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L-DOPA-induced behavioral sensitization of motor activity in the MPTP-treated common marmoset as a Parkinson's disease model

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ABSTRACT

L-DOPA is the gold standard for treatment of Parkinson's disease (PD). However, the drug produces some serious side effects, including dyskinesia, which is characterized by repetitive involuntary movements—including chorea. In the present preclinical study using a nonhuman primate model, dyskinesia caused by repeated L-DOPA administration was investigated in the context of behavioral sensitization by objectively quantifying motor activity in the common marmoset of PD model (the Parkinsonian marmoset). Twelve male Parkinsonian marmosets previously treated with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and six intact male marmosets were used. The motor activity of each marmoset was measured using infrared sensors attached to each individual living cage. Parkinsonian marmosets (n = 6) exhibited behavioral sensitization (enhanced motor activity) in 10 weeks upon oral administration of L-DOPA (10 mg/kg per day on 3 days/week). These animals also exhibited dyskinesia characterized by repetitive rapid movements including chorea in 6–10 weeks. Neither behavioral sensitization nor dyskinesia was observed in Parkinsonian marmosets given vehicle and in intact marmosets given L-DOPA at the same dose (both n = 6 each). Behavioral sensitization was detected sensitively and objectively on motor activity only in Parkinsonian marmosets given repeated L-DOPA at a similar dose used in PD patients. The behavioral feature of the marmosets was dyskinesia similar to that of PD patients but appeared earlier than would be manifested in humans. In spite of statistically significant behavioral sensitization, some marmosets did not exhibit dyskinesia in the present limited L-DOPA administration period. Although both commonalities and differences may exist between behavioral sensitization and dyskinesia, behavioral sensitization is considered to be an objective, quantitative, sensitive and predictive measure of behavioral mechanism underlying dyskinesia in preclinical studies in evaluating compounds.

1. Introduction

Parkinson's disease (PD) is a movement disorder caused by degeneration of the nigrostriatal dopaminergic neurons of the brain, for reasons that are not understood. These neurons, when healthy, play an important role in smoothing both voluntary and involuntary movements. Thus, degeneration of the neurons has behavioral consequences including immobility, tremor, positional dysfunction, and muscle rigidity, which, together, are termed Parkinsonism or PD-signs. L-DOPA, a therapeutic drug, has served as the "gold standard" for PD treatment for half a century (Fehling, 1966; Fox et al., 2011). However, it was reported in the 1960s that long-term medication of this drug caused development of abnormal and excessive involuntary movements termed dyskinesia (Cotzias et al., 1969; Mones et al., 1971; Treciokas et al., 1971; Huot et al., 2013), as well as other side-effects including wearing-off and on–off phenomena (Lesser et al., 1979; Zappia et al., 1999). Dyskinesia is a collection of various complex abnormal movements characterized by chorea, dystonia, and athetosis (Ellrichmann and Russ, 2007; Jackson et al., 2004) and is distinct from Parkinsonism although the etiology of dyskinesia is not wholly understood.

As definitive knowledge of the causative mechanism of dyskinesia is lacking, much effort has been devoted to develop efficacious PD treatments by drugs that do not have side-effects including dyskinesia (Adamiak et al., 2010; Ahlskog and Muenter, 2001; Muller, 2012, 2013; Kalinderi et al., 2011; Sprenger and Poewe, 2013). Treatments other than pharmacotherapy have also been employed; these include pallidotomy (Alvarez et al., 2009), deep brain stimulation (Fox et al., 2011; Terzic and Abosch, 2012; Miocinovic et al., 2013), and nerve cell transplantation (Dunnett and Rosser, 2011). Recently, dopamine neurons derived from induced pluripotent stem (iPS) cells are expected to transplant into the brains of PD patients. Such therapy carries great promise, because iPS cells can develop into dopamine neurons and these are unlikely to be rejected by the brain tissues if generated from the stem cells of the same individual (Nishimura and Takahashi, 2013).

In cooperating with the above undertakings, sensitive and valid animal models of PD are essential for preclinical testing of drug candidates to explore possible therapeutic effects with minimum side effects.

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Nonhuman primates treated with peripheral 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are generally recognized as valid PD models because the primates develop long-lasting Parkinsonism caused by dopaminergic neural degeneration in the brain (Langston et al., 1984; Fox and Brotchie, 2010). Notably, the MPTP-treated common marmoset, a small primate equivalent in body size to an adult rat, is considered to be a useful, valid, and sensitive model for preclinical evaluation of drug efficacy (Jenner, 2009; Eslamboli, 2005).

The particular characteristics and advantages of the marmoset model have been discussed in detail elsewhere (Ando et al., 2008, 2012). Among them, a normal marmoset is behaviorally active during light-on daytime but not at night showing the similar circadian rhythm as humans but not as rodents. After MPTP administration, daily spontaneous motor activity count in individual living cage measured by a motion sensor decreases markedly and continuously over several months or more. The decreased level of motor activity as a stable behavioral baseline is considered to be an objective and quantitative measure of immobility, one of the main PD-signs. By administration of L-DOPA, for example, the decreased level temporarily increases to normal level at doses comparable to clinical ones. Thus, improving the effect of L-DOPA on immobility could be detected objectively and quantitatively by motor activity measure in combination with subjectively evaluated visual inspection based on score items. At higher doses of L-DOPA, however, motor activity increases much over the normal level with the manifestation of hyper-excitability. Based on these facts, a question arises whether L-DOPA at comparable clinical doses when given repeatedly causes hyper-excitability leading to dyskinesia. If this excitability can be detected as behavioral sensitization of motor activity quantitatively and objectively (Wise and Leeb, 1993), it may help for understanding, measuring and predicting dyskinesia in the preclinical evaluation of new compounds.

