

Safety, Tolerability and Pharmacokinetics of Open Label Sarcosine Added on to Anti-Psychotic Treatment in Schizophrenia – Preliminary Study

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ABSTRACT

Background: Hypofunction of NMDA receptor-mediated neurotransmission might play a critical role in schizophrenia. Sarcosine, N- methylglycine and inhibitor of the glycine transporter-1 (Gly-T1), has been suggested as a novel treatment for schizophrenia.

Methods: Open label sarcosine was added to 22 stabilized patients: 5 patients received 2 gm/d, and 17 received 4gm/d. Pharmacokinetics samples, clinical and cognitive parameters using PANSS, CGI and MCCB were collected for all patients.

Results: Significant improvement was observed after one week of treatment on PANSS sub-scale of 'positive symptoms' ($Z = -2.68$; $P = 0.007$) and 'general psychopathology' ($Z = -3.02$; $P = 0.003$), an improvement in PANSS total score and CGI-S showed a trend ($Z = -2.72$; $P = 0.06$; $Z = -2.69$; $P = 0.08$). Speed of processing (MCCB subscale) improved significantly ($Z = -2.13$; $P = 0.03$). Sarcosine exhibited linear kinetics, with a T_{max} and $t_{1/2}$ of $\sim 1\frac{1}{2}$ - $2\frac{1}{2}$ hr and ~ 1 hr, respectively.

Limitations: This was a short period, open label pilot study with small sample size per dosage group.

Conclusions: Sarcosine is a safe compound and might be efficacious in the treatment of schizophrenia.

INTRODUCTION

The positive and negative symptoms of schizophrenia, as well as the cognitive impairment that is common in the illness, can be mimicked by the administration of agents that block the NMDA receptor, such as the street drug PCP ("angel-dust") (1). It has thus been proposed that the symptoms of schizophrenia might arise from a hypofunction in NMDA neurotransmission. Therefore, enhancing NMDA neurotransmission is predicted to improve symptoms and cognition in schizophrenia.

Initial studies of this theory have been conducted using direct NMDA glycine-site agonists, such as glycine and D-serine. These compounds, while crucial as proof-of-principle treatments, are limited by poor CNS penetration and endogenous inactivation processes. Nevertheless, most clinical studies (2, 3), as well as one meta-analysis (4), have shown significant effects of these compounds, particularly on negative symptoms.

An alternative approach to the stimulation of NMDA receptor-mediated neurotransmission in the brain is use of glycine transport inhibitors. Glycine (GLYT1) transporters are localized with brain NMDA receptors and regulate local glycine levels (5). Several recent small-scale studies have shown significant beneficial effects of sarcosine as adjunct to typical or atypical antipsychotics (6, 7). Improvements were observed in both positive and negative symptoms, and efficacy appears superior to that seen with the NMDA agonist D-serine (7). In addition an animal study showed that sarcosine infusion increased brain levels of glycine. This study also showed that dosage elevation of sarcosine up to an equal dose of 8gm/d in human elevated glycine levels (8).

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However, all of the published studies on sarcosine in schizophrenia have been conducted at a single site in Taiwan and there were no replications of their findings. Furthermore, although two studies reported improvements in PANSS cognitive symptoms items (6, 7), the effect of sarcosine on cognition has not been directly tested using an assessment tool designed and validated as a primary measure of cognition in schizophrenia, such as the MATRICS Consensus Cognitive Battery (MCCB) (9).

The current study was originally designed as a preliminary trial in order to establish safety, tolerability and pharmacokinetics of 2, 4 and 8gm of sarcosine. Our intention was to complete six weeks of treatment with 8gm of sarcosine open label treatment in order to establish the efficacy of sarcosine in a larger randomized controlled trial.

The recruitment was terminated due to the following safety issue: An article published in *Nature* in February 2009 reported that sarcosine, added to tissue culture of prostate cancer cells, induced invasive phenotype in prostate epithelial cells (10). This report suggested a potential role for sarcosine in prostate cancer progression. Due to this potential safety concern this study was terminated. This finding has been questioned and parts of it were not replicated (11). We hereby present the results of the first and the second stage of the study conducted prior to recruitment termination.

METHODS

PATIENTS

Patients were recruited from the inpatient and the outpatient wards of Sheba Medical Center in Israel between July 2009 and June 2010. The trial was approved by the local IRB and conducted according to ICH-GCP guidelines. In addition to the local IRB approval, the trial was monitored by the NIMH Data Monitoring and Safety Board. Each participant received oral and written explanations of the study, and gave written informed consent prior to inclusion.

The patients who participated in the study were aged 18-64 years and met DSM-IV criteria for schizophrenia or schizoaffective disorder as confirmed by modified SCID (12). Subjects were stabilized on any antipsychotic drug for at least four weeks prior to enrollment and sarcosine add on. Patients' antipsychotic medication was as follows: 7 risperidone, 4 quetiapine, 4 IM zuclopenthixol depo, 3 olanzapine, 2 IM fluphenazine, 2 paliperidone. Patients who were taking clozapine were not included. In order to participate in the study a total PANSS score of >60 was required, with at least 18 points on the negative symptoms

subscale (13, 14). Medical history, routine clinical blood and urine tests were collected and physical examinations were performed prior to enrollment. Patients' confidentiality was maintained during all stages of the study.

