The Anti-fatigue Effect of 20(R)-Ginsenoside Rg3 in Mice by Intranasal Administration

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20(R)-ginsenoside Rg3 (20(R)-Rg3) has shown multiple pharmacological activities and been considered as one of the most promising approaches for fatigue treatment. However, 20(R)-Rg3 has a low bioavailability after oral administration in human due to the first-pass effect. Recently, nasal route has gained increasing interest as it can avoid first-pass effect for its lower enzymatic activity compared with the gastrointestinal tract and liver. In order to provide an animal experimental evidence of 20(R)-Rg3 intranasal administrated preparation, the anti-fatigue effect of 20(R)-Rg3 after intranasal administration was investigated. Two weeks after 20(R)-ginsenoside Rg3 was administrated intranasally to mice at three different doses, the anti-fatigue effect of 20(R)-Rg3 was evaluated by the weight-loaded swimming test and biochemical parameters related to fatigue, such as serum urea nitrogen (SUN), lactic dehydrogenase (LDH), superoxide dismutase (SOD), malondialdehyde (MDA), blood lactic acid (LA) and hepatic glycogen. The results showed that compared with the negative control group, the intermediate-dose and the high-dose groups significantly prolonged the weight-loaded swimming time (p<0.05; p<0.01), and also increased the hepatic glycogen levels (p<0.05); SUN levels were decreased considerably in three 20(R)-Rg3-treated groups (p<0.01). In addition, the low-dose group obviously decreased the content of blood LA (p<0.05). However, the levels of LDH, SOD and MDA did not show a significant change. Our results predicted a benefit of 20(R)-Rg3 as an anti-fatigue treatment by intranasal administration. The mechanism was related to the increase of the storage of hepatic glycogen, and the decrease of the accumulation of metabolite such as lactic acid and serum urea nitrogen.

Key words 20(R)-ginsenoside Rg3; intranasal administration; anti-fatigue

Panax ginseng has been frequently used in traditional Chinese medicine to treat many disorders, such as debility, ageing, stress, diabetes and insomnia.1) The major biological active components of ginseng are ginsenosides.2) 20(R)-ginsenoside Rg3 (3β,12β,20(R)-dihydroxydammar-24-ene-3-O-β-d-glucopyranosyl(1→2)β-d-glucopyranoside), a minor ginsenoside from the Panax ginseng, has shown multiple pharmacological activities, including anti-tumor,3) immunity enhancement4) and memory improvement.5)

However, the plasma concentration of 20(R)-ginsenoside Rg3 (20(R)-Rg3) after oral administration in human is very low. Pang et al.6) obtained some human pharmacokinetic (PK) parameters, including Cmax of (16.00±6.00) ng/ml and t1/2 of (0.66±0.10) h at 3.2 mg/kg from the oral experiments. The short half-life and low plasma concentration of 20(R)-Rg3 from the study indicated that 20(R)-Rg3 might be metabolized quickly after oral administration. Recently, interest arises from different possible advantages presented by the nasal cavity,7) such as: the epithelium with a relatively large surface area available for drug absorption, the porous endothelial basement membrane, the direct transport of absorbed drugs into the systemic circulation thereby avoiding the first-pass effect in oral administration, the lower enzymatic activity compared with the gastrointestinal tract and liver, the convenience for administration when there is no water. For all these reasons the nasal route can be considered as a useful alternative both to parenteral and oral routes.8,9)

However, no reports on the anti-fatigue effect of 20(R)-Rg3 by intranasal administration were found. In order to provide an animal experimental evidence of 20(R)-Rg3 intranasal administrated preparation, in this study, the anti-fatigue effect of 20(R)-Rg3 after intranasal administration was investigated.

MATERIALS AND METHODS

Materials. Animals Male Kunming mice weighing 18—22 g (The Animal Experimentation of The Second Military Medical University, Shanghai, China; Animal Certificate No.: SCXK(hu)2007-0005) were used in these experiments. They were housed at a room temperature of 23±1°C with a 12/12 h light–dark cycle (lights on from 7:00 a.m. to 7:00 p.m.). Food and water were available ad libitum. Mice were treated in accordance with provisions in the Guidelines for Animal Care and Use by National Institutes of Health.

