Inhibition of Glycine Transporter-I as a Novel Mechanism for the Treatment of Depression

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Background: Antidepressants, aiming at monoaminergic neurotransmission, exhibit delayed onset of action, limited efficacy, and poor compliance. Glutamatergic neurotransmission is involved in depression. However, it is unclear whether enhancement of the *N*-methyl-D-aspartate (NMDA) subtype glutamate receptor can be a treatment for depression.

Methods: We studied sarcosine, a glycine transporter-I inhibitor that potentiates NMDA function, in animal models and in depressed patients. We investigated its effects in forced swim test, tail suspension test, elevated plus maze test, novelty-suppressed feeding test, and chronic unpredictable stress test in rats and conducted a 6-week randomized, double-blinded, citalopram-controlled trial in 40 patients with major depressive disorder. Clinical efficacy and side effects were assessed biweekly, with the main outcomes of Hamilton Depression Rating Scale, Global Assessment of Function, and remission rate. The time course of response and dropout rates was also compared.

Results: Sarcosine decreased immobility in the forced swim test and tail suspension test, reduced the latency to feed in the novelty-suppressed feeding test, and reversed behavioral deficits caused by chronic unpredictable stress test, which are characteristics for an antidepressant. In the clinical study, sarcosine substantially improved scores of Hamilton Depression Rating Scale, Clinical Global Impression, and Global Assessment of Function more than citalopram treatment. Sarcosine-treated patients were much more likely and quicker to remit and less likely to drop out. Sarcosine was well tolerated without significant side effects.

Conclusions: Our preliminary findings suggest that enhancing NMDA function can improve depression-like behaviors in rodent models and in human depression. Establishment of glycine transporter-I inhibition as a novel treatment for depression waits for confirmation by further proof-of-principle studies.

Key Words: Anxiety, elevated plus maze, forced swimming test, glutamate, *N*-methyl-D-aspartate, sarcosine

Major depressive disorder is a common mental disorder, with a lifetime prevalence of approximately 15% (1,2). Almost all the antidepressants are monoaminergic agents; however, 50–60% of patients treated with antidepressants have incomplete recovery, significant side effects, or poor compliance (3). Recently, *N*-methyl-D-aspartate receptor (NMDAR)-mediated neurotransmission has been implicated in the pathophysiology of depression. The NMDA hypothesis of depression originated from an unexpected observation that D-cycloserine, a partial agonist of the NMDAR, has antidepressant activity (4,5). Also, NMDA-enhancing treatment results in a significant reduction in depressive symptoms in patients with schizophrenia (6–9). Similarly, a reversible glycine transporter inhibitor, SSR504734, and an NMDAR coagonist, D-serine, have been shown to have antidepressant/anxiolytic effects in depression/anxiety models (10,11). Finally, expression of NMDAR 1 and 2A subunit is decreased in postmortem brains of patients with major depression (12), and NMDAR binding is also reduced in suicide victims (13). These findings imply that hypofunction at NMDAR contributes to the pathophysiology of depression.

Subsequent preclinical reports indicated that drugs antagonizing the NMDAR, such as ketamine and MK-801, have antidepressant-like effects in animal models of depression and in patients with major depression and bipolar depression (14–16). These findings imply that hyperfunction of NMDAR may also be involved in the pathophysiology of depression.

On the basis of the findings on both NMDA agonists and antagonists, depression may have complex neural substrates in that both upregulation and downregulation of NMDA function are involved, and both agonists and antagonists can be antidepressant therapies. NMDARs are widely distributed in corticolimbic circuitries, and it is not surprising that a complex behavioral disorder such as depression involves multiple circuitries with NMDA components that have opposite directions of regulation.

Systematic investigation of the antidepressant effect of NMDA enhancement is still in the early phase. Until now, animal and clinical studies of depression focused on NMDA antagonists and partial agonists (14–16). To test the hypothesis that NMDA enhancement plays a significant role in depression, we investigated the antidepressant/anxiolytic-like effects of sarcosine, a glycine transporter-1 (GlyT-1) inhibitor, in animal behavior models of the forced swim test (FST), the tail suspension test (TST), the elevated plus maze test (EPM), the novelty-suppressed feeding test (NSFT), and the chronic unpredictable stress (CUS)/anhedonia test. We also compared sarcosine and citalopram, as an active control, in efficacy, time course, attrition, and safety for patients with major depression. We hypothesized that NMDA

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enhancement will induce a more rapid and robust response than the citalopram control because of the comprehensive neurobiological roles that NMDA neurotransmission plays in neurocognition and plasticity.

Methods and Materials

Animals

A brief description of the materials and methods is presented in this section. See Supplement 1 for a full and detailed description.

