Translational Medicine

Yang, et al., Transl Med (Sunnyvale) 2014, 4:2
DOI: 10.4172/2161-1025.1000127

Research Article Open Access

A Pilot Open-Label Trial of Use of the Glycine Transporter I Inhibitor, Sarcosine, in High-Functioning Children with Autistic Disorder

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Rec date: Apr 01, 2014; **Acc date:** Apr 17, 2014; **Pub date:** Apr 22, 2014

Abstract

This open-label trial examined the efficacy and safety of a glycine transporter I inhibitor, sarcosine, in the 24-week treatment of high-functioning children with autistic disorder. Four children (three boys, one girl, 9-11 years of age; average intelligence quotient 80.5) completed the 24 weeks of study. Sarcosine administration was at 30 mg/kg/day in the form of a capsule in two divided doses. The outcome measures were. Autism Diagnostic Observation Schedule (Module 3), parent and teacher-reported Adaptive Behavior Assessment System-II, parenting stress index, Conners' Continuous Performance Test, Wisconsin Card Sorting Test, child behavior checklist and Swanson, Nolan and Pelham IV hyperactivity attention scales. Safety assessments included monthly recorded vital signs, body weight, body height and adverse events. Statistical analysis found no significant treatment effect on all the outcome measures using the Wilcoxon Signed Rank test and generalized estimating equations analysis. However, an activation effect was reported by caregivers, and was corroborated by clinician's observation. Details were reported as a case-series in the text. We concluded that sarcosine was well tolerated. Though the data are too preliminary to draw any definite conclusions about efficacy, they do suggest this therapy to be safe, and worthy of a double-blind placebo-controlled study with a focus on a certain subgroup of children with autism spectrum disorders.

Keywords: Glutamate; Autism; Clinical trial; Sarcosine; Glycine

Introduction

Autism Spectrum Disorders (ASDs), including autistic disorder, Asperger's syndrome, and pervasive developmental disorder not otherwise specified, are developmental disorders characterized by dysfunction in several core behavioral dimensions: social communication/interaction deficits and specific behavior characteristics. The social deficit dimension involves deficits in reciprocal social interactions, lack of eye contact, diminished ability to carry on conversation, and impaired daily interaction skills. The communication domain can include problems ranging from lack of verbal language to fluent but odd speech with little comprehension of pragmatics. The behavioral domain involves compulsive behaviors, unusual attachments to objects, rigid adherence to routines or rituals, repetitive motor mannerisms and unusual reactions to sensory stimuli [1]. Genetic studies and human pathological and model system studies have all led to the hypothesis that molecular pathways involved in brain synaptic growth, development, and stability are perturbed in ASDs [2-4]. Molecules targeting brain cell dendritic spine regulation for the purpose of promoting its maturation and restoring spine stability are thus proposed to be of therapeutic potential in ASD. Because glutamate and its ionotropic N-methyl-D-Aspartate (NMDA) receptors have long been known to be associated with synaptic plasticity, glutamate and NMDA receptors-mediated signaling have become targets of interest in exploring the pharmacological treatment of ASDs [5,6].

It has been suggested that direct stimulation of NMDA receptors can cause neuronal injury through excessive excitotoxicity. An indirect approach to enhancing the NMDA neurotransmission is through increasing the availability of synaptic glycine, the "co-transmitter" of glutamate, by the attenuation of the glycine reuptake through glycine transporter 1 (GlyT-1) [7]. Sarcosine, also known as N-methylglycine with the chemical formula CH3NHCH2COOH, is a potent endogenous inhibitor of GlyT-1 and can enhance NMDA neurotransmission [8-10]. Sarcosine was firstly isolated and named by German chemist Justus von Liebig in 1847. It is a non-proteinogenic amino acid that occurs as an intermediate product in the synthesis and degradation of the amino acid glycine. This molecule has been shown to be effective in clinical trials involving the negative and cognitive symptoms of chronic patients with schizophrenia [11], in acutely ill persons with schizophrenia [12,13], in patients with obsessive–compulsive disorders[14], to temporarily relieve the depressive and neuropsychiatric symptoms of patients with Parkinson-related dementia [15] and to improve depressive-like behavior in a rodent model and inhuman depression [16]. To the authors' knowledge, there is currently no report on using sarcosine for the treatment of patients with ASD. In the present study, we hypothesized that sarcosine is a therapeutic agent for children with ASD due to its activity as an NMDA receptor and glutamate transmission enhancement.

