Anti-fatigue Effect of Ginsenoside Rb1 on Postoperative Fatigue Syndrome Induced by Major Small Intestinal Resection in Rat

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Ginsenoside Rb1 (GRb1), one of the principle active ingredients of *Panax ginseng*, exerts multiple pharmacological activities to fight fatigue. In the present study, we investigate the anti-fatigue effect of GRb1 on postoperative fatigue syndrome (POFS) in a rat model induced by major small intestinal resection. GRb1 (10 mg/kg) was administrated intraperitoneally once daily for 1, 3, 7, and 10 d from the operation day. Anti-fatigue effect was assessed by grasping test and biochemical parameters in blood or skeletal muscle were determined by autoanalyzer or commercially available kits. Transmission electron microscope was applied to observe the ultra microstructure of skeletal muscles. The results revealed that GRb1 significantly enhanced rat maximum grip strength with POFS. Similarly, negative alterations in biochemical parameters (lactic acid, hepatic glycogen, muscle glycogen and malondialdehyde) of POFS rats were improved by GRb1. In addition, GRb1 also increased the activity of lactate dehydrogenase and superoxide dismutase in POFS. No significant differences of levels of blood urea nitrogen and ultra microstructure of skeletal muscles were found between the POFS and GRb1 treatment rats. The potent anti-fatigue effect of GRb1 on POFS might be achieved through improvement of energy metabolism and suppression of skeletal muscle oxidative stress.

Key words ginsenoside Rb1; postoperative fatigue syndrome; energy metabolism; oxidative stress; animal experiment

*Ginseng*, the dried root of *Panax ginseng* C. A. Meyer (Araliaceae), has been used as a tonic to treat various disorders in Chinese traditional medicine. It is considered the king of herbs since the earliest Chinese pharmaceutical monograph “Shen Nong Ben Cao Jing.” Currently, *ginseng* is used worldwide as a popular herbal medicine, especially in China, Korea and Japan. Ginsenosides are thought to be the main active ingredients of *ginseng* with multiple pharmacological activities including anti-aging, anti-carcinogenic, anti-oxidation, anti-inflammation, and anti-fatigue. Modern science has identified more than 50 kinds of ginsenosides. Ginsenoside Rb1 (GRb1), one of the ginsenosides, belongs to the protopanaxadiol group of steroidal saponins. Accumulating evidence indicates that GRb1 exerts a protective effect against stress in various conditions.

Currently, one of the major tasks of surgery is to enhance the recovery after operation. Postoperative fatigue syndrome (POFS) is a common complication after surgery, especially major abdominal and cardiac procedures. For affected patients, POFS manifests itself as a feeling of malaise, lethargy, loss of energy, concentration difficulties and debilitating fatigue. It can persist for up to one month after abdominal operations and gradually resolves in as long as 3 months after uncomplicated gastrointestinal surgery. Patients who suffer from POFS have a prolonged recovery to normal daily life. In addition, POFS increases health-service costs, burdens patients themselves, their families, hospitals and society greatly. Unfortunately, the etiology of the syndrome has not been fully explained. Much of the published research suggests that POFS is an endocrine-metabolic response to surgical stress. Biological, psychological and social factors are responsible for the development and progression of POFS. Due to its unclear etiology, there are few effective interventions for POFS. Methods to relieve fatigue and shorten the rehabilitation course after surgery are popular pursuits in modern surgery.

However, much of the published research evaluating properties of *ginseng* with respect to fatigue has been done under various stress conditions. Little information exists about the anti-fatigue effects of *ginseng* on POFS followed by surgical stress. In our previous study, we successfully established a new model for POFS by major small intestinal resection in rats. Based on this model, we found that enteral nutrition combined with GRb1 can improve POFS in a dose-dependent manner, which may be explained by the fact that it can strengthen postoperative nutrition, restrain hypermetabolism, and increase improve immunity.

In this study, we used a grasping test and typical biochemical parameters of energy metabolism and oxidative stress to further study the anti-fatigue effect of GRb1 on POFS induced by major small intestinal resection in rats.

MATERIALS AND METHODS

**Animals** The protocol for the animal experiment was approved by the Institutional Animal Committee of Wenzhou Medical University. Adult specific-pathogen-free male Sprague-Dawley rats (weighing 220±10 g) were obtained from Shanghai SLAC Laboratory Animal Co., Ltd. in China. All rats received human care throughout the experiment in ac-
cordance with “Guide for the Care and Use of Laboratory Animals.” The rats were maintained under specific pathogen-free conditions, controlled temperatures (20–22°C), humidity (45–55%) and light (12 h light/12 h dark cycle) conditions with a standard rat chow and water made available *ad libitum*, except for one day of fasting before and after the operation.