The experiments of animals were carried out in two phases as follows:

Phase I. In the acute study, the FST, EPM, and TST were used to determine the antidepressant-like effect of sarcosine. The rats used in the acute study were naive.

Phase II. In the chronic study, the lowest efficacy dose of sarcosine in the acute study of FST (560 mg/kg per day) was given for 21 days. The NSFT was used in the chronic sarcosine administration nonstressed study, and the sucrose preference test (SPT) and NSFT were also conducted at the end of chronic sarcosine administration in CUS study (see Supplemental Methods and Figure S1 in Supplement 1).

Patient Participants

Patients were recruited from the outpatient clinic at the Department of Psychiatry, China Medical University Hospital, Taichung, which is a major medical center in Taiwan. The study was approved by the institutional review board and conducted in accordance with Good Clinical Practice procedures and the current revision of the *Declaration of Helsinki*. Patients were evaluated by the research psychiatrist after a thorough medical and neurological workup. The Structured Clinical Interview for DSM-IV (17) was conducted for the diagnosis.

Han Chinese patients with 17-item Hamilton Rating Scale for Depression (HAMD-17) ≥18 (18) were enrolled in this study. The inclusion and exclusion criteria are listed in Supplement 1.

Study Design

All patients were randomly, double-blindly, assigned to receive a 6-week trial of citalopram or sarcosine. Both efficacy and safety were evaluated at baseline and at the end of weeks 2, 4, and 6. Twenty milligrams of citalopram or 500 mg of sarcosine was packed with the same additives and capsule. The dose was initiated at 1 capsule per day in the first 2 weeks, and titrated to 1 capsule b.i.d. in weeks 3–4, and 1 capsule in the morning and 2 capsules before sleep in weeks 5–6 if clinically indicated. The dosing strategy of citalopram was to decrease the likelihood of exposing the subjects to high-dose citalopram, for which the Food and Drug Administration (FDA) issued a warning against dose-dependent QT-interval prolongation and Torsade de Pointes. The dosing strategy for sarcosine was based on the experiences in patients with schizophrenia (7,8,19–21) and pilot findings from patients with major depression that showed clinical efficacy between 500–1500 mg/d. Patients were randomly assigned in blocks of six subjects to receive citalopram or sarcosine in a 1:1 ratio.

