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ENDURANCE EXERCISE PROMOTES CARDIORESPIRATORY REHABILITATION WITHOUT NEURORESTORATION IN THE CHRONIC MOUSE MODEL OF PARKINSONISM WITH SEVERE NEURODEGENERATION

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Abstract

Physical rehabilitation with endurance exercise for patients with Parkinson's disease has not been well established, although some clinical and laboratory reports suggest that exercise may produce neuroprotective effect and restore dopaminergic and motor functions. In this study, we used a chronic mouse model of Parkinsonism, which was induced by injecting male C57BL/6 mice with 10 doses of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (25 mg/kg) and probenecid (250 mg/kg) over five weeks. This chronic Parkinsonian model displays a severe and persistent loss of nigrostriatal neurons resulting in robust dopamine depletion and locomotor impairment in mice. Following the induction of Parkinsonism, these mice were capable to sustain an exercise training program on a motorized rodent treadmill at a speed of 18 m/min, 0° of inclination, 40 min/day, 5 days/week for 4 weeks. At the end of exercise training, we examined and compared their cardiorespiratory capacity, behavior, and neurochemical changes with that of the probenecid-treated control and sedentary Parkinsonian mice. We found that the resting heart rate after 4 weeks of exercise in the chronic Parkinsonian mice was significantly lower than the rate before exercise, whereas the resting heart rate at the beginning and 4 weeks afterwards in the control or sedentary Parkinsonian mice were unchanged. Exercised Parkinsonian mice also recovered from elevated electrocardiogram R-wave amplitude that was detected in the Parkinsonian mice without exercise for 4 weeks. The values of oxygen consumption, carbon dioxide production, and body heat generation in the exercised Parkinsonian mice before and during the Bruce maximal exercise challenge test were all significantly lower than their sedentary counterparts. Furthermore, the exercised Parkinsonian mice revealed a greater mass in the left ventricle of the heart and an increased level of citrate synthase activity in the skeletal muscles. The

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amphetamine-induced, dopamine release-dependent locomotor activity was markedly inhibited in the sedentary Parkinsonian mice, and remained to be inhibited in the exercised Parkinsonian mice. Finally, neuronal recovery from the loss of nigrostriatal tyrosine hydroxylase expression and dopamine levels in the severe parkinsonian mice after exercise was not evident. Taken all together, these data suggest that 4 weeks of treadmill exercise promoted physical endurance resulting in cardiorespiratory and metabolic adaptations in the chronic Parkinsonian mice with severe neurodegeneration without demonstrating a restorative potential for the nigrostriatal dopaminergic function.

Keywords

electrocardiogram; dopamine; MPTP; Parkinson's disease; probenecid; rodent treadmill

INTRODUCTION

Parkinson's disease (PD) is a neurological disorder involving a progressive degeneration of the nigrostriatal dopaminergic neurons. Patients with PD typically exhibit impaired motor functions including muscular rigidity, a reduced ability to initiate voluntary movements, resting tremor, and postural instability resulting in frequent falls (Colcher and Simuni, 1999). Nonmotor related dysfunctions affecting the behavioral, cognitive, cardiorespiratory, sleep, and sensory systems are also importantly contributing to the PD disability (Adler, 2005).

Early interventions with exercise and/or balance training have been reported in a number of clinical studies showing overall improvement in muscle strength, balance, daily activities, motor performance, and ambulation in PD patients (Miyai et al., 2000; Bergen et al., 2002; Hirsch et al., 2003). Although clinical findings suggest that lack of motivation and regular physical activities in PD patients would cause further motor deterioration, and adequate exercise training would benefit their motor performance especially during early disease stages, more research is necessary for validating the long-term benefits of aerobic exercise in PD. Furthermore, it is not known whether the improvement of PD symptoms after exercise/balance training is simply due to physical rehabilitation or is contributed by a complex system involving neuronal adaptation and recovery.

Using the rat model of Parkinsonism induced by 6-hydroxydopamine, either voluntary running or treadmill-paced exercise has attenuated dopamine (DA) loss in the striatum with or without significant recovery of behavioral deficits (Tillerson et al., 2003; Mabandla et al., 2004; Poulton and Muir, 2005). The neuronal recovery in 6-hydroxydopamine-treated rats triggered by exercise is shown to be associated with an increase of the striatal glial cell-line derived neurotrophic factor (Cohen et al., 2003). In an acute mouse model of Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), it is shown that treadmill exercise ameliorates behavioral deficits and reverses several striatal dopaminergic indices including the loss of DA, tyrosine hydroxylase (TH), and DA transporter levels when compared with the sedentary Parkinsonian (PK) animals (Tillerson et al., 2003). In another study, high-intensity treadmill exercise in the acute MPTP-treated mice leads to behavioral recovery; however, the striatal expression of DA transporter is down-regulated and the expression of TH is not changed (Fisher et al., 2004). It is conceivable that differences in the exercise results obtained from animal models of Parkinsonism could be due to experimental variables such as the age and species of the animal, the method and severity of the induced nigrostriatal lesion, the type and intensity of the exercise regimen.

In our laboratory, we have developed and characterized a chronic mouse MPTP/probenecid model of Parkinsonism with severe symptoms resembling those seen in the advanced PD (Lau

et al., 1990; Petroske et al., 2001). In this model, we have observed a robust depletion of striatal DA and its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC). Striatal terminal DA uptake is also markedly inhibited. These effects persist throughout a survival period of at least 6 months that are in contrast to other acute and subacute MPTP mouse models of PD, in which the loss of striatal DA and reduced striatal DA uptake tend to reverse spontaneously over time. In this chronic PK model, the TH immunoreactivity in the substantia nigra pars compacta (SNpc) cells is down regulated and the number of DA neurons is markedly reduced (Petroske et al., 2001). Morphologically, early signs of neuroapoptosis associated with microglial infiltration are detected (Novikova et al., 2006) that is followed by an increasing number of abnormal inclusion bodies found in the degenerative SNpc neurons (Meredith et al., 2002). These inclusions are granular and filamentous in appearance and are immuno-positive to α -synuclein and ubiquitin. At the ultrastructural level, we further confirm that these inclusions contain the dense and granular core similar to that of the classical Lewy bodies. In addition, numerous lobulated, secondary lysosomes filled with lipofuscin are observed in the cytoplasm of α -synuclein-immunoreactive neurons (Meredith et al., 2002). Therefore, this chronic PK model affords a suitable system for examining the impact of endurance exercise on behavioral, cardiorespiratory, and neuronal functions.

