Prevention of Postoperative Fatigue Syndrome in Rat Model by Ginsenoside Rb1 via Down-Regulation of Inflammation along the NMDA Receptor Pathway in the Hippocampus

Wei-Zhe Chen, a,b Shu Liu, a,b Fan-Feng Chen, a Chong-Jun Zhou, a Jian Yu, a Cheng-Le Zhuang, a Xian Shen, a Bi-Cheng Chen, a,c and Zhen Yu a,b,

a Department of Gastrointestinal Surgery, The First Affiliated Hospital of Wenzhou Medical University; Wenzhou 325000, China; b Department of Surgery, Shanghai Tenth People’s Hospital Affiliated to Tongji University; Shanghai 200072, China; and c Wenzhou Key Laboratory of Surgery, The First Affiliated Hospital of Wenzhou Medical University; Wenzhou 325000, China.

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Postoperative fatigue syndrome (POFS) is a common complication which decelerates recovery after surgery. The present study investigated the anti-fatigue effect of ginsenoside Rb1 (GRb1) through the inflammatory cytokine-mediated N-methyl-D-aspartate (NMDA) receptor pathway. A POFS rat model was created by major small intestinal resection and assessed with an open field test. Real-time quantitative polymerase chain reaction, western blot analysis, high performance liquid chromatography and a transmission electron microscopic analysis were used to determine typical biochemical parameters in the hippocampus. Our results showed that POFS rats exhibited fatigue associated with an increased expression of inflammatory cytokines and NMDA receptor 1, higher (kynurenine)/(tryptophan) and (kynurenine)/(kynurenic acid) on postoperative days 1 and 3, and an increased expression of indoleamine 2,3-dioxygenase (IDO) on postoperative day 1. Degenerated neurons were found in the hippocampus of POFS rats. The NMDA receptor antagonist MK801 had a significant effect on central fatigue on postoperative day 1. GRb1 had no effect on IDO or tryptophan metabolism, but exhibited a significant effect on POFS by inhibiting the expression of inflammatory cytokines and NMDA receptor 1. These data suggested that inflammatory cytokines could activate tryptophan metabolism to cause POFS through the NMDA receptor pathway. GRb1 had an anti-fatigue effect on POFS by reducing inflammatory cytokines and NMDA receptors.

Key words ginsenoside Rb1; postoperative fatigue syndrome; N-methyl-D-aspartate receptor; inflammatory cytokine; tryptophan

Ginseng is the dried root of Panax ginseng C. A. Meyer (Araliaceae) which has been known as one of the most widely recognized traditional Chinese medicines and used in many diseases for thousands of years. Ginsenosides are the major biological active ingredients of ginseng. It has been reported that many different types of ginsenosides have a wide range of therapeutic and pharmacological actions. Accumulating evidence indicates that ginsenoside Rb1 (GRb1), one of the ginsenosides, provides diverse benefits against inflammation, fatigue and oxidation.

