Effect of Intraperitoneal Rotenone Injection on Neurodegeneration and Neurochemical Modulation: A Dose Exploration Study

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Abstract

Earlier studies have proved that chronic subcutaneous exposure to the mitochondrial toxin like rotenone has induced features of Parkinson's disease (PD) in rats. It includes dopaminergic neuron degeneration with decreased tyrosine hydroxylase activity. The aim was to identify the optimum dose of ip rotenone for inducing PD in rat model. The male Wistar rats were injected IP with three doses of rotenone 1, 2 or 3 mg/kg daily for 21 days. The IP administration of 3 mg/kg rotenone produced motor impairment and In addition, it caused a significant decrease in the number of tyrosine hydroxylase-immunoreactive neurons in the substantia nigra and dopamine concentration in the striatum. The 1 mg/kg dose did not indicate any damaging effect on body weight after 21 days of daily rotenone IP injections. Moreover, rats in the 3 mg/kg group showed a decreased locomotor activity and shorter latency to fall from a rotarod than control rats. The histopathological and immunohistochemical changes identified in the nigrostriatal neurons, was also evident at this dose.

KeyWords: Parkinsonism, Rotenone, Tyrosine Hydroxylase
DOI Number: 10.14704/NQ.2022.20.12.NQ77204 **NeuroQuantology2022;20(12): 2278-2282**

Introduction

Parkinson's disease (PD) is the age related second common neurodegenerative disorder after Alzheimer's disease (1). PD affects approximately 0.5-1% of the entire population over 60 years of age (2). It is estimated that 8.7 to 9.3 million elderly people (above 60 years) will be affected worldwide by 2030 (3). The epidemiological studies on the prevalence of PD have shown that 0.3% of the world population among the developed nations is suffering from the disease.

PD is characterized by motor deficits like resting tremor, rigidity, bradykinesia and postural abnormalities as recommended by Movement disorder society (MDS). It is also associated with pathological findings such as reduction in the dopaminergic neurons of substantia nigra pars compacta (SNpc) and also appearance of alpha synuclein in the cytoplasm of the neurons (4). PD is also accompanied with neurochemical changes such as reduction of tyrosine hydroxylase and decreased striatal dopamine (DA) levels in striatum. The experimental evidence confirms that deficiency of complex I of the mitochondrial electron transport chain (ETC) plays a key role in the pathogenesis of PD (5).

The exact etiology of Parkinson's disease (PD) remains unidentified and is likely to be multifactorial. The environmental factor such as exposure to pesticide is one of the common cause for PD development. Rotenone, a pesticide is a potent inhibitor of complex I of the mitochondrial ETCresulting in behavioral and neuropathological changes in rat. The various literature evidence shows that chronic rotenone exposure in rat is associated with pathogenesis of PD similar to humans (6,7).

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It has exhibited alteration in calcium signaling, oxidative stress production and apoptosis, reduced tyrosine hydroxlase, proteasomal dysfunction, and the formation of cytoplasmic inclusions such as ubiquitin and alpha-synuclein (8). Furthermore, studies have revealed that exposure to rotenone have produced motor symptoms, such as muscular rigidity, bradykinesia, posture and gait abnormality (9,10). The other chemicals used for producing PD model in rat are 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA). But, these models replicate only the acute symptoms in PD. The 6-OHDA is administered by local stereotactic injector method and cannot cross the blood–brain barrier, which makes it less applicable (11).

There are various modes of rotenone administration to establish PD model in rat. The first established rotenone model was produced by Stereotaxic injection in the year 1985 (12). Related to systemic administration of rotenone, Stereotaxic injection had a minimum adverse effects with less mortality rate but reported to have an acute toxicity effect. Due to the direct administration, it may avoid physical and metabolic changes caused by rotenone, thus not suitable for eliciting the premotor changes in PD. Moreover, α-synuclein expression in the neuronal cytoplasm is also not evident (11).

The systemic administration of rotenone was done through different routes such as intravenously, subcutaneously, and intraperitoneally. It was all found to be successful in producing chronic degeneration in PD. Even though administration of rotenone via osmotic minipump was producing neuronal damage, the implantation of the minipump itself caused death in rats (13).

Intraperitoneal injection of rotenone is gaining interest due to its reproducibility and simplicity. Though, the doses of rotenone through intraperitoneal injection in inducing PD model vary widely (1.25–3 mg/kg/day) (14), limiting the objective evaluation of this method. A standard dose of rotenone for inducing PD in rats should be established. To the best of our knowledge, very few studies have addressed this issue. Hence, the aim of the present study was to investigate the effects of different doses of intraperitoneal injection of rotenone to identify the optimum dose for replicating the features of PD. In this study the experimental details of intraperitoneal injection at various dose to induce PD model and its histological, neurochemical, and behavioral features are discussed.

Methodology

Experimental animals

The study was approved by Institutional Animal Ethics Committee (Approval number – SU/CLAR/RD/006/2018) and rats were treated in accordance with the guidelines of CPCSEA. The animals were housed in groups of four/cage under appropriate room temperature with water and food given ad libitum.

