Glycine Transporter I Inhibitor, N-methylglycine (Sarcosine), Added to Clozapine for the Treatment of Schizophrenia

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Background: *Agonists at the N-methyl-D-aspartate (NMDA)-glycine site (D-serine, glycine, D-alanine and D-cycloserine) and glycine transporter-1 (GlyT-1) inhibitor (N-methylglycine, or called sarcosine) both improve the symptoms of stable chronic schizophrenia patients receiving concurrent antipsychotics. Previous studies, however, found no advantage of D-serine, glycine, or D-cycloserine added to clozapine. The present study aims to determine the effects of sarcosine adjuvant therapy for schizophrenic patients receiving clozapine treatment.*

Methods: *Twenty schizophrenic inpatients enrolled in a 6-week double-blind, placebo-controlled trial of sarcosine (2 g/day) which was added to their stable doses of clozapine. Measures of clinical efficacy and side-effects were determined every other week.*

Results: *Sarcosine produced no greater improvement when co-administered with clozapine than placebo plus clozapine at weeks 2, 4, and 6. Sarcosine was well tolerated and no significant side-effect was noted.*

Conclusions: *Unlike patients treated with other antipsychotics, patients who received clozapine treatment exhibit no improvement by adding sarcosine or agonists at the NMDA-glycine site. Clozapine possesses particular efficacy, possibly related to potentiation of NMDA-mediated neurotransmission. This may contribute to the clozapine's unique clinical efficacy and refractoriness to the addition of NMDA-enhancing agents.*

Key Words: Glutamate, GlyT-1, N-methyl-D-aspartate, sarcosine, schizophrenia, treatment

In addition to dopaminergic neurotransmission, glutamatergic neurotransmission has been implicated in the pathophysiology of schizophrenia (Olney and Farber 1995; Tsai and Coyle 2001). N-methyl-D-aspartate (NMDA) receptor, a subtype of ionotropic glutamate receptor, plays an important role in neurodevelopment and cognition. Glutamate and glycine (or D-serine) serve as co-agonists at the NMDA receptor with activation of both the glutamate and glycine sites required for channel opening (Thomson et al 1989). The glycine transporter-1 (GlyT-1) plays a pivotal role in maintaining the concentration of glycine within synapses at a sub-saturating level. The anatomical distribution of GlyT-1 is parallel to that of the NMDA receptor (Smith et al 1992). Supporting the critical role GlyT-1 plays in NMDA neurotransmission, GlyT-1 inhibitor, a sarcosine analogue, N[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine (NFPS) and the GlyT-1 mutant mice have been shown to enhance NMDA neurotransmission and antipsychotic properties (Bergeron et al 1998; Chen et al 2003; Kinney et al 2003; Tsai et al 2004). The potency of a series of GLYT-1 antagonists for inhibiting phencyclidine (PCP)-induced hyperactivity in vivo correlated significantly with their potency in antagonizing GlyT-1 in vitro (Javitt et al 1999).

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The most compelling link between the NMDA system and schizophrenia concerns the mechanism of action of the psychotomimetic drug PCP, which is a NMDA antagonist (for reviews, see Tsai and Coyle 2001; Halberstadt 1995; Javitt and Zukin 1991). The psychosis induced by NMDA antagonists causes not only positive symptoms similar to the action of dopaminergic agonists but also negative symptoms and cognitive deficits associated with schizophrenia (Grotta 1994; Herrling 1994; Javitt and Zukin 1991; Kristensen et al 1992; Krystal et al 1994). Deutsch et al (1989) was the first to propose glycinergic interventions at the strychnine-insensitive glycine binding site on the NMDA receptor complex (NMDA-glycine site) for the treatment of schizophrenia.

Several studies had demonstrated the clinical benefits of treatment for schizophrenia targeting the NMDA-glycine site and no significant side effect was noted. These included D-serine (Heresco-Levy et al 2005; Tsai et al 1998a, 1998b), glycine (Heresco-Levy et al 1996, 1999, 2004), D-alanine (Tsai et al 2006) and D-cycloserine (Goff et al 1999a; Heresco-Levy et al 2002; van Berckel et al 1996) and the GlyT-1 inhibitor sarcosine (Lane et al 2005; Tsai et al 2004). These add-on treatments usually improve the symptoms of chronically stable schizophrenia. However, not all studies are positive; several recent reports did not demonstrate clinical efficacy include those by Carpenter et al (2004), Duncan et al (2004), and Goff et al (2005). Our recent study suggests that add-on treatment with sarcosine to stable antipsychotic regimens improves all the critical symptom clusters of chronically stable schizophrenia patients on atypical (risperidone) or typical antipsychotics (Tsai et al 2004). D-serine, D-alanine and sarcosine can also improve positive symptoms in chronic schizophrenia patients on stable doses of typical or atypical antipsychotics, but excluding clozapine (Heresco-Levy et al 2005; Tsai et al 1998a, 2004, 2006). A very recent study suggests that sarcosine, superior to D-serine, can benefit acutely ill schizophrenia patients with concurrent risperidone therapy (Lane et al 2005). This finding indicates that a GlyT-1 inhibitor may be more efficacious than NMDA-glycine site agonists for adjuvant treatment of schizophrenia (Lane et al 2005).