Postoperative fatigue syndrome (POFS) is a series of clinical symptoms which decelerates recovery after major abdominal or cardiac surgery. POFS can be divided into peripheral fatigue and central fatigue, the former manifests itself as the decline of skeletal muscle motor function; the latter mainly shows up as the changes of spirit and mood state. POFS reduces the quality of life after surgery, increases medical costs, has a great influence on patients, their families, hospitals even society. Unfortunately, there is not a full explanation to the etiology of POFS, even its central mechanism. There are few effective interventions to improve POFS because of its unclear mechanism. As is playing an important role in neurons cellular plasticity, learning and memory processes, N-methyl-D-aspartate (NMDA) receptor has been associated with the pathophysiology of a variety of neurological and psychiatric diseases such as major depression, anxiety, chronic pain syndrome, Alzheimer’s disease, Parkinson’s disease and Huntington’s disease. NMDA receptor-mediated neuro-transmission is one of the etiologies of stress-related cognitive impairment and behavioral abnormalities. Severe stress response will release large amounts of glutamate to excessively activate NMDA receptor, and then neurons will be damaged to the death with intracellular calcium overload, finally produces neuro-toxicity. Indoleamine 2,3-dioxygenase (IDO) widely exists in the brain endothelial cells, astrocytes and microglia cells, which is activated by inflammatory cytokines such as interleukins-1β (IL-1β), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ). IDO can decompose tryptophan (TRP) into kynurenine (KYN). KYN is then metabolized into quinoline acid (QA) which is a strong agonist of NMDA receptor and can lead to neuronal damage, or kynurenic acid (KYNA) which is known as an antagonist of NMDA receptor and reduces NMDA overstimulation to protect neurons. A recent research by Morimoto et al. indicated that NMDA receptor antagonist can improve central mental fatigue induced by intraperitoneal injection of lipopolysaccharide (LPS) in mice. However, the detail mechanism of GRb1 is still unknown.
and there is little information about the effect of NMDA receptor on central fatigue of POFS. So we aim to find out whether GRβ1 had an effect on central fatigue of POFS through the inflammatory cytokines mediated NMDA receptor pathway. In this study, based on a POFS rat model induced by major small intestinal resection, we used open field test and typical biochemical parameters of inflammatory response, tryptophan metabolism and changes of NMDA receptor in the hippocampus to further study the central mechanism of POFS and the anti-central fatigue effect of GRβ1 on POFS.

MATERIALS AND METHODS

Animals Adult specific-pathogen-free male Sprague-Dawley rats (weighing 230±10g) were obtained from Shanghai SLAC Laboratory Animal Co., Ltd. in China. The rats were maintained under specific pathogen-free conditions, at a temperature of 21±1°C, humidity 45–55% and 12:12 h light:dark cycle with a free access to tap water and standard laboratory rat chow. All animals received humanitarian concern in accordance with “Guiding Principles for the Care and Use of Laboratory Animals.” The protocol for the animal experiment was approved by the Institutional Animal Committee of Wenzhou Medical University.

Animal Grouping and Administration After an adaptation period for one week, rats were randomized into 5 different groups as follows: control group (CG), POFS rat model group (MG), GRβ1-treated POFS model group (GG), NMDA receptor antagonist group (NG) and GRβ1-treated control group (RG). The POFS rat model was induced by major small intestinal resection as described previously. Briefly, rats in the MG, the GG and the NG group had 70% of the length of small intestine resected. The length of the small intestinal mesenterium starting from 10 cm below the ligament of Treitz was measured and defined as the small intestinal length. The CG and RG group went through the same procedure, but without any small intestinal resected. Rats in the GG and RG group received intraperitoneal injection of GRβ1 at a dose of 15 mg/(kg×3 d) 3 d before surgery and every day after surgery until sacrificed. GRβ1 (purity over 98%, purchased from Shanghai Tauto Biotech Co., Ltd., Shanghai, China) was dissolved in the saline with a concentration of 3.75 mg/mL. Rats in the NG group received intraperitoneal injection of NMDA receptor antagonist MK801 (purchased from Sigma, St. Louis, MO, U.S.A.) at a dose of 15 mg/kg once immediately after surgery. The open field test was conducted after the drug injection.

Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR) Assays Real-time quantitative PCR were performed for quantitate expression of inflammatory cytokines (IL-6, IL-1β, TNF-α) and NMDA receptor 1 (n=8 for the CG, MG and GG group). β-ACTIN gene, which is a housekeeping gene, was chosen as an internal gene control. The total RNA was extracted from partial hippocampus tissues with 1 mL TRIzol reagent (Invitrogen Life Technologies, Grand Island, NY, U.S.A.) and the integrity of RNA was identified by the method agarose gel electrophoresis. RNA concentration and purity were quantified by microplate reader (Thermo Scientific, Bremen, Germany). Then 1 µL total RNA was reverse transcribed to 50 µL cDNA with reverse-transcription (RT) kit (TOYOBO Co., Ltd., Japan). Real-time quantitative PCR experiments were performed using ABI Prism 7500HT (Applied Biosystems, Carlsbad, CA, U.S.A.) with SYBR Green RT-PCR Master Mix (TOYOBO Co., Ltd.). The reaction condition for 3 min preheating at 95°C, followed by 40 cycles of 15 s at 95°C, 15 s at 60°C, 45 s at 72°C and then melting curve was used. mRNA expression levels were expressed as relative to control and were compared as fold change using the comparative Ct method described previously. The sense sequence and the anti-sense sequence of each gene were shown in Table 1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense Sequence (5′–3′)</th>
<th>Product length (bp)</th>
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<td>β-ACTIN</td>
<td>Sense: TACCAAACTGGGACGATAGT</td>
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<tr>
<td></td>
<td>Anti-sense: GTGCGCTTTAGGGTTCAGAG</td>
<td></td>
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<tr>
<td>IL-6</td>
<td>Sense: AAGGACCAAGACCATCCCA</td>
<td>129</td>
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<tr>
<td></td>
<td>Anti-sense: ACCACAGTGGAGAATGTCCA</td>
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<tr>
<td>IL-1β</td>
<td>Sense: GCATCCAGCCTTCAAATCTCA</td>
<td>108</td>
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<tr>
<td></td>
<td>Anti-sense: ACCGGCAAGACATAGGTAGC</td>
<td></td>
</tr>
<tr>
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<td>Sense: GAGATGTGGAACCTGGCAGAG</td>
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<tr>
<td></td>
<td>Anti-sense: ACCAGGAGATGGAAGAGGCT</td>
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<td>NMDA receptor 1</td>
<td>Sense: CAAAAGGAGTGGAACCGAATG</td>
<td>200</td>
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<tr>
<td></td>
<td>Anti-sense: TGCTCTGAAAAGGCTGCAAA</td>
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Western Blot Analysis

Western blots were performed on proteins from hippocampus tissue homogenates (n=8 for the CG, MG and GG group), which was homogenized in RIPA lysis buffer (Beyotime Biotechnology, China). Protein concentrations were determined and quantified using the bicinchoninic acid (BCA) protein assay kit (Beyotime Biotechnology, China). Proteins samples (30 μg) were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by electrophoresis performed according to standard procedures. After the electrophoresis, the gel was separated from the glass plates and the proteins were electrotransferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, U.S.A.) in a wet transfer system (Bio-Rad, U.S.A.). The quantification of proteins was performed by calculating the density of each individual band sample and was compared as fold change to internal control. Using Quantity One software 4.6.2 (Bio-Rad, Hercules, CA, U.S.A.), the resulting labeled bands were quantified with a rabbit anti-NMDAR1 primary antibody (1:1000; Cell Signaling Technology, U.S.A.) or a rabbit anti-indoleamine 2,3-dioxygenase antibody (1:50; Abcam plc, Cambridge, U.K.). Glyceraldehyde-3-phosphate dehydrogenase (rabbit anti-GAPDH primary antibody; 1:1000; from Goodhere Biotechnology, U.K.) or a rabbit anti-indoleamine 2,3-dioxygenase antibody (1:50; Abcam plc, Cambridge, U.K.) were used as an internal control. After three washings with TBST over 10 min, the membranes were incubated at 25°C for 1 h with horseradish peroxidase (HRP) conjugated anti-rabbit immunoglobulin G (IgG) secondary antibody (1:5000; Bioworld Technology, Inc., U.S.A.). Followed by exposure to X-ray films (Kodak), the resulting labeled bands were quantified with Quantity One software 4.6.2 (Bio-Rad, Hercules, CA, U.S.A.). The quantification of proteins was performed by calculating the density of each individual band sample and was compared as fold change to internal control.

