Schizophrenia Research 121 (2010) 125–130

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

High dose D-serine in the treatment of schizophrenia

Joshua T. Kantrowitz [a], Anil K. Malhotra [b,c], Barbara Cornblatt [b,c], Gail Silipo [a], Andrea Balla [a], Raymond F. Suckow [d], Cyril D'Souza [e], John Saksa [e], Scott W. Woods [e], Daniel C. Javitt [a,*]

[a] Schizophrenia Research Center, Nathan Kline Institute for Psychiatric Research/New York University Langone School of Medicine, Orangeburg, NY, United States
[b] Department of Psychiatry, Albert Einstein College of Medicine, Bronx, NY, United States
[c] Department of Psychiatry, Zucker Hillside Hospital, Glen Oaks, NY, United States
[d] Analytical Psychopharmacology Dept., New York State Psychiatric Institute, New York, NY, United States
[e] Department of Psychiatry, Yale University, New Haven, CT, United States

ARTICLE INFO

Article history:
Received 12 March 2010
Received in revised form 6 May 2010
Accepted 10 May 2010
Available online 11 June 2010

Keywords:
Schizophrenia
NMDA
D-serine
Negative symptoms
Cognition

ABSTRACT

Background: D-serine is an allosteric modulator of the brain *N*-methyl-d-aspartate (NMDA) receptor and a potential novel treatment of schizophrenia. Double-blind studies have been performed at 30 mg/kg/day (~2 g/day) with encouraging results, but no formal dose escalation studies have been performed. We describe the first evaluation of the efficacy and safety of d-serine at doses >30 mg/kg/day; a 4-week, open-label trial of adjunctive D-serine (30, 60 or 120 mg/kg/day).

Methods: 42 antipsychotic-stabilized patients with schizophrenia or schizoaffective disorder participated. PANSS was obtained bi-weekly and neuropsychological (MATRICS) was obtained pre- and post medication phase. The pharmacokinetics/pharmacodynamics (PK/PD), and safety of doses ≥ 30 mg/kg was also evaluated.

Results: Significant improvement in symptoms and neuropsychological measures was noted across doses. On the PANSS, improvement was observed for positive ($p = 0.006$;$d = 0.46$), negative ($p < 0.001$;$d = 0.68$), general ($p = 0.001$;$d = 0.53$), and total ($p < 0.0001$;$d = 0.74$) symptoms. On MATRICS, while only non-significant improvement was noted at 30 mg/kg, highly significant, large effect size improvement was noted on the composite score ($p < 0.01$; $d = 1.0$) for doses ≥ 60 mg/kg, leading to a significant dose-by-time interaction ($p < 0.01$).

In PK analyses, significant dose-dependent increases in plasma D-serine levels were seen during the study, predictive of significantly increased brain levels. Furthermore, increases in plasma levels correlated with improved symptomatic and neuropsychological function.

Discussion: These findings support double-blind investigation of D-serine at doses ≥ 60 mg/kg/d, and suggest effectiveness in treatment of both persistent symptoms and neurocognitive dysfunction.

1. Introduction

Phencyclidine (PCP) and related compounds induce a psychotic state that closely resembles schizophrenia by blocking *N*-methyl-d-aspartate (NMDA)-type glutamate receptors (Javitt and Zukin, 1991). Agonists of the NMDA positive allosteric modulatory site, such as glycine and D-serine, reverse behavioral effects of PCP preclinically, suggesting potential therapeutic utility. Disturbances in D-serine metabolism are also reported (Kantrowitz and Javitt, 2009). To date, several studies of D-serine in schizophrenia have been conducted using a dose of ~30 mg/kg with positive results across studies (Javitt, 2008). Nevertheless, ideal doses of D-serine in clinical studies remains to be determined. In

* Corresponding author. Nathan Kline Institute for Psychiatric Research, 140 Old Orangeburg Rd Orangeburg, NY 10962, United States. Tel./fax: +1 845 398 6534.
E-mail address: javitt@nki.rfmh.org (D.C. Javitt).

0920-9964/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.schres.2010.05.012

this study, we evaluated clinical effects of D-serine at doses >30 mg/kg/d with emphasis on both safety and efficacy.

