Ameliorating Effect of Dimethylsulfoniopropionate on the 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-Induced Parkinson’s Disease of Mice

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Summary  The effect of dimethylsulfiniopropionate (DMSP) on the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson’s disease (PD) of mice was examined for 5 d. The distilled water (the control group) and the DMSP solution at 5×10⁻⁴ M (the DMSP group) were supplemented ad libitum to six mice each in two groups for 2 wk. An appropriate amount of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) solution (20 mg/kg body wt) was then intraperitoneally injected into all the test mice once a day initially for 3 d, which definitely made the control mice similar to the PD-model mice. The moving ability (running power) of the mice in both groups was measured using an automatic Wheel Running Instrument. The immobility duration of the upside-down mice in both groups was estimated by a newly developed polygraph (RMP-6008M, Nihon Koden Co., Ltd., Japan). The results indicated that the mice in the DMSP group showed a stronger moving ability and a shorter immobility duration compared to the mice in the control group during the experimental period. Furthermore, the amounts of catecholamines (dopamine and norepinephrine) in the brains except for the cerebellums of all the test mice were estimated 2 d after the last MPTP injection, which demonstrated that the brains of the mice in the DMSP group accumulated larger amounts of catecholamines, especially dopamine, than in the control group. Accordingly, the administration of low concentrations of DMSP proved to prevent and/or ameliorate the decreased mobility and the typical immobility (Akinesia) of the MPTP-induced PD-model mice probably due to increased amounts of dopamine in the brains of the DMSP group mice.

Key Words  dimethylsulfiniopropionate, Parkinson’s disease, mice, catecholamines, immobility

Parkinson’s disease (PD) has been known to result from the degeneration of the substantia nigra in the central nervous system of especially the aged, which causes a deficiency in dopamine in the substantia nigra, and later, the degeneration and/or inactivation of the noradrenergic-, serotonergic- and cholinergic-nervous systems in the central nervous system (1–3). The decrease of dopamine appears to be also caused by various origins, i.e., oxidative stress, environmental pollution, workplace conditions or dietary intake involving in the intake of isoquinoline derivatives (4). However, the amelioration of the disease is reported not to be completely affected even by the addition of L-dopa, a precursor of dopamine (2, 4). Although L-dopa is proven to be the most effective among the drugs used for curing this disease at present (1, 2). In contrast, the ingestion of dimethylsulfiniopropionate (DMSP), which widely occurs in seaweeds (5), especially green sea algae (6), proves to stimulate the mobility of rats and mice, and simultaneously accumulates large amounts of catecholamines in the brains and livers of rats (7) and mice (unpublished data) based on our previous experiments. We therefore examined the effects of catecholamines accumulated by the supplementation of DMSP on the PD-model mice induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrimidine (MPTP).

Materials and Methods

DMSP was synthesized by refluxing 3-bromopropionate and dimethylsulphide at 23°C for 24 h, which was then washed with chilled ethylether and crystallized from methanol. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyrimidine chloride (MPTP) was purchased from Wako Pure Chemical Industries, Ltd., Japan. All other chemicals were of the best quality available.

Twelve C57BL6 male mice at 15 wk of age (30.4±2.1 g) were purchased from CLEA Japan, Inc., Japan, raised at 23°C and a relative humidity of 60% for 1 wk.
Effect of Dimethylsuloniopropanionate on Parkinson’s Disease-Model

Fig. 1. The mobility of the mice with or without DMSP after the injection of MPTP. The DMSP solution at $5 \times 10^{-4} \text{M}$ and the distilled solution were given ad libitum to two groups of test mice for 2 wk. An appropriate amount of the MPTP solution was then intraperitoneally injected into all the mice once a day initially for 3 d. The mobility (running power) of the mice in both groups was determined by a Wheel Running Instrument and expressed in terms of turning numbers (means±SD, n=6). The values at the start of the experiments were 7.6±1.51 and 10.5±1.41 of the control and DMSP groups (means±SD, n=6), respectively. The marks, ○ and □, show the values of the mobility in the control and DMSP groups after the start of the experiments, in which the values obtained by the 1st, 2nd, 3rd and 4th determination in this order are shown on the vertical axes at 0, 1, 2 and 5 d. The symbols, * and **, show significant differences (p<0.05) from the control values at the indicated times and the corresponding start values, respectively. For experimental conditions see details in Materials and Methods.