Methods

This study was a pilot 24-week open label trial with the aim of gaining preliminary experience with sarcosine for the treatment of

children with autistic disorders. The participants were a convenient sample of outpatients children aged between 9 to 12 years old and of both genders. They were recruited from the Department of Psychiatry, Kaohsiung Medical University Hospital and Kaohsiung Armed Forces General, which are major medical centers in Taiwan.

The diagnosis of autistic disorder was made using DSM-IV-TR criteria [17] with clear documentation of each criterion in the history record or current interview. All the interviews were conducted by an expert child and adolescent psychiatrist (P.Y). Assessments were performed by a child psychologist trained to use the Autism Diagnostic Observation Schedule [18]. Each participant's mother and a designated teacher were responsible for filling out the required questionnaires. The level of intelligence was considered as an exclusion criterion, in that for each participant the Full Scale Intelligent Quotient (FSIQ) or the subscale of the Perceptual Reasoning Index (PRI) as ascertained by the Wechsler Intelligence Scale for Children Fourth Edition-Chinese version [19] had to be above 70. For children receiving other medication treatments, the drugs had to be at a stable dose for at least 2 months before entering the study and remained unaltered throughout the clinical trial. Concomitant educational, occupational, physical, communicational or behavioral treatment was permitted, but no new treatment was allowed to be added. Special care was taken to exclude children with seizure disorders or known genetic syndromes. The research protocol was approved by the Institutional Review Boards (Registration number: 101-023) of the hospital mentioned above. Sarcosine is regulated as a food supplement in Taiwan and an Investigational New Drug (IND) application was not required. After a description of the study, informed consent was obtained from parents and the children themselves gave their assents. This trial was performed in 2012 and 2013.

Four children with autistic disorder enrolled and completed the open-label study. The dosages of sarcosine were equivalent to those used in earlier studies [12,13] and that were effective as add-on therapy in chronic adult patients with schizophrenia (i.e. 30 mg/kg/day). Sarcosine was provided by Natural Pharmacia International Inc (Belmont Massachusetts). Purity of more than 99% was confirmed by high performance liquid chromatography.

The children were followed up in person every month. Telephone contact with the family was made every week to monitor treatment compliance and side effect. The overall sarcosine adherence was excellent. The primary outcome measures were the Autism Diagnostic Observation Schedule (ADOS)-Module 3[18] and the parent and teacher-reported Adaptive Behavior Assessment System-II [20] (the above outcomes were measured at pre-intervention and at the end of 24 weeks of intervention). The secondary outcome measures were the Conners' Continuous Performance Test (CPT) [21], the Wisconsin Card Sorting Test, parental reported Child Behavior Checklist (CBCL) and Teacher Report Form (TRF) in the Achenbach system of Empirically Based Assessment [22], parent and teacher reported version of the Swanson, Nolan and Pelham IV (SNAP-IV) hyperactivity attention scales [23] (the above outcomes were measured at pre-intervention, at 4 , 8, 12 and 16 weeks after the beginning of intervention, and at the end of 24 weeks of intervention) and the Parenting Stress index [24] (baseline and at the end).

Data analysis

Statistical analysis found no significant treatment effect for all the outcome measures using the Wilcoxon Signed Rank test for baseline and final state comparison and generalized estimating equations analysis for some of the outcome measures. Nevertheless, observation of subjects by parents and clinicians noted that sarcosine administration seemed to have an activating effect. Details were reported as the case-series as below. Please see Table 1 for the summary of the characteristics and primary outcome measures of the four subjects.

