Effects of d-cycloserine on MPTP-induced behavioral and neurological changes: Potential for treatment of Parkinson’s disease dementia

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Glutamatergic dysfunction has been implicated in the neurodegeneration seen in Parkinson’s disease (PD). Sub-chronic intraperitoneal injection with d-cycloserine (DCS), a partial agonist at the glycine binding site of the N-methyl-D-aspartate (NMDA) receptor, at dosages of 30, 100, or 200 mg/kg/day, was used to evaluate the role of NMDA receptors in neuronal and behavioral changes in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD rat model. Starting one day after intra-nigral infusion of MPTP, transient disturbance of motor function in the rotarod test was observed. This impairment spontaneously recovered to control levels 6 days after MPTP lesioning and DCS treatment facilitated recovery. MPTP lesioning also caused deficits in working memory and anxiety-like behavior in the T-maze and elevated plus-maze tests, respectively. Further, object recognition was disrupted in MPTP-lesioned rats, and interleukin-2 levels in the striatum, amygdala, and non-prefrontal cortex were increased, both changes being restored by DCS treatment. Furthermore, MPTP lesion-induced dopaminergic degeneration, microglial activation, and cell loss in the hippocampal CA1 area were all improved by DCS treatment. These results suggest that NMDA receptors are involved in PD-related neuronal and behavioral dysfunctions and that DCS may have clinical potential in the treatment of dementia associated with PD.

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to neuronal excitation. Thus, excessive glutamate release in the brain has been implicated in excitotoxic neuronal death following MPTP lesioning [15,77,79]. Impairment of executive and visuospatial functions is observed not only in patients with PDD [16,22], but also in people exposed to MPTP [91]. In addition, cognitive impairment has been observed in MPTP-lesioned rats in the two-way active avoidance task [26] or Morris water maze test [18,25,60], as well as in monkeys in object recognition tasks [84]. Our previous study demonstrated that the behavioral deficits observed in MPTP-lesioned rats may be similar to the behavioral symptoms seen in PDD patients [92,98]. The above data suggest that dysfunction of glutamatergic activity may be involved in the neuroinflammation, neurodegeneration, and cognitive deficits in PD. Thus, drugs modulating the function of NMDA receptors may have beneficial effects in PDD therapy.

NMDA receptors in the hippocampus mediate not only learning and memory [88], but also object recognition [31]. D-cycloserine (DCS), a partial agonist of the glycine binding site of the NMDA receptor, improves spatial navigation and learning deficits in aged rats [6,7,81], anxiety-like behavior in rats [33,100], and object recognition in MPTP-lesioned monkeys [84]. DCS can also improve brain damage-induced impairment of long-term potentiation (LTP) in the hippocampus [103] and restore impairments in neurodegeneration and episodic-like memory in MPTP-lesioned rats [96]. Intra-hippocamal injection of DCS has been reported to reverse MK-801-induced memory deficits in rats [43]. However, it is not known whether DCS treatment affects MPTP-induced emotional and cognitive deficits in rats. In order to further evaluate the potential of DCS in PDD therapy, we examined its effects on motor behavior, working memory, emotional behavior, and object recognition in rats after MPTP lesioning using a battery of behavioral tests. In addition, we analyzed IL-2 levels and neurohistological changes in the brain. Our results showed that DCS treatment improved MPTP-induced behavioral and neurological deficits. We therefore suggest that DCS may have beneficial effects in PDD therapy.

2. Materials and methods

2.1. Animals

Male Wistar rats (415.9 ± 4.0 g; National Laboratory Animal Center, ROC) were housed in groups of five in acrylic cages (35 cm × 56 cm × 19 cm) in an animal room with a 12 h light-dark cycle (lights on at 07:00 h) with food and water available ad libitum. Each animal was handled for 5 min/day on 3 consecutive days, starting one day after arrival. All experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of Chung Shan Medical University (IACUC approval no.: 434).

2.2. General procedure

All animals underwent stereotaxic surgery and bilateral infusion of MPTP–HCI (1 μmol in 2 μl of saline; Sigma, MO, USA) or vehicle on day 0 into the substantia nigra pars compacta (SNc) [see surgery section below], as in previous reports [17,26,92,96,98]. One day after surgery, the rats received daily intraperitoneal (i.p.) injections of DCS (30, 100, or 200 mg/kg/day; Sigma, USA) or saline in a volume of 1 ml/kg (n = 21–22 per group) at 18:00 h for 13 days. The rationale of using these dosages in the present study was based on our previous studies, where the treatment of DCS at the dosages of 10 or 30 mg/kg caused behavioral changes in the elevated plus-maze [100] and episodic-like memory [96] tests. For analyzing whether higher dose of DCS has higher or even reversed effect, two higher doses were applied in this study. The rats were subjected to a battery of behavioral tests performed as in our previous studies [92,98]: the rotarod test was performed on days 1–7, the T-maze test on days 8–10, the elevated plus-maze test on day 11, and the object recognition test on day 12. Animals were handled for 5 min/day on 3 consecutive days, starting one day after exposure to DCS, then, 5 min after the last exposure session, a test session was performed. Four different object made of transparent glass, paper, porcelain, or metal (all about 10 cm × 10 cm × 10 cm) were used for each rat. All objects were unfamiliar to the rats before the experiment. The three of the objects (“A”, “B”, and “C”) were fixed to the floor 27 cm from three corners of the arena. Starting on day 11 after MPTP lesioning resolution of the VIAS was set to 0.7 cm and the image processing capability was higher than 14 pictures/sec. Some of the animals were randomly assigned to the rotarod test (n = 12–12 for each group) or T-maze test (n = 11–12 for each group), and the behavior in these two tests was scored manually by a trained observer blind to the treatment conditions. The test equipment and objects used in this study were cleaned using 20% ethanol and thoroughly dried before each test trial. On day 14 after MPTP lesioning, the rats were euthanized by exposure to CO2 from gas canister, transcardially perfused with phosphate-buffered saline, and the brain immediately removed for histological examination and IL-2 assay.

