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# Neuron-protective effect of subanesthestic-dosage ketamine on mice of Parkinson's disease

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ABSTRACT

**Objective:** To discuss the neuron-protective effect and possible mechanism of subanesthestic-dosage ketamine on Parkinson's disease mice induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

**Methods:** A total of 30 mice were divided equally into three groups, model control group (MC group), ketamine treatment group (KT group), and blank control group (BC group), respectively. The Parkinson's disease mice of MC group and KT groups were established by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (20 mg/kg/d), while mice in KT group were treated by intraperitoneal injection of subanesthestic-dosage ketamine (8 mg/kg). Differences on behaviors and the number of nigra dopaminergic neurons of mice in each group were compared through the behavioral test and tyrosine hydroxylase immunohistochemistry experiments after the treatments. Furthermore, Western blot was used to test the expression of autophagy-related gene LC3-Ⅱ, Beclin1, Parkin, PINK1, and mTOR.

**Results:** Compared with the BC group, the neuroethology scores were lower and the amount of TH positive cells were less both in MC and MT groups; In KT group, the neuroethology scores were higher and the amount of tyrosine hydroxylase positive cells were significantly more than that in MC group ($P < 0.05$). Moreover, expression levels of autophagy-related proteins LC3-II, Beclin1, Parkin, and PINK1 were higher, while the mTOR expression level was lower than that in MC group.

**Conclusions:** The subanesthestic-dosage ketamine has some protective effects on the coordinating ability of movement and cognitive ability of Parkinson's disease mice induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. This is probably due to that the autophagy activity of cells is activated by subanesthestic-dosage ketamine and that the neurons are protected.

## 1. Introduction

Parkinson's disease (PD) is a neurological disease with long-term degenerative disorder of central nervous system that mainly affected the motor system. It has four essential characteristics, such as static tremor, rigidity, bradykinesia and postural unsteadiness [1], and the primary pathogenesis is that the level of dopamine is reduced by progressive death of neurons in the substantianigra pars compacta (SNpc) [2]. At present, genetic studies suggested that PD morbidity was closely associated with genetic factor, environment, abnormal accumulation of proteins, oxidative stress, and other factors [3–6], but the exact cause and pathogenesis was still not clear. Levodopa drugs are mainly used to improve PD's symptom clinically, but long-term use of levodopa can cause adverse reaction such as disorders in movement and cognition or 'on-off' phenomenon [7]. Therefore, it is the focuses of current research that seeking for the new neuron-protective drugs to treatment PD.

As a commonly-used antalgic anesthetic, ketamine is also the non-competitive antagonist of the *N*-methyl-D-aspartic acid receptor. For its complex pharmacological effects, different dose

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and usage have different functions on the neuronal system [8]. The latest research showed that subanesthestic-dosage ketamine could promote the synthesis and transport of dopamine, protect neurons of PD rats and reduce disorders in the cognitive function after surgeries [9]. Currently, there are few studies on the effects of long-term use of subanesthestic-dosage ketamine on PD patients. In this study, C57BL/6 mice were treated by 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intraperitoneal injection to establish the PD models, and intervened by subanesthestic-dosage ketamine at the same time. Then, according to the results of behavioral tests and biochemical experiments, the influences of subanesthestic-dosage ketamine on behavior, cognition, and nigra dopaminergic neuron of PD mice were discussed.

## 2. Materials and methods

### 2.1. Animals

A total of 30 6–8 weeks old male C57BL/6 mice at SPF level (provided by Laboratory Animal Center of Henan Province, license number: SCXK (Yu) 2015-0004) were selected. The mice were randomly divided into three groups, model control group (MC group), ketamine treatment group (KT group), and blank control group (BC group), with 10 in each group.

### 2.2. Experiment methods

#### 2.2.1. Modeling method

MPTP was used to establish the PD mice model. First day, each mouse in KT group received intraperitoneal injection of ketamine 8 mg/kg, while the same amount of normal saline was injected to each mouse in MC group. In the following 7 d, 8 mg/kg ketamine was injected to each mouse in KT group and the same amount of normal saline was injected to each mouse in MC group (the same as first day), 20 min later, each mouse was injected with 20 mg/kg MPTP in the two groups. For the BC group, 8 mg/kg normal saline was injected to each mouse for 8 d. Then 1–2 h later after the last injection, the behavioral tests and biochemical experiments were conducted in succession.