**Drugs** GRb1 (purity over 98%) was purchased from Shanghai Tauto Biotech Co., Ltd., Shanghai, China. All chemicals and reagents were procured from local suppliers and were of analytical grade.

**Animal Grouping and Administration** After an adaptation period for one week, 96 rats were randomly divided into 3 groups: a control group (CG), a POFS model group (MG), and a GRb1-treated POFS model group (GG), with 32 in each. The rat model of POFS was induced by major small intestinal resection as described in the previous study. Briefly, rats in the MG and the GG group had 70% of the length of small intestine removed, which was defined by the length of the small intestinal mesentery starting from 10 cm below the Ligament of Treitz. The CG group went through the same procedure, but without any small intestinal resection. Rats in the CG and MG group were intraperitoneally administrated saline at a dose of 10 mL/kg one hour before surgery and once daily after surgery. Rats in the GG group were administrated GRb1 dissolved in saline at a dose of 10 mg/kg by the same method as the other two groups. On postoperative day 1, 3, 7 and 10, eight rats were randomly selected from each group to measure the maximum grip strength by grasping test. The rats were then immediately anesthetized by subcutaneous injection of 2% pentobarbital sodium (3.5 mL/kg). Samples of blood, liver and skeletal muscle tissues were prepared.

**Grasping Test** The grip strength of rats in the present study was measured by a dynamometer (YLS-13A, Jinan Yiyan Technology and Development Co., Ltd., Jinan, China). This was carried out as described in the literature. Briefly, animals were gently placed on the grid of the dynamometer and pulled by their tails in the opposite direction. The maximum grip strength exerted by the rat before losing grip was recorded. We calculated the mean of three measurements, allowing 30 s of recovery time between each of them. All rats were trained to be adapted to the grasping test preoperatively.

**Determination of Lactic Acid (LA), Lactic Dehydrogenase (LDH) and Blood Urea Nitrogen (BUN)** Blood samples were obtained from the inferior vena cava. The serum was prepared by centrifugation at a speed of 2500 rpm, at 4°C for 15 min, and the levels of LA, LDH and BUN were determined using an automatic biochemical analyzer (Model 7600, HITACHI, Tokyo, Japan).

**Determination of Hepatic Glycogen, Muscle Glycogen, Malondialdehyde (MDA) and Superoxide Dismutase (SOD)** Immediately after the blood was obtained, the liver and the left gastrocnemii were dissected quickly from the rats, washed with physiological saline and dried with absorbent paper. The levels of hepatic glycogen, muscle glycogen, MDA and SOD in skeletal muscle, were analyzed with commercially available kits from the Nanjing Jiancheng Biocmpany.

**Histological Observation** The left gastrocnemii (size 1×1×2 mm³) were also fixed with 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.2) for 48 h at 4°C. They were then rinsed and postfixed with 1% osmium tetroxide in 0.1 mol/L phosphate buffered saline (PBS) for 1.5 h at room temperature, dehydrated through a graded series of ethanol to propylene oxide and embedded in epoxy resin. Regions of interest were localized and characterized with a light microscope on 1-μm sections stained with toluidine blue. Ultrathin sections were cut and examined with a transmission electron microscope (H-7500, HITACHI, Tokyo, Japan).

**Statistical Analysis** Data were expressed as mean± standard deviation (S.D.). Statistical analyses were performed using the SPSS for Windows (version 16.0) statistical program. After being analyzed by homogeneity test for variance, all the data were analyzed. The significance of the mean difference was determined by one-way ANOVA, followed by *post hoc* tests (using Least Significant Difference test, LSD-*) for multi-group comparisons. Differences were considered significant if the *p* value was lower than 0.05.

**RESULTS**

**Effects of GRb1 on Grasping Test** The results are shown in Table 1. On postoperative day 3 and 7, the maximum grip strength of the MG group and the GG group significantly decreased (*p<0.05*), compared with that of the CG group. On postoperative day 10, while the maximum grip strength of the MG group was still significantly lower than that of CG (*p<0.05*), that of the GG group returned to the level of the CG group. In addition, on postoperative day 7 and 10, the maximum grip strength of the GG group was significantly improved (*p<0.05*), compared with that of the MG group.