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To date, the NMDA-glycine site partial agonist (D-cycloserine) or full agonists (glycine and D-serine) appear ineffective for schizophrenia patients who are receiving clozapine therapy. Paradoxically, D-cycloserine worsens negative symptoms of clozapine-treated patients (Goff et al 1996, 1999b). As a partial agonist, D-cycloserine may antagonize NMDA receptor neurotransmission when glycine or other agonist(s) saturate the NMDA-glycine site (Watson et al 1990). Findings of NMDA-enhancing agents on clozapine-treated patients are not consistent. Adjunctive glycine, at 30-g/day, also exacerbates positive symptoms of clozapine-treated patients (Potkin et al 1999). In comparisons, high-dose (60-g/day) glycine (Diaz et al 2005) or D-serine (Tsai et al 1999) does not influence clinical manifestations of clozapine recipients. Exceptionally, in a Javitt et al (2001) study, high dose (up to .8 g/kg/day) glycine treatment benefits patients receiving typical or atypicals ($n = 12$), including clozapine ($n = 4$); however, a separate analysis for the clozapine receivers were unavailable.

It has been proposed that clozapine can booster NMDA activity, maybe contributing to its unique clinical efficacy (Goff et al 1999b; Tsai et al 1999). In a recent study, clozapine significantly inhibits glycine uptake into rat brain synaptosomes (Javitt et al 2005). If the effect of clozapine reaches a ceiling for clozapine-treated patients, sarcosine (a Gly-T-1 inhibitor), albeit superior to D-serine for patients taking other atypical antipsychotics (Lane et al 2005), will be still ineffective for patients receiving clozapine treatment. The current study aimed to testify this hypothesis by comparing adjuvant sarcosine and placebo therapy in clozapine-treated schizophrenia patients.

Methods and Materials

Participants

Subjects were recruited from the inpatient unit of China Medical University Hospital, which is a major medical center in Taiwan. The research protocol was approved by the institutional review board (IRB). Sarcosine is a natural amino acid. It is regulated as food supplement and investigational new drug (IND) application is not required in Taiwan. Written informed consent was obtained from all participants after a detailed description of the study, which was approved by the IRB of China Medical University. Patients were evaluated by the research psychiatrists after a thorough medical and neurological workup. The Structured Clinical Interview for DSM-IV (American Psychiatric Association 1994b) was conducted for the diagnosis. Patients with Axis I diagnosis other than schizophrenia, significant depressive symptoms (Hamilton Depression Rating Scale > 25), or serious medical or neurological illness were not included. All the enrolled patients had a normal physical exam, neurological exam and laboratory screening tests.

Twenty Taiwanese schizophrenia inpatients were enrolled, and all completed the double-blind, placebo-controlled study. Demographic information of the patients is shown in Table 1. All patients fulfilled the DSM-IV diagnosis of schizophrenia (American Psychiatric Association 1994a) and the criteria of primary deficit syndrome (Kirkpatrick et al 1989). The patients were treatment resistant to at least two different classes of antipsychotics, in doses equal to at least 400-600 mg of chlorpromazine per day for at least 8 weeks. Also, patients received adequate trials of clozapine but without satisfactory response with total scores 70 or higher on the Positive and Negative Syndrome Scale (PANSS) (Kay et al 1987). They kept clinically stable with their clozapine doses remaining constant for at least 3 months prior to the enrollment. The same doses were also continued throughout the period of sarcosine trial.

