D-serine adjuvant treatment alleviates behavioural and motor symptoms in Parkinson’s disease

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Abstract
Parkinson’s disease (PD) manifestations include motor symptoms and behavioural deficits that resemble schizophrenia negative symptoms. The N-methyl-D-aspartate subtype of glutamate receptor (NMDAR) represents a novel pharmacological target in PD. D-serine (DSR) allosterically modulates in-vivo NMDAR-mediated neurotransmission and has been shown to improve negative and antipsychotic drug-induced parkinsonian symptoms in schizophrenia patients. This pilot study assessed DSR effects in ten PD patients who completed a 6-wk double-blind, placebo-controlled, crossover adjuvant treatment trial with 30 mg/kg.d DSR. Primary outcome analyses consisted of separate repeated-measures multivariate analyses of variance for Unified Parkinson’s Disease Rating Scale (UPDRS), Simpson–Angus Scale for Extrapyramidal Symptoms (SAS), Abnormal Involuntary Movement Scale (AIMS), and Positive and Negative Syndrome Scale (PANSS) scores. DSR treatment was well tolerated and resulted in increased DSR serum levels (p=0.001) and significantly reduced UPDRS (p=0.02), SAS (p=0.009) and PANSS (0.05) total scores. These preliminary findings suggest that DSR treatment may be beneficial in PD. Larger-sized studies with optimized DSR dosages are warranted.

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Key words: D-serine, NMDAR, PD, treatment efficacy.

Introduction
Idiopathic Parkinson’s disease (PD) is a common and debilitating disorder, in which progressive loss of nigral dopaminergic neurons results in declining dopaminergic innervation of basal ganglia and limbic system structures (Braak & Braak, 2000). Rest tremor, rigidity, hypokinesia and loss of postural balance are the hallmarks of PD. Moreover, common PD manifestations include impairments in motivation, drive, initiation and emotional reactivity (Isella et al. 2002).

This type of dysfunction is transdiagnostic and overlaps with the concept of negative symptoms that is used in schizophrenia phenomenology in relation to apathy, flat affect and isolation (Kay et al. 1987). Within the framework of PD, apathy is estimated to affect from ~16.5% to 42% of patients and refers to a symptoms constellation that includes reduced interest and participation in normal purposeful behaviour, lack of initiative and concern and flattening of affect (Pluck & Brown, 2002).

The ability of the N-methyl-D-aspartate class of glutamate receptors (NMDARs) to modulate neurotransmission throughout the basal ganglia indicates that these receptors may represent an innovative therapeutic target for PD. NMDARs are highly expressed throughout basal ganglia and the limbic...
system, and their subunit composition differs in the various structures (Dunah & Standaert, 2003). Striatal NMDARs are crucial for dopamine-glutamate interactions (Starr, 1995) and NMDAR stimulation has been shown to enhance dopamine release and synthesis within the striatum (Chéremy et al. 1998). Furthermore, the abundance, structure and function of striatal NMDARs are altered by dopamine depletion and further modified by presently used PD dopaminergic medications (Hallett & Standaert, 2004).

Although NMDAR antagonists have shown promise in reversing motor symptoms in animal PD models, clinical studies with this type of compound failed to demonstrate consistent antiparkinsonian efficacy (Johnson et al. 2009; Starr, 1998). Furthermore, recent findings suggest that the naturally occurring amino acids glycine and D-serine (DSR), that act as full agonists at the NMDAR-associated glycine site, may be beneficial for the treatment of both behavioural and motor domains relevant to PD. Glycine (0.8 g/kg.d) and DSR (30 mg/kg.d) were recently assessed, in controlled trials, as adjuvants to the antipsychotic drugs regimens of schizophrenia patients who also exhibited antipsychotic drug-induced motor side-effects (Heresco-Levy et al. 2004, 2005). In these studies, highly significant 17–30% improvements in negative symptoms, as measured by the Positive and Negative Syndrome Scale (PANSS; Kay et al. 1987), were observed, indicating clinically relevant effectiveness against this symptom domain. Furthermore, antipsychotic drug-induced parkinsonian symptoms also decreased by ~30% during NMDAR agonist treatment, as measured by Simpson–Angus Scale for Extrapyramidal Symptoms (SAS; Simpson & Angus, 1970), but were unchanged during treatment with placebo. Similar improvements were registered in dyskinetic symptoms as measured by the Abnormal Involuntary Movement Scale (AIMS; Guy, 1976).

In view of these converging concepts and clinical observations, we hypothesized that adjuvant DSR treatment may alleviate behavioural and motor symptoms in PD. DSR allosterically modulates in-vivo NMDAR function and was selected for this pilot investigation because it is more potent than glycine in activating NMDAR and, unlike glycine, is not known to directly affect any other neurotransmitter system (Mustafa et al. 2004).