**Effects of GRb1 on LA, LDH and BUN** The results are shown in Table 2. On postoperative day 3, the level of LA of the MG and GG groups significantly increased (*p<0.05*), compared with that of the CG group. On postoperative day 7, while the level of LA of the MG group still significantly increased (*p<0.05*), that of the GG group returned to the level of the CG group. In addition, on postoperative day 3 and 7, the level of LA of the GG group significantly decreased (*p<0.05*), compared with that of the MG group. Simultaneously, on postoperative day 7, the level of LDH of the GG group significantly increased (*p<0.05*), compared with that of the CG group and the MG group. However, there were no

<table>
<thead>
<tr>
<th>Table 1. Effects of GRb1 on Grasping Test</th>
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<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>CG</td>
</tr>
<tr>
<td>MG</td>
</tr>
<tr>
<td>GG</td>
</tr>
</tbody>
</table>

*CG (control group), MG (POFS model group), GG (ginsenoside Rb1-treated POFS model group), POD (postoperative day). Values are expressed as the mean±S.D. *p<0.05 and **p<0.01 means the significant of MG and GG compared with CG at the same time point, and *p<0.05 means the significance of GG compared with MG at the same time point, respectively.*
Fig. 1. The transmission electron microscopy analysis showed higher level of SOD than that of the MG group (postoperative day 10, the level of SOD of the GG group was still higher compared with that of the CG group. However, the GG group showed a significant difference in comparison with both the MG group and the GG groups (postoperative day 3 and 7, the level of MDA significantly increased in the three groups on postoperative day 7. On postoperative day 10, the level of muscle glycogen of the MG group significantly increased (postoperative day). Values are expressed as the mean±S.D. *p<0.05 and **p<0.01 means the significance of MG and GG compared with CG at the same time point, and #p<0.05 means the significance of GG compared with MG at the same time point, respectively.

Table 2. Effects of GRb1 on LA, LDH and BUN

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Time</th>
<th>LA (mmol/L)</th>
<th>LDH (U/L)</th>
<th>BUN (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>8</td>
<td>POD 1</td>
<td>4.5±1.4</td>
<td>283±56</td>
<td>5.7±0.6</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>POD 3</td>
<td>2.7±0.7</td>
<td>368±76</td>
<td>6.2±1.1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>POD 7</td>
<td>2.4±0.6</td>
<td>444±86</td>
<td>5.7±0.7</td>
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<tr>
<td></td>
<td>8</td>
<td>POD 10</td>
<td>2.1±0.4</td>
<td>392±83</td>
<td>5.4±0.6</td>
</tr>
<tr>
<td>MG</td>
<td>8</td>
<td>POD 1</td>
<td>5.7±2.0</td>
<td>303±89</td>
<td>6.1±0.8</td>
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<tr>
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<td>POD 3</td>
<td>4.5±0.9**</td>
<td>389±79</td>
<td>7.0±1.4</td>
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<tr>
<td></td>
<td>8</td>
<td>POD 7</td>
<td>3.7±0.8**</td>
<td>466±105</td>
<td>6.2±0.8</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>POD 10</td>
<td>2.5±0.6</td>
<td>417±86</td>
<td>5.9±1.0</td>
</tr>
<tr>
<td>GG</td>
<td>8</td>
<td>POD 1</td>
<td>5.5±1.2</td>
<td>339±64</td>
<td>6.1±0.8</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>POD 3</td>
<td>3.6±0.7*</td>
<td>431±91</td>
<td>6.4±1.3</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>POD 7</td>
<td>2.8±0.7**</td>
<td>582±120*</td>
<td>6.1±0.8</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>POD 10</td>
<td>2.3±0.6</td>
<td>448±91</td>
<td>5.5±0.8</td>
</tr>
</tbody>
</table>

CG (control group), MG (POFS model group), GG (ginsenoside Rb1-treated POFS model group), POD (postoperative day). Values are expressed as the mean±S.D. *p<0.05 and **p<0.01 means the significance of MG and GG compared with CG at the same time point, and #p<0.05 means the significance of GG compared with MG at the same time point, respectively.

