AWARD NUMBER: W81XWH-09-1-0091

TITLE: A Randomized, Placebo-Controlled Trial of D-Cycloserine for the Enhancement of Social Skills Training in Pervasive Developmental Disorders

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REPORT DATE: November 2015

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
                           Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)* November 2015	**2. REPORT TYPE** Final Report	**3. DATES COVERED** *(From - To)* 1Mar2009 - 31Aug2015

4. TITLE AND SUBTITLE
A Randomized, Placebo-Controlled Trial of D-Cycloserine for the Enhancement of Social Skills Training in Pervasive Developmental Disorders

5a. CONTRACT NUMBER
W81XWH-09-1-0091

5b. GRANT NUMBER
W81XWH

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Indiana University Health
Indianapolis, IN 46202

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSORING/MONITORING AGENCY REPORT NUMBER

12. DISTRIBUTION AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The main objective of this application is to determine whether D-Cycloserine (DCS) can enhance the efficacy of social skills training (SST) in the treatment of children and young adolescents with autism spectrum disorders (ASDs). We evaluated the efficacy, tolerability, and last effects of DCS given one hour prior to each of 10 weekly SST sessions for the treatment of social impairment in 68 children and young adolescents (ages 5-11 years) with ASDs during a randomized placebo-controlled trial. The safety and tolerability of DCS and durability of treatment response was also examined.

Objective: Researchers have demonstrated that D-Cycloserine (DCS) can enhance the effects of behavioral interventions in adults with anxiety and enhance prosocial behavior in animal models of Autism Spectrum Disorders (ASD). This study extended upon this background by combining DCS with behavioral social skills therapy in youth with ASD to assess its impact on the core social deficits of ASD. We hypothesized that DCS used in combination with social skills training would enhance the acquisition of social skills in children with ASD.
Method: A 10-week, double-blind, placebo-controlled trial of DCS (50 mg) given 30 minutes prior to weekly group social skills training was conducted at two sites. Children with ASD were randomized to receive 10 weeks (10 doses) of DCS or placebo in a 1:1 ratio.
Results: No statistically significant difference attributable to drug treatment was observed in the change scores for the primary outcome measure, the Social Responsiveness Scale (SRS), total score (p=0.45) or on secondary outcome measures. At week-22 follow-up, analysis of the SRS total raw score using a robust ANCOVA demonstrated a significant

maintenance of treatment effect in the DCS group when compared to the placebo group. The least squares (adjusted) means for the two groups shows that the SRS total for the placebo group was 5.9 points (SE=2.9) greater than that for the DCS group (95% CI=0.1–11.7, p=0.048).

Conclusions: The results of this trial demonstrated no drug-related short-term improvement on the primary outcome measure, or any of the secondary outcome measures. However, an overall significant improvement in SRS total raw score was observed from baseline to end of treatment for the entire group of children with ASD. The findings of long-term follow-up data suggest that DCS may help youth with ASD maintain skills gained during social skills training. Additional larger-scale study with longer follow-up will be necessary to further understand the long-term impact of short-term DCS dosing paired with structured skills training. This suggests a need to further study the efficacy of the social skills training protocol.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPONE NUMBER** (*Include area code*)
U	U	U	UU	48	

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1. **INTRODUCTION:** The main objective of this study was to determine whether D-Cycloserine (DCS) can enhance the efficacy of social skills training (SST) in the treatment of children and young adolescents with autism spectrum disorders (ASDs). We evaluated the efficacy, tolerability, and last effects of DCS given one hour prior to each of 10 weekly SST sessions for the treatment of social impairment in 68 children and young adolescents (ages 5-11 years) with ASDs during a randomized placebo-controlled trial. The safety and tolerability of DCS and durability of treatment response was also examined.

2. **KEYWORDS:** Autism spectrum disorder, social skills training, d-cycloserine

OVERALL PROJECT SUMMARY: This study has been successfully completed and all work outlined in the SOW has been accomplished. The long-range goal of this research was to identify better treatments for the core social and communication impairment of autistic disorder (autism) and other pervasive developmental disorders (PDDs). The main objective of this application was to determine whether D-Cycloserine (DCS) can enhance the efficacy of social skills training (SST) in the treatment of children and young adolescents with autism and other pervasive developmental disorders (ASDs). The central hypothesis of this project was that DCS will enhance learning of social skills over the course of 10 weeks of SST. This study of DCS combined with SST was novel in that it is an approach to studying a promising drug that may enhance learning of social skills. We evaluated the efficacy of DCS given 30 minutes prior to each of 10 weekly SST sessions for the treatment of social impairment in 68 children and young adolescents (ages 5-11 years) with ASDs during a randomized placebo-controlled trial. Fifty-two youth with ASDs were recruited at Indiana University School of Medicine/Riley Hospital for Children and 16 youth with PDDs were recruited at the Cincinnati Children's Hospital Medical Center site. Thirty-four typically developing peers were also recruited to participate as neurotypical peer models, but did not take the study medication. Subjects with ASDs were randomized to either DCS or placebo in 1:1 ratio after they were determined to meet all eligibility criteria through screening. Each SST group had two subjects on placebo and two subjects on DCS, in addition to the two neurotypical peers.

Extensive baseline characterization of participants consisted of a thorough developmental, medical, and psychiatric history, as well as a complete physical and mental status exam. This included a detailed history of previous psychotropic drug treatments. Information regarding previous psychosocial treatments including social skills training, ABA or behavioral therapy, and speech therapy were collected at screening. These variables were used to assess between groups prior to statistical analysis to determine their potential for acting as covariates between groups. DCS or placebo were administered 30 minutes prior to each of the ten SST sessions. The timing of this dose was based on preliminary evidence that dosing just prior to learning leads to a greater response. The dose of DCS was 50 mg, which approximated the range of 1-3 mg/kg for all participants depending on their weight. All participants then completed 10 2-hour social skills training groups following a manualized curriculum developed for the study.

Over the course of the funding period, we were able to successfully enroll the projected 68 participants with ASDs. Several changes to the protocol and personnel occurred over the course of the study. Below is a summary of protocol modifications that occurred:

Amendments #	Approved	Changes
3	8/10/2010	Allows the use of up to 2 concomitant medications if on the same drug for at least 8 weeks prior to randomization. Includes the use of the ADOS. Change of PI to Erickson.
5	9/14/2010	Changed the Exclusion Criteria. Protocol now allows for enrollment of subjects with seizures, if seizure free for 6 months and is on an anti-convulsant for at least 4 weeks.
7	4/1/2011	Add eye-tracking and a Parent Survey
8	4/19/2011	Radio Ads
A002	11/10/2011	TSSA Cutoff Score 70%. Clarification of length of stable dose for concomitant medication prior to randomization. Eye-tracking clerical error corrected (Week 11)
A005	5/21/2012	Change PI from Erickson to Minshawi
A008	9/18/2012	Added second study site at Cincinnati, increasing overall study n to 106. Added characterization of typical peers. SRS completed at screen for subjects with PDD. Added repetition of VABS if screen and baseline occur more than 30 days apart. Added Plawecki as key personnel. Clarified when CGI-I and CGI-S are administered.
A010	4/29/2013	Protocol amended to include vital signs taken at all study visits (not just screen) and to remove statement that study coordinator will call family monthly between week 11 and 22 visits
A013	7/9/2013	Consent and assent amended to say that the drug does not have to be swallowed whole; the capsules can be opened and taken with food or drink.

Demographics:

Thirty-four participants with ASD were randomized to the DCS treatment group and 33 with ASD were randomized to the placebo group. One subject who was randomized to the placebo group dropped out of the study before taking any medication and subsequently was excluded from analysis. There were no statistically significant difference in age, sex, SB-V scores, the VABS-II Expressive Language subscale, the CGI-S, concomitant medications, or concomitant therapy treatments at baseline (Appendix A, Table 1). Therefore, no potential confounders were adjusted for as covariates in all subsequent analyses. Furthermore, no significant differences were noted between the two sites (Cincinnati and Indiana University) on demographic variables (Appendix A, Table 2).

Primary Outcome Analyses:

No statistically significant difference attributable to drug treatment was observed in the change scores for the SRS total score (p=0.45). Additionally, no significant differences were identified between groups in the change scores for the secondary outcome measures were identified (Appendix A, Table 3). In addition, teacher-rated ABC data was returned for 23.5% of the DCS group and 30.3% of the placebo group with no significant difference noted for any of the ABC subscales (Irritability p=0.623, Social Withdrawal p=0.845, Stereotypy p=0.434, Hyperactivity p=0.833, and Inappropriate Speech p=0.959) between groups . Teacher-rated SRS data was available for 26.4% of the DCS group and 27.2% of the placebo group, and again no significant difference was found between groups (p=0.59).

