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Research report

Environment-contact administration of rotenone: A new rodent model of Parkinson's disease

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HIGHLIGHTS

- A new model of PD was developed using environment-contact administration of rotenone in mice.
- The new model reproduces progressive nonmotor and motor deficits relevant to human PD.
- The new model induces many of the neuropathological features of human PD.
- The new model is not associated with overall animal ill health and systemic toxicity.
- The new model mimics the common ways of pesticides entering the human body.

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ABSTRACT

Epidemiological studies suggest an association between pesticides and the incidence of Parkinson's disease (PD). Individuals are likely to be exposed to numerous natural or synthetic environmental agents by ingestion, inhalation, or skin contact. Here, we describe a novel environment-contact administration of rotenone model, in which male C57BL/6 mice (15 per group per time-point) were placed in one bedding-free, rotenone-applied cage for 2 h every day over a period of 2–6 weeks, mimicking the common ways a person may be exposed to pesticides. Our results showed that rotenone exposure had no detrimental effect on body weights of mice during 6 weeks, nor did it cause systemic toxicity (HPLC analysis of rotenone in blood and brain, as well as complex I activity measurements in brain and muscle), but it caused significant impairments in motor function (open field test, pole test, and rotarod test) from 4 weeks that were responsive to apomorphine. Accordingly, rotenone caused significant dopamine depletion from the striatum (HPLC analysis), nigrostriatal degeneration (quantitative tyrosine hydroxylase immunohistochemistry and western blot), and accumulation of α-synuclein in the substantia nigra and striatum (α-synuclein immunohistochemistry) in a time-dependent manner. In addition, rotenone-exposed mice also developed deficits in gastrointestinal and olfactory function (fecal pellet output and buried food pellet test) prior to the motor dysfunction. Furthermore, we observed that α-synuclein accumulated in the anterior olfactory nucleus and the enteric nervous system at 2 weeks. In summary, this novel rotenone model was able to reproduce many key aspects of PD progression. Therefore, it provides new insight into how environmental factors could trigger PD and provides a useful tool for studying PD pathogenesis and testing neuroprotective strategies.

Abbreviations: PD, Parkinson's disease; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; TH, tyrosine hydroxylase; SNpc, substantia nigra pars compacta; GI, gastrointestinal; ENS, enteric nervous system; OB, olfactory bulb; AON, anterior olfactory nucleus.

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1. Introduction

Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder. It is characterized by relatively selective degeneration of dopaminergic neurons in the substantia nigra and loss of dopamine (DA) in the striatum resulting in resting tremor, rigidity, bradykinesia and postural instability [1,2]. A pathological hallmark of PD is the accumulation of filamentous, cytoplasmic inclusions consisting mainly of α-synuclein aggregations in the form of Lewy bodies or Lewy neurites [3,4]. While the causes of sporadic PD, which accounts for about 90% of cases, remain unknown, multiple epidemiological studies suggest an association between pesticides and the incidence of PD [5].

Many studies have used different pesticides and different routes of administration in order to reproduce pathological and clinical findings of PD in animals [6,7]. One of the most promising models to have emerged in recent years uses systemic injection of rotenone, a member of a class of compounds found in pesticides, and in natural products, that disrupts mitochondrial activity and results in pathology that is strikingly similar to that seen in PD (e.g., selective nigrostriatal neurodegeneration, accumulation and aggregation of α-synuclein and ubiquitin), in rats [8]. Despite several attempts of improving the model (see [9,10] for recent reviews), recently developed rotenone models provide limited information on the risks of environmental exposure, including oral, dermal and inhalation routes [11], because they use non-natural pathways of pesticide exposure, such as intraperitoneal, subcutaneous, or intravenous injection. On the other hand, although previous publications demonstrated that acute or subchronic administration of rotenone was not able to cause the neuropathological characteristics of PD in mice [12,13], many researchers reported that chronic oral administration of rotenone at 30 mg/kg caused specific nigrostriatal DA neurodegeneration in C57BL/6 mice [14,15]. This C57BL/6 strain has been frequently used for PD-related transgenic or knockout mice. Therefore, rotenone exposure in genetic mouse models of PD may allow investigations of possible interactions between environmental and genetic factors. In contrast, the rat model does not allow such investigations, because transgenic rat models of PD are not yet available.