STUDY DESIGN

The study was performed in two dose-level phases. Following enrollment, in the first stage 5 patients received 2gm/d of sarcosine for one week and in the second stage 17 patients received 4gm/d for one week. Five patients from each group underwent a 24-hour pharmacokinetics (PK/PD) session on the first day of treatment.

CLINICAL ASSESSMENTS

Cognitive effects of sarcosine were evaluated using the MATRICS Consensus Cognitive Battery (MCCB) (9), which was specifically designed to assess changes in cognition in schizophrenia. Neurocognitive assessment was conducted using a Hebrew translation of 9 out of the 10 MCCB sub-tests: Category fluency (15), Brief Assessment of Cognition in Schizophrenia (BACS)- symbol coding (16), Trail making A (17), Continuous Performance Test - Identical Pairs (CPT-IP) (18), University of Maryland-Letter-Number Span (19), WMS III - Spatial span (20), Rey Auditory Verbal Learning Test (RAVLT) (21), Brief Visuospatial Memory Test-Revised (BVM-T-R) (22), Neuropsychological Assessment Battery (NAB)-Mazes (23).

The Hopkins Verbal Learning Test (HVLT) (21) is the task for assessment of verbal learning used in the MCCB. Since this test has not been translated or validated into Hebrew, we used the Rey Auditory Verbal Learning Test (RAVLT) (21), which has a validated Hebrew version commonly used in clinical and research settings (24).

The cognitive battery was administered at baseline and at day 8. Since only 9 of the 10 MCCB subtests were administered, the subtest scores were converted into Z-scores to allow for comparison, and a mean of these scores was used as an estimated total MCCB score and served as primary neurocognitive outcome.

For positive and negative symptoms, the primary rating instrument was the Positive and Negative Symptom Scale (PANSS) (13). Other assessments included: Clinical Global Impression (CGI) Scale (25); Simpson-Angus Extrapyramidal Symptom Rating Scale (SAS) (26); Abnormal Involuntary Movement Scale (AIMS) (27); Calgary Depression Scale for Schizophrenia (CDSS) (28) and side effects checklist. The PANSS, CDSS, SAS, AIMS and CGI ratings were administered at baseline and after one week of treatment with sarcosine.

PLASMA SARCOSINE LEVEL DETERMINATION

Sarcosine analysis was performed using a Biochrom 20 plus amino acid analyzer (Biochrom Ltd UK). Separation was achieved by Liquid chromatography on an ion exchange column. The separation occurred by using five different buffers of lithium citrate. Each buffer that was run sequentially through the column was at different Ph and ionic strength. Some of the buffers had the addition of varying amounts of an organic solvent. The run times of the buffers and the temperature of the columns were varied to optimize the separation. After elution from the column the eluates were mixed with Ninhydrin in dimethylsulphoxide heated to 135°C and the resultant color read at 570nm and 440nm (29).

RESULTS

STAGE 1 - 2GM/D

Five patients were recruited for the 2gm/d stage of the study. All patients were males, mean age was 41.0 (31-48years). Due to the small sample size (N=5), statistical analysis for the patients who received 2gm/d was not performed. All patients in this group tolerated the medication very well and had CGI \geq 3.

Table 1. Clinical and cognitive assessments at baseline and after 8 days*

Test	Baseline	Day 8	Z	P	Cohen's D
PANSS Positive	16.61 \pm 7.45	13.47 \pm 4.67	-2.68	0.00**	-0.91
PANSS Negative	19.83 \pm 5.03	18.82 \pm 5.28	-1.27	0.20	-0.62
PANSS General	35.66 \pm 11.28	31.29 \pm 9.06	-3.02	0.00**	-0.78
PANSS Total	71.00 \pm 21.82	63.58 \pm 16.39	-2.72	0.06	-0.79
CGI-S	3.89 \pm 0.73	3.78 \pm 0.71	-2.69	0.08	-0.36
MCCB speed of processing	26.95 \pm 18.18	35.63 \pm 16.29	-2.13	0.03**	0.38
MCCB Attention	23.95 \pm 12.76	23.73 \pm 17.35	-0.57	0.57	-0.01
MCCB Working memory	27.65 \pm 12.84	24.21 \pm 21.05	-0.85	0.39	-0.27
MCCB Verbal learning	31.45 \pm 7.36	30.57 \pm 9.99	-0.21	0.83	-0.13
MCCB Visual learning	29.70 \pm 14.63	34.22 \pm 16.46	-1.49	0.13	0.44
MCCB Reasoning & Problem solving	33.55 \pm 7.61	34.78 \pm 9.40	-0.71	0.47	0.21
MCCB Total	28.87 \pm 9.36	30.42 \pm 9.89	-0.88	0.37	0.28

* We used Wilcoxon signed ranks test

** Statistically significant

STAGE 2 - 4GM/D

On the second stage we recruited 17 patients diagnosed with schizophrenia, mean age 41.89 (26-56 years), 12 males and 5 females.