Methods

This randomized double-blind, controlled crossover trial used the methodology previously described for DSR treatment assessment in schizophrenia (Heresco-Levy et al. 2005). The study was approved by the Institutional Review Boards of Herzog Memorial and Haemek hospitals in Israel and by the Israel Ministry of Health. Written informed consent was obtained from patients after the study has been described to them orally and in writing. Participants were medicated outpatients, aged ≥40 yr, with an established diagnosis of idiopathic PD of at least 5 yr duration and a rating of at least stage II (bilateral disease) on the modified Hoehn–Yahr scale of PD severity (Hoehn & Yahr, 1967). Patients having an identifiable cause of parkinsonism, a history of psychiatric disorder, drug or alcohol abuse, uncontrolled medical or neurological disorder other than PD, clinical dementia precluding assessment or clinically significant laboratory abnormalities at screening were not eligible. Subjects were required to have been treated with unchanged clinically determined optimal PD medication doses for at least 6 wk.

After a 2-wk baseline assessment period, subjects were randomly allocated to receive either DSR or placebo for 6 wk, in addition to their regular medication, the dose of which remained fixed throughout the study. After completion of the first treatment phase, in order to minimize possible carry-over effects, patients underwent a 3-wk experimental treatment (DSR/placebo) washout period, following which they received the alternate experimental treatment for a final 6-wk treatment phase. DSR and placebo were administered orally in identical capsules according to the same dose escalation schedule. Clinical and research staff, patients and their families were unaware of and could not determine the study drug assignment by appearance or otherwise. Experimental treatment was initiated at a 10 mg/kg.d dose and was increased after the first and second weeks of treatment to 20 mg/kg.d and a fixed 30 mg/kg.d dose, respectively. The range of fixed absolute daily DSR doses was 1.6–2.6 g (mean ± s.d., 2.0 ± 0.3 g). Daily experimental treatment was administered in three divided doses.

Symptoms and side-effects were assessed bi-weekly throughout the treatment phases using the Unified Parkinson’s Disease Rating Scale (UPDRS) which incorporates mental, motor and activities of daily living (ADL) components (Fahn & Elton, 1987), PANSS, SAS and AIMS. The Hamilton Rating Scale for Depression (HAM-D) and the Schwab–England Scale (SE) that provides a global rating (0–100%) of independence and ADL performance in PD were administered at baseline and end of treatment phases. All assessments were performed 2–4 h after the subjects had taken their morning ongoing PD medication dose. Systemic side-effects were recorded using the Udvalg for
Kliniske Undersgelser (UKU) Side Effects Rating Scale. According to the study protocol, patients requiring medication or dose changes, as evidenced by side-effects or a UPDRS total score increase of ≥20% had to be withdrawn from experimental treatment. DSR serum levels were obtained at baseline and end of treatment phases. DSR levels were determined using HPLC analysis as described previously (Shleper et al. 2005); basal DSR levels <10 μM were not detectable by this method. Safety laboratory assessments (SMA 20, CBC, UA) were obtained bi-weekly.

Both completers and last observation carried forward (LOCF) analyses were performed. Primary outcome analyses consisted of separate repeated-measures multivariate analyses of variance (MANOVAs) for UPDRS, SAS, AIMS and PANSS scores. Effect sizes (d) were calculated based upon symptom changes during DSR vs. placebo treatment and were interpreted using Cohen’s criteria (Cohen, 1998). Secondary analyses evaluated change during treatment with DSR or placebo using Student’s t tests and differential response rates using likelihood-ratio $\chi^2$ analysis. All statistical tests were two-tailed and were performed at $\alpha = 0.05$ level of significance using SPSS for Windows (SPSS Inc., USA). Values are reported as mean ± S.D., followed by 95% confidence intervals (CIs).

Fig. 1. Study flowchart.
### Table 1. UPDRS, SAS, AIMS and PANSS scores by treatment and week