Experimental design

Male Wistar albino rats (n=24), weighing approximately 200–250 g were used for the study. Stock solution of rotenone was prepared using Dimethylsulfoxide (DMSO) and it was diluted with olive oil to obtain a final concentration of 3 mg/5 ml rotenone. The rotenone solution was made fresh and stored in an amber bottle to avoid light exposure. The solution was vortexed before intraperitoneal injection to eliminate the possibility of settling. Rats were divided into four group, each receiving intraperitoneal injection daily for 3 weeks as follows: 1) vehicle control (n =6; 1 ml/kg olive oil); 2) 1 mg/kg rotenone (n =6); 3) 2 mg/kg rotenone (n =6); 4) 3 mg/kg rotenone (n =6).

Histopathological Assessment

The wistar rats were anesthetized and intracardially perfused with physiological saline as pre-rinse solution and subsequent perfusion with fixative solution of 10% neutral-buffered formalin (NBF). The corpus striatum part of the brain section was removed and post fixed with 10% neutral-buffered formalin (NBF) for about 48 hrs. After the fixation, tissues were dehydrated within ascending grades of alcohol then embedded in paraffin wax and cut into 5–7 µm thick sections using a rotary microtome. The paraffin sections are then subjected to hematoxylin & eosin staining (Bancroft and Gamble 2008). The histopathological assessment in corpus striatum of brain tissue was examined under the BX51 Olympus multi head microscope (15).

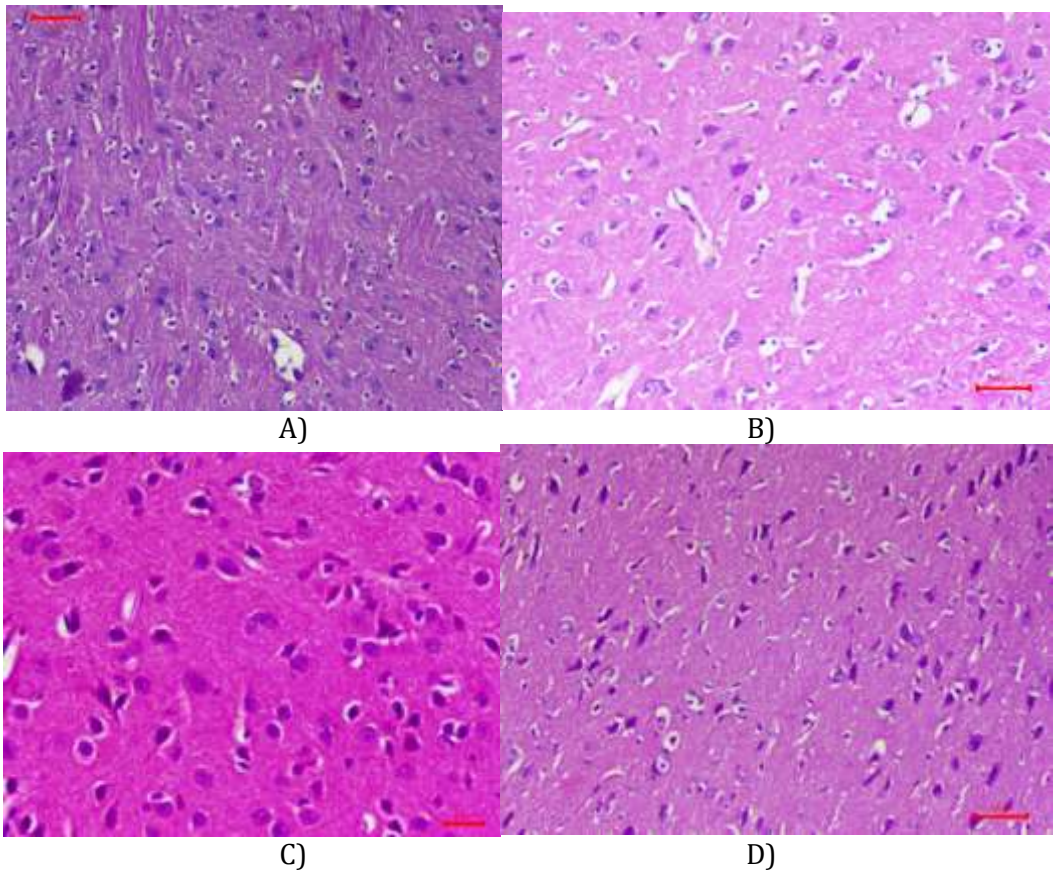
Immunohistochemical Analysis

The sections of corpus striatum (approximately 0.20 mm relative to bregma) was dissected by microtome and were rinsed in PBS and incubated

in 1% H2O2 for 30 min to block the endogenous peroxidase activity. After washing in PBS, the sections were incubated in blocking serum (10% normal horse serum and 0.1% Triton X-100 in PBS) for 60 min, followed by incubation in anti-TH mouse monoclonal antibody solution (1:500) for 24 h at room temperature. The sections were then incubated for 1 h in biotinylated anti-mouse IgG secondary antibody (1:300). The sections were subsequently incubated with avidin-biotin-peroxidase complex for 1 h at room temperature. The immunoreactivity was visualized by Incubating the sections 3oluteon consisting of 0.05% 3,3-diaminobenzidine (DAB) and 0.02% H2O2 in 50-mM Tris buffer (pH, 7.6) for 3 min. (14)

