



The Influence of Aerobic Exercise on Hippocampal Glial Cells and Inflammatory Factor Expression in the Aging Process of Rats

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Abstract: The objective is to analyze the impact of aerobic exercise on the activation of hippocampal glial cells and the expression of inflammatory factors in the aging process of rats. Methods: Thirty male SPF (Specific Pathogen Free) rats at 10 weeks of age were selected and divided into three groups: the sedentary group (A), the aging group (B), and the aging exercise group (C), with 10 rats in each group. The sedentary group (A) was raised for 6 weeks without exercise or aging intervention; the aging group (B) was continuously injected with D-galactose for 6 weeks without exercise intervention; the aging exercise group (C) was continuously injected with D-galactose for 6 weeks while simultaneously implementing exercise intervention, with 60 minutes of unassisted swimming per day, three times a week (swimming pool size 0.5m x 0.8m, water temperature $35\pm 2^{\circ}\text{C}$). All groups were harvested at the end of 6 weeks. Compared with the sedentary group, the GFAP immunohistochemical staining in the hippocampal area of the brain was stronger in both the aging group and the aging exercise group, with more GFAP expression, darker positive reaction color, and higher average fluorescence intensity than the sedentary group; compared with the aging group, the aging exercise group had less GFAP expression, lighter positive reaction color, and lower average fluorescence intensity. All differences were significant ($P < 0.05$). In the comparison of GSH levels in the hippocampal tissue of rats, the aging group had the lowest levels, indicating a decrease in the activation level of glial cells in the hippocampus, while the aging exercise group had higher GSH levels than the aging group, proving that exercise intervention in the aging process can effectively improve the activation level of hippocampal glial cells in rats.

Keywords: aerobic exercise; rats; aging process; hippocampal glial cells; inflammatory factors

1. Introduction

With the development and progress of modern society in our country, the trend of population aging is becoming more and more in-depth. Delaying aging and improving the resistance and immune level of the elderly population have become one of the research directions in the field of modern medicine. At present, research on the mechanism of aging is relatively abundant, such as linking it with mitochondrial damage, programmed aging, etc., and some research believes that human aging is affected by the aging of the nervous system, gradually showing chronic low-grade inflammation. This kind of nervous system aging problem will increase the activity of nerve glial cells with age, leading to an imbalance of the body's inflammatory state, and then accelerate aging, causing brain tissue damage or neurological dysfunction, cognitive disorders, and other specific manifestations of aging. Overall, aging belongs to a relatively macro concept category, and the core of the core is that the function of the hippocampus in the brain gradually decreases during the aging process, the original cognitive function and skill strength are weakened, and neurodegenerative diseases occur. Some studies have shown that appropriate exercise is beneficial to improve the body's anti-aging level, improve cognitive function and learning and memory ability, and delay the decline of the regeneration function of neurons in the hippocampus. Aerobic exercise can further inhibit the oxidative damage produced by the aging of the nervous system, improve the condition of cerebral blood vessels and brain energy metabolism, and also effectively improve the level of inflammatory factors in the body through the form of aerobic exercise, achieving the goals of delaying aging, improving cognitive ability, and reducing the decline of neuronal function.

2. Materials and Methods

2.1 Materials

Thirty male SPF rats at 10 weeks of age were selected for the experiment. The SPF (Specific Pathogen Free, SPF) breeding environment, with a temperature of $20\pm 2^{\circ}\text{C}$, humidity of 50% to 70%, and a 12-hour cycle of light and dark, allows the rats to drink and eat freely. The sedentary group (A), the aging group (B), and the aging exercise group (C) were established, each with 10 rats, all fed with ordinary feed.

The sedentary group (A) was raised for 6 weeks without exercise or aging intervention; the aging group (B) was

continuously injected with D-galactose for 6 weeks without exercise intervention; the aging exercise group (C) was continuously injected with D-galactose for 6 weeks, and at the same time, exercise intervention was implemented, with 60 minutes of unassisted swimming per day, three times a week (swimming pool size 0.5m x 0.8m, water temperature 35±2°C). All groups of rats were harvested at the end of 6 weeks.

2.2 Methods

2.2.1 Establishment of Aging Model and Harvesting

D-galactose is a metabolic product. If the intake of D-galactose is excessive and the content of D-galactose in the body is too high, it will further produce aldose reductase, which is reduced into galactitol with certain cytotoxicity, causing over-oxidation of proteins, lipids, etc., in the body, affecting the structure of mitochondria, and enhancing the level of amyloid proteins in the body, causing various diseases such as cognitive and metabolic disorders. The application of D-galactose in the establishment of the aging model is ideal and has good application advantages. When injecting D-galactose into rats, the back of the neck needs to be selected, and different groups of rats are injected continuously for six weeks, with an injection volume of 150mg/kg/day, among which the sedentary group (A) needs to be injected with an equal amount of physiological saline at the same position on the back of the neck, and also injected for 6 weeks. The successful sign of the aging model is that the rat's fur is dark and dull, appetite is reduced, food intake is reduced, weight is reduced, and reaction and neural activity are slow. After the last exercise of the rat, it is allowed to eat and drink freely, and after fasting for 12 hours, it is harvested. Water chloral hydrate can be injected into the rat's abdominal cavity for anesthesia, and then the brain is perfused, the hippocampus is separated, and it is frozen in the refrigerator for later use.