<table>
<thead>
<tr>
<th>Treatment assignment</th>
<th>Treatment × time (d.f. = 3, 16)</th>
<th>Effect sizeb (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week within treatment phase</td>
<td></td>
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<tr>
<td></td>
<td>0</td>
<td>2</td>
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<tr>
<td>Total UPDRS score</td>
<td>D-serine</td>
<td>43.0 ± 12.4</td>
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<tr>
<td></td>
<td>Placebo</td>
<td>40.8 ± 9.8</td>
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<tr>
<td>Mental component (I)</td>
<td>D-serine</td>
<td>3.7 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>2.7 ± 1.5</td>
</tr>
<tr>
<td>ADL component (II)</td>
<td>D-serine</td>
<td>16.3 ± 4.1</td>
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<tr>
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<td>Placebo</td>
<td>15.5 ± 3.6</td>
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<tr>
<td>Motor component (III)</td>
<td>D-serine</td>
<td>23.0 ± 8.6</td>
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<td>Placebo</td>
<td>22.6 ± 6.8</td>
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<tr>
<td>SAS</td>
<td>D-serine</td>
<td>12.0 ± 5.1</td>
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<td>Placebo</td>
<td>10.2 ± 5.1</td>
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<td>AIMS</td>
<td>D-serine</td>
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<td>4.2 ± 6.1</td>
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<tr>
<td>Total PANSS score</td>
<td>D-serine</td>
<td>55.8 ± 13.9</td>
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<td>Positive symptoms</td>
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<td></td>
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<td>7.1 ± 0.3</td>
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<tr>
<td></td>
<td>Placebo</td>
<td>28.1 ± 7.7</td>
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</table>

UPDRS, Unified Parkinson’s Disease Rating Scale; ADL, Activities of Daily Living; SAS, Simpson–Angus Scale; AIMS, Abnormal Involuntary Movement Scale; PANSS, Positive and Negative Syndrome Scale.

a Data are reported as mean ± s.d. unless otherwise indicated (n = 10).
b Effect size (Cohen’s d) based upon change score from week 0 to week 6.

### Results

Twenty-five patients were screened and 13 patients entered the study. Three subjects were withdrawn from the study due to non-compliance: two at week 1 of the second treatment phase (one received DSR and one placebo during the first phase); one at week 2 of the second treatment phase while receiving placebo. Ten subjects (six women, four men, mean age 64.3 ± 7.4 yr) completed both treatment phases (Fig. 1) and were included in the primary completers analysis. Subjects were in Hoehn–Yahr stages 2–4 (mean 3.0 ± 0.8) and their mean disease duration was 8.9 ± 5.4 yr. Baseline mean UPDRS, PANSS and SE scores were 41.9 ± 10.4, 52.2 ± 12.5 and 59.5 ± 18.3, respectively. Six (60%) patients reported having dyskinesias; the proportion of the waking day in which subjects were in the ‘off’ state ranged from 0% to 75% (Supplementary Table S1, available online). All patients were on L-dopa treatment (mean daily dose 612.5 ± 416.0 mg), five (50%) were receiving dopamine agonists and two (20%) amantadine. For all subjects, symptoms were stable for at least 2 wk prior to study initiation. Six patients were randomized to receive DSR during the first treatment phase; four received placebo first.

Throughout the study DSR treatment was well tolerated and no experimental treatment side-effects were registered. Serum DSR levels at baseline and post-placebo treatment were ≤10 μM, while following 6 wk DSR treatment they increased ~12-fold to 120.0 ± 52.4 μM (Z = −3.43, p = 0.001).

Significant, large effect size DSR effects were observed in the completers’ analysis for overall PD symptomatology, and extrapyramidal symptoms as assessed by UPDRS and SAS total scores (Table 1). DSR beneficial effects were registered for both mental and motor UPDRS subscales as well as for PANSS total and negative symptoms scores. A significant DSR treatment effect was registered for SE scores (F = 6.2, d.f. = 1, 18, p = 0.02) which did not change significantly during placebo treatment but improved by ~10% following DSR administration. Overall, the proportion of the waking day in which subjects had dyskinesias or were in the ‘off’ state decreased under DSR treatment (Supplementary Table S1). No significant treatment effects were registered.
for AIMS (Table 1) and HAMD ($F = 0.03, \text{d.f.} = 1, 10, p = 0.80$) scores.

An additional LOCF analysis included the data from all 13 patients that participated in the study. Significant DSR effects vs. placebo were also registered in this analysis in terms of UPDRS total ($F = 4.0, \text{d.f.} = 3, 18, p = 0.007$), SAS ($F = 5.6, \text{d.f.} = 3, 18, p = 0.007$), PANSS total ($F = 3.5, \text{d.f.} = 3, 18, p = 0.007$), and PANSS general psychopathology ($F = 3.3, \text{d.f.} = 3, 18, p = 0.007$) scores. A favourable trend under DSR treatment was observed for PANSS negative symptoms scores ($F = 2.9, \text{d.f.} = 3, 18, p = 0.007$).