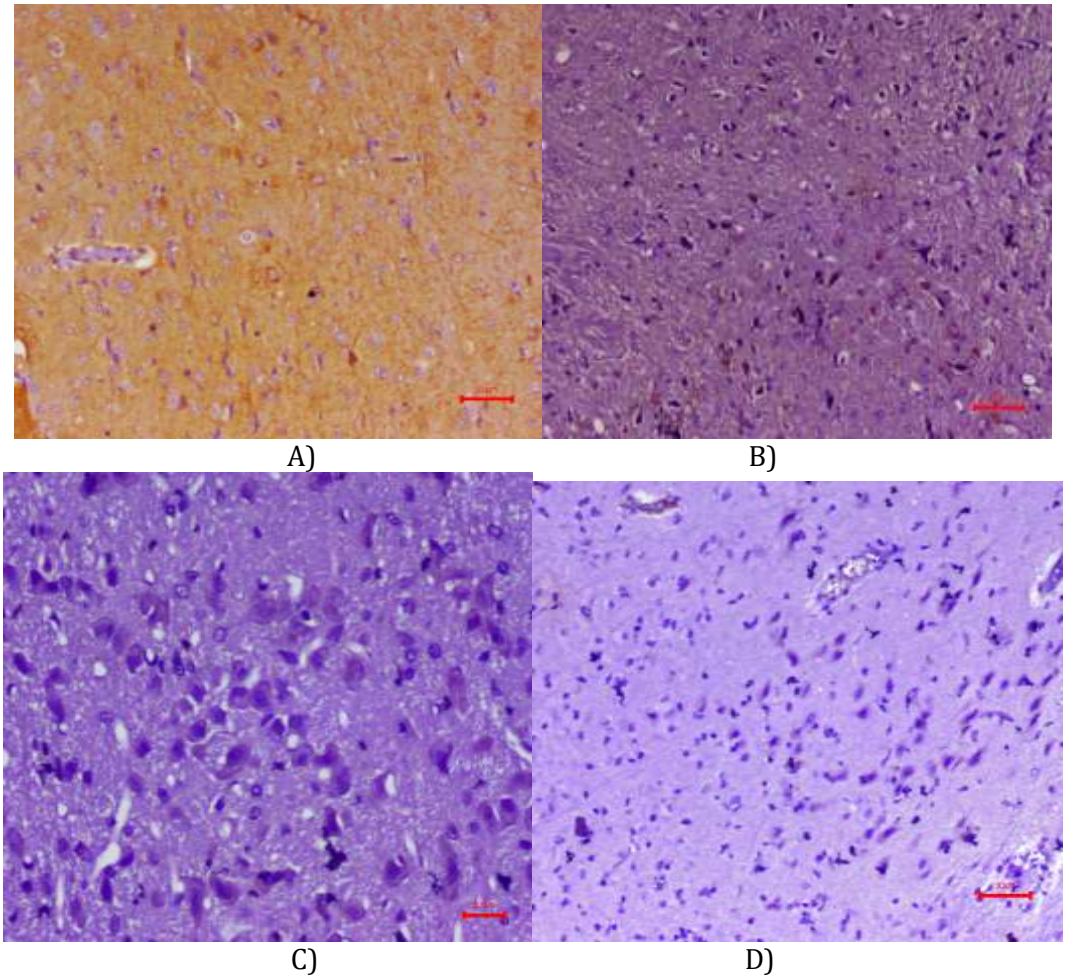
Results & Discussion

Histopathological images of corpus striatum stained with hematoxylin and eosin (H&E). (A) Vehicle control (B) PD-1 mg/kg rotenone (C) PD-2 mg/kg rotenone (D) PD-3 mg/kg rotenone (scale bar 20 mm).



A) B) C) D)

Microscopic images from coronal sections of corpus striatum, showing TH immunoreactivity. (A) control, showed normal TH expression indicated in brown (B) PD-1 mg/kg rotenone (C) PD-2 mg/kg rotenone (D) PD-3 mg/kg rotenone (scale bar 20 mm). Substantia nigra shows percentage of cells showing positivity: 70 %, Average intensity of staining was strong, and the pattern of staining was in cytoplasm and nuclear. The striatum showed percentage of cells showing positivity around 60 %, Average intensity of staining was strong and the pattern of staining was in cytoplasmic and nuclear.



A) B)

C) D)

Conclusion

The striatal neurons showed alpha synuclein aggregations as well. Although novel, subcutaneous rotenone model of PD is tedious and extremely labor-intensive. The current paper demonstrates that the same features of PD can be reproduced by intraperitoneal injection of rotenone. Concurrently, our data indicate that IP injection of 3 mg/kg rotenone for 21 days enables the reduction of TH neurons with associated clinical features of PD, while maintaining less mortality in rats.

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