

Anti-fatigue Effect of L-arginine Complex Preparation on Mice

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Abstract: Objective: To investigate the anti-fatigue effect of L-arginine complex preparation on mice.

Methods: The experimental mice were divided into a blank group, low dose group, medium dose group and high dose group. L-arginine complex preparation mice were intragastrically administered for 30 days, and the mice were tested for exhaustive swimming time. At the same time, contents of plasma lactic acid, lactate dehydrogenase, urea nitrogen and hepatic glycogen were measured. **Results:** Compared with the blank control group, the weight-bearing swimming time and hypoxia-tolerant survival time of the low, middle and high dose groups was significantly increased ($P<0.05$). Whereas, the serum urea nitrogen levels and lactose content were significantly decreased ($P<0.05$). However, compared with the blank control group, the liver glycogen content of the middle and high dose groups was increased significantly ($P<0.05$), and there was no significant difference in the low dose group.

Conclusion: The L-arginine complex preparation has an anti-fatigue function in mice.

Keywords: L-arginine, Complex, Anti-fatigue

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1 Introduction

Fatigue is a sub-health state and a complication of many diseases. Diabetes, liver and kidney diseases can cause fatigue, which leads to low immunity and easy to cause pathogenic bacteria. Long-term fatigue will not only affect the quality of life and work efficiency but also leads to death^[1-3]. At present, China's sub-healthy population has reached 70%, chronic fatigue is in urgent need of prevention and improvement. Currently, anti-fatigue drugs are mostly cerebral cortex excitatory drugs, such as caffeine, amphetamine, methylphenidate and others. Most of these anti-fatigue drugs have certain side effects and addiction which greatly limits its clinical application. The L-arginine complex preparation is an anti-fat health food prepared by using essential amino acids as the main component and has the advantages of natural safety and reliability. In this study, the anti-fatigue effects of L-arginine complex preparation were examined by measuring the weight-bearing swimming time, hypoxia tolerance, serum urea nitrogen, blood lactate and liver glycogen in mice.

2 Material

2.1 Experimental animals and test conditions

The experimental clean grade male BALB/C mice with a weight of 19g-22g were used. Environmental conditions were tested including temperature ranging from 20°C to 24°C, relative humidity ranging from 60% to 70%. The mice were acclimated for 5 days in the

animal house environment prior to the test.

2.2 Experimental reagents

L-arginine complex preparation (Shandong Mingren Freda Pharmaceutical Co., Ltd., batch number 2016L05105), urea nitrogen detection kit, lactic acid detection kit, glycogen detection kit were purchased from Beijing Suobaolai Technology Co., Ltd.

2.3 Main instruments

Swimming box (50cm x 50cm x 40cm), electronic balance, lead skin, centrifuge, homogenizer, constant temperature water bath and others.

3 Methods

3.1 Animal grouping and treatment period

Each experimental project was set up with different doses of experimental groups and the blank control group. The experimental group was divided into low dose group, middle dose group and high dose group according to the dosage of 0.15g/kg, 0.325g/kg and 0.7g/kg respectively. The gavage volume was 0.1mL/10g body weight once a day for 30 days while the blank control group was given the same volume of distilled water.

3.2 Weight-bearing swimming test

Forty mice were randomly divided into 4 groups with 10 in each group. After continuous administration for 30 days and 30 minutes after the last gavage, the mice were tested to swim in water with 30cm depth, the temperature of 25±0.5°C. A lead of 5% of body weight was put at the root of the rat tail. The exhaustion time of mice was recorded from as the time of floating on the surface of the water before sinking into the water for 10 seconds after start swimming.

3.3 Anti-hypoxia test

Forty mice were randomly divided into 4 groups with 10 in each group. After continuous administration for

30 days and 1 hour after the last gavage, the mice were placed in a 250ml jar containing 10g of sodium lime with one per bottle. The stopper is then sealed with Vaseline before starting record the time. The time of mice to survive hypoxia as the time before the death of the mice was observed and recorded.

3.4 Biochemical tests analyses

Forty mice were randomly divided into 4 groups with 10 in each group. Continuous administration for 30 days and 30 minutes after the last gavage, the mice were swim in water at 30°C for 90 minutes prior to immediate eyeballs withdrawn. The blood and serum were collected by centrifugation while serum urea nitrogen was determined using a urea nitrogen test kit. After the mice were sacrificed, the liver was withdrawn, homogenized and centrifuged to determine hepatic glycogen using a glycogen test kit. After the sample was given for 30 minutes at the final time, the mice were loaded with 2% body weight to swim in water at 30°C. Fifteen minutes later, the blood was collected and blood lactate was determined using a lactic acid test kit.

3.5 Statistical analysis

Single factor multivariate analysis of variance and LSD test were performed using GraphPad Prism 8 statistical software. The results were expressed as ±s and the significance level was $P<0.05$.