In the present study, we tested the hypothesis that endurance exercise training in the chronic PK mice leads to behavioral and functional recovery of the nigrostriatal dopaminergic system. The experimental design of this research was to first establish an optimal treadmill exercise program for the chronic PK mice that would allow them to run and demonstrate an improved capacity of cardiorespiratory and metabolic outcome measures comparable to human conditions of physical fitness. We then analyzed behavioral and neuronal functions in the exercised group of chronic PK mice and made comparisons with the probenecid-treated controls and the sedentary group of chronic PK mice.

EXPERIMENTAL PROCEDURES

Animals

Ten to twelve week-old, male, C57BL/6 mice (Charles River Laboratories, Inc., Wilmington, MA, USA), weighing between 25–27 g at the beginning of the study, were housed in single cages with food pellets and water available *ad libitum*. The room was maintained at a constant temperature and humidity on a 12-h/12-h light/dark cycle. A total of 58 mice were used in the present study. All animal treatments were carried out strictly according to the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23, revised 1996) and were approved by the Institutional Animal Care and Use Committees from the University of Missouri-Kansas City, the University of Kansas Medical Center and the University of Houston. This investigation was conducted with assurance that a minimal number of animals was used and the experimental procedures did not significantly cause animal suffering.

Chronic mouse model of Parkinsonism with severe neurodegeneration

To prepare the chronic PK model with severe neurodegeneration, mice were injected with a total of 10 doses of MPTP hydrochloride (25 mg/kg in saline, s.c.) in combination with an adjuvant, probenecid (250 mg/kg in dimethyl sulfoxide, DMSO, i.p.) as previously described (Petroske et al., 2001). The 10 doses were administered on a five-week schedule with an interval of 3.5 days between consecutive doses. Probenecid is known to promote MPTP accumulation in the brain and to potentiate its neurotoxic effect by impeding the renal excretion and neuronal clearance of MPTP and its toxic metabolites (Lau et al., 1990; Lau, 2005). MPTP hydrochloride and probenecid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Safety

precautions for the use of MPTP during chemical preparation and animal injections were taken according to the procedures previously described (Lau et al., 2005).

Locomotor activity and analysis

A probenecid-treated control or MPTP/probenecid-treated mouse was individually placed in a clear open-top Plexiglas box (40 × 40 × 40 cm) seven days after the chronic treatment. The mouse was videotaped in the transverse plane after initial exploration in the cage environment for fifteen minutes. The pattern of the horizontal movement in the mouse was recorded on video for the next thirty minutes. The video recording was downloaded to a high resolution 720 × 480 video capture card (Avermedia Inc., Milpitas, CA, USA) and the position of mouse was manually digitized for every frame of the downloaded video. The Euclidian distance formula was used to calculate the resultant position of the mouse relative to the origin based on the digitized x and y pixel coordinates. Approximate entropy (ApEn) was used to determine the consistency of the mouse's movement (Pincus, 1991; Pincus & Goldberger, 1994). ApEn is a regularity statistic that evaluates the likelihood that similar patterns in the time series will be present at a later time period. A time series with a more consistent pattern has an ApEn value closer to zero, while a time series with a less consistent or irregular pattern has an ApEn value closer to two (Pincus, 1991; Pincus & Goldberger, 1994; Stergiou *et al.*, 2004). A higher ApEn has previously been associated with locomotive dysfunctions found in the elderly and individuals with PD (Stergiou *et al.*, 2004).

A detailed description of the ApEn algorithm can be found in Pincus (1991) and Pincus & Goldberger (1994). Briefly, the ApEn was calculated using the following equation, where m is the length of segments compared in the time series, r is the similarity criterion, and $C_m(r)$ is the number of segments of length m that are found to be similar in the time series.

$$ApEn(m, r) = \ln \left[\frac{C_m(r)}{C_{m+1}(r)} \right]$$

The two segments of the time series are considered similar if their difference is less than the similarity criterion. For this investigation, we calculated constancy of the mouse's movement with a segment length of two and a similarity criterion of 0.2. Similar values have been used in previous investigations that have used ApEn to investigate the consistency of movement patterns in subjects that have movement disorders (Stergiou *et al.*, 2004; Georgoulis *et al.*, 2006). A 2 × 3 repeated measures ANOVA with a Tukey post-hoc was used to find statistical differences at the 0.05 alpha level in the ApEn values.

In addition, we measured the amphetamine (3 mg/kg, i.p.)-challenged, DA release-dependent locomotor activities in the open-field with an automated behavioral monitor equipped with an X-Y arrangement of infrared photoreceptor beams (16 per side) (Optovarimax, Columbus Instruments, Columbus, OH, USA). Mice were placed in a clear open-top Plexiglas box (40 × 40 × 40 cm) within the monitor in a subdued lighting environment. After an initial 15 min exploratory period, ambulatory locomotor activity that measures the beam breaks only while moving in an X-Y direction was recorded by a computer for 6 successive 5-min periods and a mean score was calculated for each period. Total distance (m) traveled by each animal with either saline or amphetamine treatment was cumulated after every 5 min for the entire 30 min of observation period. The specific amphetamine-stimulated movement activity was plotted by subtracting the results of saline treatment from that of amphetamine treatment.

