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Exercise attenuates neuronal degeneration in Parkinson's disease rat model by regulating the level of adenosine 2A receptor

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Abstract

Parkinson's disease occurs due to loss of dopaminergic neurons, which alters the behavioural changes. The present study evaluates the effect of exercise on neurodegeneration against Parkinson's disease (PD) rat model and postulates its effect on novel molecular pathway. Rotenone was administered at 1 mg/kg s.c. every 48 h for 18 days for the induction of PD and exercise was given to rats for a period of 2 weeks after the confirmation of PD. Moreover, PD rats also received CGS 21680 (adenosine A2A receptor agonist, 0.5 mg/kg, i.p.) with exercise for a period of 2 weeks after confirmation of PD. The effect of exercise was assessed for motor and cognitive function in PD rats. The level of inflammatory cytokines and neurotransmitters was estimated in brain tissue of PD rats. Data of investigation reveal that exercise attenuates cognitive and motor function in PD rats, the exercise + CGS 21680 group shows reverse in the behavioural changes compared to exercise-treated PD rats. The level of inflammatory cytokines and neurochemical level ameliorated in the exercise-treated group compared to the PD group of rats, which is reversed in the exercise + CGS 21680 group. In conclusion, exercise protects neurodegeneration in PD rats by reducing aggregation of α-synuclein and activity of adenosine 2A receptor.

Key words: *Parkinson's disease, exercise, adenosine A_{2A} receptor, inflammation, oxidative stress.*

Introduction

Parkinson's disease (PD) is a chronic degeneration of dopaminergic neurons which decrease the level of dopamine in the substantia nigra [2]. Parkinson's disease is characterised by classical symptoms such as tremor, bradykinesia, and rigidity, which is also associated with cognitive dysfunction [6]. Parkinson's disease is associated with a number of pathogenic pathways including aggregation of α-synuclein (α-syn) in the neurons, contributes to the activation of inflammatory mediators and oxidative stress leads to neurodegeneration [17].

The literature reveals that adenosine A2 receptor (AA2) emerged as a promising target for the management of PD, antagonists of which reverse the altered motor and non-motor function in PD rats [5]. Moreover, AA2 receptor regulation also emerges for the beneficial effect of neuronal dysfunction such as Alzheimer's disease, stress disorders and epilepsy [24]. AA2 receptor modulation reported for α-syn-mediated neurotoxicity, which alters the synaptic function leads to neuronal death [18]. AA2 receptor regulation stimulates aggregation of α-syn, activates the microglia which stimulates the neuronal inflammatory pathway by regulating TLR-2 pathway, which leads to neuronal death [16].

There are several therapies conventionally used for the management of PD which modulates the motor function and reduces the progression of disease. However, these medicines also have a number of limitations. It is well documented that exercise improves multiple

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organ functions, which protects PD clinically [1]. Reported studies suggest that exercise promotes motor function by improving the level of dopamine and protects dopaminergic neurons [21]. Exercise downregulates the expression of AA2 receptor, which is reported to alter the neurochemical level in the brain tissue [22]. Exercise promotes the level of dopamine in striatum, which could improve the motor function [8]. Thus, the present report evaluates the effect of exercise against PD.

Material and methods

Animals

Sprague Dawley rats (either sex, age: 8 weeks, 250-300 gm) were housed under standard environmental conditions (22 ±2°C, humidity 55-60%, light-dark cycle of 12 hours each) and fed with standard pellet diet and water. The protocol was reviewed and approved by the Beijing Xiaotangshan Hospital animal ethical committee, JN. No. 202001230b0601211.

Experiments

All the animals were separated into two different groups like the control group (n = 6), PD groups (n = 18) which received rotenone (1 mg/kg) s.c. every 48 h for 18 days for the induction of PD [13]. PD groups were further separated into three groups such as the negative control group which did not receive any treatment or exercise for next 2 weeks; the exercise-treated group received two weeks' exercise after the induction of PD; the AA2 group received exercise along with CGS 21680 (adenosine A_{2A} receptor agonist; Sigma-Aldrich Ltd., USA; 0.5 mg/kg, i.p.) for a period of two weeks after the induction of PD (Fig. 1).

