The Egyptian Journal of Hospital Medicine (October 2017) Vol.69(1), Page 1628-1633

Granulocyte-Colony Stimulating Factor Prevents Motor Dysfunction and Histological Damages In A Rat Model Of Parkinson's Disease

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ABSTRACT

Aim of the work: Parkinson's disease is the most common neurodegenerative movement disorder worldwide. The main motor clinical manifestations of Parkinson's disease are resting tremors, bradykinesia, rigidity, and postural instability. In the present study, we aimed to evaluate the effects of granulocyte-colony stimulating factor (G-CSF) on the body weight, motor function, and brain histology of rotenone-treated rats.

Material and methods: Rats were divided into four groups, as follows: **Group 1** the control rats, **Group 2** the rotenone-treated rats (2 mg/kg, 28 days), **Group 3** the rotenone + G-CSF (20 µg/kg, 28 days)-treated rats and **Group 4** the rotenone + G-CSF (40 µg/kg, 28 days)-treated rats. Body weight was measured on weekly basis. Postural instability was evaluated at the end of the study and the motor behavior was monitored. Then, rats were decapitated and brain histology was examined. **Results:** Rotenone resulted in body weight loss, bradykinesia/akinesia, rigidity, postural instability, and histological damages. All these deficits were prevented by G-CSF at 40 µg/kg. Therefore, G-CSF may be a potential neuroprotective agent in Parkinson's disease.

Keywords: G-CSF, rotenone, body weight, postural instability

INTRODUCTION

Parkinson's disease, the most common neurodegenerative movement disorder, remains a major disabling progressive condition affecting mainly elderly people. The clinical manifestations of Parkinson's disease can be divided into motor and nonmotor symptoms. Resting tremors, bradykinesia/akinesia, rigidity, and postural instability are the main motor symptoms of Parkinson's disease[1]. The nonmotor symptoms include cognitive impairment (mainly dementia), autonomic dysfunction, mood disturbance (depression), psychosis, sleep disturbance (such as excessive daytime sleepiness, disturbed nocturnal sleep, and rapid eye movement sleep behavior disorder [RBD])[2].

The two major neuropathological hallmarks of Parkinson's disease are dopaminergic neuronal loss in the substantia nigra and accumulation of α-synuclein-positive intraneuronal inclusions, namely Lewy bodies (LB)[3]. The non-motor symptoms are likely related to pathology in brain areas rather than the nigrostriatal projection, particularly the hippocampus and cerebral cortex. Currently, most treatments of Parkinson's disease provide pharmacological dopamine replacement and symptom control. However, none of these treatments can delay the disease progression.

Epidemiological studies have suggested that environmental factors, such as pesticide exposure, constitute a major risk factor for Parkinson's disease[4-6]. One of these pesticides related to Parkinson's disease is rotenone[4]. Rotenone is the most potent member of the rotenoid family of neurotoxins[7]. It is a well-characterized inhibitor of mitochondrial complex I, an activity that results in ATP depletion and oxidative stress[8]. Owing to its lipophilicity, rotenone can readily cross the blood-brain barrier. Betarbet *et al*. first showed that chronic systemic rotenone exposure could induce almost all the features of Parkinson's disease in rats[9]. In the present study, a rat model based on administration of low-dose rotenone (2 mg/kg) for 28 days was used to reproduce the features of Parkinson's disease.

Similar to erythropoietin, whose receptors were found to be expressed by the neurons and which displayed neuroprotective effects[10, 11], granulocyte-colony stimulating factor (G-CSF) receptors (G-CSFR) were found to be expressed by neuronal cells; in addition, G-CSF displayed neuroprotective effects in numerous neurological conditions[12-14]. With regard to Parkinson's disease, G-CSF was shown to increase tyrosine hydroxylase-expressing nigral neurons and striatal dopamine content in the acute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model[15]. In this study, we aimed to assess the effects of two doses of G-CSF on the motor function and brain histology of rotenone-treated rats.

Received: 5 / 6 /2017
Accepted:14 /6 /2017
DOI: 10.12816/0040111

MATERIALS AND METHODS

Animals

Twenty-eight male Wistar rats, weighing 220 g on average, were housed in cages at controlled temperature and humidity conditions. They were provided with food and water *ad libitum*. All experiments involving animals were conducted according to the Institutional and International Ethics Guidelines for the care and use of laboratory animals**.** The study was approved by the Ethics Board of Ain Shams University. Rotenone and G-CSF were purchased from Sigma (St. Louis, MO, USA). Rotenone suspension was prepared in sunflower oil, whereas G-CSF was diluted with 5% dextrose to an injectable volume. All other chemicals were commercially available.