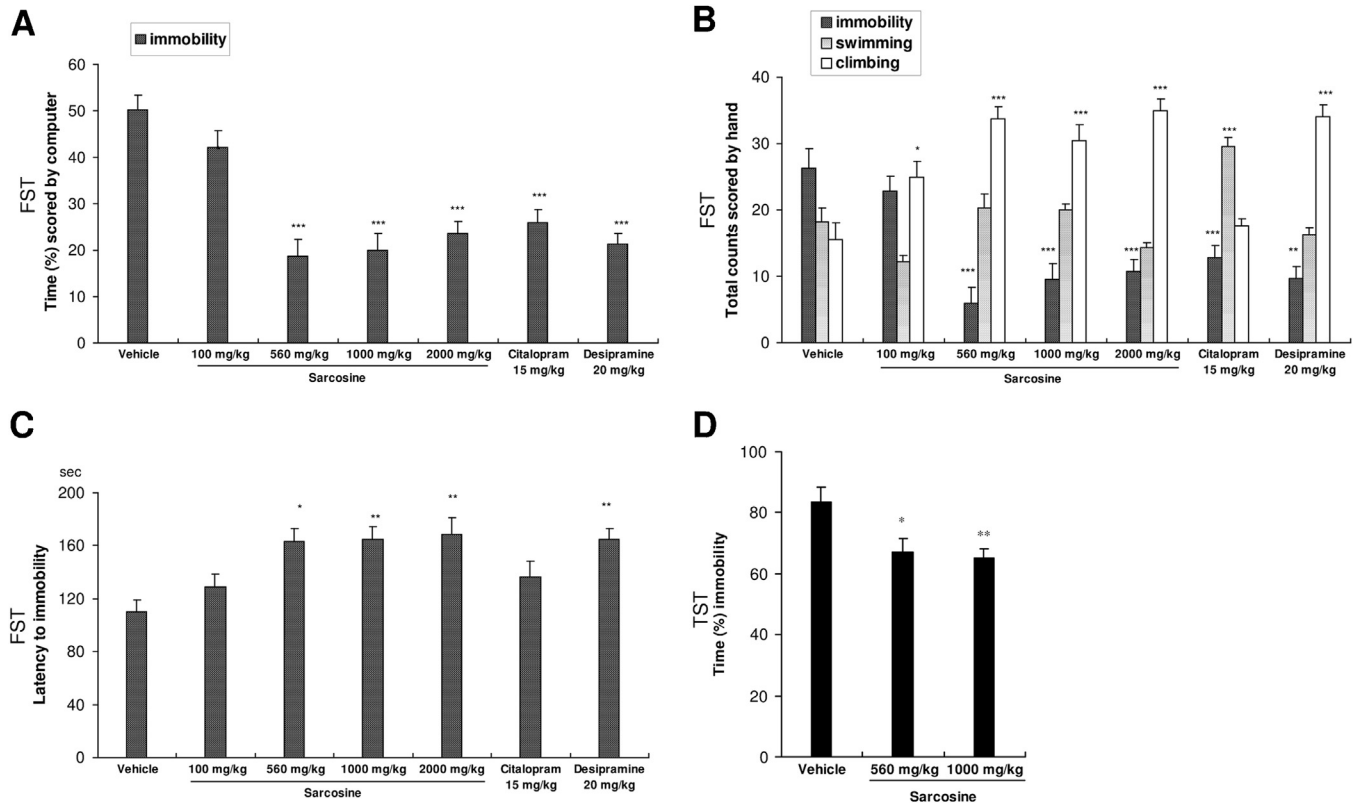

Figure 1. Effect of acute administration of vehicle, citalopram, desipramine, and different doses of sarcosine on the **(A)** percentage of immobility time scored by computer, **(B)** the frequency of behaviors (immobility, swimming, climbing) scored manually, **(C)** the latency to first immobility in the forced swim test (FST), and **(D)** the effect of acute administration of vehicle and sarcosine at 560, 1000 mg/kg on the percentage of immobility time in the tail suspension test (TST) [$n = 8$–10 per dose group, $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$ vs. vehicle (mean ± SEM)].

Patients, caregivers, and investigators (except the investigational pharmacist) were all masked to the assignment. Medication was provided in coded containers. Patient compliance and safety were closely monitored by caregivers and pill-counting by the study staff.

Assessments

The primary outcome measures were clinical improvement measured by HAMD-17 total score, Global Assessment of Function (GAF, Axis V in DSM-IV), and remission (defined as a score of <7 on the HAMD-17) (22). The secondary outcome measures were treatment success rates as measured by response rate, dropout rate, and clinical improvements measured by Clinical Global Impression of Severity (CGI-S) (23) and three factors of HAMD-17 (24). Response was defined as a reduction of ≥50% of the HAMD-17 score. Of the three factors of HAMD-17, the mood factor consists of seven items (depressed mood, feelings of guilt, suicide, work and activities, psychomotor retardation, somatic energy, and libido), the sleep/psychic anxiety factor of five items (initial insomnia, middle insomnia, delayed insomnia, agitation, and psychic anxiety), and the somatic anxiety/weight factor of three items (somatic anxiety, hypochondriasis, and weight loss) (24).

Clinical ratings were performed by a research psychiatrist (H.-Y.L.), who was trained and experienced in the use of the scales. Systemic side effects of treatments were evaluated by means of physical and neurological examinations and laboratory tests and reviewed by application of the Udvalg for Kliniske Undersogelser Side Effects Rating Scale (25).

Data Analysis

See Supplement 1 for a full and detailed description for the statistical analysis.

Results

Preclinical Study

Antidepressant-Like Effects of Sarcosine. For the acute study, the treatment of desipramine, citalopram, and sarcosine at the dose range of 560–2000 mg/kg significantly decreased immobility time of computer-scored FST ($F_{6,63} = 15.0$, $p < .001$; Figure 1A). However, sarcosine at 100 mg/kg did not decrease immobility significantly ($p = .55$). Similar findings were also observed when immobility was scored manually ($F_{6,63} = 11.5$, $p < .001$; Figure 1B). Furthermore, the differential effects on active (swimming and climbing) behaviors in the FST are shown in Figure 1B. Sarcosine at 100–2000 mg/kg and desipramine increased climbing behavior significantly ($F_{6,63} = 15.8$, $p < .001$) and citalopram increased swimming behavior significantly ($F_{6,63} = 15.9$, $p < .001$). Sarcosine at 560–2000 mg/kg and desipramine also prolonged latency to the first immobility in the FST when compared with the vehicle group (Figure 1C) ($F_{6,63} = 5.0$, $p < .001$). Additionally, to examine whether these compounds could increase spontaneous locomotor activity and yield a false-positive result in FST, the total closed arm entries and distance moved in EPM were measured as an indicator of general activity (26). None of the drug treatments increased general activity (Figure S2F in Supplement 1), and acute treatment of sarcosine at the doses of 560 and 1000 mg/kg significantly decreased immobility time of TST ($F_{2,23} = 5.88$, $p < .01$; Figure 1D).

