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NEUROPROTECTIVE EFFECTS OF SWIMMING TRAINING IN A MOUSE MODEL OF PARKINSON'S DISEASE INDUCED BY 6-HYDROXYDOPAMINE

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Abstract—Parkinson's disease (PD) is characterized by progressive dopamine (DA) depletion in the striatum. Exercise has been shown to be a promising non-pharmacological approach to reduce the risk of neurodegeneration diseases. This study was designed to investigate the potential neuroprotective effect of swimming training (ST) in a mouse model of PD induced by 6-hydroxydopamine (6-OHDA) in mice. The present study demonstrated that a 4-week ST was effective in attenuating the following impairments resulting from 6-OHDA exposure: (i) depressive-like behavior in the tail suspension test; (ii) increase in the number of falls in the rotarod test; (iii) impairment on long-term memory in the object recognition test; (iv) increase of the reactive species and interleukin 1-beta (IL-1β) levels; (v) inhibition of the glutathione peroxidase (GPx) activity; (vi) rise of the glutathione reductase (GR) and glutathione S-transferase (GST) activities and vii) decrease of DA, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) levels. The mechanisms involved in this study are the modulation of GPx, GR and GST activities as well as IL-1β level in a PD model induced by 6-OHDA, protecting against the decrease of DA, DOPAC and HVA levels in the striatum of mice. These findings reinforce that one of the effects induced by exercise on neurodegenerative disease, such as PD, is due to antioxidant and anti-inflammatory properties. We suggest that exercise attenuates cognitive and motor declines, depression, oxidative stress, and neuroinflammation induced by 6-OHDA supporting the hypothesis that exercise can be used as a non-pharmacological tool to reduce the symptoms of PD.

Key words: Parkinson's disease, exercise, 6-hydroxydopamine, cognitive impairment, oxidative stress, neuroinflammation.

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Abbreviations: 6-OHDA, 6-hydroxydopamine; ANOVA, analysis of variance; BW, body weight; CAT, catalase; CS, citrate synthase; DA, dopamine; DAT, dopamine transporter; DCHF-DA, 2′,7′-dichlorofluorescein diacetate; DCF, 2′,7′-dichlorofluorescein; DOPAC, 3,4-dihydroxyphenylacetic acid; EDTA, ethylenediaminetetraacetic acid; GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione S-transferase; H_2O_2, hydrogen peroxide; HVA, homovanillic acid; IL-1β, interleukin 1-beta; LTM, long-term memory; NADPH, nicotinamide adenine dinucleotide phosphate-oxidase; OFT, open-field test; ORT, object recognition test; PD, Parkinson's disease; RS, reactive species; SAP, swimming adaption period; ST, swimming training; STM, short-term memory; TST, tail suspension test.

INTRODUCTION

Parkinson's disease (PD) is characterized by progressive degeneration of dopaminergic neurons in the nigrostriatal system and dopamine (DA) depletion in the striatum. While the pathogenesis of PD is not clear, damage of dopaminergic neurons by oxygen-derived free radicals is considered to be an important contributing mechanism (Kabuto and Yamanushi, 2011). At the time of diagnosis, patients typically display an array of motor impairments including bradykinesia, resting tremor, rigidity, and postural instability. Although most of the typical motor impairments are due to the loss of nigrostriatal dopaminergic neurons, PD affects multiple neuronal systems both centrally and peripherally, leading to a constellation of non-motor symptoms including olfactory deficits, affective disorders, memory impairments, as well as autonomic and digestive dysfunctions (Noyce et al., 2012; Dasuri et al., 2013).

6-Hydroxydopamine (6-OHDA) is used to produce an animal model of PD, and is considered an endogenous toxin which is found in urine from parkinsonian patients (Andrew et al., 1993). The toxicity of 6-OHDA is thought to be related to its ability to produce free radicals and to cause oxidative stress, which may lead to the induction of inflammation and finally cell death (Koprich et al., 2008; Shobana et al., 2012). 6-OHDA is susceptible to autooxidation, resulting in the formation of 6-OHDA quinine and hydrogen peroxide (H_2O_2), superoxide radical ($^{\cdot}O^{2-}$), and hydroxyl radical ($^{\cdot}OH$) (Opacka-Juffry et al., 1998). These active oxygen forms are neurotoxic because of their strong oxidizing potential (Kabuto and Yamanushi, 2011). The unilateral intrastriatal injection of 6-OHDA induces pronounced behavioral alterations as well as biochemical and neurochemical deficits similar to PD (Tadaiesky et al., 2008; Santiago et al., 2010; Shobana et al., 2012). However, the bilateral lesions caused severe impairments on behavior analysis resulting in a high mortality rate of animals (Iancu et al., 2005).