Experimental groups: Rats were categorized into four groups (n = 7 each) as follows:

- **Group 1:** control rats received the vehicles of G-CSF and rotenone.
- **Group 2:** rats received rotenone SC at a dose of 2 mg/kg daily for 28 days.
- **Group 3:** rats treated with G-CSF SC at 20 µg/kg, as well as rotenone for 28 days.
- **Group 4:** rats treated with G-CSF SC at 40 µg/kg, as well as rotenone for 28 days.

The body weight of rats in all groups was measured on weekly basis. In addition, we evaluated the motor behavior and postural instability of rats. Rats were then decapitated and brains were dissected out and fixed in 10% formol-saline for 24 h.

Body weight

The body weight of rats in all experimental groups was measured on weekly basis using an animal balance. Body weight changes over the study period were evaluated in all groups.

Motor behavior

Motor behavior of rats in all experimental groups was evaluated by a blinded observer at the end of the study. Any deviation from the normal motor behavior was reported.

Postural instability test

The test was conducted as previously described by Woodlee *et al.*[16]. Breifly, rats were held in almost a vertical upside-down ("wheelbarrow-like") position over a sandpaper alongside a ruler. This rough surface (sandpaper) was used to induce stepping, rather than bracing or dragging, as a response to the imposed weight shifts. When viewed from above, the nose tip of the rat was aligned at the zero line of the ruler. One forelimb was gently restrained against the rat's torso while the animal was moved forward over the single unrestrained forelimb until it made a "catch-up" step in order to regain its center of gravity. The new position of the nose tip was recorded as an indicator of the displacement needed to trigger a "catch-up" step in the unrestrained forelimb. Three trials were performed for each forelimb, and the average of both forelimbs was calculated.

Histopathological examination

After fixation of the isolated brains, washing was done with tap water and then serial dilutions of alcohol were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 °C in hot air oven for 24 h. Paraffin bees wax tissue blocks were prepared. Slices were sectioned at 4 µm using a sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained with hematoxylin and eosin (H & E) stain. The slides were then examined using a light microscope. This method was adopted from **Bancroft *et al.*[17].**

The study was approved by the Ethics Board of Ain Shams University.

Statistical analysis

Parametric data (body weight) were expressed as the mean ± standard deviation, and analyzed by ANOVA followed by Tukey's Post Hoc Test. Non-parametric data (postural instability) were expressed as the median and interquartile range, and analyzed using Kruskal-Wallis test followed by Dunn's Post Hoc Test. All statistical analyses were carried out using GraphPad Prism (version 5.0).

RESULTS

Body weight changes

As shown in **table 1**, rats in the control group showed weight gain from approximately 218 g on day 1 to 295 g on day 28. However, rotenone administration resulted in weight loss by approximately 19%, compared to the average body weight on day 1. G-CSF (20 µg/kg) prevented body weight loss, wherein rats in this group maintained nearly the same initial weight. G-CSF treatment at 40 µg/kg resulted in an increase in the average body weight from approximately 228 g on day 1 to 261 g on day 28.

Table 1: changes in average body weight of rats in different experimental groups over the study period

Group	Average weight on day 1	Average weight on day 28	Weight change (%)
Control group	217.509	295.251	35.742 ± 4.377
Rotenone-treated disease group	227.714	184.114	-19.147 ± 2.52 [a]
Lower dose G-CSF-treated group	220.17	227.526	3.341 ± 1.789 [ab]
Higher dose G-CSF-treated group	228.286	260.666	14.184 ± 0.8871 [abc]

The body weight of rats in all experimental groups was measured on weekly basis, starting from day 1. Results are expressed as the means ± standard deviation ($n = 7$). [a]$P < 0.05$ versus the control group, [b]$P < 0.05$ versus the disease group. [c]$P < 0.05$ versus the lower dose G-CSF-treated group.

Monitoring of the motor behavior

As shown in **table 2**, 100% of rotenone-treated rats exhibited bradykinesia or akinesia and approximately 71% of them showed muscle rigidity. Rats treated with G-CSF (20 µg/kg) did not markedly differ from those receiving rotenone only. However, G-CSF (40 µg/kg) was able to significantly prevent rotenone-induced motor dysfunction.