For the chronic study, 21-day treatment of sarcosine with the lowest effective dose (560 mg/kg) in the FST significantly reduced the latency to feed in the NSFT for nonstressed rats (Figure 2A) ($t = 3.1$, $df = 14$, $p = .008$). In the CUS with chronic sarcosine administration study, CUS-exposed rats exhibited a reduction in SPT and increased the latency to feed in the NSFT compared with the unstressed control rats (Figure 2B,C) ($F_{2,27} = 3.918$, $p < .05$; $F_{2,27} = 12.711$, $p < .001$, respectively). Chronic (21 days) administration of sarcosine reversed the CUS-induced behavioral deficits in both SPT and NSFT (Figure 2B,C). However, chronic administration of sarcosine did not restore the loss of body weight in stressed rats (data not shown).

Clinical Study

One hundred fourteen patients were screened, and 40 were deemed eligible and provided informed consent. Among these 40 subjects, 20 each were randomly assigned to the sarcosine or citalopram therapy, respectively (Figure S3 in Supplement 1). Demographic data, education level, age at illness onset, number of previous major depressive episodes, severity of the mental

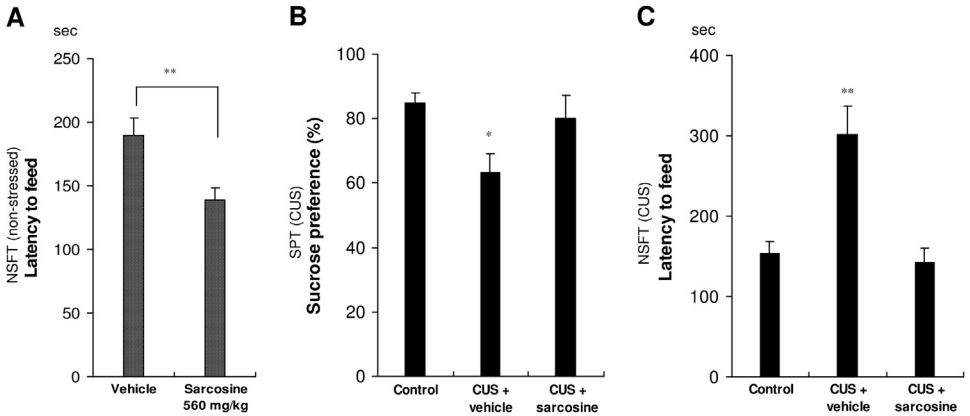

Figure 2. Effect of chronic administration of vehicle and sarcosine at 560 mg/kg for 21 days on **(A)** the latency to feed in the nonstressed novelty-suppressed feeding test (NSFT). For chronic administration in the chronic unpredictable stress (CUS) test, rats are handled daily (home cage control) or subjected to the CUS procedure for 35 days. Animals are administered saline or sarcosine (560 mg/kg) for the 21 last days of the experiment. The efficacy of CUS or sarcosine on sucrose preferences test (SPT) and NSFT are measured. The SPT was conducted 1 day later and NSFT on 2 days after drug treatment. **(B)** The percentage of sucrose preference in SPT with CUS and **(C)** the latency to feed in the NSFT with CUS. [$n = 10$ per dose group, $^*p < .05$, $^{**}p < .01$, vs. vehicle **(A)** or control **(B, C)** (mean ± SEM)].

Table 1. Demographic and Clinical Characteristics of Patients with Major Depression Assigned to Citalopram or Sarcosine Treatment

Characteristics	Study Group		*p* Value
	Citalopram ($n = 20$)	Sarcosine ($n = 20$)	
Female Sex, No. (%)	9 (45)	6 (30)	.33[a]
Age (yr)	35.7 (9.5)	37.2 (11.3)	.66[b]
Body Weight (kg)	62.1 (14.5)	63.2 (10.0)	.77[b]
Body Mass Index (kg/m^2)	23.6 (4.7)	24.1 (3.8)	.57[c]
Education Level (yr)	13.7 (2.9)	12.6 (2.9)	.25[c]
Age At Illness Onset (yr)	32.6 (10.2)	33.4 (10.2)	.79[b]
No. Of Previous Episodes	1.3 (1.3)	1.6 (1.0)	.55[c]
Dose (mg)	27.0 (11.3)	900 (502.6)	
HAMD-17 Score	24.5 (5.3)	23.7 (5.8)	.63[b]
CGI-S	4.3 (0.7)	4.5 (0.7)	.48[c]
GAF	51.0 (7.4)	49.5 (7.1)	.53[b]

Except in sex ratio, all data are expressed as mean (SD).

CGI-S, Clinical Global Impression of Severity; GAF, Global Assessment of Function (Axis V in DSM-IV); HAMD-17, 17-item Hamilton Rating Scale for Depression.

[a]χ^2 test.

[b]Two-sample *t* test.