4 Results

4.1 Weight-bearing swimming time and anti-hypoxia ability test

Compared with the blank control group, the weight-bearing swimming times of the low dose, middle dose and high dose groups were significantly increased ($P<0.05$). Whereas, compared with the blank control group, the anti-hypoxia survival times of the low dose, middle dose and high dose groups were significantly increased ($P<0.05$).

Table 1. Comparison of weight-bearing swimming time and hypoxia tolerance of mice in each group ($\bar{x}\pm s$, n=10)

Groups	Swimming time (min)	Anti-hypoxia survival time (min)
Blank control group	56.41±11.69	33.41±3.69
Low dose group	108.73±16.55*	46.21±5.96*
Medium dose group	116.52±26.94*	51.48±2.84*
High dose group	113.22±21.58*	49.33±2.98*

4.2 Biochemical tests results

Compared with the blank control group, the serum urea nitrogen levels in the low dose, middle dose and high dose groups were significantly decreased ($P < 0.05$). Whereas, compared with the blank group, the blood lactate contents in the low dose, middle

dose and high dose groups were significantly lower ($P < 0.05$) while the hepatic glycogen contents in the middle dose and high dose groups were significantly higher ($P < 0.05$) than that of the blank control group, but there was no significant difference in the low dose group.

Table 2. Comparison of various biochemical tests in the blood of mice in each group during exercise ($\bar{x} \pm s$, $n=10$)

Groups	Serum urea nitrogen (mg/100ml)	Blood lactate (mg/100ml)	Hepatic glycogen (mg/100g)
Blank control group	18.56±1.68	38.06±4.24	1011.3±173.8
Low dose group	13.59±2.31*	22.47±5.16*	1125±209.6
Medium dose group	12.06±2.06*	20.09±4.55*	1308±298.4*
High dose group	13.11±1.98*	22.11±3.97*	1258±311.7*

5 Discussions

Fatigue is a sub-health state and a complication of many diseases. If it is not detected and controlled early, it can easily develop into a disease state called “chronic fatigue syndrome” and severe fatigue can lead to death. L-arginine complex preparation is an anti-fatigue health food independently developed by Shandong Mingren Furuida Pharmaceutical Co., Ltd. It is prepared from L-arginine, L-citrulline, taurine, vitamin C, vitamin E powder and folic acid in a certain ratio. Current research shows that fatigue is closely related to nitric oxide (NO). In the peripheral fatigue mechanism, high levels of NO can increase the skeletal muscle blood flow, reduce oxygen consumption, maintain a high oxygen uptake rate and help to delay the occurrence of exercise fatigue. In the central nervous system, NO can reduce the mRNA expression of endothelin-1 (*et-1*) in the brain tissue caused by strong exercise load, thereby improving cerebral ischemia and hypoxia which is beneficial to regulate the production of central fatigue^[4]. Current research has shown that L-arginine has a significant effect on the body’s ability to produce NO as a whole, especially in the heart and other organs. Therefore, supplementation with L-arginine can increase endurance during exercise. Long-term supplementation of L-arginine can increase NO content in the skeletal muscle and muscle glycogen storage, improve exercise endurance and thus prolong exercise time and finally render anti-fatigue effect^[5]. Studies have shown that L-citrulline is converted into the essential amino acid L-arginine in the body and plays an important role in maintaining NO metabolism in normal cardiovascular function. Therefore, supplementation of citrulline can

also improve the body’s fatigue status, maintain healthy cardiopulmonary function and have a good effect in sport health^[5]. Literature has shown that taurine has a significant anti-fatigue effect and inhibitory effect on the oxygen consumption of the heart in mice with increased isoproterenol. It can significantly prolong the swimming time of mice^[6]. Vitamin C and vitamin E are important anti-oxidants in the human body and play an important role in eliminating free radicals. They not only can delay fatigue during exercise and improve exercise performance, but also help in recovery from fatigue^[7-8]. Folic acid is an essential nutrient for human body. Studies have shown that folic acid can enhance the proliferation of neural stem cells (NSCs) to a certain extent and improve its anti-oxidant capacity. In addition, folic acid can improve the intracellular anti-oxidant defense system and has direct anti-free radical action^[9].

This experimental study is based on the “Functional Evaluation Procedures and Test Methods of Healthy Supplement” in determining the rules for the result of the anti-fatigue experiment: “If there are positive results in more than 1 exercise tests (weight-bearing swimming test or climbing rod test) and more than 2 biochemical tests (blood lactate, serum urea nitrogen, liver glycogen), it can be determined that the test sample has anti-fatigue effect.” The results showed that L-arginine complex preparation can significantly prolong the swimming time of time, prolong the survival time of mice under normal pressure and hypoxia. It can significantly reduce serum urea nitrogen and blood lactate after exercise, and increase the liver glycogen content during exercise. It indicates that the L-arginine complex preparation has an anti-fatigue effect. However, its exact anti-

fatigue peripheral and central mechanisms need further investigation.

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