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Behavioral Evaluation of rotenone model of Parkinson's disease in male Wistar rats

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Abstract

Introduction: Parkinson's disease (PD) is a prevalent neurodegenerative disorder in elderly people. The condition is associated with damage to the dopaminergic neurons in the substantia nigra pars compacta, a great decrement of dopamine neurotransmitter in the striatum, and a deficiency of tyrosine hydroxylase; the rate-determining enzyme during dopamine formation. In rats, rotenone has been administered to induce a syndrome that mimics the behavioral and pathophysiological finding of Parkinson's disease.

Aim of the work: To evaluate the behavioral aspects of PD in rotenone-treated Wistar rats.

Materials and Methods: Rotenone at 2 mg/kg was injected subcutaneously to male adult Wistar rats in the rotenone-treated group once a day for 4 weeks for induction of PD. Open Field test, object recognition test, Rota-rod test, footprint test and forced swim test were performed at the end of induction. Tyrosine hydroxylase levels in the brain were measured.

Results: Our data showed deteriorated behavioral aspects after rotenone treatment, with a decrease in exploration and locomotion, impaired motor coordination, asymmetrical impaired gait, increased depressive-like behaviors, reduction in short-term memory and reduction in tyrosine hydroxylase levels.

Conclusion: in this study, rotenone subcutaneously at a dose of 2 mg/kg daily for 4 weeks, in adult male Wistar rats, caused the appearance of the motor and the non-motor features of PD with a marked drop-off tyrosine hydroxylase levels in the striatum.

Keywords: Rotenone, Parkinson's disease, Wistar Rats.

Introduction

Parkinson's disease (PD) is considered one of the commonly occurring neurodegenerative diseases, affecting 1% of people older than 55 years (1). This condition is illustrated for the loss of up to seventy percent of the dopaminergic neurons in the substantia nigra pars compacta (SNc), a huge decrease in dopamine (DA) in the striatal neurons (1). Tyrosine hydroxylase (TH) is a rate-determining enzyme during the process of dopamine biosynthesis. The disease is featured for an increasing decline in TH levels, following the loss of dopaminergic neurons in the substantia nigra (2). The most accepted presentation of PD involves; tremor, rigidity, bradykinesia, and gait abnormality; however, patients may be presented by these motor manifestations, in addition to non-motor symptoms such as olfactory

disorders, sleep disturbances, and psychological manifestations (3).

The complete PD process is still not fully known up till now. Despite of the different animal models of PD. During the last few decades, various models of PD in animals have been grown (4).

Rotenone is used as an herbicide and/or an insecticide. It is a strong toxin that belongs to the family of rotenoid toxins, that are naturally present in tropical plants (5). Chronic liability to little doses of rotenone; causes the inactivation of the electron transport chain in the rat brain mitochondria (5). In rats, injections of rotenone have caused behavioral and neurological deficits (6). The amazing creativity of the rotenone model is that it appears to mimic the whole picture of PD, with the appearance of α-synuclein aggregation and Lewy-like body synthesis (7). Chronic susceptibility to small doses of rotenone; caused complex-I inhibition paralleled to that observed in patients, it generated a strongly discriminative nigrostriatal breakdown (8). Rotenone rat model despite being very promising, has not been widely used and studied, compared to other older well-established models of PD. In this work, we have focused on the behavioral evaluation of movement disorders and extra kinetic aspects of the PD in adult male Wistar rats.

Materials and Methods:

Sixty adult male Wistar rats with body weight 250±25 gm, 8-12 month age; were purchased from Faculty of Science, Sohag University and were housed in Medical Animal Laboratory in Sohag Faculty of Medicine. Animals freely introduced to chew and tap water. Rats were housed in metal cages (20×32×20 cm3) at normal light/dark cycle and room temperature. The study was officially approved by the Research Ethics Committee regarding the manipulation of laboratory animals. Rats were blindly separated into 2 groups; control and rotenone treated Groups (n= 30 in each). Rotenone at a dose of 2 mg/kg in 1ml\kg vehicle was injected subcutaneously, once a day for 4 weeks (9). At the end of the induction period, behavioral tests were performed.

Behavioral tests

1- Open field test (OFT).

Open Field was a circular arena, made of plastic; the floor was divided by a marker into compartments with a central area. Ordinary laboratory lighting was used. Rats were put in the middle of the field, let to examine it for 5 minutes, for the first time, with video-camera recording. the field was swept with a 70% ethanol solution and drained with paper tissue after each time (10).

Each rat was then scored for ***the locomotor activity***, which was expressed as the sum of lines crossed plus the number of balances. A ***score for exploratory behavior*** was given, which was the sum of the number of times the rat passed through central area plus the duration of time the rat stayed in the central area, finally, an ***anxiety score*** was calculated, equal to the sum of urine and feces spots (10).

2- Object recognition test (ORT).