2. Methods

2.1. Subjects

2.1.1. Inclusion criteria

1) Aged 18-60; 2) SCID diagnosis of schizophrenia/schizoaffective disorder (First, et al., 1997); 3) Positive and Negative Symptom Scale (PANSS) (Kay, et al., 1987) negative symptom score >20 and total score between 60-110; 4) Stable Clinical Global Impression Scale (CGI) (Guy, 1976) for two consecutive weeks; 5) Simpson Angus Scale (SAS) (Simpson and Angus, 1970) score ≤12; 6) Calgary Depression Inventory for Schizophrenia (CDDS) (Addington, et al., 1994) score ≤10 and suicide less than moderate (<2); 7) No medication changes within 4 weeks prior to study; 8) Chlorpromazine (CPZ) equivalent ≤1500 mg (Woods, 2003).

Exclusion criteria: 1) Treatment with ≥2 antipsychotics/clozapine; 2) Renal impairment; 3) Alcohol/substance abuse and use within past month or dependence and use within past six months; 4) Known neurological disorder.

2.2. Study Design

The study was performed in 3 dose-level phases. Following enrollment, patients underwent a 24-hour pharmacokinetics/pharmacodynamics (PK/PD) session on Day 1 of treatment. Subjects then received four weeks of open-label D-serine. A 4-hour, PK/PD session was completed at study end in the 60 and 120 mg/kg phases. Safety measures (urinalysis, chemistry, CBC, LFT's) were obtained weekly, with a follow-up two weeks after the last D-serine dose. Inter-rater reliability was maintained by biweekly teleconferences.

2.3. Procedures

2.3.1. Clinical Assessments

The predesignated primary clinical outcomes were the PANSS total and the CGI (http://clinicaltrials.gov/ct2/show/NCT00322023). Individual PANSS subscales, the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1982), SAS; the Abnormal Involuntary Movement Scale (AIMS) for tardive dyskinesia (TD) (Guy, 1976); Barnes Akathisia Scale (BAS) (Barnes, 1989); and CDDS were secondary outcomes.

2.3.2. Neurocognitive

The predesignated neurocognitive rating outcome was the composite T-score of the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) battery (Nuechterlein, et al., 2008) which was obtained at baseline and end-of-treatment.

2.4. Statistical Analysis

Baseline data was compared by a Chi-square test and a one-way ANOVA for categorical and continuous data, respectively. Despite baseline differences in symptoms and cognition between sites, reflecting greater impairments in inpatients, there were no significant site-by-time effects. Within-dose baseline-final comparisons were made with a paired t-test, and across dose/site–by-time comparisons with a repeated measure ANOVA (rmANOVA). Within-group effect sizes (Cohen's d) were calculated for the baseline-final change scores (Cohen, 1988). Exploratory pharmacodynamic relationships were determined by Spearman's correlation coefficients (r_s). Reported values are Mean ± SD.

2.5. Plasma D-serine level determination

D- and L-serine levels were determined using liquid chromatography with fluorescence detection (Hashimoto, et al., 1992). Intra-assay variability did not exceed 6.4% for the 7 calibration concentrations (n = 8 for each concentration). Inter-assay variation, measured using two sets of quality controls at 50 and 500 nM/mL of D-serine prepared in drug-free plasma, did not exceed 9.1% and 6.0% for the respective concentrations (n=13 days).

2.6. Subject Disposition

42 individuals were enrolled. 34 individuals completed at least one phase, with eight completing at least two [three participating in the 60 and 120 mg/kg) and five others in all three], yielding 47 evaluable phases (Table 1). For repeaters, there were a mean 7.0 months and a mean 10.8 months between the 30 and 60 and the 60 and 120 mg/kg trials, respectively.

Baseline scores did not differ between completers and non-completers ($p > 0.05$). Of the non-completers, two withdrew after the PK/PD session, two for reported side effects, three for minor laboratory abnormalities and two for reasons unrelated to study medication.

Table 1
Study Demographics for Study Completers.