In addition to the primary endpoint of the study at Week 11, the SRS total score was also measured at Week 6. A linear mixed effects model was fitted to further test the treatment effect over time using data at all three visits. Again, there was no significant difference between the two treatment groups ($p = 0.502$). The repeated measures of SRS total scores are depicted in Appendix A, Figure 1.

Secondary Responder Analysis:
A responder analysis was conducted based on CGI-I scores at 11-week follow-up. For the responder analysis, 33.3% of participants in the DCS group were classified as responders to treatment based on the CGI-I, as compared to 32.3% in the placebo group, which showed no significant difference in rate of response between groups ($p = 0.927$). Based on the observed trend of improvement in both treatment groups, subjects were combined to assess whether SRS total score changed significantly from baseline to week 11. A paired t-test for all 67 subjects with ASD showed a mean change score of -15.14 with 95% confidence interval (-19.90, -10.38), $p < 0.0001$.

Week-22 Follow-Up Analysis:
Week-22 analysis of the SRS total score using a robust ANCOVA identified five outliers, 3 in the placebo group and 2 in the DCS group, who were removed from subsequent analyses. With the outliers removed, there was a significant maintenance of treatment effect noted in the DCS group in comparison to subjects treated with placebo, with lower ratings on the SRS total score at week-22 in the DCS group. The least squares (adjusted) means for the two groups shows that the SRS total for the placebo group was 5.9 points (SE=2.9) greater than that for the DCS group (95% CI=0.1–11.7, p=0.048). There was no statistically significant difference noted between groups on the social awareness, social communication, social motivation, and autistic mannerism subscales of the SRS (Table 2). The social cognition subscale appears to drive the difference in the SRS total score as this subscale was significantly different between groups (DCS mean score of 15.6 (SE=0.7), placebo mean score 18.4 (SE=0.7), p=0.002). On the SRS total score there was also a statistically significant age group effect (p=0.024) for the difference between the adjusted means. It is noteworthy that all of the outliers in the placebo group were in the younger age group and both of those in the DCS group were in the older age group. As a result, if these five outliers were retained then a statistically significant treatment group by age group interaction effect would be present, with the younger age group demonstrating greatest improvement with DCS treatment.

As a result of the statistically significant treatment group by SRS total score at week-11 interaction, the difference between the adjusted means for the two treatments depends on the SRS total score at week-11 (Appendix B, Figure 2). This is typical of ANCOVAs that show a significant treatment by baseline interaction term. In this analysis the SRS total score at week-11 is playing the role of baseline. So in this case, when the SRS total score at week-11 is 90 or below, then the DCS group has a statistically significantly smaller least squares mean than that of the placebo group (Appendix B, Table 3). If the SRS total score at week-11 is greater than 90 and less than 140, there are no statistically significant differences between the adjusted means. If the SRS total score is equal to 140, the placebo group has a slightly smaller adjusted mean than the DCS group (p=0.049). Therefore, it appears that subjects who responded to therapeutic intervention at week-11 were more likely to maintain treatment effects at week-22 if they had received DCS. Subjects who demonstrated little improvement at week-11, with high SRS values

at week-11, were unlikely to demonstrate improvement at week-22. DCS in this study may function to enhance durability of the social skills treatment effects, but has little impact if the treatment was not successful initially.

There was no statistically significant difference between treatment groups at week-22 on the ABC, CGI-I, CGI-S, and VABS-II secondary outcome measures (Appendix B, Table 2). The parent rated TSSA scores were significantly higher (p=0.014) in the DCS group at week-22, mimicking the improvement noted on the parent-rated SRS total raw scores.

Eye Tracking Analysis:

The pilot eye-tracking measure was completed in 38 subjects, 21 in the DCS and 17 in the placebo group. There was no statistically significant difference identified between these groups in age, gender, diagnostic subtype, or concomitant medications. Analysis for outliers was not completed for this subgroup. Results demonstrate that the placebo group had increased percent time looking at mouth and nose, but decreased percent time looking at face as a whole (p<0.0001) compared to the DCS treatment group. There was no difference in percent time spent viewing the eye region between groups. This data indicates that the DCS treated group was potentially more socially motivated to view the faces in the eye tracking paradigm at week-22 as a result of treatment with active drug.

Adverse Events Analysis:

Appendix A, Table 4 shows the number of subjects who reported an adverse event, as well as all categories of AEs where at least 10% of either group (DCS or placebo) reported experiencing that AE. Fisher's Exact tests were utilized to derive p values. No category of adverse event showed a statistically significant difference between groups. The DCS group experienced more emesis than the placebo group (17.6% vs 6.1%, p=0.26). Overall, more patients in the DCS group reported at least one adverse event compared to the placebo group (94.2% vs. 84.8%) although this difference was not significant (p=0.21). The placebo group had a higher number of total adverse events (149 vs. 138) (p=0.87). Finally, only one serious adverse event (one instance of making a suicidal comment at school when angry) was reported in the placebo group.

3. **KEY RESEARCH ACCOMPLISHMENTS:**
   - Posters presented at three major international professional conferences
   - The primary outcome manuscript was written and submitted to the journal Molecular Autism in February 2015.
   - Long-term follow-up and eye tracking data were analyzed and results were submitted to the Journal of the Academy of Child and Adolescent Psychiatry in October 2015.

4. **CONCLUSION:** The results of this trial demonstrated no drug-related short-term improvement on the primary outcome measure, or any of the secondary outcome measures. However, an overall significant improvement in primary outcome measure, Social Responsiveness Scale total raw score, was observed from baseline to end of treatment for the entire group of children with PDD. The week-22 data demonstrating significantly enhanced durability of treatment effect in those subjects who received weekly DCS is remarkable in light of multiple therapeutic intervention trials demonstrating limited durability of treatment. In this analysis, DCS appears to enhance

durability of the social skills gains made during short-term group therapy resulting in parent-reported increased social cognition and objectively measured improved focus on facial viewing in our pilot eye tracking paradigm. This suggests a need to further study the efficacy of the social skills training protocol. There are several possible explanations for the lack of pharmacological treatment effect in this study. When examining the characterization of the sample, the majority of subjects enrolled in this study were diagnosed with PDD-NOS and Asperger's Disorder. These diagnoses indicate an overall milder symptom presentation, which may have potentially introduced a ceiling effect whereby there was less room for improvement with the treatment. Another potential reason for lack of drug effect in the current study is that social interactions, and therefore social deficits, are difficult behaviors to objectively quantify due to the ways in which social behavior changes in different settings and circumstances and over time. The future goal is to examine the efficacy of the social skills training curriculum compared to a control condition in order to assess the value of the social skills training as a stand-alone treatment.

5. **PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:**

   a. List all manuscripts submitted for publication during the period covered by this report resulting from this project. Include those in the categories of lay press, peer-reviewed scientific journals, invited articles, and abstracts. Each entry shall include the author(s), article title, journal name, book title, editors(s), publisher, volume number, page number(s), date, DOI, PMID, and/or ISBN.

      (1) Lay Press: Nothing to report
      (2) Peer-Reviewed Scientific Journals:

      Minshawi, N. F., Wink, L., Shaffer, R., Plawecki, M., Posey, D., McDougle, C.J., Swiezy, N., & Erickson, C. (under review). A randomized, placebo-controlled trial of D-Cycloserine for the enhancement of social skills training in Autism Spectrum Disorders. *Molecular Autism.*

      Wink, L., Minshawi, N. F., Shaffer, R., Plawecki, M., Posey, D., McDougle, C.J., Swiezy, N., & Erickson, C. (under review). D-Cycloserine for Enhanced Durability of Social Skills Training in Autism Spectrum Disorders: Week-22 Follow-up after 10 Week Clinical Trial. *Journal of Child and Adolescent Psychiatry.*

      (3) Invited Articles: Nothing to report
      (4) Abstracts: Nothing to report

   b. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

      *Wink, L.K., Minshawi, N.F., Shaffer, R., Hurwitz, S., Plawecki, M., McDougle, C.J., & Erickson, C. (May, 2014). A randomized, placebo-

controlled trial of D-Cycloserine for the enhancement of social skills training in Autism Spectrum Disorders: Initial Results. Presented at the International Meeting for Autism Research (IMFAR), Atlanta, Georgia.