Endurance exercise in mice

In the present investigation, the effect of treadmill exercise training in chronic PK mice was studied without adding any anti-Parkinsonian medications such as L-dopa, anti-cholinergic drugs and dopamine receptor agonists. A six-lane motorized rodent treadmill (Columbus

Instruments, Columbus, OH, USA) was utilized for exercise training. Following the chronic MPTP/probenecid treatment, the exercised group of animals was introduced to the treadmill slowly over the course of a week with initial orientation and walking on the moving treadmill. The 4 week exercise protocol did not begin until mice could run at a speed of 18 m/min. The training protocol was individualized for each animal aimed at running for 40 min/day for 5 days/week at a speed of 18 m/min with 0° of inclination. Sedentary mice did not exercise; however, they were transported daily to the training room so that they were exposed to the same environment as the exercised group of animals.

In order to monitor the ability of mice to run on a daily basis, the average daily running scores were tabulated for the exercised group. A score of 4 indicated that the animal ran with little assistance for the full 40 minutes. A score of 3 indicated that the animal ran well, but required some manual encouragement, typically consisting of brushing. An animal that required continual prodding to maintain the running speed received a score of 2, and a score of 1 indicated that the animal did not complete the full 40 minute protocol, in which case the length of the run was recorded.

Electrocardiogram recording

Electrocardiograph (ECG) recordings were obtained before the initiation (week 0) and again at the completion (week 4) of exercise program in conscious mice from probenecid-treated control, sedentary PK and exercise-trained PK groups (5–6 animals/group). ECG recordings (leads I, II, III, aVR, aVL, and aVF) were captured with an electrocardiograph (model VS4, Cambridge Instruments; Cambridge, UK). All leads were established by surface electrodes. Heart rates from a period of 1–2 min were analyzed based on ECG tracings and cardiac R wave amplitudes were analyzed from lead I only.

Calorimetric measurements

Indirect calorimetry was carried out using an open circuit small animal calorimeter (Eco-Oxymax) attached to an enclosed modular treadmill (Simplex II, Columbus Instruments, Columbus, OH, USA). The volume of oxygen consumption (VO_2 , L/kg/h), carbon dioxide production (VCO_2 , L/kg/h), and body heat generation (kcal/h) were measured while at rest and during a maximal exercise test, which was modified according to the Bruce protocol designed to evaluate human cardiovascular fitness or coronary disorder under cardiac stress conditions with maximal physical exertion (Bruce et al., 1963). Fresh ambient air was drawn through the chamber at a flow rate of 1 L/min. Each mouse was placed on the stationary treadmill in an enclosed chamber and the resting values were recorded during the first 12 min. The treadmill was then motorized at a speed of 6 m/min, 0° of inclination. Increases in inclination of 5° were made every 12 min. Three m/min stepwise increases in the treadmill speed were made every 6 min. The test was halted when an animal was no longer able to run. The maximal speed reached was 24 m/min; however, many animals failed to reach that speed and stopped running earlier. Since not all animals completed the maximal exercise test beyond 15 m/min with 15° of inclination, calorimetry data above these settings were not analyzed.

Citrate synthase activity assay

Citrate synthase is routinely used as a marker for expressing the muscle metabolic capacity (Holloszy et al., 1970). At the end of exercise study, cardiac, gastrocnemius and soleus muscles (5–6/group) were dissected immediately and stored at -80°C . Frozen tissues were weighed and cut into thin strips with a scalpel or scissors. Muscles were homogenized using a glass homogenizer with Teflon pestle on ice in a buffer consisting of 0.1 M Tris-HCl, pH 8.1, 150 mM NaCl, 0.1% Triton X-100, 1 mM EDTA, and 0.2 mM phenylmethylsulphonyl fluoride at a ratio of 1:20 (w/v). Homogenates were centrifuged at 16,000 $\times g$ for 15 min at 4°C . Supernatants were collected and the extracted protein concentrations in the supernatants were

measured by using a Protein Assay kit (BioRad, Hercules, CA, USA) on an MRX microplate reader (Dynex Technologies; Chantilly, VA, USA).

The citrate synthase activity assay was conducted according to the method described by Siu et al. (2003). Citrate synthase activity was performed in 96-well plates and expressed as optical density at 405 nm (OD_{405}) per minute per mg of total protein. Purified commercial citrate synthase (Sigma, St. Louis, MO, USA) was used as a standard to verify the specificity and for extrapolating the activity of citrate synthase in samples. The citrate synthase activity was carried out in duplicates from three random sampling areas per muscle.

Assays for dopamine content and tyrosine hydroxylase expression

As previously described (Petroske et al., 2001), tissue DA and DOPAC contents were determined by high performance liquid chromatography with electrochemical detection. Tissue preparation and analysis of TH expression by Western blot procedures have been described in detail and published by our group in a previous study (Lau et al., 2005).

Statistical analysis

All data are presented as mean $\pm$ S.E.M. Statistical comparisons of values between two groups of animals were carried out by using the unpaired two-tailed Student's t-test. When more than two groups of means were compared, a one way analysis of variance (ANOVA) with the non-parametric Mann-Whitney U test was applied. In all cases, a *P*-value of less than 0.05 was considered to be significant.

RESULTS

Locomotor deficit and endurance exercise training

The spontaneous movement in chronic PK mice was recorded and compared with the probenecid-treated control mice. We detected a marginal change, though not statistically significant increase in the inconsistency of movement pattern (ApEn) in mice 1, 3 and 7 days after the last injection with MPTP/probenecid. Our analyses indicated no significant differences in the main effect for Group ($F(1,4) = 1.76$, $p = 0.26$) and for Day ($F(2,8) = 1.49$, $p = 0.28$). However, there was a significant interactive effect for Group $\times$ Day ($F(2,8) = 13.35$, $p = 0.003$), which was further confirmed by the post-hoc analysis showing a significant difference ($p < 0.05$) in the consistency of the movement pattern between control and chronic PK mice on Day 7 post-treatment (Figure 1A). This observation was also supported by the qualitative features of the movement pattern displayed by the control and chronic PK mice. It is apparent that the probenecid-treated control mouse explored the perimeter of the cage with a more consistent movement pattern than the chronic PK mouse (Figure 1B). The movement of the chronic PK mouse appeared to have a less consistent or irregular pattern that may be related to neurodegeneration incurred in the nigrostriatal region (Figure 1C).

