adverse events other than extrapyramidal symptoms were also similar in the three groups (Table 3). These systemic side-effects were all mild, and did not warrant medical treatment. The routine blood cell count, chemistry, and EKG after treatment remained unchanged and were all within the normal ranges (data not shown). No dropout was due to side-effects.

Discussion

The efficacy profile of sarcosine is similar to that of the pilot study on the sarcosine add-on treatment for chronically stable patients (Tsai *et al*. 2004*b*), where sarcosine was better than placebo in all symptom profiles. Importantly, the present study further indicates that sarcosine can improve QOL and general functioning. D-serine's efficacy does not appear evident when analysed by the GEE model, the more stringent analysis (Table 2), this is consistent with the study in acute patients (Lane *et al*. 2005). However, it may require more power to show efficacy of D-serine treatment. Similarly, the effect sizes of D-serine treatment are smaller than those of sarcosine treatment in all the measurements including negative symptoms. Consistent with the similar comparison study (Lane *et al*. 2005) in acutely ill patients, the present study suggests that the GlyT-1 inhibitor can be more efficacious than the NMDA/glycine site agonist for the treatment of schizophrenia, at the tested dosages (2 g/d, which is the only dose tested so far).

However, previous D-serine add-on trials (Heresco-Levy *et al*. 2005; Tsai *et al*. 1998) of patients with chronically stable schizophrenia also showed comprehensive symptom improvement. The discrepancy of findings in D-serine efficacy is probably due to the difference in the concomitant antipsychotics; patients were treated by an atypical antipsychotic in the present trial whereas the majority of patients were treated with conventional antipsychotics in our first trial (Tsai *et al*. 1998). Nevertheless, Heresco-Levy *et al*. (2005) reported significant effect sizes in multiple symptom domains, in which D-serine was added on to olanzapine or risperidone. Moreover, this study is limited in sample size. Although the sarcosine group was numerically superior to the D-serine group in all outcome domains, the differences did not reach statistical significance after Bonferroni correction. Therefore, the superior efficacy of sarcosine over D-serine should be considered preliminary. The optimal doses for sarcosine and D-serine can be different; a higher dose of D-serine may be required to reach the same efficacy as sarcosine.

To date, little data are available for comparisons between NMDA-enhancing agents (Heresco-Levy & Javitt, 2004; Lane *et al*. 2005). The results of the present study and the antecedent one (Lane *et al*. 2005) suggest that GlyT-1 may be a more effective target to enhance NMDA function than the NMDA/glycine site itself. This difference may due to the fact that sarcosine acts by blocking the re-uptake of released glycine whereas NMDA/glycine site agonists tonically stimulate the receptor. Further, transporter inhibitors may be more efficacious than the transmitter itself. Similarly, serotonin transporter inhibitors are superior to tryptophan (a neurotransmitter precursor, albeit not a neurotransmitter) for the treatment of depression (Shaw *et al*. 2002). It should be borne in mind that we only compared one dose of D-serine and sarcosine. A detailed

parallel, fixed dose-finding study can resolve the issue clearly.

NMDA neurotransmission regulates synaptic plasticity, memory, and cognition (Coyle, 1996). This cognition-enhancing effect is supported by the positive finding in executive function of our D-serine study (Tsai *et al*. 1998). Because cognitive deficiency in schizophrenia is increasingly viewed as a core factor for functional outcome (Green *et al*. 2004), the positive findings for the short-term QOL and general functioning of the present trial support the notion that NMDA-enhancing agents can improve functional outcome. Taken together, the findings from the trials of the NMDA-enhancing agent added to dopamine/5-HT receptor antagonists, sarcosine provides additional benefits not only for symptom reduction during both acute and chronic phases but also for the short-term functioning outcome. This novel approach represents a new avenue to improve the function and QOL of patients with schizophrenia who often suffer from lifelong functioning disability. Nevertheless, more meticulous research is required to test these agents before any conclusion can be drawn for the therapeutic effect of NMDA-enhancing agents on cognitive domains and long-term functional outcome in the community.

In earlier studies (Heresco-Levy *et al*. 2005; Lane *et al*. 2006; Tsai *et al*. 1998, 2004), sarcosine or D-serine did not worsen the side-effects of other antipsychotics. The present study replicated these findings; the few side-effects reported by the patients were minimal and did not differ significantly among groups, including the placebo group. Sarcosine is a naturally occurring amino acid in humans and food. Toxicological profiles of sarcosine have not been thoroughly examined. Supporting the safety of using sarcosine as a therapeutic agent to enhance NMDA neurotransmission, sarcosinaemia due to defective sarcosine dehydrogenase is generally benign (Eschenbrenner & Jorns, 1999; Levy *et al*. 1984) and the phenotype of sarcosine dehydrogenase mutant mice is unexceptional (Harding *et al*. 1992). However, Gly-T1 homozygous knockout mice cannot survive (Tsai *et al*. 2004). Complete blockade of Gly-T1 may be toxic for the rodent development due to the excessively inhibitory glycinergic drive to the respiratory neurons (Gomeza *et al*. 2003). A thorough human toxicology study, therefore, is necessary. After the present study was completed, sarcosine was identified as a differential metabolite that was detected as being greatly increased in urine during prostate cancer progression to metastasis (Sreekumar *et al*. 2009). Sarcosine is the major donor of the methyl group. Although this is not a direct proof of the carcinogenicity of sarcosine, and possibly elevated levels of sarcosine can be the result rather than the cause of the tumour progression, it is important to monitor the risk of prostate cancer during the treatment of sarcosine. On the other hand, sarcosine has protective effects against hepatoma; animals missing glycine *N*-methyltransferase that synthesizes sarcosine develop liver cancer whereas transgenic mice overexpressing glycine *N*-methyltransferase are resistant to aflatoxin B1-induced liver cancer (Martínez-Chantar *et al*. 2008; Yen *et al*. 2009). In rodents, D-serine selectively damages renal proximal tubule cells (Williams & Lock, 2004). In humans, toxicological properties of D-serine have not been fully elucidated. However, D-serine at a dose of $\sim 2\,g/d$ was safe for the patients in all four trials (Heresco-Levy *et al*. 2005; Lane *et al*. 2005; Tsai *et al*. 1998, 1999).

The present study was limited by the fixed-dose comparison without parallel, fixed dose-finding trials. The definitive difference of Gly-T1 inhibitors *vs*. NMDA/glycine site agonists and their clinical application requires further study. However, this study together with the one for acutely ill patients (Lane *et al*. 2005) and the single-agent study (Lane *et al*. 2008) indicate that sarcosine, a GlyT-1 inhibitor, represents a novel therapeutic approach that is worthy of further investigation (Javitt, 2008). Optimizing pharmacotherapy for schizophrenia can be achieved by a combination treatment of atypical antipsychotics and a Gly-T1 inhibitor.

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Statement of Interest

Sarcosine and D-serine are protected by US patent 6228875, 6667297, 6420351, 6974821 for which G.E.T. is an inventor.

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