2.3. Surgery

Brain surgery was performed as in our previous report [92,96,98]. Briefly, the rats were anesthetized using Zoletil (20 mg/kg, i.p.; Virbac, Carros, France), then MPTP–HCl (1 μmol in 2 μl of saline) was bilaterally infused into the SNc with the following coordinates adapted from the rat brain atlas [72]: AP: −5.0 mm, ML: ±2.0 mm, DV: −8.0 mm from the bregma, midline, and skull surface, respectively. Controls were subjected to the same procedure, but were infused with 2 μl of saline instead of MPTP. Immediately after the test, the rats were injected with penicillin G procaine (0.2 ml, 20,000 IU, IM) and housed individually in plastic cages (25 cm × 41 cm × 19 cm) for a week, then they were re-grouped in their home cages (rats from the same home cage underwent the same treatment). During the first 5 post-operative days, 1% sucrose solution was provided ad libitum to prevent weight loss after surgery and reduce mortality [17,25].

2.4. Behavioral tests

2.4.1. Rotarod test (n = 11–12 for each group)

Motor function was assessed using an automated rotarod (Rotarod, RT-02, Sigma, Taiwan), as described previously [95]. Briefly, the rats were trained daily on the rotarod at constant speed of 18 rpm; rod diameter 9 cm) for 3 consecutive days before MPTP lesioning, at which time the rats were able to keep walking on the rod for at least 30 s (taken as the maximum time). Starting one day after the MPTP lesion, the rats were tested daily on the rotarod for 7 consecutive days. Each test included 3 trials (maximum of 30 s per trial) with a 30 s inter-trial interval. Latency to falling off the rod was recorded and the data are presented as the percentage of the maximum walking time (30 s) on the rotarod.

2.4.2. T-maze test (n = 11–12 for each group)

The construction of the T-maze and the test procedures were similar to those described previously [4]. Briefly, the T-maze was constructed of black polyvinyl plastic. A stem alley (60 cm long × 15 cm wide × 30 cm high) was connected to an arm alley (95 cm long × 10 cm wide × 30 cm high) in a T shape. At the entrance of each arm, there was a sliding door that could be closed, thus forcing rats to run into the other arm. The training session consisted of 9 trials, each composed of two parts. The first part was the forced run in which one of the arms, left or right according to a random order, was closed by a sliding door and the rat was put into the stem alley and allowed to explore the maze, i.e., the stem and open arm. A food cup containing chocolate balls (Kellogg’s, Taiwan) was located at the end of the arm as a reward. After entering the arm and getting the reward, the rat was removed and put in a cage adjacent to the T-maze. The second part was the choice run, carried out 30 s later, when the rat was again allowed to run in the maze, but with both arms open. Choosing the newly opened arm, the opposite to that used in the forced run, was the correct response to get the reward. The forced run and choice run were performed one by one for 9 times for each rat in 2 consecutive training days, then, on the following day, 3 forced-choice–choice run trials were made 2 choices following a single forced run, and correct responses in the 6 choice runs were recorded. On the day before T-maze training, the rats were partially food restricted, the diet only being provided for 1 h, while, on the 2 training days, the diet was provided for only 1 h after the behavioral observation on that day.

2.4.3. Elevated plus-maze test (n = 20–22 for each group)

The construction of the elevated plus-maze and the testing procedures were identical to those in our previous report [33]. The following three measurements were recorded: (1) arm time: the time spent in the open and enclosed arms, (2) enclosed arm activity: the number of times the animal crossed a virtual line dividing an arm into a proximal and a distal half, and (3) rearing number: the number of times the animal reared up on the hind legs during the test. Entry into any of the compartments was defined by the center of the body being placed in the compartment.