Treadmill exercise

Rodent treadmill was used to provide exercise training to the rats for a period of 14 days after the confirmation of PD. Training was provided for 40 min every day with an inclination of 0 degree at a speed of 8 m/min, i.e. first 5 min at a speed of 2 m/min, next 5 min at 5 m/min and last 30 min at a speed of 8 m/min. Biochemical, neuronal and behaviour activities were estimated within 48 h after the last session of exercise.

Estimation of motor function

Motor function and muscle balance were estimated using the rotarod apparatus. All the animals were mounted on the apparatus which rotates at a speed of 18 RPM and time to fall was recorded as the latency period.

Behavioural studies

Apomorphine-induced rotation behaviour was assessed to determine the lesions of the dopaminergic system in PD rats. Apomorphine depletes the dopamine level which alters the number of rotations in PD rats. Contralateral rotation was induced by the administration of 3 mg/kg, s.c. and changes in behaviour were monitored for the duration of 1 h.

Morris Water Maze test

Cognitive function in rats was estimated using the Morris Water Maze (MWM) test as per previously published reports [25]. MWM apparatus' dimensions are as follows: circular pool: 120 cm; height: 50 cm; and depth: 30 cm. The pool was separated into four different quadrants and a platform was placed in one quadrant, trials were given on 5 consecutive days. The platform was removed on the 6th day and the time spent in the target quadrant was observed.

Preparation of brain tissue homogenate

Brain was isolated from each animal after sacrificing them and brain tissue was homogenized in pH = 7.4, 0.1 M phosphate buffer. Brain tissue was centrifuged at 3000 rpm for 15 min and supernatant was separated to determine the neurochemical and biochemical estimation.

Estimation of dopamine and glutamate

The levels of glutamate and dopamine were estimated in the brain tissue homogenate using their respective assay kits as per the directions given by the manufacturer of kits.