Nominal relative D-serine dose (mg/kg/d)	30 mg/kg (n=12)	60 mg/kg (n=19)	120 mg/kg (n=16)
Age	41.7 ± 11.4	43.5 ± 9.4	43.2 ± 9.6
Male (%)	75%	95%	88%
Schizophrenia (n)	9	16	13
Schizoaffective (n)	3	3	3
Inpatient (n)	5	8	6
Highest Education	12.1 ± 2.3	11.7 ± 1.9	11.9 ± 2.8
Age of 1st Treatment	21.9 ± 5.4	21.1 ± 6.4	20.8 ± 8.9
Age of 1st Hospitalization[1]	22.3 ± 4.5	22.6 ± 5.4	24.0 ± 8.6
Antipsychotic dose (CPZ Equivalents/d)	468.8 ± 252	602.6 ± 295	493.7 ± 319
Atypical (%)	67%	68%	75%
Dose Repeaters	N/A	5	8
Body weight (kg)	88.9 ± 17.2	99.8 ± 17.5	99.5 ± 21.0
Achieved absolute D-serine dose (g/d)	2.58 ± 0.31	5.58 ± 0.45	11.1 ± 1.1

Except for number of dose repeaters, there were no significant baseline differences on a one-way ANOVA (all $p > 0.29$).
[1]Data unavailable for one patient in 30 mg/kg dose and one in 120 mg/kg dose.

3. Results

3.1. PANSS

Across doses, significant, moderate effect-size improvements were observed on PANSS total and subscales (Fig. 1 a-d and Supplement 1). Significant improvement was seen on the total PANSS within all three phases ($p<0.05$), and across all three subscales for the 120 mg/kg dose.

3.2. MATRICS

Baseline scores were ~2 sd below normative levels on the MATRICS, suggesting clinically significant baseline cognitive deficits (Fig. 2). There were no significant differences in baseline mean composite ($F_{2,44}=1.5;p=0.23$) or individual MATRICS domain scores by dose, except for a significantly lower baseline Reasoning & Problem Solving Domain for 30 mg/kg ($F_{2,44}=5.6;p<0.01$).

For the 30 mg/kg dose, there was no significant main effect of time on the MATRICS composite score ($t_{11}=-0.89;p=0.39;d=0.25$), although a moderate improvement in the Speed of Processing Domain ($t_{11}=2.4;p=0.04;d=0.69$) was seen. In contrast, significant, statistically large treatment effects were observed at both the 60 ($t_{18}=-4.9;p<0.001;d=1.1$) and 120 ($t_{15}=-3.9;p=0.01;d=0.99$) mg/kg doses, leading to a significant dose-by-time interaction ($F_{2,44}=3.6;p=0.035$) for the three phases. Moreover, in an exploratory analysis (Fig. 2d), a significant, large effect-size improvement was seen for the composite score ($t_{34}=-6.3;p<0.001;d=1.1$), with greater improvement at ≥60 mg/kg vs. 30 mg/kg dose noted for the overall composite ($F_{1,44}=6.2;p=0.017$), and the Visual ($F_{1,44}=4.8;p=0.034$) and Verbal ($F_{1,44}=4.7;p=0.035$) Memory Domains. In addition, for ≥60 mg/kg doses, significant change was observed across all domains except Working Memory ($t_{33}=1.6;p=0.12$).

3.3 *CGI and secondary outcomes:* On the CGI-Improvement scale, 36.2% of subjects were rated as at least mildly improved ($t_{46}=-3.5;p<0.001$), with no significant difference across doses ($F_{2,44}=0.11;p=0.895$). While no significant changes were seen in lower doses (all $p>0.19$), in the 120 mg/kg phase, both total SANS and Global Affective Flattening significantly improved (all $p<0.02$). There were no significant changes in CDSS. The AIMS showed a modest, significant improvement (Table 2).

3.4. Pharmacokinetics

For all doses, D-serine exhibited linear kinetics, with a Tmax and $t_{½}$ of ~1-2 hr and ~3.3 hr, respectively (Fig. 3a and Table 3). Furthermore, mean levels observed during PK assessments at week 4 were significantly greater than those at week 1 ($t_{46}=4.7;p<0.001$), suggesting accumulation over time (Fig. 3b and 3c).