Table 2: motor function of rats in different experimental groups

	Control group	Rotenone-treated disease group	Lower dose G-CSF-treated group	Higher dose G-CSF-treated group
Normal	100%	-	14.286%	85.714%
Brady-kinesia /akinesia	-	100%	85.714%	14.286%
Muscle rigidity	-	71.429%	71.429%	-

Values represent the percentage of rats in each group showing a certain motor feature.

Postural instability

As shown in **figure 1**, rotenone resulted in a significant increase in the distance moved by the rats in order to regain their center of gravity, compared to the control group. The lower-dose G-CSF-treated rats did not significantly differ from the disease group. However, the distance moved by the rats treated with G-CSF (40 µg/kg) was significantly shorter than that moved by the rotenone-treated rats.

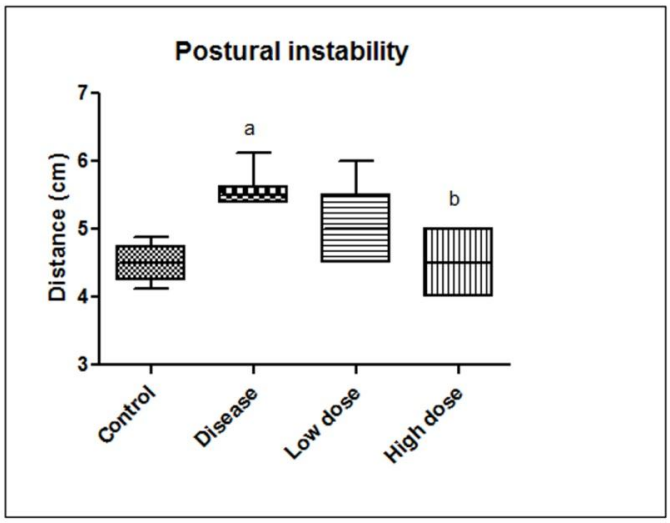

Figure 1: measurement of postural instability of rats in different experimental groups
Postural instability was assessed based on the displacement needed to trigger a "catch-up" step in the unrestrained forelimb when the rat was held in almost a vertical upside-down ("wheelbarrow-like") position over a sandpaper. Data are expressed as the median and interquartile range. [a]$P < 0.05$ versus the control group and [b]$P < 0.05$ versus the disease group.

HISTOPATHOLOGY

As shown in **figures 2 and 3**, the control rats had normal histological structure of the hippocampus and cerebral cortex. Rats in the rotenone group showed nuclear pyknosis and degeneration in the hippocampus, as well as focal gliosis and neurodegeneration in the cerebral cortex. Rats pretreated with G-CSF (20 µg/kg) showed normal histological structure of the hippocampus; however, neuronal degeneration was observed in some of the neurons of the cerebral cortex. G-CSF treatment at 40 µg/kg could preserve the normal histological structure of both the hippocampus and cerebral cortex.

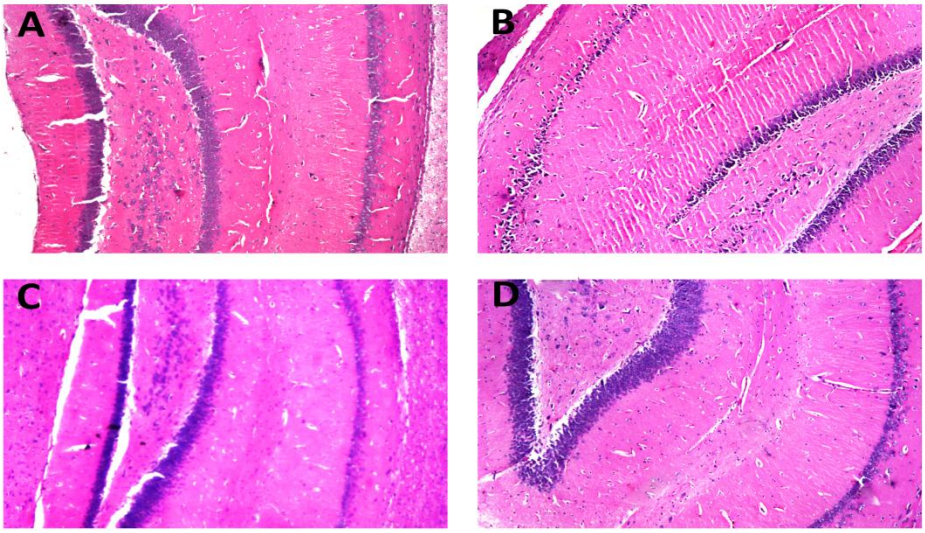

Figure 2: H & E-stained sections of the hippocampi of rats in the A) control, B) disease, C) lower dose-treated, and D) higher dose-treated groups. (160×)