Drugs 20(R)-Ginsenoside Rg3 (purity: 93.58%) was purchased from Dalian Fusheng Pharmaceutical Company, Dalian, China (No.: 070221); All the kits were purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

Methods. Animal Grouping and Administration Mice weighed before experiment, were divided into 4 groups at random (30 mice/group), including one low-dose group (0.05 mg/kg), one intermediate-dose group (0.1 mg/kg), one high-dose group (0.5 mg/kg), and one negative control group (normal saline). The dose range was determined by preliminary tests and previous studies.10) All the mice were administrated intranasally with 10 μl of drug solution per day via a transferpettor, 5 μl each nostril.

Body Weight Change Changes in body weight of the mice were observed during initial and terminal stages of the test. One hundred and twenty male mice were used for the following tests performed in a randomized double-blind manner.

Weight-Loaded Swimming Test Briefly, 30 min after the last intranasal administration, the mice (10 mice per group) were dropped individually into an acrylic plastic pool.
(90×45×45 cm) filled with fresh water maintained at 30±1 °C, approximately 35 cm deep so that mice could not support themselves by touching the bottom with their tails.

A lead block (5% of body weight) was loaded on the tail root of the mice. The total swimming time of mice was calculated from the moment they were dropped into the water till they were completely exhausted as evidenced by sinking into water and drowning.

**Measurement of the Contents of SUN, LDH, Hepatic Glycogen, SOD and MDA** Thirty minutes after the last administration, the mice (10 mice per group) were forced to swim for 90 min without weight loading, after 60 min of rest, the mice were anesthetized with ether and whole blood samples were collected in tubes by heart puncture. Blood samples were placed for about 1 h at 4 °C and centrifuged for 10 min at a speed of 3000 rpm. The supernatant was collected and contents of SUN and LDH were analyzed with commercial kits. In addition, immediately after the blood had been collected and contents of SUN and LDH were analyzed with commercial kits. The blood LA levels were analyzed with commercial kits.

**Statistical Analysis** Results were expressed as the mean±standard error (S.E.). Firstly, the data were analyzed by homogeneity test for variance. If the data were homoscedasticity, the significance of the mean difference was determined by one-way ANOVA, followed by a LSD-t test. Otherwise, it was determined by Tamhane’s T2 test. All statistical analyses were performed using SPSS v10.0 statistical analysis software. A value of *p*=0.05 was considered to indicate statistical significance.

**RESULTS AND DISCUSSION**

**Effects on Body Weight Change** The results were shown in Table 1. The one-way ANOVA results indicated that there were no significant differences in the body weight of the mice in the 20(R)-Rg3 groups, in comparison with the negative control group during initial and terminal stages in this test (*F*=0.708, *p*=0.549 (>0.05)).

**Effects on Weight-Loaded Swimming Test** The results were shown in Fig. 1. The weight-loaded swimming time in each group was significantly different through one-way ANOVA test (*F*=4.55, *p*=0.009 (<0.05)), and the significance of the mean difference between every two groups was analyzed by LSD-t test. The results showed that the weight-loaded swimming time of the mice was obviously prolonged by 20(R)-Rg3 in the intermediate-dose and high-dose groups, compared with which in the negative control groups (*p*=0.03 (<0.05); *p*=0.002 (<0.01)).

**Effects on SUN, LDH, Hepatic Glycogen, SOD and MDA** The results were shown in Table 2. The significance of the mean difference of SUN and hepatic glycogen levels were determined by Tamhane’s T2 test. The results indicated that compared with the negative control group, 20(R)-Rg3 in the other three groups obviously reduced the SUN levels in mice (*p*=0.000 (<0.01); *p*=0.000 (<0.01); *p*=0.000 (<0.01)), and 20(R)-Rg3 in the high-dose group increased the hepatic glycogen level (*p*=0.015 (<0.05)). The one-way ANOVA and LSD-t test results showed that, compared with negative control, the effects of three dose groups on LDH (*p*=0.065; *p*=0.409; *p*=0.127), MDA (*p*=0.323; *p*=0.365; *p*=0.341) and SOD (*p*=0.356; *p*=0.173; *p*=0.209) levels were not significant.