Although the chronic PK mice have exhibited dopaminergic locomotor deficit, this deficit did not apparently deter the mice from enduring the daily treadmill exercise. The chronic PK mice were somewhat different for the acute PK mice, which showed an initial handicap in running speed and duration (Fisher et al., 2004). In fact, exercise performance in the chronic PK group had an average daily running score (3.59 ± 0.17) that was significantly better than the running score for the normal non-PK mice (2.49 ± 0.52 , $p < 0.01$).

Cardiac responses

We monitored the ECGs from the control, sedentary PK, and exercised PK mice at the beginning and the end of 4 weeks. The resting heart rate was not different among the three groups of animals following chronic probenecid or MPTP/probenecid treatment (Fig. 2A).

After 4 weeks, the resting heart rate was not altered in the probenecid-treated control or sedentary PK mice; however, it was significantly reduced in the chronic PK mice receiving 4 weeks of treadmill exercise ($p<0.03$) (Fig. 2A).

Our analysis of the ECGs revealed normal cardiac rhythms in all groups of mice. No abnormalities were uncovered in either R-R intervals or ST segments among animal groups (data not shown). However, a striking difference was found in the ECG R wave amplitude. The R wave amplitude was not altered in the probenecid-treated controls at the beginning and the end of 4 weeks without exercise (Fig. 2B). Interestingly, the R wave amplitude was significantly elevated in the chronic sedentary PK mice after 4 weeks with no exercise ($p<0.01$) (Fig. 2B). This phenomenon was not observed in the chronic exercised PK mice (Fig. 2B). Therefore, 4 weeks of exercise in the chronic PK mice reversed the abnormality of ECG R wave as detected in the sedentary counterparts and brought it back to normalcy as seen in the control animals.

We additionally isolated and weighed the left ventricle of the hearts from all 3 groups of animals. As shown in Fig. 3, the left ventricle mass of 4-week exercised PK mice that was expressed as mg per g of body weight raised to the power of 0.78 according to Kemi et al. (2002) was significantly greater than either probenecid-treated control or sedentary PK mice ($p<0.001$). These results indicate that aerobic exercise efficiently produced left ventricle hypertrophy in PK mice, which is consistent with the findings that mice mimic human responses to exercise by increasing the capacity, size and mass of the myocardium (Kemi et al., 2002).

Respiratory and calorimetric responses

Following four weeks with or without exercise, the respiratory gas exchange data were collected in an enclosed calorimeter for all groups of animals, which were challenged by a modified maximal exercise test according to the Bruce protocol (see Experimental Procedures). In all groups of animals (Fig. 4A), the volume of O_2 (VO_2) consumption was highest while running at 6 m/min (0° inclination) and then declined when running at the maximal speed of 15 m/min (15° inclination), which may be due to animal fatigue (Schefer and Talan, 1996). Interestingly, the sedentary PK mice had a higher VO_2 value than the control animals at rest ($p<0.05$); thus, when running at 6 (0° inclination) or 15 (15° inclination) m/min, there were only marginal increases of VO_2 due to their high basal VO_2 to begin with (Fig. 4A). The level of O_2 consumption in the exercise-trained, chronic PK mice at rest or while running at 6 and 15 m/min was not different from that of the control animals, but it was significantly lower than the sedentary PK mice ($p<0.05$).

In contrast, the resting expiratory CO_2 production (VCO_2) in all three groups of animals was statistically indifferent (Fig. 4B). The CO_2 production was similarly elevated in all 3 groups of mice when challenged by running at 6 (0° inclination) and 15 (15° inclination) m/min ($p<0.03$) (Fig. 4B). Apparently, CO_2 production was not altered in the chronic PK mice with or without 4 weeks of exercise training.

We further measured body heat production in three groups of animals before and during the maximal exercise test in the enclosed calorimeter. In all groups of mice, only marginal release of body heat was recorded when running at 6 (0° inclination) and 15 (15° inclination) m/min (Fig. 4C). It was noticeable that the overall body heat generation in exercise-trained PK mice was correspondingly and significantly lower than the sedentary PK group at rest or running at 6 (0° inclination) or 15 (15° inclination) m/min ($p<0.01$) (Fig. 4C).

Skeletal and cardiac muscle citrate synthase activity

To further confirm that the 4-week treadmill exercise program used in this study reached an endurance level, we determined the citrate synthase activity in the gastrocnemius, soleus, and cardiac muscles. As expected, the citrate synthase activity in the gastrocnemius muscle of the exercised PK mice was significantly higher than either the probenecid-treated controls or sedentary PK mice ($p < 0.001$) indicating that the state of aerobic fitness was metabolically achieved in the chronic PK mice following 4 weeks of exercise (Fig. 5). Similar findings were also found in the soleus muscle; however, the citrate synthase activity in the cardiac muscle was indifferent among all three groups of animals (data not shown).

Dopaminergic locomotor behavior

Locomotor deficit is a hallmark of Parkinson's disorder, which can be demonstrated in laboratory animals by monitoring their ambulatory activities in an open-field apparatus. In the present study, we monitored the amphetamine (3 mg/kg, i.p.)-challenged movement, which is dependent on the indirect action of amphetamine to release the endogenous neuronal DA. It is noteworthy that 4 weeks after chronic MPTP/probenecid treatment, we detected a significant decline of amphetamine-stimulated ambulatory movement in the chronic PK mice when compared to the probenecid-treated control animals ($p < 0.01$) suggesting a long-term motor deficit accompanied by the loss of DA neurons in the basal ganglia of the chronic PK mice (Fig. 6). In the exercised PK mice, the amphetamine-induced locomotor activity was still significantly lower than the probenecid-treated controls ($p < 0.02$) and not significantly different from the sedentary PK mice (Fig. 6).