6. **INVENTIONS, PATENTS AND LICENSES:** Nothing to report

7. **REPORTABLE OUTCOMES:** Nothing to report

8. **OTHER ACHIEVEMENTS:** Nothing to report

   For each section, 4 through 9, if there is no reportable outcome, state "Nothing to report."

9. **REFERENCES:** List all references pertinent to the report using a standard journal format (i.e., format used in *Science*, *Military Medicine*, etc.).

Minshawi, N. F., Wink, L., Shaffer, R., Plawecki, M., Posey, D., McDougle, C.J., Swiezy, N., & Erickson, C. (under review). A randomized, placebo-controlled trial of D-Cycloserine for the enhancement of social skills training in Autism Spectrum Disorders. *Molecular Autism.*

Wink, L., Minshawi, N. F., Shaffer, R., Plawecki, M., Posey, D., McDougle, C.J., Swiezy, N., & Erickson, C. (under review). D-Cycloserine for Enhanced Durability of Social Skills Training in Autism Spectrum Disorders: Week-22 Follow-up after 10 Week Clinical Trial. *Journal of Child and Adolescent Psychiatry.*

Wink, L.K., Shaffer, R., Hurwitz, S., Pozdol, S., Minshawi, N. F., Swiezy, N. B., Erickson, C. A., Stigler, K. A., Posey, D. J., & McDougle, C. J. (October, 2011). Initial Experiences in a Randomized, Placebo-Controlled Trial of D-Cycloserine for the Enhancement of Social Skills Training in Pervasive Developmental Disorders. Presented at the American Association for Child and Adolescent Psychiatry Annual Conference, Toronto, Canada.

Shaffer, R., Wink, L.K., Minshawi, N. F., Swiezy, N. B., Hurwitz, S., Pozdol, S., & Erickson, C. A. (May, 2012). Development of a novel social skills training curriculum. Presented at the International Meeting for Autism Research (IMFAR), Toronto, Canada.

Shaffer, R., Minshawi, N. F., Swiezy, N., Hurwitz, S., Pozdol, S., Wink. L., Erickson, C. (November, 2012). Behavioral data collection in a social skills training group. Presented at the Association for Behavioral and Cognitive Therapies (ABCT) Annual Meeting, National Harbor, Maryland.

Wink, L.K., Minshawi, N.F., Shaffer, R., Hurwitz, S., Plawecki, M., McDougle, C.J., & Erickson, C. (May, 2014). A randomized, placebo-controlled trial of D-Cycloserine for the enhancement of social skills training in Autism Spectrum Disorders: Initial Results. Presented at the International Meeting for Autism Research (IMFAR), Atlanta, Georgia.

10. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of

manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

    a. Appendix A: Manuscript of primary outcomes (under review with the journal Molecular Autism) is attached. We have submitted revisions per the editor's request and are awaiting final confirmation on the paper's status at this time.

    b. Appendix B: Manuscript of long-term outcomes (submitted to the Journal of the Academy of Child and Adolescent Psychiatry in October 2015) is attached. We are still awaiting feedback from the journal editors on the status of this paper.

A Randomized, Placebo-Controlled Trial of D-Cycloserine for the Enhancement of Social Skills Training in Autism Spectrum Disorders

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Words in Abstract: 234, Words in Text: 6059, Number of Tables: 4, Number of Figures: 1,
Supplementary Materials: 0

Abstract

Background: Researchers have demonstrated that D-cylcoserine (DCS) can enhance the effects of behavioral interventions in adults with anxiety and enhance prosocial behavior in animal models of Autism Spectrum Disorders (ASD). This study extended upon this background by combining DCS with behavioral social skills therapy in youth with ASD to assess its impact on the core social deficits of ASD. We hypothesized that DCS used in combination with social skills training would enhance the acquisition of social skills in children with ASD.

Methods: A 10-week, double-blind, placebo-controlled trial of DCS (50 mg) given 30 minutes prior to weekly group social skills training was conducted at two sites. Children with ASD were randomized to receive 10 weeks (10 doses) of DCS or placebo in a 1:1 ratio.

Results: No statistically significant difference attributable to drug treatment was observed in the change scores for the primary outcome measure, the Social Responsiveness Scale (SRS), total score (p=0.45) or on secondary outcome measures.

Conclusion: The results of this trial demonstrated no drug-related short-term improvement on the primary outcome measure, or any of the secondary outcome measures. However, an overall significant improvement in SRS total raw score was observed from baseline to end of treatment for the entire group of children with ASD. This suggests a need to further study the efficacy of the social skills training protocol. Limitations to the current study and areas for future research are discussed.

Clinicaltrials.gov   NCT01086475. Registered 10 March 2010

Keywords: D-cycloserine, Autism Spectrum Disorders, social skills training, social deficits

Introduction

Autism Spectrum Disorders (ASD), including Autistic Disorder, Asperger's Disorder and Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS), have received increasing attention from researchers, clinicians, and the public since autism was first described by Leo Kanner in 1943[1]. The diagnosis of ASD is characterized by core social and communication deficits, as well as restricted, repetitive behaviors. In recent years, the rates of ASD have escalated, with the most recent Centers for Disease Control and Prevention data estimating prevalence at 1 in 68 children in the United States[2]. While some successful pharmacological and behavioral interventions have been identified for the treatment of hyperactivity/inattention and irritability associated with ASD[3, 4], little progress has been made in the effective treatment of primary social and communication deficits. The limited success of clinical trials targeting core social impairment in ASD is likely in part due to the heterogeneity of ASD, difficulty quantitatively tracking treatment response, and high placebo response rates[5]. Regardless, the lack of viable treatments is particularly concerning given that pervasive social impairment in ASD can limit lifelong functioning and independence[6].

Research in psychiatric disorders has led to some advances in ASD research. Specifically, a parallel is frequently drawn between schizophrenia and ASD due to similarity between the negative symptoms of schizophrenia and social withdrawal seen in ASD, as well as the implication of glutamate dysregulation in both disorders[7]. Consequently, several targeted treatment trials in both ASD and schizophrenia have focused on modulating glutamate neurotransmission[5, 8]. D-cycloserine (DCS), a partial agonist of the *N*-methyl-D-aspartate (NMDA) glutamate receptor and a Food and Drug Administration-approved treatment for tuberculosis, has been researched for treatment of negative symptoms of schizophrenia with mixed results[9, 8, 10-12]. In ASD, a single-blind pilot study of DCS in children and adults (mean age of 10 years) found that DCS was associated with a clinically significant reduction in social withdrawal and increase in social responsiveness compared to a placebo control[13]. However, a double-blind, placebo-controlled trial of daily dosing of DCS in 88 children with ASD found no significant difference in measures of social withdrawal or global severity ratings during 8 weeks of daily treatment[14].

Glutamatergic neurotransmission has also been of interest in the treatment of anxiety disorders[15, 16]. A growing body of preclinical and clinical research has demonstrated the ability of DCS to enhance learning in the treatment of anxiety symptoms[17]. The mechanism believed to be responsible for this effect is the enhancement of

learned extinction of fear responses via combination Cognitive Behavior Therapy (CBT) and DCS treatment[17, 18]. Results have shown that DCS plays an augmentative role in the learning that takes place during CBT and therefore leads to greater success than when CBT is used alone.

The promising results from anxiety studies focused on extinction-based learning, as well as the role of DCS in other forms of learning, have subsequently led to the investigation of the role of DCS in the enhancement of social learning in animal models of ASD. Modi and Young [19] demonstrated that DCS combined with social learning paradigms in mice increased prosocial bonding and partner selection. This model of social learning may be similar to the social learning that takes place during social interactions and behavioral skills training in individuals with ASD. However, no studies of combined DCS plus non-drug therapy have been published in children with ASD. Based on this background, we investigated DCS treatment in combination with behavior therapy in youth with ASD. We hypothesized that DCS used in combination with social skills training utilizing the technology of Applied Behavior Analysis (ABA), the most empirically supported behavioral intervention for ASD[20], would enhance the acquisition of social skills in children with ASD. The social skills training curriculum involved a combination of didactic instruction in the form of social stories[21, 22], discussions, discrimination training tasks, as well as performance and feedback-based instruction, such as role playing and modeling of skills. Typically developing peers were also incorporated as models in all groups. Social skills training that includes the use of typically developing peers as models and agents of intervention have been shown to increase the social interactions of children with ASD[23, 24]. The social skills group curriculum utilized in this study was previously investigated via a pilot study with eight children with ASD and four typically developing peers. This pilot study used the *Triad Social Skills Assessment* (TSSA)[25] as the primary outcome measure. Children who participated in the social skills training demonstrated a significant improvement in overall social skill ability on the TSSA at post-test (p < 0.05). The children with ASD showed significant improvement in understanding emotions, initiating interactions, responding to interactions, general social competency[26]. We additionally hypothesized that children treated with DCS would show greater improvement in social functioning from social skills training than those taking placebo.