	A	B	C	D
Gender	M	M	F	M
Age	9Y9M	10Y9M	9Y10M	11Y2M
Highest subscale IQ	83	103	83	101
Sarcosine mg/day	1000	1000	900	900
Other mg/day	Concerta 54	Concerta54	X	X
ADOS-1	11	22	20	14
ADOS-2	19	21	22	13
GCS-P-1	70	77	87	72
GCS-P-2	70	81	91	87
GCS-T-1	75	89	78	88
GCS-T-2	67	86	84	76

ADOS-1: Social interaction & communication score from Autism Diagnostic Observation Schedule (ADOS) at the baseline; ADOS-2: ADOS at the end of 24 weeks of trial; GCS-P-1: Parental reported General Adaptive Composite Score (GCS-P) from. Adaptive Behavior Assessment System-II at baseline; GCS-P-2: GCS-P at the end of 24 weeks; GCS-T-1: Teacher reported General Adaptive Composite Score (GCS-T) from Adaptive Behavior Assessment System-II at baseline; GCS-T-2: GCS-T at the end of 24 weeks

Table 1: Characteristics and primary outcome measures of the subjects

Report of case-series

Child A was a 9 year-9 month old boy when he finished the trial. He was diagnosed with autistic disorder when he was 1 year-10 month old. His FSIQ was 75 (Verbal Comprehension Index, VCI: 83, Perceptual Reasoning Index, PRI: 83, Working Memory Index, WMI: 70, Processing Speed Index, PSI: 83). His main problem by now was his socially passivity, poor academic performance, inattention, hypotalkativity and rigidity with rules. In addition to sarcosine 1000 mg/day, he also received Concerta (OROS-methylphenidate) treatment at 54mg/day in this 6-month trial. However, Concerta was received only when he went to school from Monday through Friday. At the final assessment (without Concerta), he was in a good mood, talked in a whisper, engaged in imaginary play by himself (a doll kicked a ball repeatedly), and would interact with the psychologist only by request. He was not able to describe his school events, and did not know the name of the classmate sitting next to him. In the clinician's opinion, the child had worsening inattention and restlessness when performing cognitive tasks, as observed in the final visit and no change in the ability to engage in social interaction. But his mother had little concern about the inattention the clinician

observed, and stated that the child was attentive during the weekdays when Concerta was resumed.

Child B was a 10 year 9monthold boy when he finished the trial. He was diagnosed with autistic disorder when he was 3years, 4 months old. His WISC-IV intelligence profile was as follows: VCI: 58, PRI: 91, WMI: 103 and PSI: 78.His main problem was socially passivity, poor in verbal communication, self-talking, poor academic performance, and rigidity with rules. In addition to sarcosine 1600 mg/day, B also received Concerta treatment at 54mg/day from Monday to Friday in this 6 month trial. At the final visit (without Concerta), he appeared more energetic, edgy and restless than in his baseline assessment state (also without Concerta). However, B became more active and talkative during social interaction with the interviewer, but he talked mainly about his own personal concerns (e.g. time schedule). During the final ADOS evaluation, he actively and repeatedly checked the time and procedure with the psychologist; and he chatted about his prior ADOS experience 6 months ago. His mother considered him improved in social spontaneity, but she also noticed that he became more restless and hyperactive, which impaired the quality of his social interaction with other people.

Child C was a 9 year10monthold girl when she finished the trial. She was diagnosed with autistic disorder when she was 3years, 9 months old. Her FSIQ was 75 (VCI: 83, PRI: 83, WMI: 70 and PSI: 83). C's main problem was socially aloofness, poor academic performance, frequently spacing out and appearing to be in a detached and dazed state. C had no friends and no intention to play with friends at school. She received 900 mg/day of sarcosine for the trial with no other concomitant medication. At the final visit, she appeared more alert and could be on task (i.e., CPT) upon request. However, she also became more stubborn and insisted on doing the task her own way as observed in the final ADOS session. She pushed away the psychologist's hand and wanted to line up things as she desired. (In the baseline ADOS evaluation, she had little intent to touch the test items.) Her mother considered her behaviors to be different after starting sarcosine because she appeared more "reachable". However, the mother could not conclude whether the changes were for better or for worse because C also became more stubborn.