**Effects on the Variance of Blood LA Level after Swim-**
The results were shown in Table 3. According to the one-way ANOVA and LSD-t test, compared with the negative control, 20(R)-Rg3 administrated groups could reduce the variance of blood LA after swimming in some extent, especially in the low-dose group, the difference was significant ($p=0.010$ ($<0.05$)).

**CONCLUSION**

Fatigue is one of the most frequent physiological reactions. It is well accepted that the most important physiological effect of fatigue is on the energy metabolism of muscular activity, and the improvement of exercise endurance is the most powerful representation of anti-fatigue enhancement.\(^{(15)}\)

The anti-fatigue activity of 20(R)-Rg3 in the present study is measured by a weight-loaded swimming test, which is accepted as a proper experimental exercise model in previous researches.\(^{(16)}\) The length of the swimming time shows the degree of fatigue.\(^{(17)}\)

In addition, SUN, lactic acid and hepatic glycogen are representative blood biochemical parameters related to fatigue. SUN, the product of energy metabolism when moving, is a sensitive index to evaluate the bearing capability when human bodies suffer from a physical load. There is a positive correlation between the urea nitrogen in vivo and the exercise tolerance. In other words, the worse the body is adapted for exercise tolerance, the more significantly the urea nitrogen level increases.\(^{(18)}\)

The muscle produces plenty of lactic acid when it obtains enough energy from anaerobic glycolysis almost at the same time when doing high-intensity exercise. Furthermore, the increase of lactic acid level will bring about a reduction of pH in muscle tissue and blood, and also induce many side effects of various biochemical and physiological processes.\(^{(19)}\)

Therefore, rapid removal of lactic acid is beneficial to relieving fatigue. Serum LDH is known to be accurate indicators of muscle damage.\(^{(20,21)}\) It catalyzes lactic acid into pyruvate, thereby reduces the accumulation of lactic acid in muscle. Liver is the direct tissue for energy conservation and utilization.

The liver converts lactate back to glycogen and releases glycogen into the blood. Energy for exercise is derived initially from the breakdown of glycogen, and later from circulation glycogen released by the liver and from non-esterified fatty acids.\(^{(22)}\) So increasing the hepatic glycogen storage conduces to enhancing the endurance capacity and locomotory capacity.

In present study, the results showed that compared with the negative group, intranasally administrated 20(R)-Rg3 had no significant difference in body weights of mice. But 20(R)-Rg3 in the high-dose and the intermediate-dose groups prolonged the weight-loaded swimming time in mice ($p<0.05$, $p<0.01$) and the hepatic glycogen level was also increased in these two groups ($p<0.05$, $p<0.05$). 20(R)-Rg3 in the three groups reduced the SUN level obviously ($p<0.01$). In addition, in 20(R)-Rg3 groups, the variance of lactic acid after swimming could be reduced to some extent, especially in the low-dose administrated group where the difference was significant ($p<0.05$). However, the effects on the LDH, SOD and MDA levels had no significant differences.

The anti-fatigue effect of ginsenoside Rg3 is not dose-dependent. It might be because the influence of ginsenosides on central nervous system has a two-way effect, which is shown by the fact that ginsenoside Rb had a sedative effect on central nervous system, while the ginsenoside Rg, especially in the lower dose, had an exciting effect.\(^{(23)}\) The experiment results showed that medium-dose (0.1 mg/kg) and high-dose (0.5 mg/kg) of Rg3 could significantly prolong the weight-loaded swimming time of mice, but the difference between the two dose groups was not significant ($p=0.30$ ($>0.05$)). And three doses of Rg3 could significantly decrease the SUN level, however, there were no significant differences between each two dose groups(high-dose and medium-dose: $p=0.76$ ($>0.05$); high-dose and low-dose: $p=0.59$ ($>0.05$); medium-dose and low-dose: $p=0.43$ ($>0.05$)). The hepatic glycogen levels were significantly increased in medium- and high-dose groups, but the differences between these two groups were not significant ($p=0.77$ ($>0.05$)). All the results indicated that the anti-fatigue effect of ginsenoside Rg3 was not dose-dependent. Relevant references were attached.

In conclusion, the present results suggest that intranasal administration of 20(R)-ginsenoside Rg3 shows an anti-fatigue effect. The intranasal administration route may have a potential application prospect.

**REFERENCES**