Nigrostriatal tyrosine hydroxylase and dopamine levels

To determine whether treadmill exercise training would restore nigrostriatal neuronal functions and DA transmission, we measured TH protein, DA and its metabolite, DOPAC levels in the nigrostriatal tissue of chronic PK mice 4 weeks after exercise and compared the results with those of control and sedentary PK mice. With Western blot analysis, we observed a marked reduction of TH protein level in the striatum (Fig. 7A) and SN (Fig. 7B) of the severe chronic PK mice comparing to the probenecid-treated control animals. Four weeks of exercise training only caused a small but not statistically significant elevation of TH contents in the striatum and SN comparing to the sedentary PK mice (Fig. 7A and B). Consistent with our previous findings (Petroske et al., 2001), analysis of DA and DOPAC contents in the striatum revealed that the loss of DA and DOPAC was robust in the severe chronic PK mice (Fig. 8A and B). After four weeks of treadmill exercise, the striatal DA and DOPAC contents in the chronic PK mice remained unchanged (Fig. 8A and B).

DISCUSSION

Patients with PD are confronted with at least two impediments: one is the progressive degeneration of the nigrostriatal neurons, and the other is the resulting physical impairments and disabilities. In order to alleviate neurodegeneration and improve motor performance and quality of life in PD patients, elucidation of neuroprotective and neurorestorative processes and examination of strategies steering towards physical rehabilitation are highly relevant.

According to a research synthesis, it is unquestionable that managed exercise therapies in conjunction with standard medication are likely to reduce the incidences of imbalance, immobility, and fall risk in patients at early stages of PD until significant limitation to exercise is evident (de Goede et al., 2001). Aerobic exercise has been reported to benefit PD patients just as much as to those without PD (Bergen et al., 2002). However, large randomized clinical trials showing evidence-based outcome of exercise on PD rehabilitation are still limited.

Furthermore, neuroprotective and neurorestorative potential of exercise in PD has been hypothesized, but results have been highly debated.

Our present study was designed to first investigate whether 4 weeks of treadmill exercise training could attain physical endurance and cardiorespiratory fitness in the chronic mouse model of Parkinsonism with severe nigrostriatal neurodegeneration. Once physical fitness was established due to exercise, we continued to examine whether animals could recover from the loss of nigrostriatal neurons and impaired movement. Our major findings are that chronic PK mice were capable to maintain the exercise training on a motorized treadmill, and they demonstrated physical endurance, autonomic and metabolic system adaptation. However, even at the exercise level when optimal physical strength and capacity were attained, recovery of the lost neuronal innervation and function in this chronic severe mouse model of Parkinsonism was not evident.

Our observations indicated that although the chronic PK mice had persistently lost a substantial number of TH-positive neurons and most of the neurotransmitter, DA, was depleted in the nigrostriatal system (Petroske et al., 2001), these animals were not inhibited from completing the exercise program on the motorized treadmill. It is obvious that we could not expect a severe PD patient to endure the rigor of treadmill exercise even at low speed without support. Unlike the human PD, we were able to study the exercise effect in laboratory on a quadrupedal PK model, which showed inconsistent movement pattern (Fig. 1) and suppressed dopaminergic locomotion (Fig. 6) as signs of basal ganglia deficit. However, the chronic PK mice did not lose their body balance and readily adapt to 4 weeks of running on the motorized treadmill without constraint.

In PD, besides the prominent feature of distress in movement, secondary complications involving disturbances in the autonomic system have been widely reported (Adler, 2005). However, interpreting the clinical reports on autonomic symptoms in PD and analyzing changes occurred after aerobic exercise could be difficult and complicated by the factors such as the severity of disease, old age, and the use of antiparkinson's medications, which can lead to autonomic changes too. To the best of our knowledge, cardiorespiratory and musculometabolic parameters before and after endurance exercise in an animal model of Parkinsonism have not been evaluated and published. In the present study, we report that at the completion of MPTP/probenecid treatment, the chronic PK mice displayed a normal myocardial rhythmic rate. However, after 4 weeks of sedentariness, these mice showed elevated R wave amplitude from their ECGs (Fig. 2). Increased R wave amplitude has been used as an indicator for medical conditions like coronary insufficiency and left ventricular ischemia (Baron et al., 1980; Poyatos et al., 1984). Our observation on the R wave change could be related to cardiac hypoperfusion and muscle weakness as seen in patients with Parkinsonism (Mathias, 1996). Four weeks of treadmill exercise in chronic PK mice brought the R wave defect back to normal (Fig. 2). Furthermore, exercise training led to a significant reduction in the resting heart rate (Fig. 2). Our findings are consistent with the previously published results in animals and humans demonstrating a similar drop of resting heart rate following endurance exercise training (Wei et al., 1987). The exercise-induced resting bradycardia could be the consequence of slowed intrinsic rate of the pacemaker or increased cholinergic predominance on the pacemaker frequency (Badeer, 1975). Our determination of the left ventricular mass (Fig. 3) further supported the notion that exercise in chronic PK mice had caused left ventricular hypertrophy that could increase cardiac output and improve contractile function in the heart (Kaplan, et al., 1994).

It has been reported that the resting energy expenditure and O₂ consumption during exercise in PD patients are higher than their age-matched control subjects (Markus et al., 1992; Protas et al., 1996). Our calorimetric study similarly showed that O₂ consumption by the chronic PK

mice at rest and during maximal exercise test was higher than the control animals (Fig. 4). Following 4 weeks of exercise training, the O₂ demand and body heat production were declined in the chronic PK mice when compared to their sedentary counterparts (Fig. 4) suggesting that physical tolerance has developed in the exercised PK animals.