Exercise has been shown to be a promising non-pharmacological approach to reduce the risk of neurodegeneration diseases (O'Dell et al., 2012; Tuon et al., 2012). It has been demonstrated that exercise can both improve and alleviate memory loss in the elderly (Kramer et al., 2006). Despite the fact that biological and molecular bases for such benefits are inconclusive, antioxidant and anti-inflammatory properties of physical exercise may contribute for the neuroprotection in models of PD in rodents (Mabandla and Russell, 2010; Tajiri et al., 2010; Dimatelis et al.,

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2013). In several studies, implementing continuous exercise programs for individuals in the early stages of PD has resulted in improved daily activity, motor performance, ambulation and overall functional independence (Ayán and Cancela, 2012).

The neuroprotective impact of exercise and its mechanisms may be better investigated by conducting laboratory experiments with animal models. Thus, we sought to investigate the potential neuroprotective effect of physical exercise training in the striatum of mice exposed to 6-OHDA. For this purpose, in this study we verified if a swimming training (ST) program could revert behavior alterations (depression, memory and coordination activity) induced by the injection of 6-OHDA. In addition, we investigated the potential protective effect of swimming exercise training against stress oxidative (reactive species (RS) levels and activities of catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR)), neurochemical alterations (DA, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC)) and neuroinflammation (interleukin-1 beta (IL-1β)) induced by the injection of 6-OHDA in the striatum in mice.

EXPERIMENTAL PROCEDURES

Animals

Experiments were performed using male C57B/6J mice (20–30 g, 90 days old). Animals were maintained at 22–25 °C with free access to water and food, under a 12:12-h light/dark cycle, with lights on at 7:00 a.m. All manipulations were carried out during the light phase on that day. All efforts were made to minimize animal suffering and to reduce the number of animals used. The procedures of this study were conducted according to the guidelines of the Committee on the Care and Use of Experimental Animal Resources and with the approval of the Ethics Committee for Animal Use (CEUA protocol # 038/2012).

Experimental design

Mice were randomly assigned into four groups (n = 10 per group): (1) vehicle/sedentary; (2) 6-OHDA/sedentary; (3) vehicle/exercise and (4) 6-OHDA/exercise. In this experimental design (Fig. 1), exercise groups were submitted to ST for 4 weeks with a progressive increase time and constant intensity. Sedentary groups were maintained in physical inactivity. After 24 h of the last bout of 2-week adaptation training, mice received stereotaxic surgery injection of 6-OHDA or vehicle. Four days after stereotaxic surgery injection, start of ST for 4 weeks and after day mice underwent cognitive behavioral tests for 3 days and, finally, submitted to euthanasia. The striatum and quadriceps femoris muscle were removed for assays.

ST protocol

Mice in the exercise groups were submitted to a 4-week ST program, adapted from Huang et al. (2010). Before 2 weeks of stereotaxic surgery, mice were acclimatized to the unfamiliar activity (swimming adaption period – SAP) in a 250-L water-filled tank with the temperature kept at 31 ± 2 °C, in order to decrease the stress of swimming activity. In the SAP, animals could swim or stand in the tank with a water depth of 5 cm. In the beginning of the second week, water depth was increased to 20 cm, so that the hind limbs of the animals could not reach the bottom of the tank. Progressively, larger weights were attached to the proximal portions of animal's tails in order to increase the exercise intensity; the weights were 2%, in correspondence to body weight (BW). This intensity is considered below to anaerobic threshold for ST, in which was demonstrated by the literature that workloads of up to 6% BW for rats (Gobatto et al. (2001) and 4% BW for mice (Almeida et al., 2011) can be considered 'sub-threshold' and is indicated to the improvement of aerobic capacity (Gobatto et al., 2001). The ST bouts were performed five times per week, and animals swam individually in a group of 10 animals. After each daily ST bout, animals were towel dried and placed near a heater until the hair dried.

Stereotaxic surgery injection of 6-OHDA

Surgery was performed under anesthesia with 10 mL/kg of 1% ketamine (Bela-Pharm, Vechta, Germany) and 0.2% xylazine (Bayer HealthCare, Leverkusen, Germany). 6-OHDA (Sigma-Aldrich, St. Louis, MO, USA; 5 μg in 2 μL of 0.9% NaCl with 0.2 μg/lL ascorbic acid) was injected slowly (0.5 μL/min) into the right striatum (0.9 mm anterior and 1.8 mm lateral from bregma, 3.0 mm ventral from the dura). After the

Fig. 1. Experimental study design. Object recognition test (ORT); short-term memory (STM); long-term memory (LTM); tail suspension test (TST); motor tests (open field, rotarod and cylinder tests).