3.5. Correlational analyses

Despite low baseline D-serine levels (1.51±1.0 nmol/ml) a significant range (0-5 nmol/ml) was observed across subjects. Pretreatment levels were significantly correlated with the

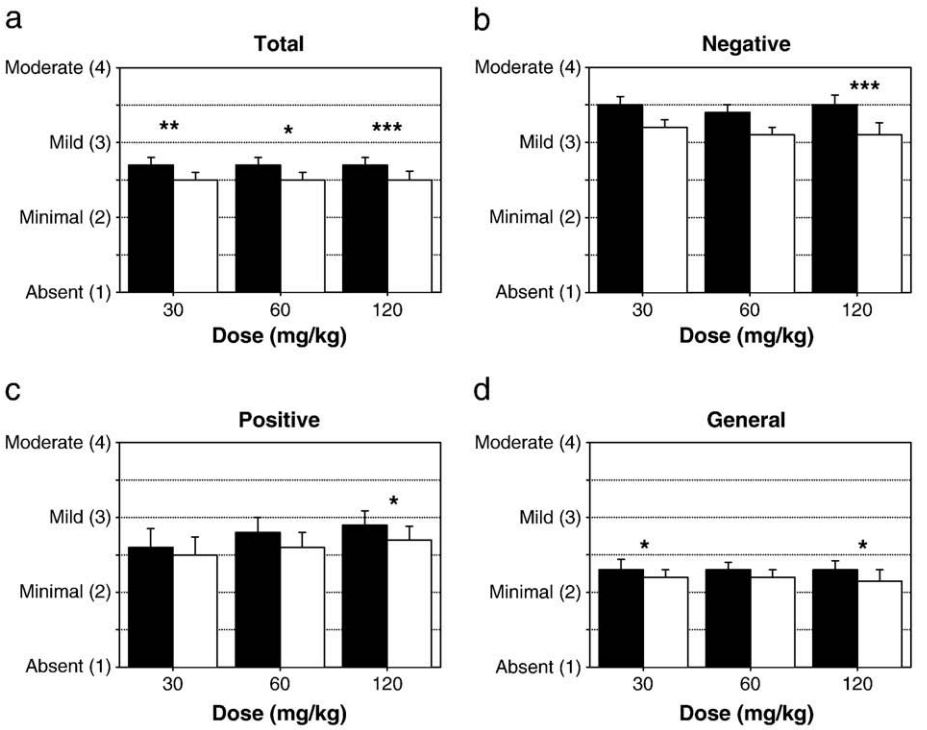

Fig. 1. *Total and PANSS outcomes.* Baseline (filled bars) and final (open bars) mean item scores for (a) total, (b) negative, (c) positive and (d) general symptoms on the PANSS. For the study as a whole, PANSS, improvement was observed for positive ($p=0.006$; $d=0.46$), negative ($p<0.001$; $d=0.68$), general ($p=0.001$; $d=0.53$), and total ($p<0.0001$; $d=0.74$) symptoms.*$p<0.05$, **$p<0.01$, ***$p<0.001$ paired t-test, baseline vs. final.