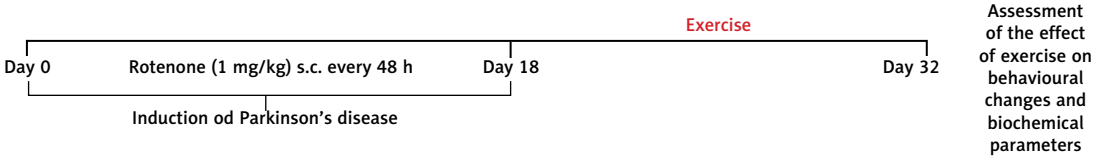

Fig. 1. Diagrammatic representation of experimental protocol design

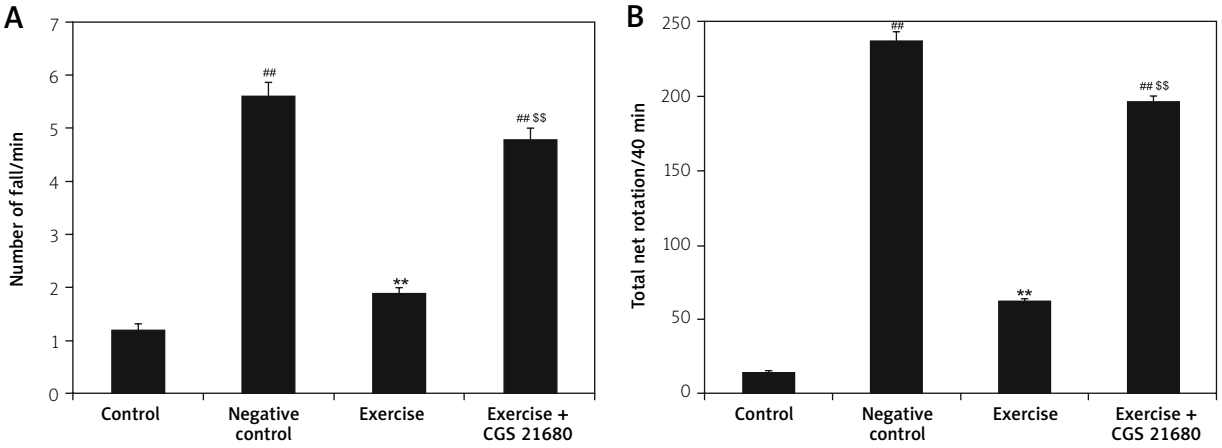

Fig. 2. Effect of exercise and adenosine A_{2A} receptor agonist (CGS 21680) on motor function in rotenone-induced PD rats. **A**) Estimation of the number of falls per min using the rotarod apparatus; **B**) Estimation of total net rotations per 40 min using apomorphine-induced rotation behaviour. Mean ±SD (n = 6); $^{\#\#}p < 0.01$ compared to the control group, $^{**}p < 0.01$ compared to the negative control group, $^{\$\$}p < 0.01$ compared to the exercise group

Estimation of inflammatory cytokines and oxidative stress parameters

The level of interleukin (IL)-1β, IL-6, and tumor necrosis factor α (TNF-α) was estimated in the tissue homogenate of Parkinson's disease rat using the ELISA kit as per the direction given by the manufacturer of the kit. Oxidative stress parameters such as lipid peroxidation (LPO) and reduced glutathione (GSH) were determined in brain tissue homogenate of the PD rat. The malondialdehyde (MDA) level was estimated in the brain tissue as per Ohkawa method at 532 nm. GSH content was determined in the hippocampal tissue by estimating the absorbance at 412 nm.

Statistical analysis

Data are expressed as mean ±SD (n = 6). One-way analysis of variance (ANOVA) followed by post-hoc Tukey test was used to compare the various groups. $P < 0.05$ was considered statistically significant.

Results

Exercise ameliorates the motor behaviour

Motor function was estimated in rotenone-induced PD rats treated with exercise and CGS 21680 using the rotarod apparatus and apomorphine-induced rotation behaviour as shown in Figure 2A, B. Effect of exercise was determined by the number of falls per min using the rotarod apparatus. There was a significant increase in the number of falls per min (rotarod apparatus) and total net rotations per 40 min (apomorphine-induced rotation behaviour) in the negative control group compared to the control group of rats. The exercise group had a significantly ($p < 0.01$) reduced number of falls per min and total net rotations per 40 min than the negative control group, however treatment with CGS 21680 reverses the positive effect of exercise in PD rats.

Exercise ameliorates cognitive dysfunction

Effect of exercise and CGS 21680 was estimated on learning and memory (cognitive function) in rotenone-induced PD rats using Morris water maze apparatus. Percentage of time spent in the target quadrant and the number of crossings was reduced and escape latency was enhanced in the negative control group compared to the control group of rats. It was observed that exercise increases the time spent in the target quadrant and the number of crossings and decreases escape latency in rotenone-induced PD rats. However, treatment with CGS 21680 reverses the effect of exercise on PD rats (Fig. 3).

Exercise ameliorates the level of neurochemicals

The level of neurochemicals such as dopamine and glutamate was estimated in the brain tissue of exercise- and CGS 21680-treated PD rats as shown in Figure 4. There was a significant reduction in the level of dopamine and the level of glutamate increases in brain tissue of the negative control group compared to the control group of rats. The glutamate level was reduced, and the dopamine level was improved significantly in tissue homogenate of the exercise-treated group com-