Table 1. Characteristics of Schizophrenic Patients Assigned to Placebo and Sarcosine Treatment

	Sarcosine ($n = 10$)	Placebo ($n = 10$)	Difference[a]
Gender (female/male)	3/7	3/7	ns
Age (years)	36.7 (10.1)	35.5 (6.6)	ns
Education (years)	11.8 (2.5)	11.3 (3.6)	ns
Age of Onset (years)	22.3 (7.1)	20.1 (5.7)	ns
Age of First Admission (years)	25.4 (9.6)	25.0 (5.3)	ns
Subtype			
Paranoid	5	7	
Disorganized	3	2	
Undifferentiated	2	0	
Residual	0	1	
Smoker/Non Smoker[b]	3/7	2/8	ns
Clozapine Dose (mg)	306 (159)	305 (55)	ns

Standard deviations in parentheses.
[a]As assessed by two sample *t*-test, or χ^2 test where appropriate.
[b]Smoker, smoking >=10 cigarette per day; non smoker, smoking 0 cigarettes per day.

All patients were randomly assigned under double-blind conditions to receive a 6-week trial of placebo or 2 grams of sarcosine daily, which has been effective for add-on therapy in typical antipsychotics or risperidone-treated schizophrenia patients (Lane et al 2005; Tsai et al 2004). Placebo and sarcosine were packed with the same additives and capsules. To ensure concealment of the randomization assignment, medication was provided in coded containers with supply of identical-appearing capsules of placebo or sarcosine. The research pharmacist implemented random allocation and masked treatment assignment was communicated by telephone to study staff. Patients, caregivers, and investigators (except the investigational pharmacist) were all masked to the assignment. Patient's compliance and safety were closely monitored by the research psychiatrists and the nursing staff. All patients took medication under the supervision of medical/nursing staff. There was no drop out or compliance issue.

Assessments

Clinical ratings were performed using PANSS (Kay et al 1987) by a research psychiatrist (H-YL), who was trained and experienced in the use of the scale. PANSS positive, negative, general (Kay et al 1987), cognitive, and depressive subscales (Lindenmayer et al 1995) were utilized for the measurement of various symptom domains. The PANSS-cognitive subscale is a symptomatic measure correlated with verbal memory and verbal intelligence (Ehmann et al 2004). Both efficacy and safety were evaluated at baseline and at the end of every 2-week period. Side-effect assessments included the Simpson-Angus Rating Scale for extrapyramidal side-effects (Simpson and Angus 1970a), Abnormal Involuntary Movement Scale (AIMS) for dyskinesia (Simpson and Angus 1970b), and the Barnes Akathesia Scale (Barnes 1989). Systemic side-effects of sarcosine treatments were reviewed by administering the Udvalg for Kliniske Undersogelser (UKU) Side-effects Rating Scale (Lingjaerde et al 1987).

Data Analysis

Demographic characteristics and clozapine doses between groups were compared by Student's two-sample *t*-test for continuous variables and by χ^2 tests for categorical variables.

For efficacy assessment and possible side-effects, linear mixed-effects models (Lange and Ryan 1989) were fit to all normally distributed outcomes, with main effects for treatment (sarcosine or placebo), time (0, 2, 4, 6 weeks), and the treatment-by-time interaction. Significance of treatment effects over time was assessed by the significance of the treatment (sarcosine vs. placebo)-by-time interaction while controlling for the baseline values. For the outcome variable with a nonnormal distribution, Mann-Whitney tests between pairs of treatments were utilized. All hypothesis tests were two sided and conducted at the .05 alpha level.

The effect size and power value were evaluated by the SamplePower package (SPSS Inc., Chicago, Illinois, 2005).

Results

As shown in Table 1, demographic data, characteristics of the schizophrenic illness, and clozapine doses were similar in the patients who received sarcosine and the patients who received the placebo (Table 1). Both groups had similar ratios of paranoid versus nonparanoid subtypes of schizophrenia. The average dose of clozapine used in this study is similar with that in earlier Taiwanese studies (Chang et al 1997; Lane et al 1999, 2001; Lu et al 2004). The two treatment groups were comparable in terms of PANSS total scores and all clinical domains (positive, negative, cognitive, depressive, and general psychiatric symptoms) at baseline.

Clinical Outcomes

The two treatment groups were comparable in terms of PANSS total scores and all clinical domains (positive, negative, cognitive, depressive, and general psychiatric symptoms) at weeks 2, 4, and 6 of adjuvant treatment (Table 2). As in Table 2, sarcosine showed no trend to be better than placebo when co-administered with clozapine. The effect size for PANSS total was −.25 (95% confidence interval [CI], −1.13–.63) at week 6. The power of PANSS total score at week 6 was 13%. For the previous study of sarcosine added to nonclozapine antipsychotics (Tsai et al 2004), the effect size for PANSS total was .63 (CI, −.05–1.30) at week 6, and the power was 60%.