2.4.4. Object recognition test (n = 21–22 for each group)

The test apparatus, an open box (10 cm long × 100 cm wide × 60 cm high), and the test procedure for the object recognition test were identical to those in our previous reports [92,98]. Each rat was subjected to 3 exposure sessions at 24 h intervals, then, 5 min after the last exposure session, a test session was performed. Four different objects made of transparent glass, paper, porcelain, or metal (all about 10 cm × 10 cm × 10 cm) were used for each rat. All objects were unfamiliar to the rats before the experiment. Three of the objects (“A”, “B”, and “C”) were fixed to the floor 27 cm from three corners of the arena. Starting on day 11 after MPTP lesioning
(5 min after the elevated plus-maze test), the rat was allowed to explore the objects for 5 min in the open box on 3 consecutive days, then, 5 min after the last exposure session, object “B” was replaced by a novel object “D” and the animal was returned to the open box for a 5 min test session. The time spent exploring the objects and the number of rearings during the exposure and test sessions were recorded. Exploration of an object was defined as the rat approaching it and making physical contact with it and lifting its nose and/or forepaws. The percentage of exploration time spent on object B or D in the session \([\text{Time}_{\text{B or D}} / \text{Time}_{\text{all objects}}] \times 100\%\) was calculated. The difference of percentage of time spent exploring object “B” in exposure 3 and on the novel object “D” served as a measure of recognition memory for the familiar object.

2.5. Measurement of IL-2 levels

The prefrontal cortex, non-prefrontal cortex (the cortex tissue excluding the prefrontal cortex), amygdala, striatum (ventral and dorsal part), and hippocampus were dissected on an ice-cold plate and stored at –80°C until use (n = 12–14 for each group). The procedures for IL-2 measurement were identical to those used in our previous reports [34,98]. Briefly, IL-2 levels in a sample containing about 30–40 μg of total protein were measured using an enzyme linked immunosorbent assay (ELISA) kit using monoclonal anti-rat IL-2 antibody (CytoSets™, BioSource, CA, USA) according to the manufacturer’s instructions.

2.6. Histological assay and image analysis

For histological assessment, 4 randomly selected rats per group were perfused intracardially with 4% paraformaldehyde in phosphate-buffered saline, then the brains were rapidly removed and post-fixed in 20% sucrose solution containing 4% paraformaldehyde at 4°C until use. To detect DAergic degeneration and microglial activation, frozen coronal brain sections (30 μm) were cut and immunostained at 4°C overnight with mouse monoclonal antibodies against rat tyrosine hydroxylase (TH) (1:2000; Zymade, USA) or rat MHC class II (OX-6; 1:200; BD Biosciences Pharmingen, CA, USA), a method identical to that used in our previous reports [92,96,98]. In sections containing the hippocampus, Nissl staining was used to identify neurons.

The stained brain sections, identified according to the rat brain atlas [72], were used to measure histological changes as described previously [98,102] using a microscope (ZEISS AXioskop2, Germany) coupled to a CCD (Optronics, USA) and Image Pro Plus Software 6.0 (Media Cybernetics, CA, USA). In this study, we created three square areas of interest, one 36,477 μm² in the striatum to determine the optical density of TH immunoreactivity, and one 18,769 μm² in the SNc, and another 2,354 μm² in the hippocampal CA1 area to determine neuronal density in these regions. To measure the density of DAergic neurons projections in the striatum, we converted the TH-stained images to gray-scale, then measured the gray level of a given area of interest, and subtracted the background staining, measured in the non-immunoreactive corpus callosum. Thus, the relative optical density was restricted to the values generated by the TH-reactive tissue. To measure the density of DAergic neurons in the SNc, images were captured, but not converted to gray-scale, and an area of interest was overlaid in this region and the somas of TH-immunoreactive neurons located in this area counted. The density of activated microglia in the SNc and striatum was measured in the areas of interest (measuring 18,769 and 36,477 μm², respectively). Because the neurons were tightly packed, it was difficult to directly count the number of pyramidal neurons in the CA1 area from a 30-μm-thick brain section, so we estimated neuronal density using a semi-quantitative method involving calculating the percentage of an area occupied by Nissl-stained neurons in an area of interest in the CA1 area. Although a stereological approach involving the counting of cells in a complete series of sections would provide additional data [25], calculating the cell number in representative brain sections yielded similar histological results to those reported in the literature [17].

2.7. Data analysis

Analysis of variance (ANOVA) repeated measures was used to analyze the rotarod test data. ANOVA followed by the least-significant difference (LSD) post hoc test was used to analyze the effects of DCS treatment and elevated plus-maze tests and IL-2 data. ANOVA with LSD post hoc test and paired-samples t-test were used to analyze the object recognition test data. All results are expressed as the mean ± SEM. The level of significance was defined as P < 0.05 (two-tailed).

3. Results

3.1. Rotarod test

ANOVA repeated measures revealed that motor function in the rotarod test had main effects of time \((F(7,371) = 62.43, P < 0.001)\) and treatment \((F(4,53) = 10.17, P < 0.001)\) and time-by-treatment interactions \((F(28,371) = 4.78, P < 0.001)\). Further analysis of the data for different time points by ANOVA showed that, compared to the sham-operated group, the percentage of the maximum time on the rotarod in the MPTP-lesioned group was significantly lower on days 1–4 (all P values < 0.001) and on day 5 (P < 0.05) after MPTP lesioning, but not on days 6 and 7. Daily treatment with DCS restored the MPTP-induced motor deficits starting on day 3 at the dosages of 30 and 100 mg/kg/day and at day 5 at the dosage of 200 mg/kg/day (Fig. 1).