[c]Mann-Whitney test.

illness, and global function at baseline were similar between the two groups (Table 1). Among them, 11 patients in the sarcosine group and 14 in the citalopram group were drug-naive ($p = .33$). The other patients had received medications in the past (but had been drug-free for 3 months or longer: inclusion criterion 6), and none was treatment-resistant. Our exclusion criteria included history of poor response to selective serotonin reuptake inhibitors (SSRIs) and history of severe adverse reaction to SSRIs.

The disposition and outcome of the 40 patients is shown in Figure S3 in Supplement 1. Patients who received sarcosine were more likely to stay in the study. Among the six subjects who received sarcosine and dropped out, four were remitters, one subject left the study to resume a job, and only one was a nonresponder with HAMD-17 total score reduction <50% from baseline; in comparison, all of nine subjects who received citalopram and dropped out were nonresponders (Figure S3 in Supplement 1).

Clinical Outcomes

The clinical changes in both primary and secondary outcomes are presented in Table 2. Because of the nonlinear time effect of the sarcosine group, as shown in Table 2, we treated visit as a categorical rather than continuous variable in the mixed-model analysis. Overall, sarcosine treatment produced a more rapid and greater improvement than citalopram treatment.

For the primary outcome measures, sarcosine produced greater improvements in HAMD-17 (4.7, 4.6, and 5.3 at weeks 2, 4, and 6, $p = .009$, .021, and .012, respectively) and GAF (7.5, 10.1, and 9.6 at weeks 2, 4, and 6, $p = .004$, <.001, and .002, respectively) than citalopram throughout the study, with effect sizes of .95 and 1.19 at the end of the study, respectively (Table 2). Subjects receiving sarcosine were more likely to be remitters at all post-treatment visits (Table 2). Compared with the citalopram group, Fisher's exact tests showed that a much better remission rate was found in the sarcosine group at weeks 2, 4, and 6, with *p* value = .047, .003, and <.001, respectively.

For secondary outcome measures, sarcosine produced greater improvement in CGI-S ($p = .001$, effect size = 1.26 at week 6) and in the mood factor (weeks 2, 4, and 6) and the sleep/psychic anxiety factor (week 6) but not in the somatic anxiety/weight factor of HAMD-17 (Table 2). The sarcosine group was also superior to the citalopram group in the response rates at weeks 2, 4, and 6, although the results did not reach the significant level. Furthermore, the dropout rate of the sarcosine group was lower than the citalopram group, especially at week 4 ($p = .02$). The dropout rate was higher in the citalopram group than the sarcosine group, which is similar to the studies that suggest the difference could still be attributed to the "treatment" effect (27,28).

Adverse Effects

Both sarcosine and citalopram were well tolerated. Citalopram treatment is associated with more treatment-emergent adverse events than in the sarcosine group (Table 3). Most side effects occurred within the first 2 weeks, except for the following: 1) in the citalopram group: nausea/vomiting ($n = 1$), sleepiness/sedation ($n = 1$), and reduced duration of sleep ($n = 1$) at week 4 and headache ($n = 1$) at week 6; and 2) in the sarcosine group: reduced duration of sleep ($n = 1$) and tremor ($n = 1$) at week 4 and increased dream activity ($n = 1$) at week 6. They were usually short-lived, except for nausea/vomiting ($n = 2$) and asthenia/fatigability ($n = 2$) reported at both week 2 and week 4 in the citalopram group and sleepiness/sedation (n = 2) and increased duration of sleep ($n = 1$) reported at both week 2 and week 4 in the sarcosine group. These side effects were all mild and not warranting of medical treatment. The routine blood cell count, chemistry, and electrocardiogram after treatment remained unchanged and were all within the normal ranges (data not shown). No dropout was due to side effects.

Discussion

We investigated the behavioral effects of sarcosine, a GlyT-1 inhibitor, in rodent models and in patients with major depression. We found that sarcosine expressed antidepressant-like properties in the animal models: administration of sarcosine decreased the immobility duration of FST and TST, reduced the latency to feed in the NSFT, and reversed behavioral deficits caused by CUS without stimulation of the locomotor activity in EPM, which are regarded as characteristic features of antidepressant drugs (29). Our findings are consistent with early reports about antidepressant-like activity of SSR504734, a reversible GlyT-1 inhibitor, in a chronic mild stress animal model (10) and D-serine, an NMDA coagonist, in learned helpless animal model (11). Additional behavioral studies of depressive-like behaviors (olfactory bulbectomy, etc.) and anxiety-like responses (light-dark box, etc.) can substantiate these pilot findings. At the same time, D-serine and D-alanine, full agonists of the NMDA-glycine site, also had similar behavioral effects in our preliminary studies (Huang *et al*; personal communication; July 11, 2012). It is interesting to learn that sarcosine, in addition to GlyT-1 inhibition, can prevent desensitization of NMDA function at high concentrations (30). Substantiation of GlyT-1 as a novel target for developing new antidepressants awaits further proof-of-principle studies.

