Neuroscience Letters 541 (2013) 193–198

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Effects of lifestyle modifications on cognitive impairments in a mouse model of hypercholesterolemia

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HIGHLIGHTS

- Physical exercise and probucol decreased plasma cholesterol levels in LDLr$^{-/-}$ mice.
- Physical exercise mitigates the spatial memory deficits of LDLr$^{-/-}$ mice.
- Probucol did not mitigate the spatial memory deficits of LDLr$^{-/-}$ mice.
- Exposure to EE did not mitigate the spatial memory deficits of adult LDLr$^{-/-}$ mice.
- Exposure to EE induced persistent anxyolitic-like effects in LDLr$^{-/-}$ mice.

ARTICLE INFO

Article history:
Received 4 December 2012
Received in revised form 5 February 2013
Accepted 26 February 2013

Keywords:
Familial hypercholesterolemia
LDL receptor knockout mice (LDLr$^{-/-}$)
Cognitive deficits
Physical exercise
Probucol
Environmental enrichment

ABSTRACT

Epidemiological studies indicate that high midlife plasma cholesterol levels increases the risk of Alzheimer's disease. Moreover, middle-aged familial hypercholesterolemia (FH) subjects show a particularly high incidence of mild cognitive impairments (MCI). These evidence points to hypercholesterolemia as one of the modifiable risk factors focused on prevention/treatment of cognitive deterioration. The present study draws a comparison between pharmacological (lipid-lowering drug probucol) and non-pharmacological (voluntary running wheel, RW) approaches for the management of hypercholesterolemia and cognitive impairments associated with the low-density lipoprotein receptor-deficient (LDLr$^{-/-}$) mice, a well-established rodent model of FH. We also investigated whether exposure to environmental enrichment (EE), a feasible option to increase physical activity in young mice cohort, from birth to adolescence (PN45) yields long-term behavioral changes in adult LDLr$^{-/-}$ mice (PN90). We observed that both probucol and RW significantly decreased total and non-HDL plasma cholesterol levels in LDLr$^{-/-}$ mice. Notably, only physical exercise mitigated the spatial memory deficits of LDLr$^{-/-}$ mice. In addition, we showed that exposure to EE from birth until the adolescence did not mitigate the spatial memory deficits of adult LDLr$^{-/-}$ mice in the object location task, although it induced persistent anxyolitic-like effects in the open field arena. Collectively, our results emphasize the advantages physical exercise, in comparison to lipid-lowering drugs, for the management of cognitive deficits associated with FH.

1. Introduction

Familial hypercholesterolemia (FH) is an inherited metabolic disorder characterized by high levels of plasma low-density lipoproteins (LDL) [12]. Nowadays, with a prevalence of about one in 500 individuals, FH remains the most common monogenic disorder of lipoprotein metabolism, and it is mainly due to mutations in the LDL receptor (LDLr) gene that leads to the plasma accumulation of cholesterol ester-laden LDL particles [21]. Accordingly, familial hypercholesterolemia (FH) patients with defective LDLr display high plasma levels of LDL cholesterol and are at significant risk of developing cardiovascular disease [3].

While hypercholesterolemia has been recognized for decades as a potential risk factor for cardiovascular disease development, recent studies have indicated an association between cholesterol dyshomeostasis and the pathophysiology of sporadic Alzheimer's

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http://dx.doi.org/10.1016/j.neulet.2013.02.043

disease (AD) [7]. Epidemiological studies have shown that hypercholesterolemia is an early risk factor for AD [30], and patients with FH – which are exposed to higher cholesterol levels from early life – may be recognized as a potential high-risk group for cognitive decline. Interestingly, middle age patients with FH show a particularly high incidence of mild cognitive impairments (MCI) [35], corroborated by an experimental model of FH, the ApoB100/LDLr$^{-/-}$ mouse strain [27].

Taken together, these evidence points to hypercholesterolemia as one of the modifiable risk factors focused on prevention/treatment of cognitive deterioration. Pharmacological [14] and non-pharmacological [18] approaches have been used with relative success to manage dyslipidemias. However, contraindications and adverse effects of pharmacotherapy raise interest in non-pharmacological management of diseases, such as physical exercise or diet, not only to improve health, but also the welfare and quality of life.

The present study emphasizes physical exercise as intervention strategy against cognitive impairments [1] associated with FH. Voluntary running wheel (RW) was used to assess activity-mediated cholesterol lowering and/or behavior in mature mice, and environmental enrichment (EE) was used to increase activity in young mice cohort. Moreover, we drew a comparison between physical exercise (voluntary running wheel, RW) and a lipid-lowering drug (probucol) for the management of hypercholesterolemia and cognitive impairments associated with FH. To address the aforementioned questions regarding hypercholesterolemia, physical exercise and cognitive performance, we used the low-density lipoprotein receptor-deficient (LDLr$^{-/-}$) mice, a well-established rodent model of FH [34].