2.2.2 Activation Status of Glial Cells

Immunofluorescence staining of the rat hippocampal area: OCT is used to embed the whole brain tissue, and after slicing, 0.3% Triton X-100 solution is used to break the membrane at room temperature for 10 minutes; 5% BSA solution is closed for 1 hour; then the first antibody (CD31, GFAP) is added and incubated overnight at 4°C. The next day, PBS is used to rinse, and the corresponding fluorescent second antibody is added and incubated at room temperature in the dark for 1 hour. After rinsing the second antibody with PBS, DAPI is added to re-stain the cell nucleus for 5 minutes. Observe and take photos under a fluorescence microscope.

Detection of GSH levels in rat hippocampal tissue: Take the homogenate of the hippocampal tissue, centrifuge at 4°C, 12000rpm for 10 minutes, take the supernatant, and use the BCA method to measure the protein concentration. Then, use the enzyme marker to measure the OD value of reduced glutathione (GSH) at 405nm, and finally calculate the content of GSH in the hippocampus.

2.2.3 Neuroinflammatory Level

TNF- α is an inflammatory factor that can produce a large number of inflammatory mediators, directly affecting the body's immune regulation level, and is measured by goat anti-rat TNF- α polyclonal antibody.

2.3 Statistical Methods

Data analysis was performed using SPSS26.0 statistical software. Multiple group comparisons were made using one-way ANOVA and repeated measures ANOVA, and intra-group comparisons were made using the LSD method. Data are represented in the form of mean \pm standard deviation ($\bar{x}\pm s$). The test level $\alpha=0.05$.

3. Results

3.1 Activation Status of Glial Cells

Compared with the sedentary group, the GFAP immunohistochemical staining in the hippocampal area of the brain was stronger in both the aging group

and the aging exercise group, with more GFAP expression, darker positive reaction color, and higher average fluorescence intensity than the sedentary group; compared with the aging group, the aging exercise group had less GFAP expression, lighter positive reaction color, and lower average fluorescence intensity. All differences were significant ($P<0.05$). In the comparison of GSH levels in the hippocampal tissue of rats, the aging group had the lowest levels, indicating a decrease in the activation level of glial cells in the hippocampus, while the aging exercise group had higher GSH levels than the aging group, proving that exercise intervention in the aging process can effectively improve the activation level of hippocampal glial cells in rats.

3.2 TNF- α Neuroinflammatory Level

As shown in Figure 1, the cumulative optical density value of TNF- α in the sedentary group was the lowest, followed by the aging exercise group, and the aging group had the highest cumulative optical density value of TNF- α , proving that under the influence of aging, the level of TNF- α neuroinflammation in rats significantly increased, but after aerobic exercise intervention, the level of TNF- α neuroinflammation in the aging exercise group was lower than in the aging group, with significant differences ($P < 0.05$).

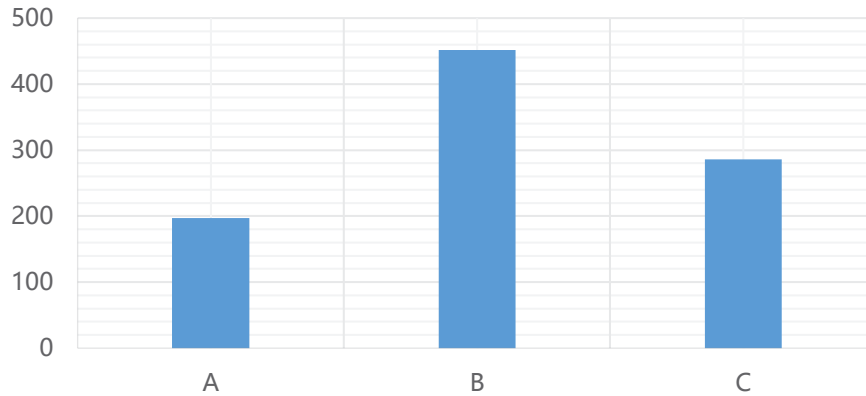


Figure 1. Cumulative optical density value (IOD) of TNF- α inflammatory factor

4. Discussion

Aging, influenced by both genetic and environmental factors, is an inevitable biological process. Prolonged inactivity can lead to health issues and reduced immunity, while regular exercise can improve health and cognitive function, and delay aging. This study used a rat aging model to investigate the effects of aerobic exercise on hippocampal glial cells and inflammatory markers. Rats were divided into three groups: sedentary (A), aging (B), and aging with exercise (C), and were subjected to six weeks of aging intervention with or without exercise. The aging model used D-galactose injections to accelerate aging. Results showed that aerobic exercise effectively controlled glial cell activation and reduced TNF- α levels, suggesting that it can mitigate neuroinflammation and cognitive impairment associated with aging, thus potentially delaying the aging process.

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