<center>Method</center>

Study Design

A 10-week, double-blind, placebo-controlled trial of low dose (50 mg) DCS given 30 minutes prior to weekly group social skills training was conducted at two sites, Indiana University School of Medicine and

Cincinnati Children's Hospital Medical Center. Children with ASD were randomized to receive 10 weeks (10 doses) of DCS or placebo in a 1:1 ratio. All children received 10 weeks of manualized social skills training. Children were further divided into two age groups, 5-7 years and 8-11 years, for the purposes of keeping social skills groups more homogeneous. Each social skills group included up to four children with ASD and two typically-developing peer models (TPs) in the same age group. The TPs participated in all group activities but did not take DCS or placebo. Adverse Events (AEs) and interval history were collected prior to dosing and outcome measures were administered at baseline, week 6, and week 11. This trial was approved by the Institutional Review Board at each site.

Participants

Sixty-seven children with ASD ages 5-11 years participated in the study along with 34 typically-developing, same-aged children who served as TPs. One subject with ASD was excluded from analyses due to early dropout prior to taking the study drug. Participants were recruited from academic autism treatment centers, local schools, and community organizations. Written informed consent was obtained from legal guardians and assent was obtained when participants were able. Diagnosis of ASD was made through administration of the *Autism Diagnostic Observation Schedule[27, 28]*, *Autism Diagnostic Interview-Revised[29]*, and clinical interview using the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* (DSM-IV-TR)[30] criteria for Autistic Disorder, Asperger's Disorder, or Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS).

Subjects with ASD were required to have an Intellectual Quotient greater than 70 on the *Stanford-Binet 5th Edition[31]* (SB-V) and a communication standard score greater than 70 on the *Vineland Adaptive Behavior Scale 2nd Edition* (VABS-II)[32] survey edition. These criteria were included to ensure that participants did not have cognitive or language deficits that could interfere with their ability to participate in group social skills training. Additional inclusion criteria included a *Triad Social Skills Assessment* (TSSA)[25] score of 70% or less on both parent questionnaire and child assessment, significant social impairment as measured by a T-score of 60 or greater on the *Social Responsiveness Scale* (SRS)[33] and Clinical Global Impression Severity (CGI-S) scale score of at least four ("Moderately Ill"). The CGI-S is a clinician-rated global assessment of symptom severity. The CGI-S item is rated on a scale from 1 to 7 (1 = normal, not at all ill; 2 = borderline ill; 3 = mildly ill; 4 = moderately ill; 5 = markedly ill; 6 = severely ill; 7 = among the most extremely ill patients). Rater training was conducted with gold standard vignettes and inter-rater reliability of 80% or greater was established.

Study participants were required to remain on stable psychotropic medication dosing targeting symptoms associated with ASD (e.g., insomnia, inattention, hyperactivity, anxiety, irritability) for a minimum of two weeks (with the exception of four weeks for fluoxetine) prior to randomization. Potential participants were excluded if they were taking more than two psychotropic medications or if they were currently taking a glutamatergic modulator (e.g., riluzole, memantine, acamprosate, topiramate, amantadine). In addition, concomitant psychosocial treatments could not include group social skills training outside of the study and all therapies were required to have been stable for at least 90 days prior to randomization.

The TPs were screened with the *Child Symptom Inventory-4*[34] to ensure that they did not have a history of psychiatric symptoms that were currently affecting social skills (e.g., attention-deficit/hyperactivity disorder, oppositional defiant disorder, schizophrenia, ASD, social anxiety disorder, and major depression). The child's appropriateness for inclusion in the social skills groups (e.g., absence of social, behavioral, or language problems) was also assessed by a trained clinician. Parents of TPs provided informed consent and TPs provided assent.

Social Skills Training

Social skills groups were conducted following a manualized curriculum adapted for use in the present study. The curriculum utilized ABA-based methodologies, including shaping, incidental teaching, positive reinforcement, and visual schedules, as well as social stories and weekly parent-mediated homework assignments. Sessions focused on a specific social skill topic each week: greetings, emotions, conversations, review, and saying good-bye. The curriculum included a variety of techniques that have been shown to help children with ASD learn social skills. A sample curriculum is provided in Table 1. Specific techniques included sorting examples of appropriate and inappropriate behaviors, reading social stories, engaging in role-plays, labeling flash cards, engaging in art activities, and playing various games. Minor modifications were made to curriculum based on the age group (5-7 or 8-11 year olds) to enhance understanding and developmental appropriateness. Social groups were facilitated by masters or doctoral-level clinicians with expertise in ASD and ABA. Therapists were trained in the curriculum by a lead therapist (the study's principle investigator) and significant therapist overlap occurred both within and across study sites to address consistency in therapist techniques. However, treatment fidelity data was not collected.

The TPs assisted in modeling and reinforcing appropriate behavior during each group session. The TPs were recruited from local school districts and via media advertisements. Prior to the start of social skills training, TPs were educated in a separate session. An introduction to behaviors associated with ASD was presented, along

with an overview of the social skills curriculum and weekly schedule. In addition, TPs engaged in role play with the clinicians to practice appropriate skills and corrective feedback was provided. A social story on ASD was also provided for the TPs to review at home with their parents prior to the first social skills group.

Primary Outcome

The primary outcome measure of the ASD phenotype and social interactions in participants with ASD was the parent-rated SRS total raw score. The SRS is a standardized, 65-item measure of the core symptoms of ASD where each item is scored on a 4-point scale, which has been used extensively in ASD research[35-38]. The SRS was administered at screen, baseline, week 6 (after 5 weeks of SST), and at week 11 (after 10 weeks of social skills training).

Secondary Outcomes

Several secondary outcome measures were included to capture different aspects of ASD that could be affected by the proposed treatment. When available, SRS data was collected from teachers of the subjects with ASD at baseline, week 6, and week 11. Additionally, all participants were evaluated using the VABS-II, Aberrant Behavior Checklist (ABC)[39], Clinical Global Impression Improvement Scale (CGI-I), and the TSSA, at baseline and week 11.

The adaptive functioning of subjects was evaluated at baseline and week 11 using the VABS-II. The VABS-II assesses adaptive functioning in four domains: Communication, Daily Living Skills, Socialization, and Motor Skills. Administered via semi-structured interview with parents or caregiver, the VABS-II provides a measure of overall functioning of children and adults. The VABS-II is a standardized, norm-referenced assessment that is used extensively in individuals with ASD[40, 41].

The ABC was collected at baseline, week 6 and week 11 to assess the impact of the treatment on symptoms relevant to ASD. The ABC is a 58-item parent questionnaire with five subscales derived by factor analysis: Irritability, Social Withdrawal, Stereotypy, Hyperactivity, and Inappropriate Speech. The ABC has been extensively used in psychopharmacological studies of ASD [42]. When available, teachers of the subjects with ASD were also asked to complete the ABC at the same time points.

The CGI-I was utilized as a clinician-rated dichotomous outcome measure to assess response to treatment. A trained clinician blind to treatment assignment rated the CGI-S at baseline and the CGI-I at each visit following randomization. Factors included in rating the CGI-I included parent report, parent-rated measures, teacher-rated

measures, and clinician-rated measures. The CGI-I provides a qualitative measure of treatment response through a rating from 1 to 7 (1 = very much improved; 2 = much improved; 3 = minimally improved; 4 = no change; 5 = minimally worse; 6 = much worse; 7 = very much worse). Rater training was conducted with gold standard vignettes and inter-rater reliability of 80% or greater was established. At the end of treatment subjects with a CGI-I of "1" or "2" were categorized as responding to the treatment and subjects with CGI-I scores of "3" or higher were categorized as nonresponders.