We also found that sarcosine increased the percentage of open-arm time and entries at a high doses (Figure S2 in Supplement 1), suggesting that NMDA enhancers can be good candidates for antidepressants because most antidepressants increase open-arm time (31) and most antidepressants also have

Table 2. Outcome Measures for the 6-Week Sarcosine Versus Citalopram Treatment

			Treatment Group				
	Scale	Study Week	Citalopram Outcome	Sarcosine Outcome	Estimate[c] (SE)	*p* Value	Effect Size
Primary outcome	HAMD-17 total score[a]	0	24.5 (5.3) ($n = 20$)	23.7 (5.8) ($n = 20$)			
		2	20.3 (7.1) ($n = 20$)	14.8 (7.8) ($n = 20$)	−4.65 (1.75)	.009[c]	.75
		4	16.3 (5.1) ($n = 12$)	12.3 (7.1) ($n = 19$)	−4.58 (1.94)	.021	.78
		6	14.0 (5.0) ($n = 11$)	9.4 (6.9) ($n = 14$)	−5.26 (2.06)	.012	.95
	GAF[a]	0	51.0 (7.4)	49.5 (7.1)			
		2	54.1 (9.8)	60.1 (10.6)	7.50 (2.54)	.004[c]	.83
		4	57.7 (6.0)	65.2 (9.4)	10.11 (2.81)	<.001	1.16
		6	60.8 (7.5)	67.8 (9.2)	9.58 (2.98)	.002	1.19
	Remission rate[b]	2	0/20 (0%)	5/20 (25%)		.047[d]	.38
		4	0/20 (0%)	8/20 (40%)		.003	.50
		6	1/20 (5%)	13/20 (65%)		<.001	.63
Secondary outcome	CGI-S[a]	0	4.3 (.7)	4.5 (.7)			
		2	4.1 (1.0)	3.4 (1.2)	−0.85 (0.27)	.002[c]	.90
		4	3.6 (.8)	2.8 (1.0)	−1.22 (.29)	<.001	1.33
		6	3.4 (.8)	2.6 (.9)	−1.06 (.31)	.001	1.26
	Dropout rate[b]	2	0/20 (0%)	0/20 (0%)			
		4	8/20 (40%)	1/20 (5%)		.020[d]	.42
		6	9/20 (45%)	6/20 (30%)		.514	.15
	Response rate[b]	2	1/20 (5%)	6/20 (30%)		.091[d]	.33
		4	3/20 (15%)	11/20 (55%)		.019	.42
		6	4/20 (20%)	14/20 (70%)		.004	.50
	HAMD-17 mood[a]	0	12.35 (2.25)	12.05 (3.46)			
		2	10.05 (2.86)	6.80 (4.24)	−2.95 (1.06)	.007[c]	.80
		4	7.83 (2.76)	5.30 (3.61)	−2.80 (1.17)	.019	.78
		6	6.55 (3.62)	4.00 (3.31)	−2.54 (1.24)	.044	.76
	HAMD-17 sleep/psychic anxiety[a]	0	6.95 (2.33)	6.60 (1.70)			
		2	5.90 (2.77)	4.45 (2.14)	−1.10 (.60)	.070[c]	.52
		4	4.83 (2.17)	4.16 (2.19)	−.88 (.66)	.190	.46
		6	4.64 (1.50)	3.07 (2.50)	−1.89 (.71)	.010	.99
	HAMD-17 somatic anxiety/weight[a]	0	3.60 (1.70)	3.35 (1.84)			
		2	2.95 (1.64)	2.75 (1.94)	.05 (.31)	.873[c]	.04
		4	2.33 (1.56)	2.21 (1.90)	−.22 (.35)	.523	.21
		6	2.00 (1.48)	1.71 (1.94)	−.45 (.37)	.226	.44

The mood factor consists of seven items: depressed mood, feelings of guilt, suicide, work and activities, psychomotor retardation, somatic energy, and libido; the sleep/psychic anxiety factor consists of five items: initial insomnia, middle insomnia, delayed insomnia, agitation, and psychic anxiety; the somatic anxiety/weight factor consists of three items: somatic anxiety, hypochondriasis, and weight loss.

CGI-S, Clinical Global Impression of Severity; GAF, Global Assessment of Function; HAMD-17, 17-item Hamilton Rating Scale for Depression.

[a]Outcome is given as mean (SD).

[b]Outcome is given as *n*/total (%).