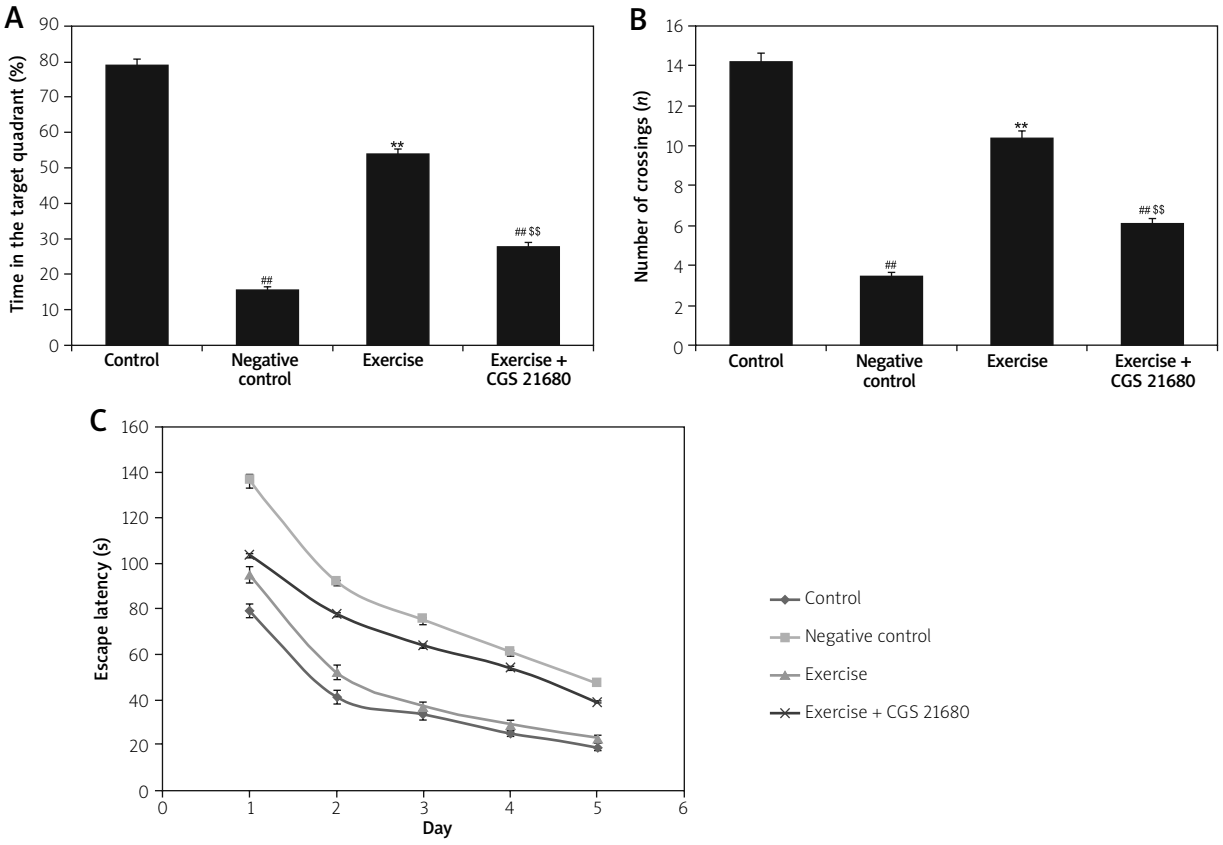

Fig. 3. Effect of exercise and adenosine A_{2A} receptor agonist (CGS 21680) on the cognitive function in rotenone-induced PD rats using Morris water maze apparatus. Mean ±SD (n = 6); $^{\#\#}p < 0.01$ compared to the control group, $^{**}p < 0.01$ compared to the negative control group, $^{\$\$}p < 0.01$ compared to the exercise group.

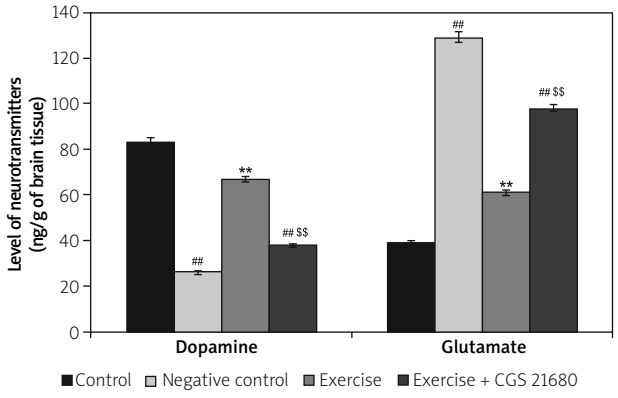

Fig. 4. Effect of exercise and adenosine A_{2A} receptor agonist (CGS 21680) on the level of neurochemicals such as dopamine and glutamate in brain tissue homogenate of rotenone-induced PD rats using Morris water maze apparatus. Mean ±SD (n = 6); $^{\#\#}p < 0.01$ compared to the control group, $^{**}p < 0.01$ compared to the negative control group, $^{\$\$}p < 0.01$ compared to the exercise group.