Accompanying the exercise effects on physical endurance and cardiorespiratory adaptations, the impact of aerobic exercise program in the chronic PK mice was further supported by the observation of increased citrate synthase activity in the skeletal muscles (Fig. 5), which is commonly expected in animals trained with aerobic exercise (Murakami et al., 1994). The exercise effect on citrate synthase activation was specific to skeletal muscles, whereas the cardiac muscle did not show any appreciable changes in the enzyme activity. It is believed that the cardiac muscle being an excitable tissue might already pace to work close to its optimal oxidative capacity; thus, exercise training could not further increase the oxidative capacity and citrate synthase activity in the myocardium (Siu et al., 2003).

In spite of the remarkable physical endurance developed in the chronic PK mice following 4 weeks of exercise, significant recovery from the loss of nigrostriatal TH expression and DA content due to chronic MPTP/probenecid treatment was not evident (Fig. 7 and 8). Behaviorally, the amphetamine-induced, DA release-dependent locomotor activity was still markedly inhibited (Fig. 6). The results from amphetamine-induced behavior and neurochemical studies collectively indicate that dopaminergic transmission and function remained deficient after 4 weeks of exercise in the chronic PK mice. Fisher et al. (2004) also reported that exercise did not elevate the TH-immunoreactivity in an acute model of Parkinsonism using young adult mice, which were treated with 4 serial injections of 20 mg/kg MPTP at 2 hr intervals and exercised on a motorized treadmill at a speed of 20.5–23.0 m/min, 2 × 30 min/day, 5 days/week for 30 days. In another study, however, Tillerson et al. (2003) found that exercise attenuated the loss of striatal TH and DA in an acute model of Parkinsonism with retired breeder mice, which were treated with 2 injections of MPTP (15 mg/kg), 12 hr apart, and exercised at a speed of 5 m/min, 2 × 5 min/day for 9 days. It is likely that the inconsistency of results reported by the above studies, including ours, could be contributed by differences in the age of animals, the model used for inducing PK neurodegeneration, and the intensity and duration of exercise paradigm.

The chronic MPTP/probenecid-treated mouse model of Parkinsonism affords a persistent level of nigrostriatal neurodegeneration with severe neuropathological symptoms resembling some of those seen in patients with PD (Petroske et al., 2001; Lau, 2005). Studying the impact of exercise in neurodegenerative disorders by using this chronic mouse model of Parkinsonism, we determined that exercise promoted physical rehabilitation resulting in cardiorespiratory and metabolic adaptation in this model. However, exercise in the severe, chronic PK animals did not demonstrate a neurorestorative potential or produce an appreciable recovery from the loss of nigrostriatal dopaminergic function.

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Abbreviations

ApEn, approximate entropy; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; ECG, electrocardiograph; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; PK, Parkinsonian; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase.

REFERENCES

- Adler CH. Nonmotor complications in Parkinson's disease. *Mov Disord* 2005;20(Suppl 11):S23–29. [PubMed: 15822106]
- Badeer HS. Resting bradycardia of exercise training: a concept based on currently available data. *Recent Adv Stud Cardiac Struct Metab* 1975;10:553–560. [PubMed: 1108127]
- Baron DW, Ilsley C, Sheiban I, Poole-Wilson PA, Rickards AF. R wave amplitude during exercise. Relation to left ventricular function and coronary artery disease. *Br Heart J* 1980;44:512–517. [PubMed: 7437190]
- Bergen JL, Toole T, Elliott RG 3rd, Wallace B, Robinson K, Maitland CG. Aerobic exercise intervention improves aerobic capacity and movement initiation in Parkinson's disease patients. *NeuroRehabilitation* 2002;17:161–168. [PubMed: 12082243]
- Bruce RA, Blackmon JR, Jones JW, Strait G. Exercising testing in adult normal subjects and cardiac patients. *Pediatrics* 1963;32:742–756. [PubMed: 14070531]
- Cohen AD, Tillerson JL, Smith AD, Schallert T, Zigmond MJ. Neuroprotective effects of prior limb use in 6-hydroxydopamine-treated rats: possible role of GDNF. *J Neurochem* 2003;85:299–305. [PubMed: 12675906]
- Colcher A, Simuni T. Clinical manifestations of Parkinson's disease. *Med Clin North Am* 1999;83:327–347. [PubMed: 10093581]
- de Goede CJ, Keus SH, Kwakkel G, Wagenaar RC. The effects of physical therapy in Parkinson's disease: A research synthesis. *Arch Phys Med Rehabil* 2001;82:509–515. [PubMed: 11295012]
- Fisher BE, Petzinger GM, Nixon K, Hogg E, Bremner S, Meshul CK, Jakowec MW. Exercise-induced behavioral recovery and neuroplasticity in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse basal ganglia. *J Neurosci Res* 2004;77:378–390. [PubMed: 15248294]
- Georgoulis AD, Moraiti C, Ristanis, Stergiou N. A novel approach to measure variability in the anterior cruciate ligament deficient knee during walking: the use of the approximate entropy in orthopaedics. *J Clin Mon Comp* 2006;20:11–18.
- Hirsch MA, Toole T, Maitland CG, Rider RA. The effects of balance training and high-intensity resistance training on persons with idiopathic Parkinson's disease. *Arch Phys Med Rehabil* 2003;84:1109–1117. [PubMed: 12917847]
- Holloszy JO, Oscai LB, Don IJ, Mole PA. Mitochondrial citric acid cycle and related enzymes: adaptive response to exercise. *Biochem Biophys Res Commun* 1970;40:1368–1373. [PubMed: 4327015]
- Kaplan ML, Cheslow Y, Vikstrom K, Malhotra A, Geenen DL, Nakouzi A, Leinwand LA, Buttrick PM. Cardiac adaptations to chronic exercise in mice. *Am J Physiol* 1994;267:H1167–1173. [PubMed: 8092282]
- Kemi OJ, Loennechen JP, Wisloff U, Ellingsen O. Intensity-controlled treadmill running in mice: cardiac and skeletal muscle hypertrophy. *J Appl Physiol* 2002;93:1301–1309. [PubMed: 12235029]
- Lau, YS. Progressive neurodegeneration in the chronic MPTP/probenecid model of Parkinson's disease.. In: Ebadi, M.; Pfeiffer, R., editors. *Parkinson's Disease*. CRC Press; Boca Raton, FL: 2005. p. 109–115.
- Lau YS, Novikova L, Roels C. MPTP treatment in mice does not transmit and cause Parkinsonian neurotoxicity in non-treated cagemates through close contact. *Neurosci Res* 2005;52:371–378. [PubMed: 15936837]
- Lau YS, Trobough KL, Crampton JM, Wilson JA. Effects of probenecid on striatal dopamine depletion in acute and long-term 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice. *Gen Pharmacol* 1990;21:181–187. [PubMed: 2332138]
- Mabandla M, Kellaway L, St Clair Gibson A, Russell VA. Voluntary running provides neuroprotection in rats after 6-hydroxydopamine injection into the medial forebrain bundle. *Metab Brain Dis* 2004;19:43–50. [PubMed: 15214505]
- Markus HS, Cox M, Tomkins AM. Raised resting energy expenditure in Parkinson's disease and its relationship to muscle rigidity. *Clin Sci (Lond)* 1992;83:199–204. [PubMed: 1327636]
- Mathias, CJ. Disorders affecting autonomic function in Parkinsonian patients.. In: Battistini, L.; Scarlato, G.; Caraceni, T.; Ruggieri, S., editors. *Parkinson's Disease*. Raven Press; New York: 1996. p. 383–391.