Rats were put in the previous arena used in the open field test, to judge the preference index for a new object. Four items were in use: A1, A2, B, and C. Training was conducted by allowing each rat to explore the arena for 5 min, with two similar items (A1 and A2). Items were put on opposing sides. To evaluate short-term memory

(STM), the rat was retested 1.5 h after training; the rats examined the arena for 5 min, with 2 Items; one well-known (A1) and one new (B). Items were placed on two adjacent sides. After each trial, the items and arena were wiped with 70% ethyl alcohol. To evaluate long-term memory (LTM), another evaluation was given one day after the training day. The same rat re-examined the field for 5 min in the presence of a well-known object A2 and a new object C. Exploration time is considered when the rat sniffed or touched any items. The percentile duration each rat took exploring any of the objects was calculated. Exploration preference was expressed as following: Training = (A2 /(A1+A2)) 100; STM= (B / (A1+B)) 100; LTM= (C / (A1+C)) 100 (11)**.**

3- Footprint test.

Gait was tested using a wood passage apparatus ending with a roofed shelter. The passage was lined with a long precut paper sheet. Rats were trained twice or until each rat could run to the shelter without rewarding. During the test, the four paws of each rat were colored with safety watercolors. Red was used for the front right paw, green for the front left paw, blue for the right hind paw and yellow for the left hind paw. The rat was then placed at the unroofed end of the apparatus and allowed to walk to the roofed end of the corridor; leaving a footprint on the paper. The rat was then washed by water and allowed to groom. The paper prints were used for PD gait analysis. For each animal, five measurements were calculated using 4 paw prints; (front stride length (FSL), front stride width (FSW), hind stride length (HSL), hind stride width (HSW) and overlap (OL)). These values were then averaged to provide gait measurements (12, 13).

4- Rotarod test.

To judge the motor coordination of the rotenone PD rat model, we used a stationary Rotarod (Harvard Apparatus, UK). The Rotarod rotated for 120 sec. at a speed of 15 RPM. A trial was ended when the rat fell down the Rotarod or when they end the 120-sec trial without falling. The mean value of the three tests was taken. the rats were trained for 5 days before the test (14).

5- Forced swim test (FST)

To measure depressive-like behavior, each rat was placed in a water cylinder, 20 cm in diameter that was filled in-depth with 50 cm water. Water temperature was adjusted within the thermo-neutral zone of temperature (at 31 ± 1 °C), suitable for rodents. The duration of immobility within a 6-min session was recorded as an immobility score. The first 2 min of the test was deleted from calculations (14).

Tissue preparation

After behavioral tests, the rats were anesthetized with Zoletil (1mg\kg i.p (Vibac Laboratories, France). Rats were subjected several times to transcardiac perfusion with 0.05 ml phosphate buffer (PBS) in saline. The brain was removed, snap freeze in liquid nitrogen, and placed for 1 hour at - 80°C. The striatum was dissected through multiple manual coronal sections with a sharp razor blade. Samples stored in Eppendorf tubes at -20°C**,** until they were extracted to measure the levels of Tyrosine hydroxylase enzyme by ELISA (Tyrosine hydroxylase (TH) rat ELISA kits (#:96791) from Glory Science Co., (Ltd, China)**).**

Statistical analysis

All data were subjected to statistical analysis using Statistical Package for social sciences (IBM-SPSS), version 24 IBM- Chicago, USA (May 2016). Data were expressed as

mean ± standard deviation. The student t-test was used to compare the means between the two groups. The statistical significance was considered at p <0.05.

Results

The impacts of rotenone-treated rats as a model of PD were studied. Rotenone at a dose of 2mg\kg\day for 5 weeks was injected S.C to induce PD in 30 healthy male rats. At the end of induction, neurobehavioral tests were performed. Tyrosine hydroxylase levels in the striatal tissue were estimated by ELISA.

Effects of rotenone on open field test parameters

Animal behaviors in the open field were assessed according to; *exploratory score, locomotor score, and anxiety score.* In the control group, the mean exploratory score was 8.8 ± 2.5, the mean locomotor score was 27.5 ± 3.7 and the mean anxiety score was 1.6 ± 0.7. There was a significant decrease in both locomotion and exploration of the rotenone-treated group compared to the control group (p<0.001), while an insignificant discrepancy between the two groups regarding anxiety was noticed *(p =1.0)*.

Effects of rotenone on the object recognition test

Object recognition was evaluated for STM and LTM. There was a significant decline in STM of the rotenone-treated group compared to the control group *(p< 0.05)*, there was a statistically insignificant discrepancy regarding TP and LTM between control & rotenone-treated groups *(p=0.69, p=0.73 respectively*).