2. Methods

2.1. Animals

LDL receptor knockout (LDLr$^{-/-}$) mice founders were purchased from Jackson Laboratory (Bar Harbor, ME), breed at Universidade Federal de Santa Catarina (Florianópolis, Brazil), and maintained under standard conditions of light (12-h light cycle, on 7:00 AM), and temperature (23 ± 1 °C). These animals display constitutive increased plasma levels of cholesterol (2–3×) in low fat diet conditions/standard [15], as used here (NUVILAB, Nuvital, Paraná, Brazil). Animals had free access to food and water. All efforts were made to minimize the number and suffering of animals. The procedures used in the present study complied with the guidelines on animal care of the UFSC Ethics Committee on the Use of Animals, which follows the "Principles of Laboratory Animal Care" from NIH.

2.2. Experimental design

2.2.1. Experiment 1

Twenty-six-weeks-old male LDLr$^{-/-}$ mice were individually housed in cages with free access to running (G1) (RW 4½″, Super Pet, USA) or locked wheels (G2) (not spinning), this last to avoid environmental bias. These wheels remained in the same cage place to avoid spatial enrichment. At same time, half of animals of G1 and G2 were treated with the lipid-lowering drug probucol. Probucol (Sigma, St. Louis, MO, USA) was added in drinking water in a final concentration of 17.5 mg/L, providing a dose of approximately 3.5 mg/kg/day for each animal. The duration of probucol treatment and exposure to running wheels (RW) lasted four weeks. After this period, animals were evaluated in the object location task (N = 9–10 animals per group). After the performance of the cognitive tasks, animals were food-deprived overnight and the blood was collected in heparinized tubes from the heart to determine the total cholesterol and non-HDL-cholesterol levels.

2.2.2. Experiment 2

Briefly, LDLr$^{-/-}$ timed pregnant mice were randomly assigned to the standard or enriched environment (EE) group 4–7 days prior to delivery. The EE-rearing protocol used was based on the previously described by Simonetti et al. [29]. One dam and its litter were placed in standard control cages (30 cm × 13 cm × 13 cm), while two dams with a combined litter size of 10 pups or more were placed in EE cages (45 cm × 30 cm × 13 cm). The EE contained many additional objects (a running wheel, plastic tubing, rubber balls, scented plush balls and a bell-ball). Items were moved to different locations within each cage every 2–3 days. Both male and female pups were used in roughly equal proportions. Mice assigned to the EE group received enrichment until adolescence (postnatal day 45, PN45). The dams were housed in the EE during the weaning period (i.e. from birth to PN21), and then they were withdrawn from the EE and the male and female pups were changed to independent EE cages. On the postnatal day 90, animals were evaluated in the object location task (N = 8 animals per group).

2.3. Behavioral test

2.3.1. Object location task

The spatial memory of mice was assessed with the object location task. The task is based on the spontaneous tendency of rodents, previously exposed to two identical objects, to later explore one of the objects – replaced in a novel location – for a longer time than they explore the non-displaced object, and has been used for the evaluation of hippocampal-dependent memories [2].

The experimental apparatus used in this study was an open-field box, made of transparent PVC, with a white floor of 50 cm × 50 cm (divide by black lines into 25 squares of 10 cm × 10 cm), and transparent walls with 40-cm high. The apparatus was placed in a dimly lit and sound-isolated room. Identical plastic rectangles (4 cm high × 4.5 cm wide) were used as objects. The mice were placed in the center of the apparatus with two identical objects for 5 min. The objects were placed 7 cm away from the walls of the open field. Exploration of the objects was recorded by a stopwatch when mice sniffed, whisked, or looked at the objects from no more than 1 cm away. After the training phase, the mice were removed from the apparatus for 180 min. After the inter-trial interval, one object was moved to a new location. The time spent by the animals exploring the objects in new (displaced) and old (familiar/non-displaced) locations were recorded during 5 min. All locations of the objects were counterbalanced among the groups. To analyze the cognitive performance of mice, a location index was calculated: (Tdisplaced × 100)/(Tdisplaced + Tnon-displaced), where Tdisplaced is the time spent exploring the displaced object and Tnon-displaced is the time spent exploring the non-displaced object [2].

2.4. Plasma lipids levels

The animals were food-deprived overnight, and whole blood was collected from the heart, immediately centrifuged at 1000 × g and the plasma frozen at −80 °C. Total cholesterol (TC), HDL-cholesterol and triglycerides (TGs) were measured in plasma using the enzymatic kit according to the manufacturer's instructions (Gold Analisa Diagnóstica Ltda, Minas Gerais, Brazil). The concentration of non-HDL-cholesterol was calculated using the equation: (LDL + VLDL + IDL) = TC − HDL.