These ideas prompted us to develop and characterize a novel rotenone mouse model that not only closely recapitulates the clinical and pathological features of PD, but also mimics the common ways of pesticides entering the human body. In the present study, C57BL/6 mice were directly exposed to rotenone dissolved in olive oil, which was called environment-contact administration. Here we present the experimental details of the model and characterization of its histological, neurochemical, and behavioral features.

2. Material and methods

2.1. Animals

Male C57BL/6 mice aged between 8 and 9 weeks were used for all experiments (Charles River Co., China). Mice were raised on a 12-h light/dark cycle, with food and water available ad libitum and were housed in groups of five per cage. All experiments were performed in accordance with the guidelines established by the National Institutes of Health for the care and use of laboratory animals and were approved by the Animal Care Committee of the Peking Union Medical College and Chinese Academy of Medical Sciences.

2.2. Experimental apparatus and rotenone exposure

The apparatus was modified from standard bedding-free laboratory mouse cage (500 sq. cm). The cage lid, wrapped with scotch tape, was inverted over the cage (Fig. 1A and C). The tape was used to prevent mice from holding on to the cage-top; gaps were left at corners for ventilation. The rotenone (Sigma, Saint Louis, MO, USA) solution was first prepared as a 50× stock in 100% dimethyl sulfoxide (DMSO) and diluted in olive oil (Torres Y Ribelles, S.A.). The solution was applied evenly over the bottom of the apparatus at 10 ml/cage and control animals received the vehicle only (2% DMSO in olive oil). Fifteen mice were placed in one cage for 2 h every day (8–10 am) in the dark (Fig. 1B and C). The crowded rotenone environment made each mouse move around and exposed to the compound sufficiently. The dose of rotenone was calculated by dividing the amount of rotenone dissolved in 10 ml olive oil by total body weights of 15 mice in one cage. In this study, rotenone was administered daily at 5 mg/kg over a period of 2–6 weeks, which was determined by the survival rate experiment described below. After rotenone exposure, mice were returned to home cage with normal density (5 mice per cage). In pilot experiments, mice became clean and tidy within the following 22 h.

2.3. Experimental design

In pilot studies (the survival rate experiment), rotenone was tested over a range of doses (5, 10, 30, and 100 mg/kg/day). Male C57BL/6 mice ($n = 15$ per group) were exposed to rotenone according to the methods described above for 14 days. Animal weights and survival rate were determined daily during the period. The survival rate of rotenone-exposed mice at 5 mg/kg for 14 days did not change during the experimental period, as similar with vehicle-exposed mice (Fig. 1D). Therefore, the dose of 5 mg/kg was used in the studies.

As shown in Fig. 1E, mice received rotenone (5 mg/kg) or vehicle administration once daily for 2 weeks ($n = 15$), 4 weeks ($n = 30$), and 6 weeks ($n = 30$), respectively. The effects of rotenone exposure on behavioral performances of the animals in open field test, pole test and rotarod test were measured at the indicated time point. After behavioral tests, mice were sacrificed and brain tissues were collected for biochemical examinations. In addition, gastrointestinal (GI) function and olfactory function were monitored after 1 and 2 week(s) of rotenone or vehicle exposure.

2.4. Behavioral examination

The body weight and general health condition were monitored daily during the period of exposure to rotenone or vehicle. No animal died during this period. All behavioral testing occurred during the light phase of the 12-h light/dark cycle.

2.4.1. Open field test

Open field test was conducted as previously described [16,17]. The apparatus consisted of a rectangular area of 50 × 50 cm surrounded by a 30 cm high wall. The area was divided into 25 squares of 10 × 10 cm. The mouse was placed in the center of the open field and its activity during the subsequent 5 min was assessed. Horizontal locomotion (number of square crossings), and frequency of rearing (sometimes termed rearing activity) were observed. The apparatus was cleaned with 70% alcohol and water between trials. The experiment was video-taped and analysis was performed by a researcher blinded to the identity of the groups.

2.4.2. Pole test

The method was adapted from the protocol previously described [17,18]. The apparatus consisted of a 50 cm high wooden pole,