3.2. T-maze

ANOVA followed by LSD post hoc test revealed that MPTP lesioning decreased the percentage of correct responses in the T-maze test \((F(4,57) = 6.59, P < 0.001)\), compared to the sham-operated group. All dosages of DCS used (30, 100, and 200 mg/kg/day) were able to reverse this deficit (Fig. 2). Further analysis, by using one-sample t-test, showed that the percentage of correct response in sham-operated rats and rats receiving MPTP lesion accompanied with DCS treatment was significantly higher than the chance level (50%) \((t = 10 or 11, all t values \geq 7.31, all P values ≤ 0.001)\). How-

![Fig. 1. Effects of d-cycloserine (DCS) on motor function in MPTP-lesioned rats in the rotarod test. MPTP (1 μmol) was bilaterally infused into the substantia nigra pars compacta, then DCS (30, 100, and 200 mg/kg/day, i.p.) or saline (1 ml/kg/day, i.p.) was administered from day 1 after MPTP lesioning. The data are expressed as the mean ± SEM for the indicated number of rats. *P < 0.05, **P < 0.01, ***P < 0.001, compared to the sham-operated group on the same day.

![Fig. 2. Effects of d-cycloserine (DCS) on the behavior of MPTP-lesioned rats in the T-maze test. Animals were treated as in Fig. 1, then the T-maze test was performed on day 10 after MPTP lesioning. The data are expressed as the mean ± SEM. **P < 0.01, ***P < 0.001, compared to the sham-operated control. ##P < 0.01, ###P < 0.001, compared to the chance level (50%).}
ever, MPTP-lesioned rats showed lowered percentage of correct response, compared to the chance level (df = 10, \( t = 4.14, P = 0.002 \)).

Independent sample t-test showed no differences in the open arm time and enclosed arm time in the elevated plus-maze test (df = 105, \( t \) values \( \leq 1.11 \), both \( P \) values \( \geq 0.27 \)) and the percentage of exploration time spent on novel object in the object recognition test (df = 104, \( t = 0.21, P = 0.835 \)) between the rats that had and had not been tested in the rotarod and T-maze tests, indicating that the rotarod and T-maze tests did not affect the behavior in the followed elevated plus-maze test and object recognition test. Thus, the data of the flowed two tests from all of the rats, irrespective of receiving or not rotarod and T-maze tests, were analyzed.

### 3.3. Elevated plus-maze test

ANOVA with an LSD post hoc test indicated that MPTP lesioning significantly decreased open arm time (\( F(4,106) = 4.66, P = 0.002 \)), compared to sham-operated controls. No deficit of open arm time was observed in MPTP-lesioned rats treated with DCS at the dosage of 30, 100, and 200 mg/kg/day. No differences were observed in the enclosed arm time, enclosed arm activity, and rearing number between the groups (Table 1).

#### 3.4. Object recognition

The method used in the test is shown in Fig. 3A. ANOVA revealed that there were no differences between the groups in total exploration time and the percentage of time exploring object “B” in the 3 exposure sessions (data not shown). ANOVA with LSD post hoc test showed that MPTP-lesioned rats receiving either saline or DCS 200 mg/kg spent a smaller percentage of time exploring object “D” (\( F(4,105) = 7.73, P = 0.002 \)) than sham-operated controls. As shown in Fig. 3B, further analysis using the paired-samples t-test showed that the sham-operated and MPTP groups treated with DCS at the dosages of 30 and 100 mg/kg/day spent a higher percentage of time exploring object “D” than exploring object “B” (df = 21, \( t \)-values

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**Table 1** Effects of \( \alpha \)-cycloserine on the behavior of MPTP-lesioned rats in the elevated plus-maze test.

<table>
<thead>
<tr>
<th></th>
<th>Sham Saline (n=21)</th>
<th>MPTP Saline (n=20)</th>
<th>DCS 30 mg/kg (n=21)</th>
<th>DCS 100 mg/kg (n=22)</th>
<th>DCS 200 mg/kg (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Open arm time (s)</strong></td>
<td>32.0 ± 5.2</td>
<td>10.5 ± 2.7**</td>
<td>22.5 ± 6.5</td>
<td>33.1 ± 9.7</td>
<td>51.0 ± 8.3</td>
</tr>
<tr>
<td><strong>Enclosed arm time (s)</strong></td>
<td>210.3 ± 13.9</td>
<td>208.1 ± 14.6</td>
<td>222.8 ± 10.4</td>
<td>221.0 ± 11.5</td>
<td>194.1 ± 12.0</td>
</tr>
<tr>
<td><strong>Enclosed arm activity (no.)</strong></td>
<td>11.0 ± 1.3</td>
<td>10.8 ± 1.5</td>
<td>10.5 ± 1.6</td>
<td>10.1 ± 1.6</td>
<td>9.6 ± 1.2</td>
</tr>
<tr>
<td><strong>Rearing (no.)</strong></td>
<td>9.3 ± 1.2</td>
<td>8.8 ± 1.1</td>
<td>8.1 ± 1.2</td>
<td>10.2 ± 0.9</td>
<td>9.3 ± 0.8</td>
</tr>
</tbody>
</table>

DCS: \( \alpha \)-cycloserine. Data are expressed as the mean ± SEM. **\( P < 0.01 \), compared to the sham-operated controls.