To assess the impact of the treatment on social skills and social knowledge, the TSSA was administered to the subjects and their parents at baseline and week 11. The TSSA is a criterion-based assessment that addresses three components of social knowledge and skills: cognitive (ability to problem-solve interpersonal conflicts), behavioral (ability to initiate and maintain interactions and respond appropriately to others), and affective (ability to understand emotions). The TSSA has been used as a supplemental descriptive measure of social skills[43], as well as in treatment planning[44]. Other outcome measures collected included eye tracking data and direct behavioral observations (not reported in the present paper).

Finally, monitoring for AEs was completed at each visit for subjects with ASD. The site physician kept a log of AEs that included the date of onset, date of resolution, severity, and relationship to study intervention (e.g., definite, probable, possible, remote, or none).

Parental satisfaction with the study and social skills training group was measured via questionnaire at the end of the study (week 11). Parents were asked to answer the question, "Participating in this group was worthwhile for my family", on a five-point Likert scale with "1" being anchored to "strongly disagree" and "5" being "strongly agree".

Statistical Analysis

Study participants' demographic and clinical characteristics were summarized and compared between the DCS and placebo groups at baseline using two-sample t-tests for continuous variables and Fisher's exact tests for categorical variables. The change scores of the primary outcome variables (SRS total score and subscales) from baseline to 11-week follow-up were also compared between the two treatment groups using t-tests. Similar analyses were conducted for the secondary outcomes including VABS-II total score and subscales, ABC subscales and TSSA parent report. In addition, a linear mixed effects modeling was used to further test the treatment effect over time using longitudinal SRS total scores measured at baseline, 6-week and 11-week visits. Responder analysis

(responders were defined as "much improved" or "very much improved" for CGI-I at 11-week follow-up) was conducted using Chi-square test. AEs during the treatment period were also analyzed. All analyses were performed using SAS version 9.2.

Results

Thirty-four participants were randomized to the DCS treatment group and 33 were randomized to the placebo group. One subject who was randomized to the placebo group dropped out of the study before taking any medication and subsequently was excluded from analysis. Comparisons between the two groups showed no statistically significant difference in age, sex, SB-V scores, the VABS-II Expressive Language subscale, the CGI-S, concomitant medications, or concomitant therapy treatments at baseline (Table 1). Therefore, no potential confounders were adjusted for as covariates in all subsequent analyses. Furthermore, no significant differences were noted between the two sites (Cincinnati and Indiana University) on demographic variables (Table 2).

Insert Table 2 here

Insert Table 3 here

No statistically significant difference attributable to drug treatment was observed in the change scores for the SRS total score (p=0.45). SRS subscale scores were evaluated as an exploratory analysis and were also not statistically significant. Additionally, no significant differences were identified between groups in the change scores for the secondary outcome measures were identified (Table 3). In addition, teacher-rated ABC data was returned for 23.5% of the DCS group and 30.3% of the placebo group with no significant difference noted for any of the ABC subscales (Irritability p=0.623, Social Withdrawal p=0.845, Stereotypy p=0.434, Hyperactivity p=0.833, and Inappropriate Speech p=0.959) between groups . Teacher-rated SRS data was available for 26.4% of the DCS group and 27.2% of the placebo group, and again no significant difference was found between groups (p=0.59). Finally, at Week 11 parental satisfaction was found to be high across both groups (96% and 88% for DCS and placebo groups, respectively rated satisfaction as a "4" or "5" on the five-point Likert scale). A Fisher's Exact Test demonstrated no significant difference in the level of parental satisfaction (p=0.33).

Insert Table 4 here

In addition to the primary endpoint of the study at Week 11, the SRS total score was also measured at Week 6. A linear mixed effects model was fitted to further test the treatment effect over time using data at all three visits. Again, there was no significant difference between the two treatment groups ($p = 0.502$). The repeated measures of SRS total scores are depicted in Figure 1.

Insert Figure 1 here

A responder analysis was conducted based on CGI-I scores at 11-week follow-up. For the responder analysis, 33.3% of participants in the DCS group were classified as responders to treatment based on the CGI-I, as compared to 32.3% in the placebo group, which showed no significant difference in rate of response between groups ($p = 0.927$). Based on the observed trend of improvement in both treatment groups, subjects were combined to assess whether SRS total score changed significantly from baseline to week 11. A paired t-test for all 67 subjects with ASD showed a mean change score of -15.14 with 95% confidence interval (-19.90, -10.38), $p < 0.0001$.

Adverse Events

Table 4 shows the number of subjects who reported an adverse event, as well as all categories of AEs where at least 10% of either group (DCS or placebo) reported experiencing that AE. Fisher's Exact tests were utilized to derive p values. No category of adverse event showed a statistically significant difference between groups. The DCS group experienced more emesis than the placebo group (17.6% vs 6.1%, p=0.26). Overall, more patients in the DCS group reported at least one adverse event compared to the placebo group (94.2% vs. 84.8%) although this difference was not significant (p=0.21). The placebo group had a higher number of total adverse events (149 vs. 138) (p=0.87). Finally, only one serious adverse event (one instance of making a suicidal comment at school when angry) was reported in the placebo group.

Insert Table 5 here

Discussion

The core social deficits seen in ASD are severely impairing and few interventions have been identified to successfully and consistently treat these impairments. Several promising studies have shown DCS to enhance

behavioral therapy outcomes in individuals with anxiety disorders, as well as demonstrating potential benefits of DCS treatment in ASD. The present study extended these lines of study by evaluating DCS mediated enhancement of the learning of social skills in children with ASD. The results of this double-blind placebo-controlled short-term trial demonstrate no drug-related improvement on the primary outcome measure, or any of the secondary outcome measures. However, an overall significant improvement in SRS total raw score was observed from baseline to end of treatment for the entire group of children with ASD.

There are several possible explanations for the lack of pharmacological treatment effect in this study. Notably, the large increase in SRS scores observed in both the DCS and placebo groups is greater than that seen in other intervention studies[45]. This may be explained by the novelty of the social skills training group, something that had previously may have been unavailable to the participants. Therefore, demonstrating the added benefits of DCS to an already high placebo response rate may be very difficult. Also, the SRS was collected immediately following the 10-week trial, which does not allow for the assessment of the durability of these findings.

Another potential explanation for the lack of drug effect may be the heterogeneity of ASD, which makes this a particularly challenging population to study and all the more difficult to find effective pharmacological and behavioral interventions. It is also important to note that since study enrollment ended, the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (DSM-5[46]) has been published with revisions resulting in a new category of diagnosis called Autism Spectrum Disorder, along with the restructuring of diagnostic criteria. However, we do not believe these diagnostic changes would have influenced the results of the current study.

Another potential reason for lack of drug effect in the current study is that social interactions, and therefore social deficits, are difficult behaviors to objectively quantify due to the ways in which social behavior changes in different settings and circumstances and over time. This study utilized the parent-rated SRS total raw score to evaluate social deficits in ASD. The SRS provides a global perspective on social deficits in ASD. However, the learning occurring during social skills training may not produce effects sufficiently robust to alter these broad, subjective social skills ratings. In the future, a more direct, objective measurement of social behavior and social interest, such as eye tracking, may be required to capture change in social interaction which occurs at a level not readily observable by caregivers and clinicians.

Several additional factors should be considered in evaluating the findings of the current study. Based on the effective dose of DCS used in studies of DCS plus therapy for treatment of phobias and social anxiety, all

subjects in this trial received 50 mg of DCS regardless of weight[47, 48]. It is possible that higher doses (potentially weight-based) may have resulted in greater improvement for the DCS group. However, the phobia study by Ressler et al. (2004) demonstrated no difference between 50 mg and 500 mg doses of DCS so it is unclear what impact dosage adjustment may have provided[47]. Longer duration and more frequent treatment may also need to be considered. Ten weekly doses of DCS and social skills training may not be sufficient to make robust changes in symptoms of social impairment and extended length of treatment and/or daily dosing may be necessary. In addition, the psychotherapy studies referenced in the development of this protocol dealt with operant conditioning via learned extinction. The current study, utilized some operant conditioning techniques (such as reinforcement), but also used other learning mechanisms in the training of social skills (such as social learning through modeling and role playing). It is possible that DCS has its greatest influence over learned extinction and our negative results may reveal the limitation of our employed learning mechanism.