Adverse Effects

Both the sarcosine and placebo groups had mild extrapyramidal symptoms at the beginning of the study. The baseline scores of Simpson-Angus (sarcosine group .8 ± 1.2, placebo group .1 ± .3), AIMS (sarcosine group 1.2 ± 2.6, placebo group 1.3 ± 3.2) and Barnes Akathesia Scale (sarcosine group .0 ± .0, placebo group .0 ± .0) were similar in both groups. Both the placebo and sarcosine groups did not significantly change their profiles of side-effects and were not different from each other after six weeks of adjunctive treatment (Simpson-Angus: sarcosine group .6 ± .9, placebo group .0 ± .0; AIMS: sarcosine group .6 ± 1.4, placebo group 1.3 ± 3.2; and Barnes Akathesia Scale: sarcosine group .0 ± .0, placebo group .6 ± 1.9).

Treatment-emergent adverse events in the placebo group included constipation (n = 1) and salivation (n = 1); the sarcosine group included insomnia (n = 2). These systemic side-effects were all short-lived and resolved spontaneously within days, not warranting medical treatment. They were likely coincidental observations. The routine blood cell count, chemistry, urine analysis, and electrocardiogram (EKG) after six weeks of sarcosine or placebo treatment remained unchanged and were all within the normal ranges (data not shown).

Discussion

Our results indicate that adjunctive treatment of sarcosine, acting as an inhibitor of the GlyT-1, has no effects on the symptom domains of schizophrenia patients who are receiving clozapine therapy. Similarly, adjunctive 2-g/day D-serine (Tsai et al 1999) or high-dose (Potkin et al 1999), long-term (up to 60-g/day, 24-week) glycine therapy (Diaz et al 2005) does not affect the patients receiving clozapine treatment.

The negative results of the current study are unlikely due to a type II error. Although we had a small number of subjects, there is "no trend" for sarcosine to be better than placebo as observed

Table 2. Clinical Measures for the Six-Week Placebo-Controlled Sarcosine Treatment

Scale	Treatment	Baseline	Week 2	Week 4	Week 6
PANSS-Total	Placebo	77.7 (12.6)	75.8 (13.0)	73.8 (13.1)	71.5 (14.4)
	Sarcosine	78.2 (6.4)	76.0 (5.9)	76.3 (6.4)	74.5 (6.6)
	t (p value)	.11 (.92)	−.12 (.91)	.77 (.44)	.96 (.34)
PANSS-Positive	Placebo	16.4 (3.3)	16.6 (4.0)	16.2 (4.2)	15.8 (3.8)
	Sarcosine	18.1 (4.4)	17.7 (3.9)	17.9 (3.9)	17.8 (3.9)
	t (p value)	.97 (.34)	−.83 (.41)	.00 (1.00)	.42 (.68)
PANSS-Negative	Placebo	26.7 (4.6)	25.5 (4.9)	25.2 (4.6)	24.4 (5.5)
	Sarcosine	26.9 (4.2)	26.2 (4.3)	26.1 (4.8)	25.0 (5.7)
	t (p value)	.09 (.93)	.51 (.61)	.72 (.48)	.41 (.68)
PANSS-General	Placebo	34.6 (6.9)	33.7 (6.5)	32.4 (6.5)	31.3 (7.2)
	Sarcosine	33.2 (5.1)	32.1 (4.9)	32.3 (4.5)	31.7 (4.9)
	t (p value)	−.53 (.60)	−.13 (.90)	.82 (.42)	1.13 (.26)
PANSS-Cognitive	Placebo	15.1 (4.3)	14.6 (4.7)	14.5 (4.3)	14.3 (4.4)
	Sarcosine	12.6 (3.4)	12.1 (3.2)	12.2 (3.4)	12.1 (2.7)
	t (p value)	−1.45 (.15)	.00 (1.00)	.32 (.75)	.49 (.63)
PANSS-Depression	Placebo	6.4 (2.2)	6.2 (2.1)	5.6 (1.6)	5.3 (1.6)
	Sarcosine	5.9 (2.1)	5.9 (2.1)	5.6 (2.0)	5.2 (2.2)
	z (p value)	−.47 (.64)	−.47 (.64)	−.15 (.88)	−.46 (.65)

Standard deviations in parentheses. Mixed-effects models were used for all normally distributed outcomes. Significance of treatment effects over time was assessed by the significance of the treatment (sarcosine vs. placebo)-by-time interaction while controlling for the baseline values. For the outcome variable with a non normal distribution (PANSS-Depression), Mann-Whitney tests between pairs of treatments were utilized.