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**Fig. 3** Effects of \( \alpha \)-cycloserine (DCS) on object recognition in MPTP-lesioned rats. Animals were treated as in Fig. 1, then the object recognition test was performed on days 11–13 after MPTP lesioning. (A) Schematic diagram of the arrangement of the objects in the test. Rats underwent 3 exposure sessions (5 min each) at 24 h intervals, then were tested for 5 min starting 5 min after the end of exposure session 3. In the test session, object “B” was replaced by a novel object “D”. (B) Percentage of time spent on exploring object “B” or “D”. (C) Rearing number in the exposure and test sessions. The data are expressed as the mean ± SEM. **\( P < 0.01 \), ***\( P < 0.001 \), compared to the sham-operated group. ***\( P < 0.001 \), ****\( P < 0.001 \), compared to the percentage time spent on object “B” (paired t-test).

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**Fig. 4** Effects of daily treatment with \( \alpha \)-cycloserine (DCS) on IL-2 levels in the brain of MPTP-lesioned rats. The brain tissue was taken 14 days after MPTP lesioning. The data are expressed as the mean ± SEM. *\( P < 0.05 \), compared to the sham-operated group.
Fig. 5. Effects of d-cycloserine (DCS) on MPTP-induced changes in dopaminergic neurons in the SNc at day 14 after lesioning. Dopaminergic neurons stained for tyrosine hydroxylase (TH) are shown in the representative coronal sections. DCS 30, 100, or 200 indicates the dosage of DCS used. Magnification, 50×; bar, 200 μm. The black square in the schematic drawings indicates the area used for measuring the density of dopaminergic neurons. *P < 0.05, **P < 0.01, compared to the Sham + saline group. ***P < 0.001, compared to the MPTP + saline group.

≥3.15, all P values ≤0.005). However, no difference in the percentage of time exploring objects “B” and “D” was observed between the MPTP-lesioned group and the MPTP-lesioned group treated with 200 mg/kg/day of DCS. In terms of rearing number in the object recognition test, ANOVA with repeated measures revealed a significant time effect (F(3,306) = 45.50, P < 0.001), but no significant treatment effect or time-by-treatment interactions (Fig. 3C).

3.5. IL-2 measurement

As shown in Fig. 4, compared to the sham-operated group, the MPTP-lesioned group showed a significant increase in IL-2 levels in the striatum, amygdala, and non-prefrontal cortex (F(4,65) ≥3.74, all P values <0.05), but not in the prefrontal cortex and hippocampus. However, the above changes were not observed in the MPTP-lesioned rats treated with DCS at dosage of 30, 100, and 200 mg/kg/day.

3.6. Histology

Representative photomicrographs of immunostained and Nissl-stained brain sections are shown in Figs. 5–9. TH immunoreactivity was observed in the cell bodies of DAergic neurons in the SNc and in DAergic processes in the striatum. ANOVA showed that rats in the MPTP-lesioned group exhibited a decreased density of DAergic neurons in the SNc (F(4,19) = 21.41, P < 0.001) (Fig. 5B and G) and a lower relative optical density of TH immunoreactivity in the striatum (F(4,19) = 14.73, P < 0.001) (Fig. 6B and G) compared to the sham-operated group. The MPTP-induced decrease in the density of DAergic neurons in the SNc compared to the sham-operated group.
Fig. 7. Effects of d-cycloserine (DCS) on MPTP-induced activation of microglia in the SNc at day 14 after lesioning. DCS 30, 100, or 200 indicates the dosage of DCS used. Magnification, 50×; bar, 200 μm. A high magnification image (200×, bar, 20 μm) of the activated microglia is shown in the insets. The black square in the schematic drawing indicates the area used for measuring the density of activated microglia in the SNc. *P<0.05, **P<0.01, compared to the Sham + saline group. ***P<0.001, compared to the MPTP + saline group.

Fig. 8. Effects of d-cycloserine (DCS) on MPTP-induced activation of microglia in the striatum at day 14 after lesioning. DCS 30, 100, or 200 indicates the dosage of DCS used. Magnification, 50×; bar, 200 μm. A high magnification image (200×, bar, 20 μm) of the activated microglia is shown in the inset. The square in the schematic drawings indicates the area used for measuring the density of activated microglia in the striatum.