[c]Mixed-model repeated-measure (MMRM) models for comparisons of treatment effects over time with treatment (citalopram or sarcosine), visit (0, 2, 4, 6 weeks), and treatment-visit interaction as fixed effects, and baseline rating score as the covariance, with auto-regressive of order 1 as the type of correlation structure and intercept as the only random effect term.

[d]Fisher's exact test.

anxiolytic effects clinically. The behavior repertoire of this NMDA enhancer in FST, TST, EPM, NSFT, and CUS/anhedonia is consistent with the principle that candidate antidepressant compounds have parallel actions on behaviors relevant to both antidepressant and anxiolytic effects.

Additionally, as shown in Figure 1, the behavioral characterization of rats receiving sarcosine was similar with rats receiving desipramine but not citalopram. The major advance of the modified FST over its traditional counterpart is that it reveals that catecholaminergic agents decrease immobility with a corresponding increase in climbing behavior, whereas serotonin–related compounds such as SSRIs also decrease immobility but increase swimming behavior (32).

Clinically, sarcosine produced greater and quicker improvement in the scores of HAMD, CGI, and GAF than did citalopram. However, we did not assess the improvement before week 2. Importantly, sarcosine treatment also induced a substantially higher remission rate than citalopram. This finding, if confirmed in a large series study, will have profound impact on the outcome of major depression because most antidepressants are delayed in onset of their effectiveness and the partial responders to antidepressant treatment are vulnerable for relapse and a protracted course of illness.

In our clinical study, superior efficacy of sarcosine over citalopram treatment was manifested by the following: 1) the effect sizes for between-group comparisons were substantial in all of the primary outcome measures and most of secondary outcome measures, 2) sarcosine treatment not only diminished the severity of symptomatology but also improved global function, 3) as compared with citalopram-treated patients,

Table 3. Treatment-Emergent Adverse Events During the Study

Adverse Event	No. of Subjects		
	Citalopram	Sarcosine	Total
Nausea/Vomiting	4	3	7
Sleepiness/Sedation	3	3	6
Asthenia/Increased Fatigability	1	1	2
Constipation	2	0	2
Increased Duration of Sleep	1	1	2
Reduced Duration of Sleep	1	1	2
Concentration Difficulties	1	0	1
Depression	1	0	1
Dystonia	1	0	1
Headache, Migraine	1	0	1
Palpitations/Tachycardia	1	0	1
Tension/Inner Unrest	1	0	1
Weight Gain	1	0	1
Weight Loss	1	0	1
Increased Dream Activity	0	1	1
Orthostatic Dizziness	0	1	1
Tremor	0	1	1
Total	20	12	32

All p values are not significant between the two study groups.

improvement was faster in sarcosine recipients, with 40% of subjects reaching remission at the end of week 4 and 65% at week 6, and 4) subjects given sarcosine were more likely to remit and less likely to drop out (Table 2; Figure S3 in Supplement 1).

Regarding the three factors of HAMD-17, sarcosine was also more efficacious than citalopram in improving the mood and the sleep/psychic anxiety factors but not for the three items in the somatic anxiety/weight factor (Table 2). These findings are consistent with the rodent study, which suggests that sarcosine may have both antidepressant-like and anxiolytic effects as demonstrated in FST, EPM, NSFT, and CUS/anhedonia test. Sarcosine, an NMDA enhancer, may represent a novel treatment for depression. Treatment for depression may be individually tailored in the future when the NMDA-enhancing agent is an option.

Our findings seem rather dissonant, with preclinical and clinical data showing that NMDA antagonists such as PCP, MK-801, and ketamine have efficacy in some animal models and clinical depression (14,15). Acute administration of ketamine also ameliorates depressive symptoms in patients with major depression or bipolar depression (15,16). Both NMDA agonists and antagonists produce a robust response in contrast to the delayed action of currently used antidepressants (15,16). Taken together, the findings of the two different approaches suggest that depression involves complex neural substrates, in that both NMDA enhancement and inhibition can result in improvement of behavioral manifestations of depression. Agents modulating NMDA function may provide benefits for depressed patients who do not respond or are partially responsive to conventional monoamine-modulating antidepressants.

The results of both NMDA agonist and antagonist exerting antidepressant-like effects seem disconcordant and counterintuitive. Nevertheless, our findings are not in contradiction with those of empirical studies about NMDA antagonists exerting antidepressant effect. First, at the cellular level, the antidepressant-like effects of ketamine were found to be selectively abolished with the use of an α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) antagonist (NBQX) before infusion (33). The antidepressant effects of ketamine are possibly mediated by means of AMPA receptor (AMPAR) activation secondary to NMDA blockade and increased glutamatergic neurotransmission (34). Meanwhile, the NMDA-enhancing agents can facilitate the activation of postsynaptic AMPAR by an increase in neurotransmitters release, which is mediated by NMDAR. The net effect of these changes is to enhance the sensitivity of the synapse to released glutamate (35). Therefore, it is theoretically plausible that both NMDA-enhancing agents and antagonists can have similar effects through the common mechanism at AMPAR.