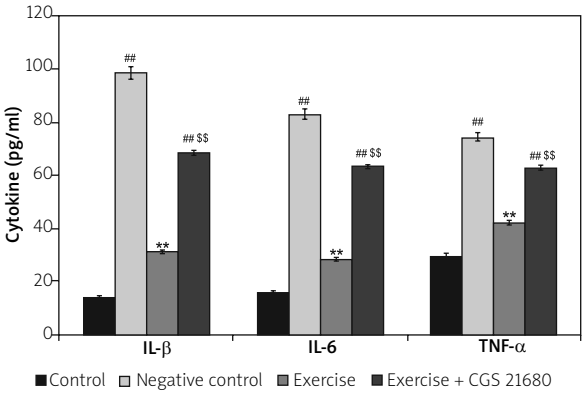

Fig. 5. Effect of exercise and adenosine A_{2A} receptor agonist (CGS 21680) on the level of inflammatory cytokines in brain tissue homogenate of rotenone-induced PD rats. Mean ±SD (n = 6); $^{\#\#}p < 0.01$ compared to the control group, $^{**}p < 0.01$ compared to the negative control group, $^{\$\$}p < 0.01$ compared to the exercise group.

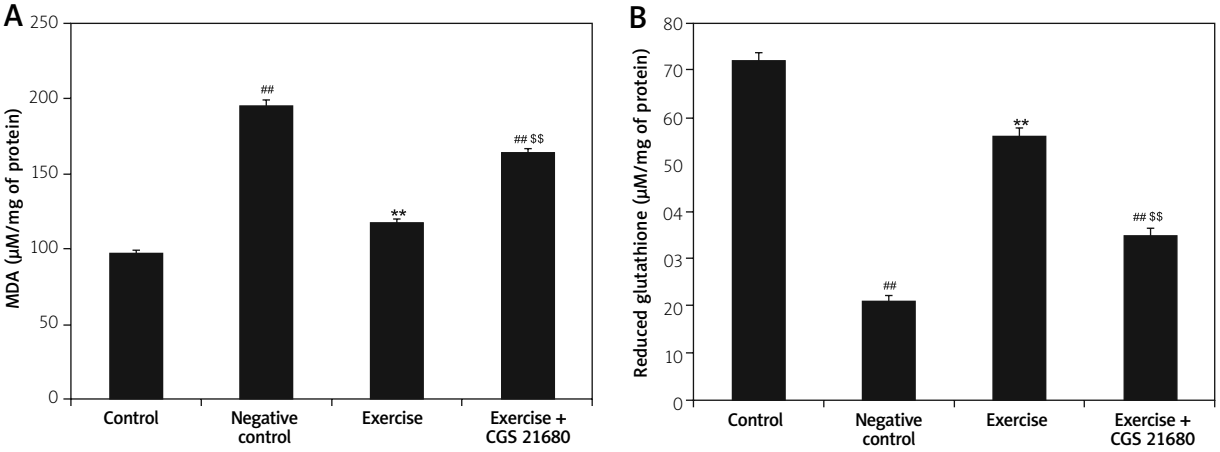

Fig. 6. Effect of exercise and adenosine A_{2A} receptor agonist (CGS 21680) on oxidative stress parameters such as MDA and GSH level in brain tissue homogenate of rotenone-induced PD rats. Mean ±SD (n = 6); $^{\#\#}p < 0.01$ compared to the control group, $^{**}p < 0.01$ compared to the negative control group, $^{\$\$}p < 0.01$ compared to the exercise group.

pared to the negative control group. Treatment with CGS 21680 reverses the positive effect of exercise against PD.

Exercise ameliorates inflammatory cytokines

Inflammatory cytokines such as IL-1β, IL-6 and TNF-α were estimated in the tissue homogenate of exercise- and CGS 21680-treated PD rats using ELISA method. The inflammatory cytokine level was significantly enhanced in brain tissue of PD rats compared to the control group. However, exercise ameliorates the altered expression of cytokines in brain tissue of PD rats and CGS 21680 treatment reverses the effect of exercise in PD rats (Fig. 5).