Table 1 summarizes the cognitive and clinical evaluations which included PANSS, MCCB, SAS and CGI, between baseline and after one week for patients receiving 4gm/d. Wilcoxon signed ranks tests were performed to compare baseline to day 8 safety, symptom and neurocognitive assessments. Our results show significant improvement in the PANSS subscale of 'positive' (Z= -2.68; P=0.007) and 'general' (Z= -3.02; P=0.003) symptoms subscale. The PANNS total score showed tendency toward significance (Z= -2.72; P=0.06) between baseline and after 8 days of treatment. Speed of processing, a subscale of MATRICS was significantly improved from baseline to day 8 of sarcosine add on.

PHARMACOKINETICS

For all doses, sarcosine exhibited linear kinetics, with a T_{max} and t_{1/2} of ~1½- 2½hr and ~1hr, respectively (Fig. 1).

SIDE EFFECTS

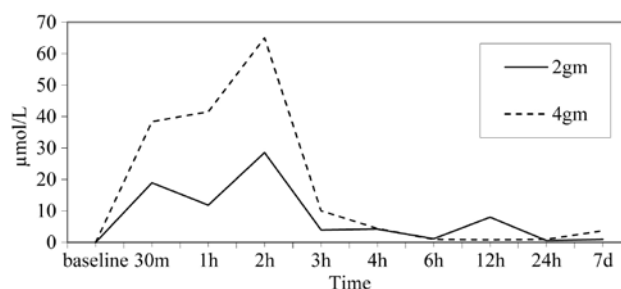
All the subjects completed the study and reported no serious side effects.

DISCUSSION

This current study has evaluated the safety, tolerability and pharmacokinetics of 2 and 4gm/d of sarcosine in order to test the possible use of this compound as add-on treatment for schizophrenia.

In general, sarcosine was well tolerated by the patients. Administration of sarcosine at 2 and 4gm/d for one

Figure 1. Pharmacokinetic chart: Blood levels at time intervals after first dose of 2gm/d and 4gm/d of sarcosine



Blood levels in µmol/L of sarcosine over a timeframe of 7 days. Sarcosine exhibited linear kinetics, with a T_{max} and t_{1/2} of ~1½- 2½ hr and ~1hr, respectively.

week appears to be safe. No significant events, abnormal laboratory results or ECG abnormalities related to the study medication were noted. As previously mentioned, early termination of this study was due to one article presenting some evidence that sarcosine can cause exacerbation of prostate cancer (10). However, later studies failed to replicate these findings and one other study has been published showing the opposite (11, 30). Furthermore, there is another ongoing NIH funded study that examines the effects of sarcosine on brain glycine concentrations (ClinicalTrials.gov identifier: NCT00538070).

To the best of our knowledge, this is the first study that presents pharmacokinetic evaluations of sarcosine. The pharmacokinetic results show that sarcosine concentration in serum reaches its peak after two hours, and the half-life of sarcosine is approximately one hour. These results indicate that sarcosine should be administered at least twice a day. In light of the above, it can be concluded that sarcosine is a safe compound, without significant side effects and with a relatively short half-life.

Although we are limited by the small number of patients, and open-label administration for only one week, the results are encouraging, showing improvements in the expected direction of reduction of positive symptoms and improvement in cognition, especially in the speed of processing, MCCB. The improvement in speed of processing, MCCB, is particularly interesting, as it is a core cognitive impairment in schizophrenia. We assume that due to the short period of time between the two evaluations, learning effect was relatively high. We hypothesize that a study with more participants and considerably longer period of time between the cognitive evaluations might reduce the learning effect.

These results are similar in direction to the several randomized controlled trials using sarcosine, that showed that 2gm/d of sarcosine as add-on therapy to antipsychotic treatment, can improve positive and negative symptoms in patients with chronically stable or acute schizophrenia (7, 31, 32).

Furthermore, Roche is now performing a phase III clinical trial on a glycine transport inhibitor and new glycine reuptake inhibitors are being developed by other pharmaceutical companies. These ongoing research projects emphasize the potential therapeutic value of sarcosine in treatment of schizophrenia (33).

In summary, we believe that sarcosine is a safe compound and further research is needed in order to evaluate its therapeutic role in schizophrenia.

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Author Contributions

Dr. Amiaz recruited the subjects, performed the clinical assessments, analyzed the data and drafted this manuscript. Dr. Kent was involved in the clinical assessments and performed blood testing. Mrs. Rubinstein was the study coordinator and performed cognitive assessments, data analysis and drafting of this manuscript. Prof. Sela was the pharmaceutical advisor to the study and was in charge of blood tests interpretation. Prof. Javitt and Prof. Weiser conceptualized the study, contributed critically to its design, and the interpretation of the data. All authors contributed to revisions and have approved the final manuscript.

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