The purpose of the present study was to examine behavioral sensitization of motor activity by repeated administration of L-DOPA at comparable clinical dose in the MPTP-treated marmoset and also to examine the relationship between behavioral sensitization and dyskinesia.

2. Methods

2.1. Ethics statement

The protocol of the present study was reviewed by the Institutional Animal Care and Use Committee and approved by CIEA (CIEA approval no. 07028A). The criteria used by the committee complied with those mandated by the Japanese Law for the Humane Treatment and Management of Animals. The present study was conducted under the principle of the three Rs (Replacement, Refinement and Reduction) of humane animal experimentation (Balls et al., 1995) and also conducted in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals, National Research Council, U.S.A.

2.2. Animals

Eighteen male common marmosets (*Callithrix jacchus*) obtained from CLEA Japan, Inc. (Tokyo, Japan), or born and reared in CIEA, were used in the present study. Among them, twelve animals were selected from MPTP-treated marmosets used in other studies where behavioral observation was performed. They had received the MPTP administration regimen (2 mg/kg s.c., once daily for 2 or 3 consecutive days) two-to-four times at intervals of several weeks during a 1.5 year period, resulting in cumulative MPTP doses of 12–22 mg/kg. During the MPTP administration regimen, and over the subsequent 2-week periods, supplements were given to compensate for anorexia and dehydration caused by acute MPTP toxicity. The details have been described elsewhere (Ando et al., 2012). These twelve Parkinsonian marmosets had never received repeated administration of a drug other than MPTP. These marmosets were used for the present study four months after the last MPTP regimen. Other than the above twelve Parkinsonian marmosets, six other marmosets were used. They were experiment naïve and were selected as intact in the present study.

During the present experiment period, each marmoset was housed in an individual stainless steel cage (30 × 50 × 48 cm). The walls and floor were made of wire mesh. Each cage was placed in a box in the same animal room. Each animal was given 50 g/day of a balanced diet (CMS-1M; CLEA Japan, Inc.). Tap water was delivered ad libitum via a valve. The temperature and humidity in the animal room, recorded each morning prior to cage washing, were 24–27 °C and 53–76%, respectively. The room was illuminated from 9 a.m. to 9 p.m.

2.3. Grouping of animals in two experiments

Twelve MPTP-treated marmosets showing typical and stable Parkinsonism (including tremor, immobility, and jerky reactions) were randomly grouped into two groups of six each. These groups were 1) Parkinsonian marmosets given L-DOPA repeatedly and 2) Parkinsonian marmosets given vehicle repeatedly. The third group was 3) intact marmosets (n = 6) given L-DOPA repeatedly. L-DOPA was given at 10 mg/kg per day on 3 days/week for 10 weeks. Vehicle was given in the same manner as L-DOPA to group 2.

The mean body weight (with standard deviation) of the 18 animals was 342.9 ± 39.6 g on the day of grouping. No statistically significant differences in body weight among the three groups were apparent upon analysis of variance (ANOVA) [$F(2, 15) = 0.444$, $p = 0.650$]. The mean (with standard deviation) ages of the Parkinsonian marmosets given L-DOPA and vehicle repeatedly were 5.6 ± 1.7 and 4.9 ± 1.3 years, respectively. These ages did not differ significantly. The mean (with standard deviation) age of intact marmosets, however, was 1.9 ± 0.1 years, thus different from the ages of the other two groups of Parkinsonian marmosets. These adult intact marmosets reached full growth at younger ages and were as healthy as the other marmosets in the 2 groups.

After completion of the repeat-dose-L-DOPA experiment (experiment 1), the same marmosets were used for the 2nd experiment to determine dose–effect and time-course–effect relationships of L-DOPA (0, 5, 10, or 20 mg/kg, tested in random order with 1 week interval between each test) using a measure of the motor activity count (as in the first experiment).

2.4. Drug, preparation and administration

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) hydrochloride powder (Sigma-Aldrich, St. Louis, MO) was dissolved in physiological saline at concentrations such that each marmoset received a dose of 2 mg/kg s.c. in a volume of 1 ml/kg.

L-DOPA (L-3,4-dihydroxyphenylalanine) solution was purchased as Neodopaston combination tablets (Sankyo Co., Ltd., Tokyo, Japan, at the time of purchase; presently Daiichi Sankyo, Co., Ltd., Tokyo, Japan). Each tablet contained 100-mg L-DOPA and 10-mg carbidopa. L-DOPA solutions were prepared immediately before administration by crushing a tablet into minute particles followed by suspension in 2.5% (w/v) Gum Arabic solution at concentrations such that animals received oral doses of 5, 10, or 20 mg/kg in a volume of 2 ml/kg. Oral administration was performed using an intra-gastric tube (designed for use with human infants), which was passed via the mouth into the stomach of the marmoset. A pencil was placed in the mouth beforehand to prevent biting of the tube.

2.5. Experiment 1: repeated L-DOPA administration

The aim of this experiment was to observe whether behavioral sensitization developed in Parkinsonian marmosets given repeated L-DOPA but neither in Parkinsonian marmosets given vehicle nor in intact marmosets given L-DOPA.