In the present study, MPTP lesioning caused disturbance of motor function, impairment of working memory in the T-maze test, anxiety-like behavior in the elevated plus-maze test, and deficits in object recognition, which were improved by daily treatment with DCS.
DCS. MPTP lesioning also decreased density of DAergic neurons in the SNc and terminals in the striatum, causing massive activation of microglia in these two nuclei, decreased neuronal density in the CA1 area of the hippocampus, and increased IL-2 levels in the striatum, amygdala, and non-prefrontal cortex. The above neurological and biochemical changes were restored by DCS treatment. These results show that DCS overcomes the MPTP-induced deficits in motor, emotional, and cognitive behaviors, and reverses the neuromodulation and neurodegeneration, suggesting a potential role for DCS in the treatment of behavioral and neuronal impairments in PDD.

In line with previous studies showing transient motor dysfunction during the first week after MPTP lesioning [13,25,86,92,98], the present study shows no differences in motor function in the rotarod test between the MPTP-lesioned and sham-operated groups at 6 days after surgery. Motor recovery was further supported by the lack of differences between the groups in enclosed arm activity and rearing number in the elevated plus-maze test and in rearing number in the object recognition test, suggesting that behavioral performance in the tests was not confounded by gross motor impairment or general sickness. Striatal re-innervation following lesions of SNc DAergic neurons may provide a possible compensatory mechanism of motor recovery in rats [90], although sustained decrease of TH-immunostaining in the striatum has been reported after MPTP lesion [9]. Even if no motor recovery has been seen in the progression of PD in humans, the ability of MPTP-lesioned rats to recover motor function makes it possible to study behavioral changes in these rats as a model for the motor presymptomatic phase of PD.

Because the effects of DCS vary depending on the activity of the glutamatergic system [99], we propose that a biphasic effect may occur in the DCS treatment, namely, during the acute phase after MPTP lesioning, when the glutamatergic system is hyperactivated, DCS may act as an antagonist, while, in the later phase after MPTP lesioning, the activity of the glutamatergic system may decrease and DCS may act as an agonist of NMDA receptor. MPTP lesioning results in an increase in corticostriatal glutamatergic innervation and regional reorganization of glutamatergic thalamostriatal terminals in the striatum of monkeys [79]. Ten days after MPTP lesioning, increased glutamate concentrations are seen in the striatum of mice [15]. Further, there was a decrease in the basal level of extracellular glutamate within the striatum in the sub-chronically MPTP-treated animals compared to an increase in the acutely treated group [82]. Thus, we propose that, during the acute stage after MPTP lesioning, the glutamatergic system may be hyper-activated and thus cause excitotoxicity. However, after a period of time or in the chronic state, glutamatergic transmission may be hypo-activated, because the number of NMDA-sensitive [3H]glutamate binding sites in MPTP-treated mice is decreased 2 weeks after MPTP lesioning [101]. Based on the above data, a biphasic evolution of glutamatergic activity may appear after MPTP lesioning in the substantia nigra. Thus, there may be a turning point, where the glutamatergic tonus changes from hyper- to hypo-activity, and this point may provide a basis for determining a temporal adequacy of treatment for these two phases. However, to our knowledge, no paper has reported this turning point. Hyper-glutamatergic activity induced by MPTP lesion has been implicated in excitotoxic neuronal death in the PD model [15,85,77,79], which may be involved in cell loss in the DAergic system and microglial activation in the hippocampus of PD brains [40]. Accordingly, treatment with NMDA antagonists before and immediately after MPTP lesioning can protect DAergic neurons from MPTP-induced degeneration [83,94] and improve motor function and suppress the progression of PD [8]. Similar to the above findings, our present study provides the first data showing that chronic treatment of DCS suppressed microglial activation and protected neurodegeneration in the hippocampus and nigrostriatal system from MPTP lesioning. Since DCS acts as an antagonist at the NMDA receptors when the glutamate levels are high [99], this drug may able to prevent overstimulation of the glutamatergic system by excessively released glutamate after MPTP lesioning. This function, especially at the acute phase after MPTP lesioning, may thus have achieved neuroprotection from excitotoxicity [65,77], and caused a sustained benefit for the entire observation period. It may also be possible that, at the chronic state, DCS exerts cognition enhancing effect through the mechanisms described below.

The NMDA receptors play an important role in cognitive function, for example, spatial learning [56] and visual recognition memory [57]. DCS has promnestic effects in rats in learning tasks [38,52] and has been reported to facilitate correct responses in spatial recognition in the radial-arm maze in MK-801-treated rats [43]. Furthermore, DCS improves memory in aging rats [6,7], overcomes brain damage-induced impairments in LTP and object recognition in rats [103], and enhances episodic-like memory in MPTP-lesioned