Finally, a limitation of this study is the novelty of the social skills training curriculum and lack of a control for the social skills training group. This curriculum was previously investigated in a pilot study, but the TSSA was used as the primary outcome measure, and not the SRS or CGI. In addition, treatment fidelity data was not collected and other standardized behavioral and social skills outcome measures, such as the Social Skills Improvement System[49], or other emotion recognition measures[24] were not utilized. All children enrolled in the study received 10 weeks of social skills training and statistically significant improvements were seen across the outcome measures when drug and placebo groups were combined. These results may point to the efficacy of this social skills training protocol at improving social outcomes for children with ASD. However, this potential mechanism cannot be confirmed without controlling for other factors that potentially influenced the results, such as maturation, time with trained clinicians, attention, and access to peers. A placebo or waitlist control group should be employed in future studies to evaluate the efficacy of our social skills curriculum.

The present study provides proof of concept that a large sample study combining medication and social skills training in ASD is feasible. Few studies have been conducted in ASD combining pharmacological and behavioral interventions, despite the common blending of these interventions in clinical settings. Future research on the role of targeted drug treatments in augmenting behavioral interventions in ASD is warranted. Despite the negative result of this short-term drug augmentation analysis, we believe further work focused on durability of treatment response is needed to assess long-term outcome following initial combination treatment in this and other

similar projects. Overall, utilizing targeted drug treatment to facilitate learning and acquisition of skills during

therapy in ASD warrants additional investigation. Lessons learned in our study of DCS as a potential augmentation

strategy to social skills training lays the groundwork for such work.

Abbreviations:
Autism Spectrum Disorders (ASD)
D-cylcoserine (DCS)
Social Responsiveness Scale (SRS)
Stanford-Binet 5th Edition (SB-V)
Vineland Adaptive Behavior Scale 2nd Edition (VABS-II)
Triad Social Skills Assessment (TSSA)
Clinical Global Impression Severity (CGI-S)
Typically-developing peer models (TPs)
Adverse Events (AEs)
Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS)
Applied Behavior Analysis (ABA)
Aberrant Behavior Checklist (ABC)[39]
Clinical Global Impression Improvement Scale (CGI-I)
Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)

Author Contributions

Noha Minshawi and Craig Erickson served as primary investigators for the study and participated in all aspects of the study and in preparation of the manuscript. Logan Wink, Rebecca Shaffer, Martin Plawecki, and Sarah Hurwitz participated in participant assessments, the implementation of the study protocol, and preparation of the manuscript. Hai Lui performed the statistical analyses and helped to revise the manuscript. David Posey, Naomi Sweizy, and Christopher McDougle participated in the design of the study and helped to revise the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Competing Interests

The authors declare no competing interests

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D-Cycloserine for Enhanced Durability of Social Skills Training in Autism Spectrum Disorders: Week-22 Follow-up after 10 Week Clinical Trial

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Word Count: 2,816 words

At a Glance:

• This report details week-22 follow-up analysis after a 10-week randomized controlled trial of D-cycloserine (DCS) plus social skills training for enhancement of social skills in youth with Autism Spectrum Disorder (ASD).

• The goal of this analysis is to assess whether DCS had a measureable impact on the durability of skills gained during short-term social skills treatment.

• At week-22 follow-up, analysis of the primary outcome measure Social Responsiveness Scale (SRS) total raw score using a robust ANCOVA demonstrated a significant maintenance of treatment effect in the DCS group when compared to the placebo group. The least squares (adjusted) means for the two groups shows that the SRS total for the placebo group was 5.9 points (SE=2.9) greater than that for the DCS group (95% CI=0.1–11.7, p=0.048).

• The findings of this study suggest that DCS may help youth with ASD maintain skills gained during social skills training.

Abstract

Importance: With the growing population of individuals diagnosed with autism spectrum disorder (ASD), there is critical need for effective and durable treatments targeting the core social impairment of the disorder.

Objective: To investigate whether D-cycloserine (DCS), a partial N-methyl-D-aspartate agonist, augments social skills training durability in youth with ASD.

Design: Analysis of week-22 data following a 10-week randomized, double-blind, placebo-controlled trial of DCS coupled with manualized social skills group intervention in youth with ASD.

Setting: This study was completed at 2 academic medical centers between August 4, 2009 and January 23, 2014.

Participants: Participants in the week-22 analysis included 60 outpatient youth ages 5-11 years with ASD, intellectual quotients above 70, and significantly impaired social functioning as measured by the Social Responsiveness Scale (SRS) and Triad Social Skills Assessment. Sixty-seven participants initially entered the active treatment phase, with one withdrawing prior to week-1, two withdrawing during active treatment, and 4 leaving the study between week-11 and week-22.

Intervention: Participants completed the 10-week active treatment phase during which they each received weekly single doses of 50 mg of DCS or placebo administered 30 minutes prior to weekly manualized group social skills training sessions. Following this treatment phase, assessments occurred at week-11 and week-22.

Main Outcome and Measures: The parent-rated SRS total raw score at week-22.

Results: At week-22 follow-up, analysis of the SRS total raw score using a robust ANCOVA demonstrated a significant maintenance of treatment effect in the DCS group when compared to the placebo group. The least squares (adjusted) means for the two groups shows that the SRS total for the placebo group was 5.9 points (SE=2.9) greater than that for the DCS group (95% CI=0.1–11.7, p=0.048).

Conclusions and relevance: The findings of this study suggest that DCS may help youth with ASD maintain skills gained during social skills training. Additional larger-scale study with longer follow-up will be necessary to further understand the long-term impact of short-term DCS dosing paired with structured skills training.

Trial Registration: clinicaltrails.gov Identifier: NCT01086475

Core social impairment is a defining feature and key predictor of long-term outcome in autism spectrum disorder (ASD)[1,2]. The growing body of pharmacologic and therapy-based research targeting ASD core impairments has been complicated by the heterogeneity of the disorder. Pharmacologic studies targeting core social impairment in ASD have focused on modulation of glutamate neurotransmission as altered glutamate functioning has been consistently implicated in ASD neuropathology[3]. Genetic variants in the N-methyl-D-aspartate (NMDA) receptor subunits have been identified in humans and murine models of ASD[4], and have been linked to repetitive behaviors and social impairment. In murine models, treatment with glutamatergic modulators has been associated with core impairment rescue[4-6]. Unfortunately, human studies of glutamatergic modulators in ASD have been less promising[7-13]. Non-pharmacologic therapy-based treatments, though widely believed to be an important aspect of ASD intervention, have also demonstrated mixed results[2,14]. Both early intervention pre-school based treatments employing Applied Behavioral Analysis (ABA) techniques and adolescent social skills groups focusing on teaching specific skills consistently demonstrate immediate improvements in social and communication skills in participants[14-16]. However, recent research points to limited durability of the improvements associated with these therapies[2,14,17-19].

One possible way to improve efficacy of treatment for core impairments in ASD is to enhance the durability of social skills intervention with pharmacotherapy. D-cycloserine (DCS) is a partial agonist at the glycine site of the NMDA receptor, is approved by the United States Food and Drug Administration for treatment of tuberculosis in children and adults, and has shown promise as an adjunct to therapeutic interventions in anxiety disorders and schizophrenia[20-31]. DCS coupled with therapeutic intervention appears to impact learning by acting on memory consolidation, particularly in novel learning paradigms[20,32]. Furthermore, studies indicate that dosing of DCS has a significant effect on treatment outcomes, with data suggesting that DCS may have peak effectiveness at moderate, infrequent doses[33].

DCS is of particular interest in ASD due to parallels between ASD and the negative symptoms of schizophrenia and significant symptomatic overlap with anxiety disorders[34]. To date, DCS studies in ASD have been monotherapy pharmacologic interventions and have not demonstrated consistent, convincing improvements in core features of ASD[35-37]. Modeled after the anxiety and schizophrenia literature, our group designed a randomized, double-blind, placebo-controlled trial of low-dose DCS given prior to weekly group social skills therapy targeting social impairment in youth with ASD, week-11 results published by Minshawi et al. (2015). At the conclusion of the active treatment phase of this study, participants in both the

DCS and placebo group demonstrated notable improvement in social functioning; but there was no statistically significant improvement attributable to drug treatment on the primary or secondary outcome measures. The objective of this 22-week follow-up analysis is to assess whether DCS had a measureable impact on the durability of skills gained during short-term social skills treatment.