Among the ten patients who completed the study DSR treatment led to a significant $7.0 \pm 2.7$ (95% CI 1.40–12.61) points reduction in UPDRS total scores ($t = 2.60, \text{d.f.} = 18, p = 0.01$) (Fig. 2). SAS ($t = 2.72, \text{d.f.} = 18, p = 0.01$) and PANSS ($t = 2.60, \text{d.f.} = 18, p = 0.01$) total scores improved by $3.4 \pm 1.6$ (95% CI 0.62–4.78) and $9.2 \pm 7.7$ (95% CI 1.70–16.46) points, respectively. These symptom reductions corresponded to 17.8%, 28% and 16.5% mean reductions, under DSR treatment, in UPDRS, SAS, and PANSS scores, respectively. In contrast, no significant changes were registered in either of the assessment scales scores during placebo treatment. Five (50%) of the ten completers showed >20% improvement in total UPDRS scores during DSR treatment vs. one (10%) of ten subjects during placebo administration ($\chi^2 = 4.07, p = 0.04$). For SAS scores, seven (70%) of ten subjects had a >20% improvement during DSR treatment vs. two (20%) during placebo administration ($\chi^2 = 5.3, p = 0.02$).

**Discussion**

Six weeks’ adjuvant treatment with 30 mg/kg.d DSR was well tolerated and resulted in this preliminary investigation in significant improvements of both motor and behavioural PD manifestations. This represents, to the best our knowledge, the first observation of beneficial effects with a NMDAR full agonist in PD. DSR was previously shown to improve negative, depression and cognitive symptoms in schizophrenia patients (Heresco-Levy et al. 2005). An indirect DSR effect upon motor PD manifestations, mediated via mental symptoms alleviation, can not be excluded, although significant changes in depression symptomatology, as measured by HAMD, were not registered. Negative symptoms partially overlap with manifestations of apathy, a frequent PD feature
probably due, in many cases, to a primary motivational impairment related to frontosubcortical dysfunction (Isella et al. 2002).

Several structurally diverse antagonists of the NMDAR glycine site were reported to reverse akinesia when administered intrastriatally to monoamine-depleted mice (Stauch Slusher et al. 1994). However, since then no glycine site antagonist treatments have been established; benefits have been reported in animal PD models also for NMDAR glycine site partial agonists (Schneider et al. 2000), and overall, the translation of positive findings with NMDAR antagonist interventions from animal studies to PD clinical trials has so far proven problematic (Johnson et al. 2009; Starr, 1998).

The molecular mechanisms by which DSR may alleviate PD symptoms are highly hypothetical at this stage. One potential explanation, however, is that subpopulations of NMDARs may contribute differentially to both pathogenesis and therapeutics of PD. Thus, in terms of pathogenesis, NR2B receptors in striatum have been specifically implicated (Nash & Brotchie, 2002). Further, agents that have shown greatest preliminary effectiveness in PD are all NR2B antagonists (Nikam & Meltzer, 2002). In monkeys, NR2A and NR2B selective antagonists were observed to have differential effects, with NR2A antagonism shown to worsen symptoms of dyskinesia (Blanchet et al. 1999). NR2B receptors have numerically lower affinity for glycine than NR2A receptors, and so may be saturated under physiological conditions. Administration of NMDAR agonists such as DSR may therefore selectively activate NR2A receptors. Activation of NR2A vs. NR2B receptors by NMDAR agonists may, therefore, restore the balance between NR2A- and NR2B-containing receptors similarly and additively to the effects of NR2B antagonists.

Hypothetically, a number of parameters, including patients’ characteristics, DSR dose and treatment duration may impact upon DSR efficacy. Less symptomatic PD patients than those in the present study may respond better to DSR treatment, and a treatment duration longer than 6 wk may be optimal. Moreover, DSR doses higher than 30 mg/kg.d may be associated with increased efficacy. Recently it was reported that adjuvant treatment of schizophrenia patients with 60 mg/kg.d DSR resulted, in addition to negative symptoms reductions, in improved neurocognitive performance (Kantorowitz et al. 2010).

DSR regimens of 30–60 mg/kg.d are well tolerated by schizophrenia patients. The present study is the first to attempt DSR administration in PD patients and confirms that treatment, for at least 6 wk, with 30 mg/kg.d DSR is safe and devoid of significant side-effects. Additional studies are required to address the long-term safety issues related to the administration of DSR in PD.

The results of this pilot study suggest an innovative line of investigation in PD pharmacotherapy. Study limitations include the small sample size and the exclusive use of symptom rating scales as assessment measures. Further larger-sized studies are warranted to determine DSR efficacy and safety profiles in PD.

Note
Supplementary material accompanies this paper on the Journal’s website (http://journals.cambridge.org/pnp).

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[Clinical Trials Registration: http://www.clinicaltrials.gov/ct/show/NCT00215904.]

Statement of Interest
Dr Heresco-Levy and Dr Javitt are inventors in patents related to the use of glutamatergic amino acids for the treatment of movement disorders.

References