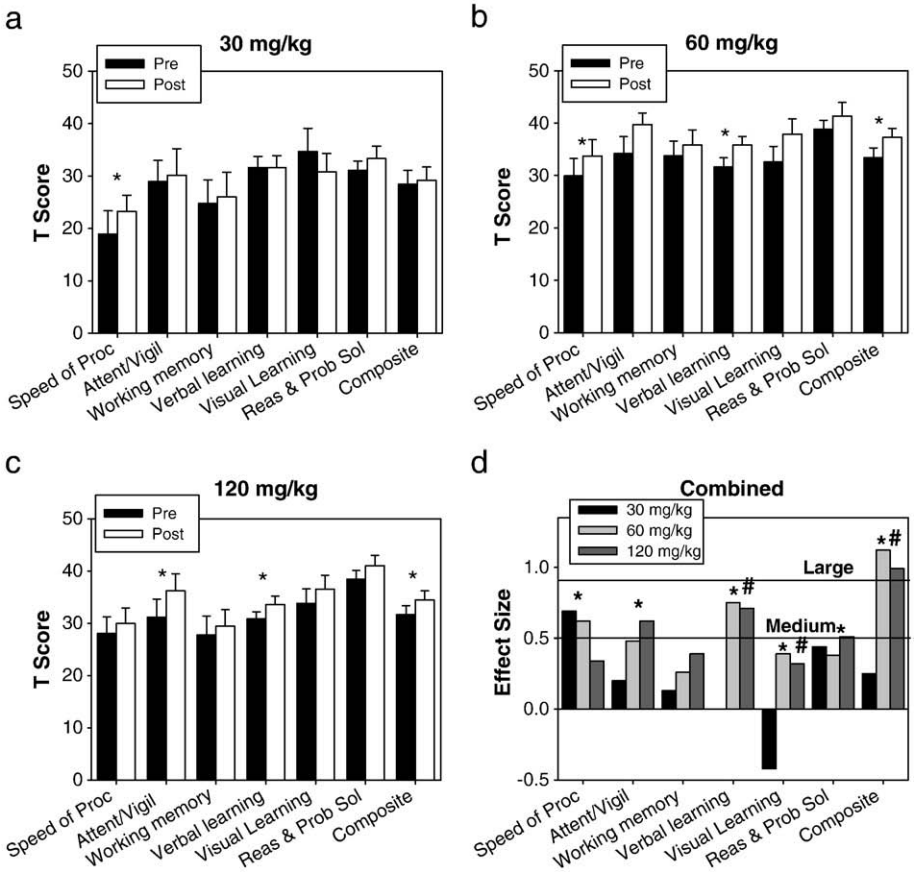

Fig. 2. *MATRICS Outcomes.* Baseline (filled bars) and final (open bars) normalized MATRICS domains and overall mean composite (mean T-score of six tested domains) for (a) 30, (b) 60, and (c) 120 mg/kg. d. Within group effect sizes for each dose. *$p<0.05$ in a paired t-test between baseline and final (a-c) or on a paired t-test for doses >60 mg/kg (d). #Significant ($p<0.05$) dose by time interaction for 30 vs. ≥60 mg/kg.

pretreatment PANSS negative symptoms for the sample as a whole ($r_s = -0.29$, $p<0.05$) (Fig. 4a). Peak D-serine at study initiation correlated with the magnitude of improvement in negative (Fig. 4b) and positive (Fig. 4c) symptoms independently. Composite final MATRICS score also correlated with peak levels (Fig. 4d). Similar relationships were observed with peak levels obtained during week 4 PK/PD studies, suggesting a continued relationship to sustained elevation in D-serine level.

Table 2
Baseline and Final extrapyramidal scales by dose.

	Baseline		Final		Change	
Dose	AIMS	SAS	AIMS	SAS	AIMS	SAS
30	1.0 ± 1.3	3.4 ± 3.4	0.7 ± 1.3	3.5 ± 3.6	-0.3 ± 0.7	0.1 ± 2.4
60	1.3 ± 2.7	3.0 ± 2.3	1.2 ± 2.6	2.4 ± 2.0	-0.2 ± 0.8	-0.6 ± 2.2
120	1.8 ± 3.1	2.3 ± 2.0	1.4 ± 3.0	1.6 ± 1.8	-0.3 ± 0.9	-0.8 ± 2.2
All	1.4 ± 2.5	2.9 ± 2.5	1.1 ± 2.5	2.4 ± 2.5	-0.3 ± 0.8*	-0.5 ± 2.2

*$p<0.05$.

3.6. Safety Measures

There were no safety issues at doses <120 mg/kg. At the 120 mg/kg dose, 1 subject showed 2+ proteinuria without glycosuria during the final week of treatment with no significant change in creatinine that resolved following d-serine discontinuation. Two patients in the 120 mg/kg phase were discontinued from this study for asymptomatic transaminitis, which resolved completely. Two additional subjects withdrew consent for reported side effects (insomnia after one dose and GI distress) in the 120 mg/kg phase.

4. Discussion

Similar to prior studies (Javitt, 2008), significant improvement was observed on PANSS positive, negative and total scores. Significant baseline correlations were observed between reduced D-serine levels and negative symptoms and between peak D-serine on day 1 and subsequent improvement in negative symptoms, consistent with prior clinical findings (Neeman, et al., 2005, Sumiyoshi, et al., 2004). As plasma D-serine levels were unknown at the time when ratings were performed, these are unlikely to reflect placebo