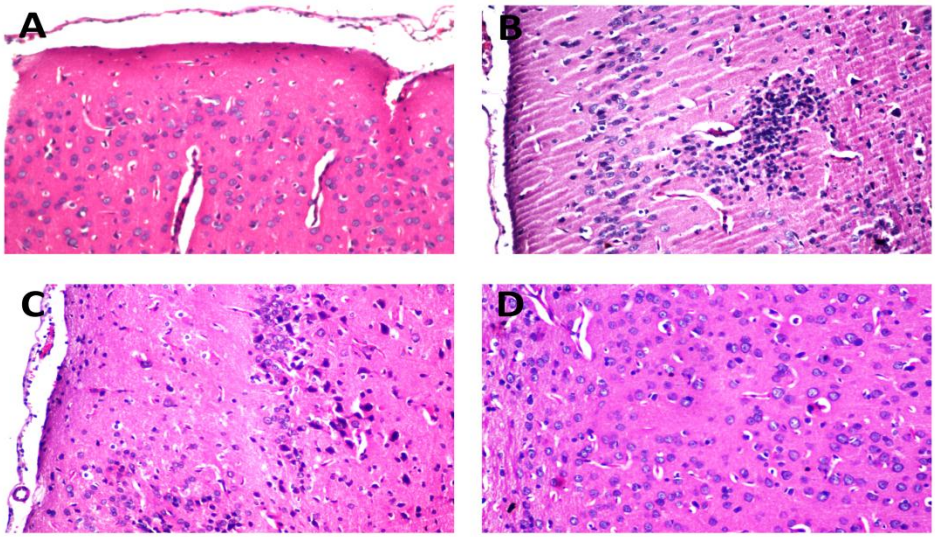

Figure 3: H & E-stained sections of the cerebral cortices of rats in the A) control, B) disease, C) lower dose-treated, and D) higher dose-treated groups. (400×)

DISCUSSION

Since Parkinson's disease is a progressive neurodegenerative disorder, recent treatment approaches aim at delaying the disease progression, rather than solely providing symptomatic control[18]. Among these approaches, neuroprotective agents have received growing attention. G-CSF has been shown to confer neuroprotection in animal models of various diseases[12, 14, 19], including Parkinson's disease, wherein it was shown to increase the number of tyrosine hydroxylase-expressing dopaminergic neurons and striatal dopamine content[15]. In the present study, we showed that G-CSF (40 µg/kg) protected the rats against rotenone-induced weight loss, motor dysfunction, postural instability, and histological damage.

Patients with Parkinson's disease exhibit motor symptoms, including bradykinesia, rigidity, and postural instability[1]. Subcutaneous rotenone administration for 28 days at a dose of 2 mg/kg could reproduce the motor features of Parkinson's disease, as evidenced by bradykinesia/akinesia and rigidity of rats and a longer displacement required to regain their center of gravity (indicator of postural instability). Our findings are in line with many previous studies[20-23]. Although the lower dose of G-CSF failed to significantly improve the motor function of rotenone-treated rats, the higher dose (40 µg/kg) prevented rotenone-induced motor dysfunction and reduced postural instability in rats.

In addition, histological examination of the brains of rotenone-treated rats revealed nuclear pyknosis and neurodegeneration in the hippocampus and cerebral cortex, as well as focal gliosis in the cerebral cortex. G-CSF (20 µg/kg) did not prevent the histological damage induced by rotenone in the cerebral cortex. However, G-CSF at 40 µg/kg nearly restored the normal histological architecture of the hippocampus and cerebral cortex. Pathology in these two brain areas could contribute to the development of nonmotor symptoms, particularly dementia, in patients with Parkinson's disease.

This study focused on evaluating the effects of G-CSF at two doses on rotenone-induced reduction of body weight, motor behavior and postural stability, as well as histological damage. However, this study has some limitations. We did not examine the effects of G-CSF on the nigrostriatal system (number of tyrosine hydroxylase-expressing dopaminergic nigral neurons and striatal dopamine content) in rotenone rat model of Parkinson's disease. In addition, the mechanisms underlying the beneficial effects of G-CSF in rotenone-treated rats are still to be investigated.

In summary, the present study highlighted the favorable effects of G-CSF in rotenone-induced Parkinson's disease in rats, in terms of improvement of the motor function and prevention of histological damage. These findings suggest that G-CSF might be a potential neuroprotective agent for management of Parkinson's disease progression.

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