Methods

Trial Design and Participants

This study evaluates week-22 follow-up data from a 10-week randomized, double-blind, placebo-controlled trial of DCS plus social skills group intervention in youth with ASD completed between August 4, 2009 and January 23, 2014 at Indiana University School of Medicine and Cincinnati Children's Hospital Medical Center. The study and follow-up analysis were approved by the institutional review board (IRB) at each participating site. Guardians of all participants provided written informed consent prior to study enrollment and, when possible, assent was obtained from enrolled youth.

Study participants were youth ages 5-11 years recruited from outpatient psychiatric clinics at both sites and via IRB approved advertising. Participants were diagnosed with autistic disorder, Asperger disorder, or pervasive developmental disorder not otherwise specified (PDD NOS) confirmed through administration of the Autism Diagnostic Observation Schedule (ADOS)[38], the Autism Diagnostic Interview-Revised (ADI-R)[39], and clinical assessment based on the Diagnostic and Statistical manual of Mental Disorders, Fourth Edition Text revision (DSM-IV-TR) diagnostic criteria[40]. Due to the demands of the social skills therapeutic intervention, subjects were required to have an Intellectual Quotient (IQ) above 70 as measured by the Stanford-Binet 5th Edition (SB-V)[41] and a Vineland Adaptive Behavior Scale 2nd Edition (VABS-II)[42] communication score above 70. At baseline, all subjects demonstrated significant social impairment with T-scores of 60 or greater on the Social Responsiveness Scale (SRS)[43] and scores of 70% or less on both the parent questionnaire and child assessment portions of the Triad Social Skills Assessment (TSSA)[44]. Included participants were also "moderately ill" based on clinician completion of the Clinical Global Impressions-Severity (CGI-S) scale[45]. Each group also enrolled two typically-developing peer models who were assessed to be free of psychiatric symptoms or developmental disability via the Child Symptom Inventory-4[46] and a medical and psychiatric interview completed by a study physician.

Concurrent psychotropic medications were permitted in study participants, though all subjects were required to remain on stable doses throughout the study. Participants were

excluded if treated with more than two psychotropic medications or known glutamatergic modulators. Participants were also required to have stable regimens of external psychosocial interventions throughout the study. Participants were excluded from the study if they were participating in concurrent social skills training programs.

For the 10-week active treatment phase of the study, subjects were sequentially randomized to either DCS or placebo in a 1:1 ratio by computer-generated randomization list accessible only by the investigational pharmacist. All participants with ASD received 10 weekly sessions of manualized social skills training following a single weekly dose of 50 mg DCS or placebo given 30 minutes prior to group therapy. Social skills intervention followed a manualized curriculum utilizing ABA techniques developed to teach skills including greetings, understanding emotions, creative play, and social conversations. Social skills groups were divided by age (5-7 years or 8-11 years), and minor modifications were made to group curriculum to accommodate the different age ranges. Groups were instructed by masters or doctorate level clinicians with specific expertise and experience working with youth with ASD.

Outcome Assessments and Statistical Analysis

Demographic data including age, gender, ASD diagnostic sub-type, IQ, level of adaptive functioning, concomitant medications and concomitant therapeutic interventions was collected for all subjects prior to randomization. The study primary outcome measure of social impairment, the parent-rated SRS total raw score, was measured at screen, baseline, week-6, week-11, and week-22. Secondary outcome measures including the Aberrant Behavior Checklist (ABC)[47], TSSA, VABS-II, Clinical Global Impression-Improvement (CGI-I) scale[45], and CGI-S were collected at baseline, week-11 and week-22. A pilot eye tracking paradigm of gaze preference employing a Tobii T120 Infrared Eye Tracker integrated with a 17-inch thin film transistor monitor controlled with Tobii Studio software (Version 3.0) was completed at week-11 and week-22 in a subset of participants beginning in year 3 of the project. Participants viewed 60 colored photographs of adult human faces from the NimStim Face Stimuli Set[48] and percent time looking at the eye, nose, mouth, or whole face regions was calculated. Monitoring for adverse events was completed by a study physician at all visits.

Demographic data, clinical characteristics, and reported adverse events/frequencies between DCS and placebo groups were compared using student's t-tests and Fisher's exact tests for continuous and categorical variables, respectively. Wilcoxon rank sum tests were completed to validate results of the Student's t-tests.

Analysis of the SRS total raw score was initiated via completion of a robust Analysis-of-Covariance (ANCOVA) in order to identify outliers. The outliers were removed and a traditional ANCOVA was then conducted in which the week-22 SRS total raw score was used as the response and the continuous covariate was the week-11 SRS total raw score. Other variables in the model were treatment group (DCS or placebo) and its interaction with the SRS total score at week-11, age group, gender, and ASD status (autistic disorder versus Asperger disorder and PDD NOS combined). The least squares means were derived for each treatment by week combination. (Least squares means were adjusted for the other variables in the model.) The contrast comparing the treatment changes between the two weeks of interest was derived; i.e. the difference between the placebo group change (week-22 minus week-11) and that of the DCS group (week-22 minus week-11) was determined. Secondary outcome measures including the ABC, TSSA, VABS-II, CGI-I, and CGI-S were evaluated using the same statistical method. (Note that the initial detection of outliers was conducted only once, on the SRS total raw score model, and those data were used for the analyses of the secondary outcomes.)

For the pilot eye-tracking data, a repeated measures ANCOVA model was conducted where the response was the percent fixation time at a particular area of interest. The continuous covariate was the baseline percent fixation time and the categorical independent variables were treatment group and week (11 or 22) as well as their interaction term. The "week" term was the repeated measure within subjects. Other covariates included the face identifier, expression, mouth (open or closed), age group, gender, and ASD status (autistic disorder versus Asperger disorder and PDD NOS combined). The least squares means were derived for each treatment by week combination. The contrast comparing the treatment change between the two weeks of interest was then derived.

Brief Review of Week-11 Outcomes

Please see paper by Minshawi et al. (2015) for full details. In brief, the 10-week active treatment phase of the study enrolled 68 children with ASD with no statistically significant treatment group differences in age, sex, cognitive functioning, adaptive skills, language skills, clinical severity of illness, concomitant medications, or concomitant therapy treatments (Figure 1). Four subjects withdrew between randomization and completion of active treatment. At week-11, both treatment groups demonstrated reduction in SRS total score, but no statistically significant difference attributable to DCS treatment on the primary or secondary outcome measures was identified. DCS was well tolerated, with irritability being the most frequently

reported adverse effect in both the DCS and placebo groups. There was no statistically significant difference in number of reported adverse events between groups (p=0.26).

Results

Of the 64 participants who completed the 10-week treatment phase, 1 subject moved from the area and 3 subjects were lost to follow-up prior to week-22. The week-22 completers had no statistically significant differences between groups in age, gender, diagnostic subtype, or concomitant medications (Table 1). Week-22 analysis of the SRS total score using a robust ANCOVA identified five outliers, 3 in the placebo group and 2 in the DCS group, who were removed from subsequent analyses. With the outliers removed, there was a significant maintenance of treatment effect noted in the DCS group in comparison to subjects treated with placebo, with lower ratings on the SRS total score at week-22 in the DCS group. The least squares (adjusted) means for the two groups shows that the SRS total for the placebo group was 5.9 points (SE=2.9) greater than that for the DCS group (95% CI=0.1–11.7, p=0.048). There was no statistically significant difference noted between groups on the social awareness, social communication, social motivation, and autistic mannerism subscales of the SRS (Table 2). The social cognition subscale appears to drive the difference in the SRS total score as this subscale was significantly different between groups (DCS mean score of 15.6 (SE=0.7), placebo mean score 18.4 (SE=0.7), p=0.002). On the SRS total score there was also a statistically significant age group effect (p=0.024) for the difference between the adjusted means. It is noteworthy that all of the outliers in the placebo group were in the younger age group and both of those in the DCS group were in the older age group. As a result, if these five outliers were retained then a statistically significant treatment group by age group interaction effect would be present, with the younger age group demonstrating greatest improvement with DCS treatment.

As a result of the statistically significant treatment group by SRS total score at week-11 interaction, the difference between the adjusted means for the two treatments depends on the SRS total score at week-11 (Figure 2). This is typical of ANCOVAs that show a significant treatment by baseline interaction term. In this analysis the SRS total score at week-11 is playing the role of baseline. So in this case, when the SRS total score at week-11 is 90 or below, then the DCS group has a statistically significantly smaller least squares mean than that of the placebo group (Table 3). If the SRS total score at week-11 is greater than 90 and less than 140, there are no statistically significant differences between the adjusted means. If the SRS total score is equal to 140, the placebo group has a slightly smaller adjusted mean than the DCS

group (p=0.049). Therefore, it appears that subjects who responded to therapeutic intervention at week-11 were more likely to maintain treatment effects at week-22 if they had received DCS. Subjects who demonstrated little improvement at week-11, with high SRS values at week-11, were unlikely to demonstrate improvement at week-22. DCS in this study may function to enhance durability of the social skills treatment effects, but has little impact if the treatment was not successful initially.