#### 2.2.2. Behavioral tests

(1) Rotarod test: The duration that each mouse stayed on the rotating bar at different rotate rate was record, which was used to assess the behavior ability (balance ability). The rotate rate was set as 19 r/min, 26 r/min, and 38 r/min respectively. The maximum duration was limited with 5 min. The test was conducted 3 times at an interval of 1 min for each time and the average was calculated. (2) Swimming experiment: This experiment was used to test the limb coordination ability of mice. Each mouse was placed in an organic-glass water tank (20 cm × 30 cm × 20 cm), with 10 cm-deep water at the temperature of 22–25 °C. Standard for evaluation: 3 score (swimming without interruption), 2.5 score (swimming with occasional floating), 2 score (floating for half of the testing time), 1.5 score (swimming occasionally), and 1 score (occasionally swimming using their hind limbs and floating around). The test was conducted for 3 min at an interval of 2 min for each time, and then the average scores were taken. (3) Water meze test: Place navigation test was conducted to evaluate the learning and memorizing ability. Each mouse was put in to the water meze from the first quartile facing to the pool wall, and the time (Escape Latency, EL) used to find the platform was record during 2 min. This test was conducted twice a day, total 3 d, the average time was obtained from the 6 times.

### 2.3. Immunohistochemistry

After the behavioral tests, 30 mice were sacrificed and brain tissues were taken out and fixed in paraformaldehyde (4%) for 48 h. Then, paraffin imbedding, slicing, and conventional dewaxing were performed. The samples were hatched for 10 min in $H_2O_2$ (3%), and then rinsed by PBS. After the tissue antigen recovery, the tissues were kept with fetal bovine serum (5%) for 30 min, and hatched overnight by rabbit-anti TH monoclonal antibody (1:300) at 4 °C. Then, the secondary antibody was incubated (1:500) for 40 min. DAB colored, and conventional sealing were performed. Five blocks were randomly selected from each group, 3 consecutive slices at the same area of each block were selected, and 3 pictures were randomly selected from each slice. The average number of SNpc TH-positive neurons in each group was calculated.

### 2.4. Western blot

The total brain tissue protein was extracted by tissue protein extraction kits, and concentration was detected by BCA. The expression of mTOR, LC3-II, Beclin1, Parkin and PINK1 were detected by Western blot. Primary antibody: mTOR polyclonal antibody, LC3 polyclonal antibody, Beclin1 polyclonal antibody, Parkin polyclonal antibody, PINK1 polyclonal antibody (1:1000), and rabbit anti-mouse β-actin antibody (Abnova) (1:2000). Second antibody: HRP goat anti rabbit IgG or HRP goat anti mouse IgG (1:3000). And relative expression quantity of each protein was calculated by Image J 2x.

### 2.5. Statistical analysis

All data was analyzed by SPSS 19.0. Measurement data was shown as average ± standard deviation (mean ± SD) and K−S was used to check whether the measurement data was consistent with the normal distribution. If yes, independent *t*-test was used to analyze the differences between two groups, and one-way ANOVA was taken to analyze the differences among three groups. If not, rank-sum test was applied to analyze the difference. $P < 0.05$ was considered that the difference was statistically significant.

## 3. Results

### 3.1. Behavior test results

The results of behavior tests were shown in Table 1. Compared with BC group, there were various degrees of neurobehavioral changes in the mice of MC and KT groups after MPTP intraperitoneal injection, such as tremor, hollow back, hypotonia, hypokinesia, which was the typical parkinsonian symptom. There were significant differences in the behavioral abilities among BC group, MC group and KT groups. Compared with MC group, mice in KT group were with significantly longer stay on the rotating bar, shorter average latency in the water meze test, and higher score in the swimming test. The results

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