- Meredith GE, Totterdell S, Petroske E, Santa Cruz K, Callison RC Jr, Lau YS. Lysosomal malfunction accompanies alpha-synuclein aggregation in a progressive mouse model of Parkinson's disease. *Brain Res* 2002;956:156–165. [PubMed: 12426058]
- Miyai I, Fujimoto Y, Ueda Y, Yamamoto H, Nozaki S, Saito T, Kang J. Treadmill training with body weight support: its effect on Parkinson's disease. *Arch Phys Med Rehabil* 2000;81:849–852. [PubMed: 10895994]
- Murakami T, Shimomura Y, Fujitsuka N, Nakai N, Sugiyama S, Ozawa T, Sokabe M, Horai S, Tokuyama K, Suzuki M. Enzymatic and genetic adaptation of soleus muscle mitochondria to physical training in rats. *Am J Physiol* 1994;267:E388–E395. [PubMed: 7943219]
- Novikova L, Garriss BL, Garriss DR, Lau YS. Early signs of neuronal apoptosis in the substantia nigra pars compacta of the progressive neurodegenerative mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid model of Parkinson's disease. *Neuroscience* 2006;140:67–76. [PubMed: 16533572]
- Petroske E, Meredith GE, Callen S, Totterdell S, Lau YS. Mouse model of Parkinsonism: a comparison between subacute MPTP and chronic MPTP/probenecid treatment. *Neuroscience* 2001;106:589–601. [PubMed: 11591459]
- Pincus SM. Approximate entropy as a measure of system complexity. *Proc Nat Acad Sci* 1991;88:2297–2301. [PubMed: 11607165]
- Pincus SM, Goldberger AL. Physiological time-series analysis: what does regularity quantify? *Am J Physiol* 1994;226:H1643–H1656. [PubMed: 8184944]
- Poulton NP, Muir GD. Treadmill training ameliorates dopamine loss but not behavioral deficits in hemiparkinsonian rats. *Exp Neurol* 2005;193:181–197. [PubMed: 15817277]
- Poyatos ME, Lerman J, Estrada A, Chiozza M, Perosio A. Predictive value of changes in R-wave amplitude after exercise in coronary heart disease. *Am J Cardiol* 1984;54:1212–1215. [PubMed: 6507292]
- Protas EJ, Stanley RK, Jankovic J, MacNeill B. Cardiovascular and metabolic responses to upper- and lower-extremity exercise in men with idiopathic Parkinson's disease. *Phys Ther* 1996;76:34–40. [PubMed: 8545491]
- Schefer V, Talan MI. Oxygen consumption in adult and AGED C57BL/6J mice during acute treadmill exercise of different intensity. *Exp Gerontol* 1996;31:387–392. [PubMed: 9415121]
- Siu PM, Donley DA, Bryner RW, Alway SE. Citrate synthase expression and enzyme activity after endurance training in cardiac and skeletal muscles. *J Appl Physiol* 2003;94:555–560. [PubMed: 12531911]
- Stergiou, N.; Buzzi, UH.; Kurz, MJ.; Heidel, J. Nonlinear tools in human movement.. In: Stergiou, N., editor. *Innovative Analyses of Human Movement*. Human Kinetics; Champaign, Illinois: 2004. p. 63-90.
- Tillerson JL, Caudle WM, Reveron ME, Miller GW. Exercise induces behavioral recovery and attenuates neurochemical deficits in rodent models of Parkinson's disease. *Neuroscience* 2003;119:899–911. [PubMed: 12809709]
- Wei JY, Li Y, Ragland J. Effect of exercise training on resting blood pressure and heart rate in adult and aged rats. *J Gerontol* 1987;42:11–16. [PubMed: 3794189]

Figure 1.

Movement deficit in chronic PK mice. (A) Comparison of approximate entropy (ApEn) values between the chronic probenecid-treated and chronic MPTP/probenecid-treated mice 7 days after treatment (N=5 per group). *Significantly higher than the probenecid-treated control animals (mean $\pm$ S.E.M., $p < 0.05$). A larger ApEn value denotes a less consistent movement pattern in the chronic PK mice. Digitized positions of a representative chronic probenecid-treated control mouse (B) and a chronic MPTP/probenecid-treated PK mouse (C) 7 days after treatment. Qualitatively it is apparent that the control mouse explored the perimeter of the cage with a more consistent movement pattern than the chronic PK mouse.