Table 3. Effects of GRb1 on Hepatic Glycogen, Muscle Glycogen, MDA and SOD

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Time</th>
<th>Hepatic glycogen (mg/g)</th>
<th>Muscle glycogen (mg/g)</th>
<th>MDA (nmol/mg prot)</th>
<th>SOD (U/mg prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>8</td>
<td>POD 1</td>
<td>16.34±3.45</td>
<td>2.74±0.96</td>
<td>0.34±0.16</td>
<td>69.32±5.83</td>
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<tr>
<td></td>
<td>8</td>
<td>POD 3</td>
<td>17.87±4.27</td>
<td>3.20±0.63</td>
<td>0.37±0.14</td>
<td>72.29±8.81</td>
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<tr>
<td></td>
<td>8</td>
<td>POD 7</td>
<td>23.84±6.45</td>
<td>3.35±0.79</td>
<td>0.29±0.12</td>
<td>81.30±10.88</td>
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<td></td>
<td>8</td>
<td>POD 10</td>
<td>23.96±7.23</td>
<td>3.64±0.85</td>
<td>0.34±0.10</td>
<td>78.22±9.51</td>
</tr>
<tr>
<td>MG</td>
<td>8</td>
<td>POD 1</td>
<td>13.72±4.07</td>
<td>2.18±0.62</td>
<td>0.50±0.17</td>
<td>72.01±9.63</td>
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<tr>
<td></td>
<td>8</td>
<td>POD 3</td>
<td>15.05±4.21</td>
<td>2.31±0.85*</td>
<td>0.77±0.21**</td>
<td>89.66±11.97**</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>POD 7</td>
<td>16.35±5.24*</td>
<td>2.86±0.61</td>
<td>0.58±0.15**</td>
<td>95.67±12.81*</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>POD 10</td>
<td>21.80±6.36</td>
<td>3.07±0.66</td>
<td>0.41±0.18</td>
<td>78.54±12.19</td>
</tr>
<tr>
<td>GG</td>
<td>8</td>
<td>POD 1</td>
<td>15.67±4.83</td>
<td>2.33±0.37</td>
<td>0.43±0.12</td>
<td>77.28±9.44</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>POD 3</td>
<td>17.85±4.57</td>
<td>3.08±0.60*</td>
<td>0.58±0.16**</td>
<td>102.59±12.92**</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>POD 7</td>
<td>23.13±6.88*</td>
<td>3.22±0.61</td>
<td>0.43±0.12**</td>
<td>110.41±13.92**</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>POD 10</td>
<td>23.61±7.66</td>
<td>3.25±0.55</td>
<td>0.36±0.11</td>
<td>91.17±8.50**</td>
</tr>
</tbody>
</table>

CG (control group), MG (POFS model group), GG (ginsenoside Rb1-treated POFS model group), POD (postoperative day). Values are expressed as the mean±S.D. *p<0.05 and **p<0.01 means the significance of MG and GG compared with CG at the same time point, and #p<0.05 means the significance of GG compared with MG at the same time point, respectively.

significant differences in the BUN among the three groups.

Effects of GRb1 on Hepatic Glycogen, Muscle Glycogen, MDA and SOD The results are shown in Table 3. On postoperative day 3, the level of muscle glycogen of the MG group significantly decreased (p<0.05), compared with that of the CG group. However, the level of muscle glycogen of the GG group significantly increased (p<0.05), compared with that of the MG group. Hepatic glycogen showed similar differences in the three groups on postoperative day 7. On postoperative day 3 and 7, the level of MDA significantly increased in both the MG group and the GG groups (p<0.05), compared with that of the CG group. However, the GG group showed a lower level of MDA than that of the MG group (p<0.05). Simultaneously, on postoperative day 3 and 7, the level of SOD of the MG and GG groups significantly increased (p<0.05), compared with that of the CG group. However, on postoperative day 10, the level of SOD of the GG group was still higher than that of the CG group (p<0.05), while the level of SOD of the MG group returned to the level of the CG group. In addition, on postoperative day 3, 7 and 10, the GG group showed a higher level of SOD than that of the MG group (p<0.05).

Skeletal Muscle Morphology The results are shown in Fig. 1. The transmission electron microscopy analysis showed no significant differences with regard to the ultra-microstructure (i.e., the appearance of cell nuclei, mitochondria and myofilaments) among the three groups.

DISCUSSION

In the present study, to evaluate the anti-fatigue effect of GRb1 on POFS induced by major small intestinal resection in rats, we used grip test to assess fatigue and typical biochemical parameters were determined. The results showed that GRb1 could improve the maximum grip strength, negate alteration in biochemical parameters of energy metabolism and skeletal muscle oxidative stress in rats with POFS.

It is well known that major abdominal operations, especially uncomplicated gastrointestinal surgeries, are commonly followed by POFS. Improvement of POFS will significantly enhance postoperative recovery. Muscle weakness and muscle fatigue is one of the main peripheral features of POFS, associated with a decline in the maximum force and a reduction in muscle endurance. Therefore, grip strength is commonly used as an objective measurement to assess fatigue in clinical research. In the present study, we employed a grasping test to determine the maximum grip strength of rats as a mea-
surement method of POFS and effects of GRb1. The results showed that maximum grip strength on day 3, 7 and 10 were reduced in the POFS rats. In addition, we also found that the mean time that rats grasped in the POFS rats was less than that of control rats. These are consistent with the typical syndromes, muscular weakness and reduced grip strength, which are present in patients suffering from POFS. GRb1 improved the maximum grip strength of POFS rats, indicating its beneficial effects on the recovery of muscular capacity.