High Performance Liquid Chromatography (HPLC)

Hippocampal samples (n=8 for the CG, MG and GG group) were processed with 5% perchloric acid for removing the impurities, then centrifuging at 18000 rpm/min at 4°C for 15 min. The supernatant solutions were measured for the concentrations of tryptophan (TRP), kynurenine (KYN) and kynurenic acid (KYN) using the HPLC-fluorescence detection system (Agilent 1260 Infinity, U.S.A.) with a Hypersil C18 column (250 mm × 4.6 mm i.d., 5 mm particle size, from Elite Analytical Instrument Co., Ltd., Dalian, China). The mobile phase consisted of 0.2 mol/L zinc acetate, 8.3 mmol/L acetic acid with 2.5% (v/v) acetonitrile. The flow rate was maintained at 1.5 mL/min. The fluorescence excitation and emission wavelengths the preparation of standard working solutions were described previously. An ultrastructural TEM analysis of hippocampal neurons was performed on postoperative day 1 (n=2 for each group). Small pieces of the hippocampus were cut into 1 mm² and immediately fixed in 2.5% glutaraldehyde at 4°C overnight. Samples were then post-fixed in 1% osmium tetroxide for 1 h and were dehydrated in a series of graded acetone (50%, 70%, 90%, 95%, 100% and 100% again, respectively) every treating for 10 min. Specimens were embedded in epoxy resin for 72 h at 40°C and for 24 h at 60°C. Semi thin sections were stained with toluidine blue. Ultrathin sections fabricated and observed were under examined by transmission electron microscopy (H-7500, HITACHI, Tokyo, Japan).

Transmission Electron Microscopic (TEM) Analysis

An ultrastructural TEM analysis of hippocampal neurons was performed on postoperative day 1 (n=2 for each group). Small pieces of the hippocampus were cut into 1 mm² and immediately fixed in 2.5% glutaraldehyde at 4°C overnight. Samples were then post-fixed in 1% osmium tetroxide for 1 h and were dehydrated in a series of graded acetone (50%, 70%, 90%, 95%, 100% and 100% again, respectively) every treating for 10 min. Specimens were embedded in epoxy resin for 72 h at 40°C and for 24 h at 60°C. Semi thin sections were stained with toluidine blue. Ultrathin sections fabricated and observed were under examined by transmission electron microscopy (H-7500, HITACHI, Tokyo, Japan).

Statistical Analysis

Data were all expressed as mean±standard deviation (S.D.). Statistical analyses were performed using the SPSS software for Windows (version 17.0)
statistical program. The significance of the mean difference was determined by one-way ANOVA, followed by post-hoc tests (using Least Significant Difference test, LSD-t) for multigroup comparisons. Differences between groups were considered statistically-significant when p-values were less than 0.05 (two-tailed).

RESULTS

Open Field Test  The results are shown in Table 2. Compared with the CG group on postoperative days 1 and 3, the mobility score of the MG group was significantly lower (p<0.05); meanwhile, the latency to leave the center, time of rest and ratio of periphery/central locomotion of the MG group significantly increased (p<0.05). Compared with the MG group, on postoperative days 1 and 3, the mobility score of the GG groups significantly increased (p<0.05) while the latency to leave the center, time of rest and ratio periphery/central locomotion of the GG group significantly decreased (p<0.05). In addition, on postoperative day 1, the latency to leave the center and ratio periphery/central locomotion of the NG group significantly decreased (p<0.05), compared with the MG group. There was no significant difference between CG and RG. In addition, there was no significant difference in the time of grooming among five groups.

mRNA Expression of Inflammatory Cytokines (IL-6, IL-1β, TNF-α) The results are shown in Fig. 1. Compared with the CG group on postoperative days 1 and 3, we found significantly increased mRNA expressions of each inflammatory cytokine in the MG group (p<0.05). The mRNA expressions of each inflammatory cytokine of the MG group returned to the level of the CG group on postoperative day 7. In addition, on postoperative days 1 and 3, the GG group showed a significantly lower mRNA expression of each inflammatory cytokine than that of the MG group (p<0.05).