Effects of rotenone on footprint test

Footprint test was used to evaluate the gait according to five values, overlap, front stride width, front stride length, hind stride width, and hind stride length. In the rotenone-treated group, rotenone caused a significantly impaired gait. When the rotenone-treated group compared to the control group, the gait showed a significant asymmetrical foot pattern as there was a statistically significant rise in OL value *(p<0.05*). The gait showed a shortened stride length; there was a significant decrease in HSL and FSL *(p<0.05)*. The gait also showed significant wide base, there was a statistically significant rise in FSW and HSW values *(p<0.05)* (table 1)

Table (1) The effect of rotenone on the gait parameters of Wister rats'

Group	I	III	P
OL	1.50±0.3	2.10±0.45	**<0.05**
FSW	4.95±1.23	4.40±1.04	0.29
FSL	11.89±1.8	9.70±1.65	**<0.05**
HSW	6.15±0.78	5.10±0.87	**<0.05**
HSL	12.65±1.6	10.1±1.46	**<0.05**

- Data were expressed as mean± SD.
- P-value calculated by t-test, p-value is considered significant if <0.05.
- n=30 in each group.
- OL; overlap, FSW; front stride width, FSL; front stride length, HSW; hind stride width, HSL; hind stride length

Effects of rotenone on motor coordination

Rota-rod test was used to assess motor coordination of rotenone treated rats. The latency time during a 120-sec trial was estimated. In the control group, the mean value of Rotarod latency time was 86.5± 15.3 sec. There was a statistically significant decrease in the latency time of the rotenone-treated group compared with the control group *(p <0.001)*.

Effects of rotenone on the forced swim test

Forced swim test was used to evaluate depressive-like behaviors in the rotenone-treated rats. There was a significant prolongation of the immobilization time of the rotenone-treated group compared to the control group ***(p<0.001)*.**

Effects of rotenone on tyrosine hydroxylase levels in the striatum

In the control group, the mean TH level in the striatum was 77.24± 7.5 mcg\ml**.** In the rotenone-treated group, rotenone injection significantly reduced the striatal TH levels of rotenone treated rats when compared to controls ***(p <0.001)*** (Fig.1).

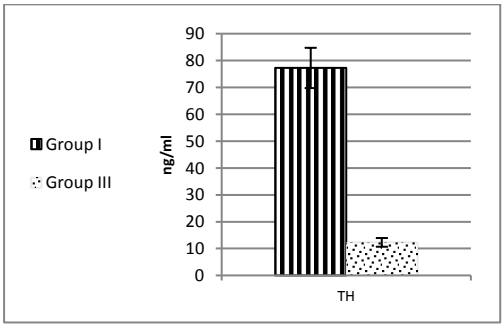

Fig 1: TH levels measured by ELISA in control and rotenone-treated rats striatum in ng\ml. TH levels significantly reduced in the striatum of rotenone-treated Wister rats (p<0.001).

Discussion

PD is a gradually progressing neurodegenerative condition affecting the elderly population all over the world. In PD, selective gradual degradation of pigmented neurons occurs in the substantia nigra (15). This results in stereotypical motor dysfunction & extra motor manifestation of PD. Rotenone is known to induce PD in rats by preferentially attacking dopaminergic neurons (6, 15). Rotenone inhibits complex-I of electron transport chain in mitochondria. It causes the accumulation of reactive oxygen species (ROS) inside the cells (16, 17). Oxidative stress has appeared to be effectively involved in the pathophysiology of PD (16, 17).

In this study, Rotenone 2 mg\kg\day was injected subcutaneously for one month to induce the rotenone rat model of PD. The rotenone-treated group showed a marked decrease in exploration and locomotion as regards the Open field test. Gait impairment recorded as; asymmetrical foot pattern, shortened stride length and widened base. Our study found a significant decline in motor coordination observed by shortened latency time on Rota-rod. Non-motor manifestation of PD also existed; a significant decrease in novel object preference in short-term memory, and a significant increase in depressive-like behaviors as prolonged immobilization time in the forced swim test. Tyrosine hydroxylase levels in the striatum significantly decreased in rotenone treated rats. Fagotti et al. (18) reported a reduction in the locomotion in the open field, in addition to a decrease in cognitive functions in rotenone-treated rats. Similar to our study, Vijayalakshmi et al. reported a remarkable decline in locomotion in Open field test in rotenone-treated Wister rats. Similarly, Valdez et al. (19) reported a 60% reduction in locomotion during the open field test in rotenone-treated rats. Sun et al. (20) reported a decline in locomotion with increased immobility time in rotenone-treated rats. Von Wrangel et al. (21) reported that rotenone-treated rats showed a reduction in latency time and a significant decline in tyrosine hydroxylase in the brain. Cannon et al. (7), who reported significantly decreased locomotion, significant gait impairment and a 50% decline in tyrosine hydroxylase activity in rotenone-treated rats. Our study agreed with Shin et al. (22) who reported a

rise in the immobility time value, during the forced swim test in rotenone-treated rats. The results of this study were in accordance with Madiha et al. (23) their data showed significantly impaired walking patterns and shortened stride length in rotenone treated rats.

Conclusion

Our data revealed a marked decline in behavioral aspects assessed during this study, after rotenone treatment. Rotenone treatment in Wistar male rats at a dose of 2 mg\kg for 4 weeks, by the subcutaneous route; caused a decrease in exploration and locomotion, impaired motor coordination, increased depressive-like behaviors, reduction in short-term memory and marked reduction in tyrosine hydroxylase levels. Rotenone-induced Parkinson's rats provide a good model for studying the effects of various drugs and interventions on the behavioral aspects of PD.

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