Alterations in the energy metabolism of skeletal muscle play a vital role in the development of peripheral fatigue. It is well accepted that the physiological response to surgical injury is characterized by hypermetabolism. Under the stress of major abdominal surgery, energy metabolism is increased and followed by high-energy demand, which can significantly influence the metabolic status of skeletal muscle. To meet the energy demand after surgical stress, glycogenolysis is increased to provide a fuel source. A postoperative increase in muscle glycogen stores has an inverse correlation with POFS, suggesting increased glycogen storage may improve POFS. Our data was also consistent with these results: POFS rats have significantly decreased levels of muscle glycogen and hepatic glycogen, but GRb1 increased the muscle glycogen and hepatic glycogen level on postoperative day 3 and 7, respectively. As another sensitive index to evaluate energy metabolism, BUN is an indicator of the metabolic outcome of protein and amino acid. However, in the present study, no significant difference of BUN was found in POFS rats, which suggested protein metabolism was not significantly influenced by POFS without major complications.

When the high-energy demand exceeds aerobic capacity in this condition, anaerobic metabolism is increased, which leads to increased LA. LDH, known as an accurate indicator of muscle injury, catalyzes LA into pyruvate, thereby reducing its accumulation in the muscle. In the present study, POFS rats have significantly increased LA levels on postoperative day 3 and 7, and increased tendency of LDH level postoperatively. However, GRb1 could decrease LA in muscle and increase LDH in plasma. The results suggested that GRb1 improves the maintenance of normal pH range in muscle tissue by reducing the accumulation of LA, and attenuates LA-induced side effects of various biochemical and physiological processes, which impair bodily performance.

In addition, hypermetabolism also has a negative impact on production of reactive oxygen species (ROS), and various studies have verified that ROS were a major cause of muscle fatigue. Under normal conditions, redox equilibrium between the production of ROS and the ability of cells to defend against them is maintained by self-regulation. In the perioperative period, ROS is increased by anesthesia, surgical trauma, inflammation and other factors. When the increased ROS overwhelms the endogenous antioxidant defense system (i.e., antioxidant enzymes), serious oxidative stress and injury occur, as reflected by the oxidative modification of macromolecules (e.g., lipids, proteins and DNA). MDA is an important lipid peroxidation production, which is a sensitive index to evaluate oxidative stress. SOD is one of the main antioxidant enzymes protecting the body from oxidative stress injury, which has an ability to clear ROS during metabolic processes; its level reflects antioxidant ability. In the present study, both MDA levels and SOD levels in skeletal muscle of POFS rats increased postoperatively, which indicated that the oxidative stress damage occurred after surgery because of increased lipid peroxidation. GRb1 showed the effects of reversion that it decreased MDA level and continuously increased the SOD level in the skeletal muscle of POFS rats. All of these findings suggest that GRb1 has an effective antioxidant activity that blocks lipid peroxidation and protects cells from oxidative stress damage in skeletal muscle. We speculate that GRb1 may exhibits the effective antioxidant activity by the chelation of metal ions (Fe²⁺, Cu²⁺, Fe³⁺), the reduction of hydroxyl radical and hypochlorous acid, or the transcriptional activation of Cu/Zn SOD genes.

In conclusion, our study demonstrates that GRb1 has an anti-fatigue effect on POFS induced by major small intestinal resection in rat, which may be related to the improvement of energy metabolism and the suppression of skeletal muscle oxidative stress. However, GRb1 has multiple pharmacological effects, such as improvement of wound healing, inflammation and organ function. Therefore, a further study is required to determine the exact mechanism of GRb1’s anti-fatigue effect on POFS.

Acknowledgments The study was supported by the National Natural Science Foundation of China (No. 81171857), the Foundation of Key Supporting Discipline of Surgical Nutrition (No. 11-ZC24), the Program of Traditional Chinese Medicine Research (No. 2011ZZ087) and Medicine & Health Platform (No. 2011ZDA018, 2013ADA014) of Zhejiang Province, China.

Fig. 1. CG (Control Group), MG (POFS Model Group), GG (Ginsenoside Rb1-Treated POFS Model Group) Representative photographs of transmission electron micrographs of gastrocnemii on postoperative day 7 (20000×). Scale bar=2µm.
REFERENCES