Expression of IDO, Activity of IDO (Ratio KYN/TRP) and Tryptophan Metabolism The protein expressions of IDO are shown in Fig. 2a. On postoperative day 1, the protein levels of IDO of the MG groups significantly increased (p<0.05), compared with that of the CG group; however, the protein expression levels of IDO were not significantly altered in the GG group compared with the MG group. The activity of IDO and the results of tryptophan metabolism are shown in Table 3. There was a tendency of increased concentrations of TRP and KYN and deceased concentrations of KYNA in the MG group compared with the CG group on postoperative days 1 and 3. However, the ratios KYN/TRP and KYN/KYNA significantly increased (both p<0.05) in the MG group compared with the CG group on postoperative days 1 and 3. In addition, there was no significant difference in the ratio KYN/TRP, ratio KYN/KYNA or concentrations of TRP, KYN and KYNA between the GG group and the MG group.

Expression of NMDA Receptor 1 The mRNA and protein expression of NMDA receptor 1 are shown in Figs. 1d and 2b, respectively. We found both significantly increased mRNA and protein expressions of NMDA receptor 1 in the MG group (p<0.01). The mRNA expressions of each inflammatory cytokine of the MG group returned to the level of the CG group on postoperative day 7. In addition, on postoperative days 1 and 3, the GG group showed a significantly lower mRNA expression of each inflammatory cytokine than that of the MG group (p<0.05).
in the GG group \((p < 0.05)\) compared with the MG group on postoperative days 1 and 3.

**Ultrastructural Change of Hippocampal Neurons**  Figure 3 shows the ultrastructure micrographs of hippocampal neurons in four groups. Hippocampal neuron in the MG group on postoperative day 1 was degenerating, in association with nuclear membrane crinkling like wave shape, chromatin condensing and gathering near the blurred nuclear membrane. In addition, we could see rarefaction and vacuoles of the mitochondrial cristae, disorderly distributed endoplasmic reticulum and reduced free ribosomes in the hippocampal neuron. In the CG group on postoperative day 1, hippocampal neuron nuclei were large and round, with the uniformly distributed chromatin inside. Plenty of normal endoplasmic reticulum, ribosomes, mitochondria and other organelles in the perikaryon could be observed. In addition, the hippocampal neurons in both the GG and NG group were relatively improved.

**DISCUSSION**

In the present study, based on the POFS model induced by major small intestinal resection in rats, we used open field test to assess fatigue and determined typical biochemical parameters to research the central fatigue mechanism on POFS, tried to explain why patients have the feeling of fatigue and
cognitive impairment after major abdominal surgery. The results indicated that the upregulated mRNA expressions of inflammatory cytokines (IL-6, IL-1β, TNF-α) could activate IDO which effected the metabolism of tryptophan in the brain, then stimulated NMDA receptor to neuronal injury in hippocampus, showed a kind of central fatigue in rats with POFS. GRβ1 could improve this central fatigue by preventing the increased expressions of inflammatory cytokines and NMDA receptor.

OFT was used to assess POFS model in the present study. Many published studies also used OFT to reflect the changes in the hippocampus.28,29) The mobility score showed an ability of locomotion. The latency to leave the center is an indication of anxiety. The time of grooming showed an ability of self-cleaning. In this open field situation, rats walked close to the walls, a behavior called thigmotaxis which was indicated by ratio of periphery/central locomotion in this study. When compared with non-emotional rats, they had fewer entries in the central part and higher stay of periphery area. It can be used as an index of timidity and a high-stress state.30) The results indicated that POFS rats had decreased levels of short-term activity, space cognition, excitability and exploratory behavior to the new environment within 3 d after surgery. These are consistent with the typical fatigue syndromes, learning and cognitive impairment and anxiety, which are also presented in patients after surgery.