Fig. 9. Effects of d-cycloserine (DCS) on MPTP-induced cell loss in the hippocampal CA1 area at day 14 after lesioning. The images show Nissl-stained pyramidal neurons in the CA1 area of the hippocampus, as indicated in the square of the schematic drawing. DCS 30, 100, or 200 indicates the dosage of DCS used. Magnification, 200×; bar, 100 μm. *P < 0.05, **P < 0.002, compared to the Sham + saline group. *P < 0.05, **P < 0.01, compared to the MPTP + saline group.
rats [96]. MPTP-induced glutamatergic dysfunction has been suggested to be involved in cognitive dysfunction after MPTP lesioning [18,25,26,60,84]. DCS may ameliorate neuronal and behavioral deficits by regulating the activity of glutamatergic NMDA receptors. As a partial agonist, DCS binds to the glycine binding site of the NMDA receptors [37] and modulates the binding of glutamate to these receptors [87,99]. DCS activates NMDA receptors, but is less effective than the endogenous ligand, glycine [87,99], therefore, at low dose, DCS increases glutamatergic transmission when glutamate is at a physiological or low concentration [87], while, at high dose, it decreases the activation of NMDA receptors by glutamate [50]. DCS at dosages of 0.5–30 mg/kg has been reported to be effective in behavioral studies in rats [33,49]. In the present study, 30 and 100, but not 200, mg/kg/day of DCS reversed the MPTP-induced impairment of object recognition. Interestingly, the effects of DCS on motor function in MPTP-lesioned rats also showed a reversed U-shaped dose response curve, which may be due to the characteristics of the partial agonist, mimicking the activity of the endogenous ligand at low dose, but antagonizing the activity of the endogenous ligand at high dose [50]. However, all the dosages used in this study (30, 100, and 200 mg/kg/day) were effective on working memory in the T-maze test and anxiety-like behavior in the elevated plus-maze test and also restored IL-2 levels and microglial activation in the brain. Thus, there may be different effective-dose windows for different measurements. This hypothesis is supported by the findings that the behavioral and biochemical effects of DCS are related to neuro-physiological activity in animals [33,100] and that the DCS–induced augmentation of effects of psychotherapy is seen in patients with anxiety disorders [35,80], but not in healthy controls [30].

The T-maze test is a kind of delayed alteration test, in which the rats need to learn a rule to make a correct choice, i.e., to choose the arm that was previously closed in forced run. Since the side of blockade of arm in forced runs was changed at random, the choice and performance in the T-maze test is regarded as working memory because of its trial-dependency [5]. The object recognition test is a kind of delayed matching to sample task [23] and is similar to the visual recognition task used in subhuman primates to assess memory dysfunction [3]. Primates and rodents show a tendency to explore new objects when a new object and a familiar object are presented together and they spend longer exploring a new object than a familiar object. Thus, the difference in the time spent exploring new and familiar objects is a measure of object recognition and/or discrimination in rats [23]. In the present study, sham-operated rats performed around 25% better than the chance level of 50% in correct responses in the T-maze test and showed successful discrimination in the object recognition test. However, MPTP lesioning significantly suppressed behavior in the T-maze and object recognition tests, indicating impairment of working memory and object recognition. Similarly, previous studies have shown that MPTP causes cognitive deficits in animals, including learning impairment in the two-way active avoidance test [17,26] and disturbance in spatial working memory and cue-based navigation, but sparing long-term spatial memory, in the Morris water maze test in rats [18,25,60], as well as a decrease in recognition response in the variable delayed-response task in monkeys [84]. It has been reported that a high percentage of patients with PDD has emotional symptoms [10,53] and that cognitively deteriorated PD patients perform more poorly in discriminating objects than healthy controls and cognitively preserved PD patients [47]. Thus, in addition to anxiety-like behavior in the elevated plus-maze test, the MPTP lesion-induced behavioral deficits in T-maze and object recognition tests may be similar to the anxiety, amnesia, and agnosia seen in PD patients. These data suggest that MPTP-lesioned rats may not only be a model for amnesia [73], but also for PDD [92,98]. Since DCS treatment significantly reversed the MPTP-induced behavioral deficits and since DCS has also been implicated as a “cognitive enhancer” in humans [36], this drug may have clinical potential in the treatment of PDD.

The hippocampus is essential for spatial navigation [106], recognition memory [11], working memory [39,85], and short-term memory associating objects and their locations [76]. Hippocampal dysfunction has been implicated in the visuospatial deficits observed in PD patients [27]. In agreement with previous reports [92,96,98], we showed that MPTP lesioning caused neuroinflammation in the brain and loss of pyramidal neurons in the hippocampal CA1 area. The decrease in neuronal density in the CA1 area may be involved in the MPTP-induced deficits in object recognition, as the improvement in this behavior after DCS treatment at the dosages of 30 and 100 mg/kg was accompanied by a protection of CA1 pyramidal cells from death. Similarly, enhancement of cognitive function by chronic DCS treatment at the dose of 30 mg/kg/day has also been observed in traumatic brain-injured-rats in the Morris water maze test [93], which requires intact hippocampal function [21]. In addition, acute treatment of DCS at the dose of 12 mg/kg increased the working memory and hippocampal-lesioned rats [85]. A previous study has reported that the involvement of hippocampal CA1 area in object recognition is delay-dependent because temporary inhibition of neuronal activity in this area impaired novel object preference in mice, after a 24 h but not a 5 min retention interval, indicating that the hippocampus is involved in encoding and/or retention of long-term object memory [31]. Since, in the current study, the intervals between object exposure sessions were 24 h, and the delay before novel object testing was 5 min, the treatment of DCS may have improved both encoding and retention of object memory. Moreover, the DCS–induced attenuation of neuroinflammation and restoration of the DAergic system may contribute to the preservation of motor and emotional behaviors and working memory in MPTP-lesioned rats, as the restoration of these behaviors by DCS treatment at all dosages used was associated with suppression of both microglial activation and the increase in IL-2 levels in the brain and with restoration of the density of DAergic neurons and terminals in the nigrostriatal system.