PANSS, Positive and Negative Syndrome Scale.

in Table 2. In addition, the power value for the PANSS total at week 6 is 60% in the study of sarcosine for nonclozapine treated patients (Tsai et al 2004), and is 13% in this study of clozapine-treated patients. The key effect size in the current study, PANSS-total, is also much lower than the previous one: −.25 (95% CI, −1.13 – .63) versus .63 (−.05 – 1.30). Therefore, it is possible that the study is negative due to the small subject size; it will require a large number of subjects to demonstrate the efficacy if there is any. At the same time, sarcosine's augmenting effects on non-clozapine antipsychotics require confirmation studies to have a clear estimation of its effect size.

Clozapine may potentiate NMDA receptor-mediated neurotransmission (Arvanov et al 1997) and adding sarcosine does not improve the patients already receiving clozapine. Neurochemical, electrophysiological and behavior studied revealed that clozapine can augment the NMDA responses (Arvanove and Wang 1999; Kubota et al 2000; Schwieler et al 2004; Corbett 1995; Geyer et al 2001). Javitt et al (2005) further reported that clozapine inhibits glycine transport. This property may contribute to its superior efficacy compared with other typical or atypical antipsychotics. These novel pharmacological mechanisms of clozapine, may explain its refractoriness to the addition of NMDA-enhancing agents, including selective GlyT-1 inhibitors, sarcosine.

In other words, patients on clozapine may already achieve the "ceiling" benefit from the enhancement of NMDA function. The reports on glycine and D-serine also support this hypothesis (Potkin et al 1999; Tsai et al 1999). Although in vitro study of GlyT-1 inhibitor, adding to physiological level of glycine, can potentiate NMDA response (Bergeron et al 1998), there is no information of the GlyT-1 effect on synaptic level of glycine in human central nervous system (CNS). This hypothesis can be tested by higher doses of D-serine, glycine, or sarcosine treatment; the higher doses can be clinically beneficial if the clozapine treatment does not reach the ceiling and the NMDA-glycine site is not yet saturated by the dosages. Conversely, higher dose of these agents will not be beneficial if clozapine treatment has reached the ceiling.

There is another possibility why sarcosine, D-serine and glycine did not improve the symptoms in schizophrenic patients treated with clozapine. Patients treated by clozapine were treatment-resistant to other antipsychotics (Kane 1992) and represented a subpopulation of severe pathology. In the current study, the patients' severity was similar to that of the subjects in the Kane study. All patients fulfilled the criteria of primary deficit syndrome (Kirkpatrick et al 1989). Although they were not re-tested by high-dose haloperidol, they had been treatment resistant to at least two different classes of antipsychotics in adequate doses for at least 8 weeks. Also, patients received adequate trials of clozapine but still without satisfactory response, with an average score of 78 on the PANSS before randomization (Table 2). Clozapine's superiority has extended to a fairly wide range of partial responders including stable outpatients with moderate symptoms (Breier et al 1993, 1994, 2000). It is thus possible that an augmenting effect of sarcosine or other NMDA-enhancing agents might be observed in less severely ill clozapine-treated subjects if the treatment effects are depending on the disease severity.

Similar to the nonclozapine patients, sarcosine does not worsen the side-effects of other antipsychotics, which are mediated by D2, 5-HT2, histamine and muscarinic receptors (Tsai et al 2006). The extrapyramidal side-effects, akathesia and dyskinetic movement were not affected by sarcosine treatment. Similarly, D-serine, glycine or D-cycloserine do not induce significant side-effects (Goff et al 1999a; Heresco-Levy et al 1999; Tsai et al 1998a, 2005). A vigorous review of systemic side-effects reveals that sarcosine treatment at a dose of 2 g/day was well tolerated in the current and previous studies (Lane et al 2005; Tsai et al 2004). The few side-effects reported by the patients were minimal and coincidental, and resolved spontaneously.

In summary, unlike patients treated with typical or other atypical antipsychotics, patients who received clozapine treatment do not obtain additional improvement with the supplementation of sarcosine. Clozapine possesses particular efficacy, possibly related to potentiation of NMDA-mediated neurotransmission. This may contribute to the clozapine's unique clinical efficacy and refractoriness to the addition of NMDA-enhancing agents, including agonists at the NMDA-glycine site and the GlyT-1 inhibitor.

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