It is well accepted that the inflammatory response is one of the pathophysiologic responses to surgical injury. Some research on the relationship between inflammation and spirit, mood and emotion showed that some inflammatory cytokines such as IL-6 and TNF-α play a vital role in causing weakness behavior and are associated with cognitive impairment and mood changes.31,32) In the present study, we found that POFS rats had a significantly increased mRNA expression of IL-6, IL-1β and TNF-α in the hippocampus on postoperative days 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>TRP (nmol/g)</th>
<th>KYN (nmol/g)</th>
<th>KYNA (nmol/g)</th>
<th>Ratio KYN/TRP</th>
<th>Ratio KYN/KYNA</th>
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<tbody>
<tr>
<td>CG</td>
<td>POD 1</td>
<td>34.34±2.78</td>
<td>5.26±0.67</td>
<td>0.321±0.023</td>
<td>0.153±0.016</td>
<td>16.36±1.51</td>
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<td>POD 3</td>
<td>39.43±4.16</td>
<td>6.27±0.79</td>
<td>0.379±0.047</td>
<td>0.159±0.011</td>
<td>16.21±1.93</td>
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<td>POD 5</td>
<td>30.97±6.80</td>
<td>4.60±0.74</td>
<td>0.303±0.085</td>
<td>0.150±0.011</td>
<td>15.51±1.45</td>
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<td>POD 7</td>
<td>30.00±3.71</td>
<td>4.23±0.56</td>
<td>0.283±0.030</td>
<td>0.141±0.007</td>
<td>15.00±1.86</td>
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<td>MG</td>
<td>POD 1</td>
<td>35.83±6.81</td>
<td>6.11±1.24</td>
<td>0.320±0.045</td>
<td>0.171±0.012*</td>
<td>19.07±2.85*</td>
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<td>POD 3</td>
<td>41.51±6.78</td>
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<td>0.175±0.013*</td>
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<td>GG</td>
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<td>35.36±4.84</td>
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<td>POD 3</td>
<td>41.61±4.50</td>
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<td>0.140±0.010</td>
<td>15.28±2.06</td>
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</table>

CG (Control group), MG (POFS model group), GG (Ginsenoside Rb1-treated POFS model group), POD (Postoperative day). TRP (Tryptophan), KYN (Kynurenine), KYNA (Kynurenic acid). Values are expressed as the mean±S.D. *p<0.05 means the significant of MG 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. 3. Representative Photographs of Transmission Electron Micrographs of Hippocampal Neuron on Postoperative Day 1 (5000× and 10000×)
CG (Control group), MG (POFS model group), GG (Ginsenoside Rb1-treated POFS model group), NG (NMDA receptor antagonist group).
and 3, which was also consistent with the result of open field test postoperatively.

NMDA receptors are abundant in the neurons of hippocampus and it is suggested that there is a close relationship between NMDA receptor and various psychiatric disorders like learning and memory impairment, anxiety and depression. As previous study suggested that the amount of excitatory NMDA receptor has a relationship with the extent of the hippocampal neuronal death, the change of hippocampal neurons morphology may be caused by NMDA receptor stimulation, the neural injury in the hippocampus can impede the neural function, and this could be a direct cause for the change of behavior in the POFS rats. In the present study, we confirmed the mRNA and protein expressions of NMDA receptor 1. The result showed that POFS rats performed postoperative fatigue associated with a significantly increased expression of NMDA receptor 1 on postoperative days 1 and 3. NMDA receptor antagonist MK801 could improve the central fatigue of POFS by reducing the ratio of periphery/central locomotion and latency to leave the center and protecting the hippocampal neurons. So we speculated that the overexpression of NMDA receptor may belong to a pathophysiologic response to surgical injury and inflammatory cytokines, which could be linked with the central fatigue of POFS.