Child D was an 11 year 2 month old boy when he finished the trial. He was diagnosed with autistic disorder when he was 4years, 8 months old. His FSIQ was 84 (VCI: 101, PRI: 87, WMI: 88 and PSI: 68). D's main problem was social passivity, poor academic performance and short, idiosyncratic ways of communication. He received 900 mg/day of sarcosine for the trial with no other concomitant medication. At the final visit, he appeared more vigilant than at baseline, and he performed much faster in non-verbal tests; specifically, he did not need to check repeatedly during the tasks. However, he still talked very briefly and was too tense to play imaginary games with the psychologist. His parents considered him improved in "spirit" and "allergic diathesis", and asked that the prescription of sarcosine to be continued when the study was officially ended. However, from a clinician's point of view, child D's behavior showed no change in the social domain.

Discussion

Our pilot trial revealed that sarcosine seemed to have activating effect as observed by the parents and clinicians. The activating effects made child B less passive, child C less detached in social interaction and child D more alert with improving mental performance speed. Nevertheless the activating effect could not be translated into improvement by statistical analysis of all the psychometric measurements we applied. A previous case report from the adult psychiatry literature also noted an activating effect after adding sarcosine 2000 mg/day for a young adult patient with schizophrenia treated on stable doses of antipsychotics treatment. The patient's activity and mood improved within 2 weeks after sarcosine add-on, but in the following 2 weeks, the patient reported increased drive, activity, libido, unpleasant inner tension, and irritability [25]. We also suspected that it was the administration of sarcosine which rendered child A and child B to be more edgy and less attentive. However, this clinical observation was not able to be corroborated by statistical analysis of teacher's and parental reports in CBCL, TRF and SNAP-IV. We hypothesized that the concomitant administration of methylphenidate and its well-established treatment efficacy for inattention and hyperactivity may contribute to the dampening of effect of sarcosine.

Up to now, only a few glutamatergic compounds have been studied in clinical trials of ASD, and the results are inconclusive [6]. Both NMDA agonist and antagonist were on the trial list because the hypothesis for scientist to design novel pharmaceutical treatment target is through glutamate mediated signaling in an attempt to modify synaptic function, but there are no validated biological markers to measure glutamate pathology in ASD.In addition, glutamate target modulation is further complicated by the large number of different receptors and transporters, the wide range of cell types expressing them, multiple regulatory controls, and a narrow concentration difference between normal synaptic function and excite-toxicity [26].

Experimental molecules for ASD treatment reported to be the NMDA antagonist include memantine and riluzole. A recent double-blind, placebo-controlled trial reported that children with autism treated with memantine as adjunctive therapy with risperidone had decreased symptoms of irritability [27]. Riluzole is thought to inhibit the release of glutamate at the presynaptic nerve cell terminus [28] and enhance glutamate reuptake [29]. Wink et al reported improvement in the severe repetitive behavior of three ASD patients by riluzole [30]. On the other hand, experimental molecules for ASD treatment focusing on enhancing and augmenting NMDA function include d-cycloserine (DCS) and GLYX-13. A preliminary, open-label study of the effects of DCS in a sample of children with ASD showed that it appeared to reduce measures of social withdrawal during a 12 week trial [31]. GLYX-13 is a monoclonal antibody fragment with partial agonist effects at the glycine modulatory site. Treatment with GLYX-13 has been demonstrated to rescue the ASD-analogous behavioral deficit in the animal model [32], but no clinical trial has been reported yet. The opposing mechanisms of memantine and DCS and the other NMDA agonists highlight the need for approaches to clinically assay NMDA function in individuals with ASD, which remains a daunting task.

To the best of the authors' knowledge, our current small sample pilot trial is the first report documenting the safety of long-term sarcosine therapy for children with ASD. Though our data are too preliminary to draw any definite conclusions about efficacy, they do suggest this therapy to be safe, and worthy of a double-blind placebo-controlled study with a focus on a certain subgroup of ASD children.

Acknowledgements

The authors have no conflicting financial interests. There are no industry-sponsored contribution nor of any corporate participation in preparing the manuscript.

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