Clinical studies on PD patients have shown that IL-2 levels are increased in the SNC [63], caudate nucleus [61], and CSF [62]. MPTP is known to increase levels of cytokines [64,66], including IL-2 [98], in several brain regions in rodents, and this effect has been proposed to be involved in neuronal cell death in the DAergic system [64]. The present data showed that MPTP lesioning caused not only massive microglial activation, but also a widespread increase in IL-2 levels in the striatum, amygdales, and non-trofical cortex. DAergic degeneration in PD also causes microglial activation [68], which may be involved in the pathophysiological processes of PD by releasing inflammatory cytokines and leading to cell death [67]. Microglial activation is observed from day 1 to day 14 after MPTP-induced degeneration of DAergic neurons in the substantia nigra in mice [45], during which period the concentration of inflammatory cytokines, for example, IL-10 and IL-12, in the CSF is increased [104]. Degenerated neurons lead to further activation of microglia and aggravate the pathological pathway [97]. In addition, IL-2 is involved in cognitive function and neuronal development in the hippocampus [79] and plays a role in psychiatric disorders [55,58]. IL-2 immunotherapy elicits cognitive impairment [14,19], and repeated injection of IL-2 induces abnormalities in novelty-induced locomotion, learning, and spatial memory in the Morris water-maze test and altered exploratory activity in rodents [48,105]. Further, another study [74] and our previous studies [34,51] have shown that IL-2 levels are related to emotional behavior and that levels of striatal IL-2 mRNA are correlated with anxiety-like behavior in the elevated plus-maze test [70]. Striatal IL-2 microinjections can modulate anxiety-like behavior in the elevated plus-maze test [71] and induce avoidance behavior in the open field test [42]. Moreover, since the MPTP-induced cytokine
increase in the CSF of mice is observed during the first 2 weeks after lesioning [104] and since our animals were sacrificed one day after the last behavioral test on day 13 after MPTP lesioning, the IL-2 changes in this study were seen during the chronic stage. All dosages of DCS used in this study corrected the deficits of anxiety-like behavior and working memory in MPTP-lesioned rats and this was associated with restoration of IL-2 levels in the brain, suggesting that the increase in IL-2 levels may be involved in the MPTP-induced behavioral deficits, notwithstanding the fact that other factors are probably also involved. In addition, the anti-inflammatory effects of DCS, such as decreasing microglial activation, may also underlie the behavioral effects, in agreement with a previous report suggesting that inhibition of neuroinflammation is an important strategy for preventing cognitive decline in PD [89].

Antagonists of NMDA receptor, for example, amantadine [29] and ketamine [24] have been demonstrated to protect DAergic neurons in the SNc in an MPTP-induced PD animal model. Although amantadine has been used in the clinic for the treatment of PD [28] and Alzheimer’s disease [41], its clinical application is still controversial [78] because NMDA receptors are involved in a number of important neurobehavioral functions. This study showed that partial agonist of the NMDA receptors, DCS, may be a promising candidate for PD treatment. Further, the biphasic changes in gluta- matic activity after MPTP lesioning can also explain why chronic treatment of DCS at low dose (30 and 100 mg/kg), may through its agonistic activity on NMDA receptors, caused better effects than higher dose (200 mg/kg) on motor function, object recognition, and density of CA1 neurons in the current study. These data suggest that, at higher dose, DCS may have antagonistic effects on NMDA recep- tors, leading to a reduction in, or even lack of, efficacy. A previous study provides supports for this view, as a single administration of DCS at low dose (0.32 and 1.0 mg/kg) significantly improved spatial short-term memory during the chronic stage in MPTP-lesioned monkeys, but a higher dose (8 mg/kg) inhibited visual discrimi- nation [84]. Thus, our study provides support for the low-dose strategy when using DCS in the clinic [35,80].

5. Conclusions

The present study shows that DCS treatment improves MPTP-induced deficits in motor, emotional, and cognitive behaviors and restores neuroinflammatory and neurodegenerative changes, suggest- ing its potential application in the prevention of the neuronal changes and dementia associated with PD. Furthermore, DCS has a good safety profile and is already in use in humans for several indica- tions [20,32,46] and this could significantly facilitate its clinical application in PD patients.

Conflict of interest

The authors declare that there is no actual or potential conflict of interest in relation to this article.

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References


Hughes RN. Responsiveness to brightness change in male and female rats following treatment with the partial agonist of the N-methyl-d-aspartate (NMDA) receptor, d-cycloserine. Behav Brain Res 2004;152:199–207.