IDO is an enzyme which is present in monocytes, macrophages and microglial cells within the brain parenchyma and converts TRP into KYN. As is well known that IDO activity is mainly stimulated by the pro-inflammatory cytokines, in the present study POFS rats had a significantly increased protein level of IDO on postoperative day 1, which was combined with up regulation of the inflammatory cytokines IL-6, IL-1β and TNF-α. Both TRP level and KYN level in the hippocampus of POFS rats increased postoperatively but there was no significant difference. However, the ratio KYN/TRP was significantly higher in POFS rats on postoperative days 1 and 3, indicating that the activity of IDO was strengthened postoperatively. In our previous study, we found that free tryptophan (f-TRP) and the ratio of f-TRP/BCAA increased in the early postoperative period in the POFS rats, so we speculated that surgical trauma may decrease the nutritional status of patients and enhance protein catabolism, thus increased TRP levels on the brain; at the same time the high transcriptional levels of IL-6, IL-1β and TNF-α may active IDO and tryptophan metabolism within 3d after surgery. Some studies summarized that the abdomen injury signal can be spread to central nervous system (CNS) through two pathways: 1) Inflammatory cytokines are released into blood and spread to CNS through the blood flow; 2) Abdominal injury signal transmit to CNS through the vagus nerve and released some cytokines. They act synergistically to give rise to postoperative fatigue symptoms. In addition, that microglia is the resident macrophages of the brain and spinal cord which secreted pro-inflammatory cytokines, and act as the main form of active immune defense in the brain. However, the present study did not determine which pathway mentioned above to stimulate the microglia in the hippocampus to synthesis inflammatory cytokines in the brain was dominant. Our further research will explain this problem.

In addition, we also found a decreased tendency of KYNA level postoperatively and a significantly increased ratio KYN/KYNA, indicating there was an imbalance between KYN and KYNA, or between QA and KYNA. Some studies have demonstrated the neurotoxicity of KYN and its metabolite QA by stimulating NMDA receptor. However, KYNA, another metabolite of KYN, is known as an antagonist of the NMDA receptor and can reduce NMDA overstimulation to protect neurons. This imbalance between neurotoxic and neuroprotective metabolites may result in a greater neurotoxic damage through NMDA receptor. Thus, the neurotoxic metabolites could overstimulate hippocampal KYNA receptor to intracellular calcium overload and neuronal injury.

Ginseng, as a Chinese traditional medicine, has been used as a tonic to treat various disorders. GRb1 is one of the active substances in ginsenosides. GRb1 exerts a protective effect in various conditions. GRb1 also showed an anti-fatigue effect on which may be associated with the improvement of energy metabolism and the suppression of skeletal muscle oxidative stress in previous study. In the present study, GRb1 decreased the levels of inflammatory cytokines (IL-6, IL-1β, TNF-α) and NMDA receptor, improved the behavioral manifestations on the postoperative days 1 and 3, indicating it could minimize the effects of central fatigue and protect the hippocampal neurons from neural injury for the POFS rats. In addition, GRb1 had no effects for the rats in RG group, indicating that GRb1 may have more beneficial effect on the pathophysiologic changes of POFS. Moreover, GRb1 had no significant effects on IDO and tryptophan metabolism. It may protect the hippocampal neurons by reducing the amount of NMDA receptor or playing neurotrophic effect. Therefore, a future study may be performed for the exact mechanism of GRb1’s anti-fatigue effect on POFS.

In conclusion, our study demonstrates that inflammatory cytokines can active tryptophan metabolic pathways on the hippocampus to cause fatigue through NMDA receptor on POFS induced by major small intestinal resection in rat. GRb1 has an anti-fatigue effect on POFS by reducing inflammatory cytokines and NMDA receptor. To our knowledge, this is the first study demonstrating that GRb1 can prevent increased both inflammatory cytokines and NMDA receptor in POFS in vivo. As 5 hydroxytryptamine (5-HT) receptor pathways play an important role in cognitive-behavioral impairment, a further study is required to determine the interaction between 5-HT receptor pathways and NMDA receptor pathways. In addition, as GRb1 has multiple pharmacological effects, we should consider whether GRb1 has an anti-fatigue effect through 5-HT pathway or whether the anti-fatigue effect of GRb1 may involve anti-oxidation or neuroprotection in hippocampus of POFS rats.

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Conflict of Interest The authors declare no conflict of interest.
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