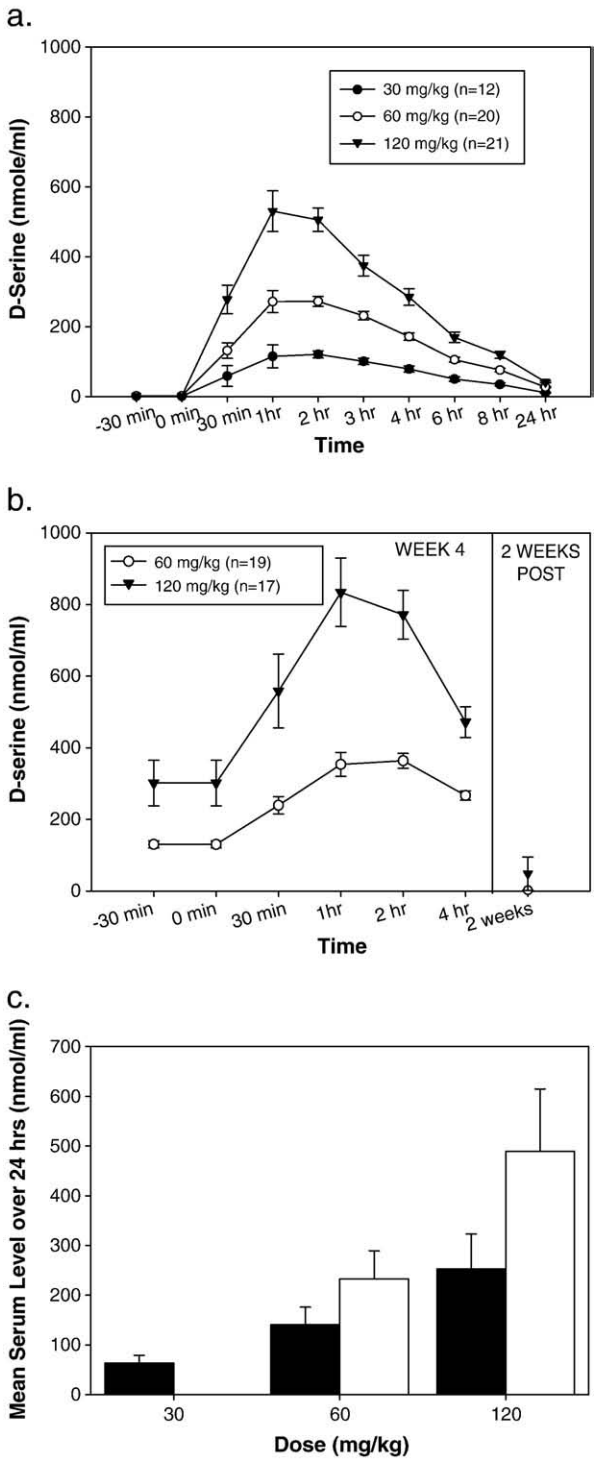

Fig. 3. *D-serine Pharmacokinetics.* a. 24-hour pharmacokinetics of an acute dose of D-serine on day 1 of treatment. b. 4-hour pharmacokinetics after 4 weeks of chronic dosing. c. Mean plasma level over 24 hours of acute and chronic D-serine during week 1 (filled bar) and week 4 (open bar) pharmacokinetics. The Week 4 pharmacokinetics session was not done in the 30 mg/kg phase.

Table 3
Cmax levels by dose.

Dose	Cmax (nmol/ml)
30 mg/kg	120.6 ± 34.6
60 mg/kg	272.3 ± 62.0
120 mg/kg	530.3 ± 266.8

response and therefore support the hypothesis of a true therapeutic response to D-serine.

Despite repeated studies of symptoms, neurocognitive data with NMDA agonists to date have been sparse. In the present study, highly significant, dose-dependent, within-subject change was observed on composite score, with large effect-size improvement ($d>1.0$) being seen at both 60 and 120 mg/kg doses. Furthermore, changes were significant not only within dose, but also relative to the 30 mg/kg dose that has been the subject of prior clinical studies. Peak D-serine levels on day 1 correlated significantly with post-treatment neurocognition scores, supporting a dose-response relationship.

As in prior studies with NMDA glycine-site agonists (Heresco-Levy and Javitt, 2004), significant improvement was noted in TD. Improvement has been noted as well in an animal model of TD (Shoham, et al., 2004). Despite the introduction of atypical antipsychotics, TD remains an issue in clinical practice (Correll and Schenk, 2008).