Figure 2.

ECG analysis of cardiac responses to endurance exercise in chronic PK mice. (A) The resting heart rate in beats per min (bpm) was recorded in each experimental group (N=5–6/group) at the beginning (Week 0) and end of 4-week period with or without exercise (Week 4) and their values (mean $\pm$ S.E.M.) were compared. There were no significant intra-group differences in the resting heart rate among the three experimental groups at Week 0. After 4 weeks without exercise, the resting heart rate of the probenecid-treated control and sedentary PK group was not changed from their respective value at Week 0. However, the resting heart rate in the 4-week exercised PK mice was significantly lower than their rate at the beginning of exercise (* $p < 0.007$). (B) The amplitude of the ECG R wave was also analyzed in the three groups of animals at the beginning of the study (Week 0) and again 4 weeks later (Week 4). There were

no significant intra-group differences in the R wave amplitude in all 3 groups of mice at Week 0. There was a significant increase in the R wave amplitude in the sedentary PK mice at Week 4 comparing to Week 0 (* $p < 0.01$). The R wave amplitude in the exercised PK mice at Week 4 was not different from the probenecid-treated control animals; however, it was significantly lower than the 4 week sedentary PK mice (* $p < 0.001$).

Figure 3.

Effect of exercise on myocardial left ventricle mass in the chronic PK mice. The left ventricle of the heart from each experimental animal was isolated and weighed. The left ventricle mass was expressed in mg per g of body weight raised to the power of 0.78, an index factor for estimating the lean body mass (Kemi et al., 2002). The left ventricle mass (mean ± S.E.M.) of the exercised PK mice (N=4) was significantly greater than the mass of either the probenecid-treated controls (N=5) or the sedentary PK mice (N=5) (*p<0.001) suggesting that 4 weeks of exercise induced cardiac hypertrophy in the chronic PK mice.

Figure 4.

Effect of exercise on respiratory and thermal responses. At the end of 4-week session with or without exercise, O₂ consumption (VO₂), CO₂ production (VCO₂), and body heat generation were measured in all 3 experimental groups of animals (N=5–6/group) in an enclosed treadmill/calorimetry apparatus before and during the Bruce maximal exercise test. (A) The sedentary PK mice had a significantly higher before-test O₂ consumption than either probenecid-treated or exercised PK mice (*p<0.05). During the maximal exercise test, VO₂ consumption was expectedly elevated in all groups of animals while running at 6 m/min (0° inclination) (ⓐ p<0.05). No further increase of VO₂ was detected while running maximally at 15 m/min (15° inclination), which might be an indication of physical fatigue. Comparing VO₂ at 6 m/min (0°

inclination) and 15 m/min (15° inclination), the exercised PK mice consumed less O₂ than the sedentary counterparts ($\phi p < 0.05$). (B) The before-test CO₂ production in all 3 groups of animals was statistically indifferent. During the maximal exercise test, VCO₂ levels were elevated in all groups of animals when running at 6 m/min (0° inclination) and at 15 m/min (15° inclination) (* $p < 0.05$). Running at 6 m/min (0° inclination), VCO₂ produced by the exercised PK mice was significantly lower than that produced by the sedentary PK mice ($^3p < 0.03$). (C) During the maximal exercise test, all groups of animals generated slightly higher but not statistically significant body heat when running at 6 m/min (0° inclination) and at 15 m/min (15° inclination). The values were indifferent between probenecid-treated controls and sedentary PK mice. However, body heat production by the exercised PK mice before and during the maximal exercise test were significantly lower than the sedentary PK mice (* $p < 0.01$).

Figure 5.

Effect of exercise on citrate synthase activity in the gastrocnemius muscle. The citrate synthase activity (mean $\pm$ S.E.M.) in the gastrocnemius muscle of the 4-week exercised PK mice (N=6) was significantly higher than the activity obtained from the probenecid-treated (N=5) or sedentary PK mice (N=6) (* p <0.001).

Figure 6.

Effect of exercise on amphetamine-induced locomotor activity. The horizontal movement expressed as cumulative distance traveled by each animal was recorded over 30 min. The amphetamine (3 mg/kg, i.p.)-induced locomotor activity in the sedentary PK mice was significantly inhibited after 4 weeks without exercise when compared with the probenecid-treated control mice (* $p < 0.01$). The amphetamine-induced locomotor activity remained to be inhibited after four weeks of exercise in the chronic PK mice when compared with the control mice (** $p < 0.02$) and not significantly different from the sedentary PK mice.

Figure 7.

Western blot analysis of TH protein expression in (A) the striatum and (B) substantia nigra of chronic probenecid-treated (N=5), sedentary PK (N=6), and exercised PK mice (N=6). The TH content was expressed as an OD ratio with reference to that of β -tubulin. Western blots revealed that there was a marked reduction of TH content in both striatum and SN of the sedentary PK mice 4 weeks after treatment when compared with the probenecid-treated mice (* P <0.001). The TH protein expressions in the striatum and SN of 4-week exercised PK mice were significantly lower than the probenecid-treated controls (** p <0.03) but not significantly higher than the sedentary counterparts.

Figure 8. Striatal DA (A) and DOPAC (B) contents in chronic probenecid-treated (N=5), sedentary PK (N=6), and exercised PK mice (N=6). Striatal DA and DOPAC levels in the sedentary PK mice 4 weeks after treatment were significantly lower than the probenecid-treated mice (* $P < 0.0001$). The striatal DA and DOPAC levels in 4-week exercised PK mice were significantly lower than the probenecid-treated controls (** $p < 0.0001$) but not significantly different from the sedentary counterparts.