Exercise ameliorates oxidative stress

Oxidative stress parameters such as MDA and GSH level were estimated in brain tissue of exercise- and CGS 21680-treated PD rats as shown in Figure 6. The level of GSH was reduced and the MDA level was increased in the tissue homogenate of the negative control group compared to the control group of rats. There was an increase in GSH and a decrease in the level of MDA in the brain tissue homogenate of the exercise group compared to the negative control group. Treatment with CGS 21680 significantly reverses the effect of exercise on the level of MDA and SOD in brain tissue of PD rats.

Discussion

Parkinson's disease is a degenerative disease of the dopaminergic neuron, which is characterized by loss of motor function [2]. There are several pathogenic pathways such as oxidative stress, neuronal apoptosis, inflammatory pathway, and deposition of α-syn around the neuron which are involved in development of PD [7]. There are several conventional drugs available for its management, which has several limitations. In the recent era, more clinical concern focuses on quality of life of the patient suffering from chronic disorders including PD. The literature reports that exercise has a beneficial effect on PD as it promotes the mobility and delays the degradation of neurodegeneration in PD [15]. Adenosine A_{2A} receptor activation is reported to be observed in PD, which is responsible for neuronal degeneration and reduction in the level of dopamine in the brain tissue [23]. Moreover, AA2 agonist (CGS 21680) is responsible for activation of inflammation and oxidative stress in PD [10]. However, the exact molecular involvement of the exercise effect on motor function and neurodegeneration in PD is yet to be explored.

Parkinson's disease is characterized by classical symptoms such as tremor, akinesia, and rigidity, which occur due to the altered motor function because of degeneration of dopaminergic neurons [6]. Motor function such as muscle coordination and locomotor activity is regulated by motor neurons, which is altered in PD [14] and this is supported by data of the study as muscle coordination and locomotor activity are reduced significantly in PD rats. Exercise improves motor function such as locomotor activity and muscle coordination in PD. Adenosine A2 receptor activation are reported to occur in PD [5] and treatment with AA2 antagonist reverses motor and non-motor function in PD. However, data of the present report reveal that

treatment with CGS 21680 reverses the effect of exercise on motor and non-motor function in PD rats. Cognitive function such as learning and memory is also altered in PD [9] and the present report reveals that exercise improves it, which is altered due to treatment with CGS 21680.

Neurochemical balance is required for the normal functioning of the nervous system and degeneration of dopaminergic neurons altered the level of neurotransmitters including reduction in the dopamine level and increase in the glutamate level in PD [11]. Data of the present study suggest that exercise attenuates the altered neurochemical level in brain tissue of PD rats, CGS 21680 treatment reverses the beneficial effect of exercise on the level of DA and glutamate in PD rats.

Oxidative stress and inflammatory pathway are involved in the pathogenesis of PD. Lipid peroxidation is an oxidative stress marker by determining the concentration of thiobarbituric acid [19]. LPO impairs the function of the cellular membrane by degrading the membrane due to oxidative degradation of polyunsaturated fatty acids [20]. Oxidative stress is one of the major pathways involved in most of the chronic disorders including PD, which causes neuronal injury in substantia nigra [4], and data of the present report also suggest that oxidative stress is enhanced in PD. Moreover, the level of reduced GSH decreases PD, which causes neuronal loss in the brain tissue [3]. Reduced GSH improves the neuronal capacity to metabolize H_2O_2, which is responsible for generation of reactive oxygen species [26]. Exercise ameliorates altered oxidative stress parameters in brain tissue of PD and treatment with CGS 21680 reverses the effect of exercise on oxidative stress parameters in PD rats.

Inflammation is involved in neuronal injury including PD, cytokines such as IL-1β, IL-6 and TNF-α are involved majorly in systemic inflammation, which is responsible for the activation of apoptosis [12]. Parkinson's disease occurs due to neuronal injury in which the inflammatory cytokine level is enhanced, which contributes to neuronal apoptosis in the brain tissue. This is supported by data of the present report and exercise ameliorates the altered level of inflammatory cytokines, which is reversed with CGS 21680 treatment.

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Disclosure

The authors report no conflict of interest.

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