There was no statistically significant difference between treatment groups at week-22 on the ABC, CGI-I, CGI-S, and VABS-II secondary outcome measures (Table 2). The parent rated TSSA scores were significantly higher (p=0.014) in the DCS group at week-22, mimicking the improvement noted on the parent-rated SRS total raw scores.

The pilot eye-tracking measure was completed in 38 subjects, 21 in the DCS and 17 in the placebo group. There was no statistically significant difference identified between these groups in age, gender, diagnostic subtype, or concomitant medications. Analysis for outliers was not completed for this subgroup. Results demonstrate that the placebo group had increased percent time looking at mouth and nose, but decreased percent time looking at face as a whole (p<0.0001) compared to the DCS treatment group. There was no difference in percent time spent viewing the eye region between groups. This data indicates that the DCS treated group was potentially more socially motivated to view the faces in the eye tracking paradigm at week-22 as a result of treatment with active drug.

Discussion

This study was designed to evaluate the impact of manualized social skills training combined with the glutamatergic modulator DCS given at a low dose prior to each training group. The trial design is novel, and to our knowledge is the first of its kind to be completed in ASD research. The week-11 results demonstrating notable improvement of both treatment groups (though no DCS treatment effect) highlight the substantial immediate impact of social skills training in youth with ASD, which is consistent with other social skills treatment studies[2]. The presently detailed week-22 data demonstrating significantly enhanced durability of treatment effect in those subjects who received weekly DCS is remarkable in light of multiple therapeutic intervention trials demonstrating limited durability of treatment[2,19]. In this analysis, DCS appears to enhance durability of the social skills gains made during short-term group therapy resulting in parent-reported increased social cognition and objectively measured improved focus on facial viewing in our pilot eye tracking paradigm. Furthermore, DCS's long track record of safe use in children, limited noted adverse effects in this study, and

demonstrated impact with sparse dosing, suggests great potential for future treatment development.

There are several limitations of our trial design that must be considered when interpreting the results of this study. Primarily, we developed a novel manualized social skills training curriculum for this study. Although the curriculum is based on validated ABA techniques, the curriculum itself has not been validated outside of this study. Future studies working to validate our curriculum, as well as studies measuring the impact of DCS coupled with validated social skills group methods should be undertaken. Furthermore, this study did not employ a control group specifically for the social skill training, limiting inferences that can be made regarding the impact of the social skills therapy itself since factors such as natural maturation of subjects over the 22-week time course and frequent interaction with peers and clinicians cannot be controlled for in this study. Also our eye tracking paradigm was piloted only in a subgroup of participants. In future studies this objective measure should be employed for all participants at multiple time points throughout the study.

It is also interesting that if the outliers were included in ANCOVA analysis of the SRS total raw score, there was a notable age group effect indicating that the younger group (age 5-7) appeared to benefit more from DCS treatment. We felt it was appropriate to remove these outliers from our analysis, with the goal of achieving the most accurate interpretation. However, it will be interesting to assess the impact of DCS plus therapeutic intervention in this younger age group through completion of larger studies.

Conclusions

Despite initial report that DCS did not augment the immediate impact of social skills intervention in this group of youth with ASD, DCS did show significant impact on durability of skills gained during social skill therapy 11 weeks after treatment cessation. This is of importance considering the body of literature suggesting limited durability of therapeutic interventions targeting core features of ASD, and suggests need for further investigation.

Acknowledgements

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Table 1. Demographic Characteristics of Week-22 Completers

Characteristics	DCS (n=32)	Placebo (n=28)	p-value
Age (years), mean (SD)	8.47 (1.83)	8.07 (1.53)	0.37
Sex, n (%) male	26 (81.35)	23 (82.14)	1.00
Diagnosis, n (%) autism	12 (37.50)	12 (52.86)	0.79
Concomitant Medications, n (%)			
Antipsychotics	6	4	0.74
Alpha-2 Agonists	6	6	1.00
Stimulants	11	7	0.57
Antidepressants	3	3	1.00
Strattera	1	1	1.00
Any Concomitant Medication	19	17	1.00
Mean Total Concomitant Medications	0.84	0.75	0.64

Table 2. Week-22 Secondary Outcome Measures^

Outcome Measures	DCS*		Placebo		Difference (DCS-P*)			
	Mean	SE*	Mean	SE	Mean	SE	t value	p value
SRS* Total Raw Score	83.5	2.3	89.2	2.5	-5.9	2.9	-2.0	**0.048**
SRS Social Awareness	11.6	0.5	12.0	0.5	-0.4	0.6	-0.7	0.510
SRS Social Cognition	15.6	0.7	18.4	0.7	-2.8	0.8	.3.3	**0.002**
SRS Social Communication	28.0	1.1	29.7	1.2	-1.7	1.4	-1.3	0.207
SRS Social Motivation	11.8	0.8	12.6	0.8	-0.8	0.9	-0.9	0.395
SRS Autism Mannerisms	15.2	0.8	16.1	0.9	-0.8	1.0	-0.8	0.430
ABC* Irritability	9.8	1.3	8.6	1.3	1.1	1.6	0.7	0.482
ABC Lethargy	7.7	1.0	7.9	1.1	-0.2	1.2	-0.1	0.895
ABC Stereotypy	4.3	0.6	4.0	0.6	0.3	0.7	0.5	0.657
ABC Hyperactivity	`4.9	1.6	17.3	1.7	-2.4	2.0	-1.2	0.230
ABC Speech	3.5	0.4	3.2	0.4	0.3	0.5	0.6	0.522
VABS-II* Adaptive Behavior Composite	85.7	2.9	88.1	3.1	-2.4	3.6	-0.7	0.512
TSSA* Parent Rating	60.3	1.7	54.9	1.9	5.4	2.1	2.6	0.014
TSSA Child Assessment	61.7	3.4	56.5	3.4	5.2	4.1	1.3	0.216
CGI-I*	2.9	0.2	2.9	0.2	0.0	0.3	-0.2	0.872
CGI-S*	3.7	0.1	3.8	0.1	0.0	0.1	-0.4	0.667

^Week-22 means adjusted for week-11, treatment by week-11, ASD group (Autism versus Asperger & PDD-NOS), gender, and age group. Five outliers were removed based on robust ANVOCA.

*DCS=D-cycloserine, P=Placebo, SE= Standard error, SRS=Social Responsiveness Scale, ABC=Aberrant Behavior Checklist, VABS-II Vineland Adaptive Behavior Scale 2nd Edition, TSSA=Triad Social Skills Assessment, CGI-I Clinical Global Impressions-Improvement Scale, CGI-S=Clincial Global Impressions-Severity Scale.

Table 3. Week-22 Social Responsiveness Scale Total Score Treatment Means (Adjusted) at Specific Values for Week-11 Scores

SRS*Total Week-11	DCS*		Placebo (P*)		Difference (DCS-P)				
	LS mean*	SE*	LS mean	SE	LS mean	SE	t value	p value	Result
60	51.9	3.4	71.0	4.7	-19.1	5.5	-3.5	0.001	C < P
70	62.3	2.8	77.0	3.7	-14.7	4.3	-3.45	0.001	C < P
80	72.6	2.4	83.0	2.9	-10.4	3.3	-3.11	0.003	C < P
90	83.0	2.3	89.0	2.5	-6.0	2.9	-2.08	0.043	C < P
100	93.4	2.6	95.1	2.5	-1.7	3.2	-0.53	0.601	ns*
110	103.8	3.1	101.1	3.0	2.7	4.0	0.67	0.506	ns
120	114.2	3.8	107.1	3.8	7.0	5.2	1.37	0.178	ns
130	124.6	4.6	113.2	4.8	11.4	6.4	1.77	0.083	ns
140	135.0	5.4	119.2	5.8	15.8	7.8	2.02	0.049	C > P

*DCS=D-cycloserine, P=Placebo, SRS=Social Responsiveness Scale, LS Mean=Least Squares means, SE= Standard error, ns= not significant.