Second, recent evidence suggests that NMDAR-induced responses depend on the receptor location: stimulation of synaptic NMDARs leads to the build-up of a neuroprotective "shield," whereas stimulation of extrasynaptic NMDARs promotes cell death (36). Altered glutamatergic function resulting in the perturbation in the balance between synaptic and extrasynaptic NMDAR activity, which plays an important role in both the pathophysiology and treatment of mood disorders and other neurodegenerative diseases (14,36). It also implies that NMDA agonist and antagonist at optimal doses may achieve the same equilibrium between the interplay of synaptic and extrasynaptic NMDARs, which leads to the same net effect for the antidepressant effect. In other words, the NMDA antagonists produce their antidepressant efficacy by preferentially blocking the excessive stimulation of extrasynaptic NMDARs. The NMDA-enhancing agents produce the similar effect by preferential stimulating the synaptic NMDARs. However, it would be interesting to compare agonists and antagonists in the same study because their behavioral effects as well as risk and benefit profiles probably are different.

A vigorous review of systemic side effects revealed that sarcosine treatment was well tolerated (7,8,19–21). The few side effects reported by the patients were minimal and coincidental, and they resolved spontaneously. The toxicology of sarcosine has been discussed in detail previously (7,8,19–21). Sarcosine was reported to activate prostate cancer cells and to indicate malignancy of prostate cancer cells. Sarcosine levels were also increased in invasive prostate cancer cell lines relative to benign prostate epithelial cells (37). However, sarcosine as a marker for prostate cancer has been disputed and cannot be replicated (38–40). In a separate trial of sarcosine treatment, the levels of prostate-specific antigen did not increase after 12 weeks of treatment (manuscript in preparation).

Our finding of an antidepressant property of sarcosine is consistent with its similar effect in the patients with schizophrenia (9,41,42). Taken together, it suggests that enhancing NMDA function to improve the behaviors of depression is not limited to major depression.

Though our study design is without a placebo group, the active control group of citalopram is ethically more acceptable to institutional review board and potential study candidates. In general, non–placebo-controlled studies may have higher response rates than placebo-controlled studies because of the high expectation (43). The superior efficacy of sarcosine over an active control SSRI drug is less likely to be demonstrated, as in this study, than by comparing sarcosine with placebo. At the same time, subjects receiving citalopram treatment who completed the study did improve significantly (HAMD-17 decreased from 24.5–14.0, Table 2).

Our dosing strategy was optimally titrating citalopram between 20–60 mg/d. Although the final dose of citalopram in our study was lower than that of some previous studies in white subjects (44,45), it is consistent with the guideline issued by the

FDA. The lower doses of SSRIs in our study can be attributed to the optimal dosing design that we started with the low recommended dose. Nevertheless, it also could be a result of the slow metabolism of citalopram, given the relatively high prevalence of the poor metabolizers of CYP2C19 in the Asian population (46). The average dose of citalopram and the response rate at the end of our study, in fact, is very close to another study in the same ethnic population, which also adopted a flexible dosing design (47).

The patients in the citalopram group had a higher dropout rate at week 4 caused by nonresponse compared with the sarcosine group. This can be partly due to the superior efficacy of sarcosine treatment that kept the patients in the study better than citalopram in a system with a high treatment discontinuation rate of patients with major depression in Taiwan (48).

Our study had more male patients. Women are twice as likely as men to have depression and four times as likely to be receiving antidepressant treatment (49). Selection of subjects who had been free of psychotropic drugs for >3 months resulted in the male > female sex distribution. In accordance, some other studies focusing on drug-free patients also enrolled more male than female patients (50,51). Nevertheless, we found that sex did not affect any of the outcome measures (data not shown). The applicability of our findings is not limited to male patients with depression.

In summary, compared with an active control of SSRI treatment, sarcosine therapy shows a fast, efficacious promise of remission of depression and a benign safety profile with minimal attrition. However, the findings must be interpreted in light of significant limitations of the absence of a placebo control group and the high dropout rate in the comparator treatment group. Future placebo-controlled, larger-sized studies are needed to fully assess the effects of sarcosine. Additionally, the preclinical experiment would be more compelling if a whole battery of studies of depression- and anxiety-related behaviors are conducted. Nevertheless, both our animal and human studies indicate that the GlyT-1 inhibitor sarcosine, or other potentials avenues for NMDAR enhancement, represent a novel therapeutic approach for the treatment of depression.

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ClinicalTrials.gov: N-Methylglycine (Sarcosine) Treatment for Depression; www.clinicaltrials.gov; NCT00977353.

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