and fed distilled water and solid diets (“M,” Oriental Yeast Co., Ltd., Japan). After 1 wk, the mice were divided into two groups; one was fed the distilled water (the control group) and the other was fed the DMSP solution at $5 \times 10^{-4} \text{M}$ (the DMSP group) for a further 2 wk. Thereafter, the MPTP saline solution (0.5 mL) was intraperitoneally injected into all the test mice in the control and DMSP groups at the ratio of 20 mg/kg body wt as a daily dose from 5:00 p.m. for three consecutive days from the day before the start of the experiments. From the next day (the first determination day) after the first injection of MPTP, the determination of the mobility (running power) of the mice was initiated at 10:00 a.m. at 1, 2, 3 and 5 d after the first MPTP injection (four determinations), using a Wheel Running Instrument (15 cm) (motor: Kato Power Pack Standard Model No. 22-011) made in-house which electrically turns at the constant rate of 10 rpm and expressed in terms of the continuous turning numbers. The estimation of the immobility duration of the mice was begun at 2:00 p.m. on the same days as those for the determination of running numbers, using a polygraph (RMP-6000M, Nihon Koden Co., Ltd., Japan) under the stated determination conditions (high and low cut: 30 and 0.3 Hz, sensitivity: 0.5 and 2 mV, determination time: 6 min), in which the test mouse was placed upside down and the tail of the mouse was suspended by connecting it to the bottom edge of a vertical spiral wire tied to a horizontal thin iron bar of a round sensor box (CP-2US10, Midori Precisions Co., Ltd., Japan) 37 cm from the floor in order to record the moving stimuli. The duration of the immobility (total times (s)/6 min) after the determination of the mobility were calculated using a computer (Think Pad 2656-41), IBM Co., Ltd., USA) and software (Bimutas II, Kissei Comtec Co., Ltd., Japan).

The running numbers and the duration time of immobility were determined 1, 2, 3 and 5 d after the initial MPTP injection and the values obtained are shown on the vertical axes at 0, 1, 2 and 5 d (Figs. 1 and 2), respectively.

To estimate the amounts of catecholamines in the brains without the cerebellum of the mice, all the mice were subjected to decapitation 2 d after the last injection of MPTP, and their brains and then cerebellums were immediately removed under cold (0°C) conditions and stored in a freezer (−20°C) before use. After thawing, an appropriate sample of the whole brain without cerebellum was suspended in a mixed solution (0.1 wet g/0.5 mL) containing ethylenediaminetetraacetate (EDTA-2Na) at 0.1 M in a 0.2 M perchloric acid solution, which was homogenized while cold (0°C) using a Kinematic Polytron (PT10-35) and an Aggregate (PCU-11) (Kinematica Ag Littau, Switzerland) for 30 s. The mixture was centrifuged at 4°C and 26,000 rpm for 10 min using a Beckman T-100. The supernatant was filtered through a Chromatodisc (pore size 0.45 μm, Kurabou Co., Ltd., Japan). The filtrate (10 μL) was used for the analyses of the catecholamines by high performance liquid chromatography (LC-6A) using a Chromatopack (SCL-6A) (Shimadzu Co., Ltd., Japan) and an electric chemical detector (Shiseido Nanosystem S1-2, Shiseido Co., Ltd., Japan) under the stated conditions (column: Type 120 (highly purified silica gel) (4.4 φ
Fig. 2. Duration of immobility of the mice with or without DMSP after the injection of MPTP. The duration of the immobility in both groups was determined for 5 d. The values are shown in terms of the ratio (%) of the total duration times (s/6 min) of the immobility at the indicated times versus those at the start of the experiments. The values at the start of the experiments were 71.6±24.0 and 41.2±19.8 s/6 min of the control and DMSP groups (means±SD, n=6), respectively. The marks, symbols and other experimental conditions are the same as described in Fig. 1. For the experimental conditions, see the details in Materials and Methods.

Fig. 3. Amounts of norepinephrine, dopamine and serotonin in the brains of the mice with or without DMSP after the injection of MPTP. The amounts of norepinephrine, dopamine and serotonin in the brains of the mice in the control and DMSP groups were estimated 2 d after the last MPTP injection. The values are shown in terms of the amounts (μg/g wet brain) of the catecholamines (means±SD, n=6). The symbols, * or ‡, show significant differences (p<0.05) (U-tests of Mann-Whitney or ANOVA and Fisher’s PLSD-tests) from the control values. The experimental conditions are the same as shown in Fig. 1. For the experimental conditions, see the details in Materials and Methods.

mm×15 cm), mobile solution: mixed solution containing 13.6 g KH₂PO₄, 0.15 g octanesulfonic acid-Na, 65 mL of 2% H₃PO₄, 10 mg EDTA-2Na, 60 mL acetonitrile in 1 L of ultrapure water, flow rate: 0.3 mL/min, attenuation: 8, detection sensitivity: 700 mA).

The care and treatment of the experimental animals were in accordance with the guidelines of the Animal Center of the National Research Council for the Care and Use of Laboratory Animals (1985) (8).

The analytical procedures were performed using the ANOVA and Fisher’s PLSD-test (Figs. 1, 2 and 3) and the U-test of Mann-Whitney (Fig. 3).