No nephrotoxicity was observed at dose <120 mg/kg/d, supporting safety. One patient did show a nephrotoxic-like pattern at 120 mg/kg, which resolved upon D-serine discontinuation.

Overall, present results are encouraging of further study of higher-dose D-serine. As our present efficacy results are from a relatively brief open-label study, they must be interpreted with caution, and replicated in larger, longer-duration, randomized, double-blind clinical investigations.

Role of funding source

This project was funded by NIH grant (U01 MH074356). Preclinical/clinical toxicology in support of the project was provided by 5R44AT000810 and a grant from the Stanley Foundation. The NIH and Stanley Foundation had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the paper for publication.

Contributors

Dr. Javitt, Woods, Malhotra, Cornblatt and D'Souza designed the study. Dr. Kantrowitz, Woods and Javitt performed the statistical analysis. Ms. Balla and Dr. Suckow performed the plasma D-serine analysis. Ms. Silipo and Dr. Saska coordinated patient recruitment. Dr. Kantrowitz wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of Interest

Daniel Javitt holds intellectual property rights for use of glycine, D-serine, and glycine transport inhibitors in the treatment of schizophrenia; he is a major shareholder in Glytech and Amino Acid Solutions; he has received consulting payments from Glytech. Scott Woods has received a use patent for a different indication; royalties devolve to Yale University to further his research and not to him personally. All of the other authors have no potential conflicts of interest relevant to this paper to report.

Acknowledgments

Funded by NIH grant (U01 MH074356) to DCJ.

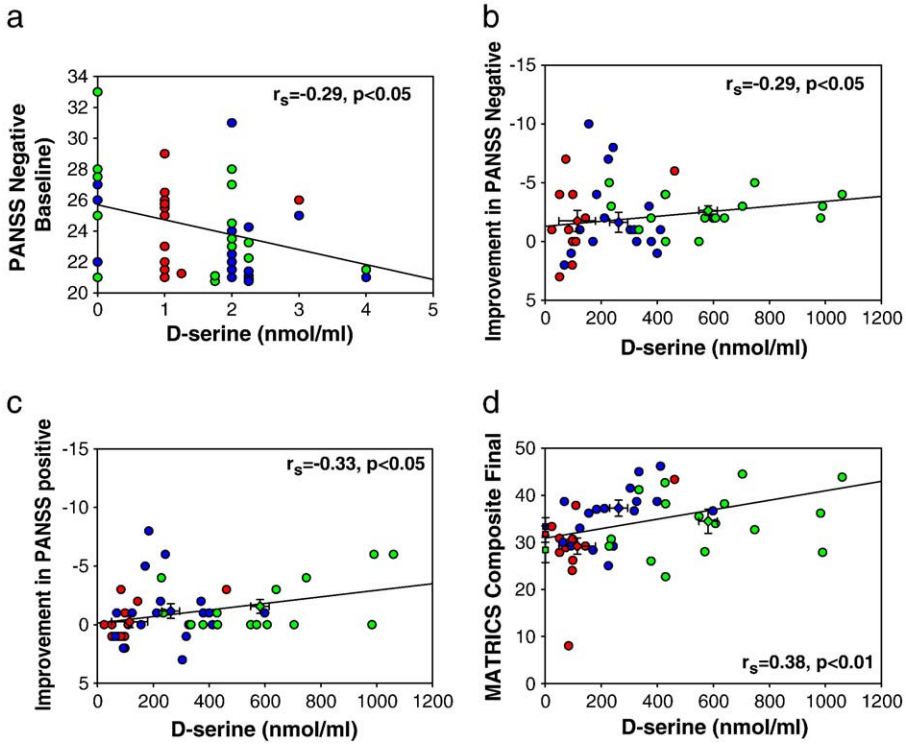

Fig. 4. *D-serine Pharmacodynamics.* Regression scatter plots of D-serine plasma level vs. behavioral outcome. Circles ●indicate an individual patient, diamonds ♦ and indicate final and baseline mean ± SEM, respectively. Red indicates 30 mg/kg, blue 60 mg/kg and green 120 mg/kg. a. Pretreatment D-serine level vs. pretreatment PANSS negative. As PANSS scores are reported as whole numbers, circles were vertically offset to avoid superimposing subjects. b and c. Peak D-serine level on Day 1 of treatment vs. improvement in (b) PANSS negative, (c) PANSS positive. d. Peak D-serine level on Day 1 of treatment vs.post-treatment MATRICS composite.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2010.05.012.

References

Addington, D., Addington, J., Maticka-Tyndale, E., 1994. Specificity of the Calgary Depression Scale for Schizophrenics. Schizophr. Res. 11 (3), 239–244.

Andreasen, N.C., 1982. Negative symptoms in schizophrenia. Arch. Gen. Psychiatry 39, 784–788.

Barnes, T.R.E., 1989. A rating scale for drug-induced akathisia. Br. J. Psychiatry 154, 672–676.

Cohen, J., 1988. Statistical Power Analysis for the Behavioral Sciences. Lawrence Erlbaum Assoc, Hillsdale, NJ.

Correll, C.U., Schenk, E.M., 2008. Tardive dyskinesia and new antipsychotics. Curr. Opin. Psychiatry 21 (2), 151–156.

First, M.B., Spitzer, R.L., Gibbon, M., Williams, J., 1997. Structural Clinical Interview for DSM-IV Axis I Disorders (SCID-IV). .

Guy, W., 1976. Early Clinical Drug Evaluation Unit (ECDEU) assessment manual for psychopharmacology Revised. National Institute of Mental Health, Bethesda, MD.

Hashimoto, A., Nishikawa, T., Oka, T., Takahashi, K., Hayashi, T., 1992. Determination of free amino acid enantiomers in rat brain and serum by high-performance liquid chromatography after derivatization with N-tert.-butyloxycarbonyl-L-cysteine and o-phthaldialdehyde. J Chromatogr Biomed Appl 582, 41–48.

Heresco-Levy, U., Javitt, D.C., 2004. Comparative effects of glycine and D-cycloserine on persistent negative symptoms in schizophrenia: a retrospective analysis. Schizophr. Res. 66 (2–3), 89–96.

Javitt, D.C., 2008. Glycine transport inhibitors and the treatment of schizophrenia. Biol. Psychiatry 63 (1), 6–8.

Javitt, D.C., Zukin, S.R., 1991. Recent advances in the phencyclidine model of schizophrenia. Am. J. Psychiatry 148 (10), 1301–1308.

Kantrowitz, J.T., Javitt, D.C., 2009. Glutamatergic Approaches to the Conceptualization and Treatment of Schizophrenia. Handbook of Neurochemistry and Molecular Neurobiology 3rd Edition.

Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr. Bull. 13 (2), 261–276.

Neeman, G., Blanaru, M., Bloch, B., Kremer, I., Ermilov, M., Javitt, D.C., Heresco-Levy, U., 2005. Relation of plasma glycine, serine, and homocysteine levels to schizophrenia symptoms and medication type. Am. J. Psychiatry 162 (9), 1738–1740.

Nuechterlein, K.H., Green, M.F., Kern, R.S., Baade, L.E., Barch, D.M., Cohen, J.D., Essock, S., Fenton, W.S., Frese 3rd, F.J., Gold, J.M., Goldberg, T., Heaton, R. K., Keefe, R.S., Kraemer, H., Mesholam-Gately, R., Seidman, L.J., Stover, E., Weinberger, D.R., Young, A.S., Zalcman, S., Marder, S.R., 2008. The MATRICS Consensus Cognitive Battery, part 1: test selection, reliability, and validity. Am. J. Psychiatry 165 (2), 203–213.

Shoham, S., Mazeh, H., Javitt, D.C., Heresco-Levy, U., 2004. Glycine and D-cycloserine attenuate vacuous chewing movements in a rat model of tardive dyskinesia. Brain Res. 1004 (1–2), 142–147.

Simpson, G.M., Angus, J.W.S., 1970. A rating scale for extrapyramidal side effects suppl Acta Psych Scand. 212, 11–19.

Sumiyoshi, T., Anil, A.E., Jin, D., Jayathilake, K., Lee, M., Meltzer, H.Y., 2004. Plasma glycine and serine levels in schizophrenia compared to normal controls and major depression: relation to negative symptoms. Int. J. Neuropsychopharmacol. 7 (1), 1–8.

Woods, S.W., 2003. Chlorpromazine equivalent doses for the newer atypical antipsychotics. J. Clin. Psychiatry 64 (6), 663–667.