Results

The mobility (running power) of the mice in the control and DMSP groups was estimated from the day after the injection of MPTP. These results are shown in Fig. 1. The mobility of the mice in the DMSP group was evidently stronger than that in the control group. The statistical analyses indicated that the mobility of the mice in the DMSP group was almost unchanged but that in the control group clearly decreased with the increasing experimental times, compared to the corresponding start values of the experiments. Furthermore, the analyses also demonstrated that the running power of the mice in the DMSP group become higher than that of the mice in the control group with the increasing times. The duration of the immobility of the upside-down mice in the control and DMSP groups was estimated for 6 min from the day after the MPTP injection. These results are shown in Fig. 2. The statistical analyses demonstrated that the values of the mice in the DMSP group were much lower than those in the control group, and further indicated that the mice in the DMSP group showed almost no increase while the mice in the control group had a fairly higher immobility duration during the experimental period. Furthermore, the amounts of catecholamines (dopamine, norepinephrine, serotonin) in the brains of the mice in the control and DMSP groups 2 d after the last injection of MPTP were determined. These results are shown in Fig. 3. The amounts of dopamine and norepinephrine, especially dopamine, in the DMSP group mice were clearly higher than those in the control group mice, showing more than 2-fold the amounts of the mice in the control group.
Effect of Dimethylsulfoxoniopropionate on Parkinson’s Disease-Model

Discussion

The effects of a low concentration (5 × 10⁻⁴ M) of DMSP, which was effectively used in the experiments with rats (7) and senescence-accelerated mice (9, 10), on the PD-model mice caused by MPTP were investigated for 5 d. The results showed that the mobility (running power) of the mice in the DMSP group remained almost unchanged compared to that at the start of the experiments, while that of the mice in the control group gradually decreased during 5 d. The statistical analyses of these facts show that the injection of MPTP produced some damage to the mobility of the mice in the control group although the injection afforded almost no effect on the mobility of the mice in the DMSP group during the 5 d. The facts may demonstrate that not the motor dysfunction (a significant property of PD) of the mice in the DMSP group but that of the mice in the control group occurred during the experimental period (1, 11, 12). The duration of immobility (Akinesia), being one of the typical characteristics of PD which have been described in many reports (1, 11, 12), was further determined by a newly developed method with a polygraph and a sensor box using the upside-down mice in the control and DMSP groups at the indicated times after the MPTP injection. The method is one of the tail suspension test frequently used in the depression-like animals (13) but developed by connecting the tail of the mouse and the sensor box with a spiral wire, which can mitigate the body movements recording not so sensitive movements as too sensitive movements of the mouse. The results indicated that the mice in the control group showed a longer immobility duration while the mice in the DMSP group exhibited almost no change with the increasing rearing times to the end of the experiments, when compared to the corresponding start values in the experiments. Moreover, the immobility duration of the mice in the control group was clearly longer than that of the mice in the DMSP group during the short experimental period. These facts may demonstrate that the mice in the control group suffered from PD by the injection of MPTP under the experimental conditions. However, the oral supplementation of DMSP to the PD-model mice fairly reinstated the behaviors (mobility and immobility) occurring in the PD-like mice.

On the other hand, the amounts of dopamine and norepinephrine in the brains without the cerebellums of the mice with MPTP and DMSP, which were intraperitoneally and orally supplemented, respectively, appeared to be greater than those in the mice in which only MPTP had been injected. The fact that the supplementation of DMSP accumulated larger amounts of dopamine in the brains of the PD-model mice may demonstrate that larger amounts of dopamine fairly reinstated the latent mobility and the characteristic symptom (Akinesia) of the PD-like mice for the short experimental period. Moreover, the amounts of serotonin in the DMSP group mice tended to be larger than those in the control group mice, which may suggest that the serotonergic system sympathetically functions with the dopaminergic systems in the PD-model mice under the experimental conditions (14). In contrast, the norepinephrine amounts of the mice in the DMSP group were larger than those of the mice in the control group. Our previous experiments indicated that the supplementation of DMSP accelerates the swimming power and the growth in fish (15) and the mobility in rats (7) and simultaneously accumulates norepinephrine in their brains. Moreover, there are reports that the destruction of norepinephrine terminals in brains decreases the locomotion activity in MPTP-treated mice (16) and that noradrenaline modulates the MPTP toxicity in norepinephrine transporter knockout mice (17). These facts may imply that the elevation of norepinephrine amounts also functions so as to increase the running power and to shorten the immobility duration in the PD-model mice of the DMSP group in the present experiments.

DMSP proves to function as an antioxidant (18, 19), an activator of immunity (20) and a stimulant by increasing the amounts of the catecholamines (7, 15). Accordingly, the prevention and/or curing of the PD-model mice by DMSP is considered to be principally attributable to the donation of dopamine and probably of norepinephrine, and to the antioxidant activity of DMSP in the brain of the mice (21). In contrast, DMSP has been customarily ingested through various seafoods (5), especially green sea algae that contain it in large amounts (6), by a number of people in the world, especially in Japan, for many years (22). The more uptake of DMSP itself and DMSP-containing foods is considered to surely exert preventative and/or ameliorating effects on the PD of humans because the characteristics of the PD-model mice induced by MPTP are reported to be very similar in mice (23, 24), monkeys (1, 25) and humans (1, 25).

A detailed experiment is needed to elucidate the ameliorating mechanisms of PD-model mice by DMSP.

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Chronic parkinsonism in humans due to a product of