

Anti-inflammatory Effect of Ginsenoside Rb1 Contributes to the Recovery of Gastrointestinal Motility in the Rat Model of Postoperative Ileus

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Ginsenoside Rb1 (GRb1), one of the principle active components of *Panax ginseng*, has been reported to reduce inflammation in various diseases. In the present study, we investigated whether GRb1 has an anti-inflammatory effect on postoperative ileus (POI) and further contributes to the recovery of gastrointestinal motility. POI was induced in rats by intestinal manipulation. The POI rats received 5, 10 and 20 mg/kg GRb1 orally *via* gavage four times before and after surgery. Gastrointestinal motility was assessed by charcoal transport. Systemic inflammation was assessed by serum tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6 and IL-10 concentrations, whereas intestinal inflammation was assessed by the activity of myeloperoxidase, and concentrations and gene expression of TNF- α , IL-1 β , IL-6 and IL-10 in the ileum tissue. The results revealed that GRb1 increased rat gastrointestinal transit with POI. The increased levels of systemic and intestinal inflammatory parameters in POI rats were also reduced by GRb1. In addition, GRb1 reduced systemic and intestinal inflammation and increased the gastrointestinal transit of POI rats in a dose-dependent manner, and with significance at doses of 10 and 20 mg/kg. These results suggest that GRb1 has a potent anti-inflammatory effect on POI and further contributes to the recovery of gastrointestinal motility. GRb1 may be a promising treatment for POI prophylaxis.

Key words ginsenoside Rb1; postoperative ileus; inflammation

Ginseng, the dried root of *Panax ginseng* C. A. MEYER (Araliaceae), has been well accepted as a tonic to treat many disorders in Chinese traditional medicine. It is considered a famous herb since the earliest Chinese pharmaceutical monograph “*Shen Nong Ben Cao Jing*.”¹⁾ Nowadays, *ginseng* has been worldwide used as a popular traditional herbal medicine, especially in China, Korea and Japan. Ginsenosides are thought to be the main active components of *ginseng* with multiple pharmacological activities including anti-inflammation, anti-aging, anti-tumor, anti-oxidation, and anti-fatigue.^{2–8)} Modern science has identified more than 50 kinds of ginsenosides. Ginsenoside Rb1 (GRb1), one of the main ginsenosides, belongs to the protopanaxadiol group of steroidal saponins. It is reported that GRb1 has been frequently used to reduce inflammatory process in various diseases.^{9–13)}

Currently, one of the major tasks of surgery is to accelerate postoperative rehabilitation. Postoperative ileus (POI) is a common and severe iatrogenic complication after abdominal surgery.^{14,15)} It is characterized by gastrointestinal dysmotility.¹⁶⁾ For affected patients, POI manifests itself as some main symptoms including abdominal bloating and distension, a mix of nausea and vomiting and delayed return of flatus and stool passage.^{17,18)} Patients who suffer from POI have a long time to recover from this gastrointestinal motility disorders. POI prolongs patient’s hospitalization, increases medical costs, and therefore affects postoperative rehabilitation.^{19,20)} However, the etiology of POI has not been fully explained.¹⁶⁾

Much of the published research suggests that many factors, such as neurogenic reflexes, inhibitory agents and inflammation, are responsible for the development and progression of

POI.^{16,21,22)} Among these factors, accumulating evidence indicates that intestinal inflammation plays an important role in the pathophysiology of POI.^{16,23,24)} The extent of intestinal manipulation during abdominal surgery causes the degree of intestinal inflammation and further determines the duration and severity of gastrointestinal dysmotility.²⁵⁾ Therefore, methods to reduce intestinal inflammation and shorten postoperative rehabilitation course are popular pursuits in modern surgery.¹⁶⁾

In the present study, therefore, we used charcoal transport and some typical inflammatory parameters to investigate whether GRb1 has an anti-inflammatory effect on POI and further contributes to the recovery of gastrointestinal motility.

MATERIALS AND METHODS

Animals The protocol for the animal experiment was approved by the Institutional Animal Care and Use Committee of Jinling Hospital, Nanjing University. Healthy adult male Sprague-Dawley rats (weighing 220 \pm 10 g) were obtained from Jinling Hospital in Nanjing, Jiangsu, China. All rats received human care throughout the experiment in accordance with “Guide for the Care and Use of Laboratory Animals.” The rats were housed in our laboratory with free access to standard rat chow and tap water made available *ad libitum*. The lights were maintained on a 12:12-h light:dark temperature controlled environment.

Drugs GRb1 (purity over 98%) was purchased from Shanghai Tauto Biotech Co., Ltd., Shanghai, China. Charcoal and gum arabic were purchased from Shanghai Boyun 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 adapta-

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tion period for one week, 48 rats were randomly divided into 6 groups: a control group (CG), a POI model group (MG), three GRb1-treated POI model groups at a dose of 5, 10 and 20 mg/kg, respectively (GG, including GG5, GG10 and GG20), and a control group treated with GRb1 at a dose of 20 mg/kg (CG20), with 8 in each. The rat model of POI was induced by intestinal manipulation as described in the previous study.^{26,27} Briefly, after full anesthesia with 2% pentobarbital sodium (3.5 mL/kg), rats in the MG and the GG groups were laparotomized through a midline abdominal incision, and then the small bowel was manipulated with moderate compression between two moist cotton applicators along its entire length. The CG and the CG20 groups went through the same procedure, but without any intestinal manipulation. Rats in the CG and the MG groups received saline orally *via* gavage once daily at 3 d, 2 d and 1 d before surgery, and at 6 h after surgery.²⁸ Rats in three GG groups were separately administrated GRb1 dissolved in saline at a dose of 5, 10 and 20 mg/kg four times by the same method as the CG group.²⁹ Rats in the CG20 group were also similarly administrated GRb1 at a dose of 20 mg/kg four times by the same method as the CG group.

Determination of Gastrointestinal Motility and Sampling To explore an appropriate investigation time point, we did a pilot experiment prior to this study, demonstrating that the levels of intestinal inflammation and motility differed significantly at 24 h after surgery, but no significant differences were found on postoperative day 2 or later (data not shown). Therefore, at 24 h after surgery, all rats in the 6 groups were selected to assess gastrointestinal motility by charcoal transport as described in the previous study.³⁰ Briefly, rats were administered with a black marker (10% charcoal suspension in 10% gum arabic, 10 mL/kg body weight) by gavage. Twenty minutes later, rats were fully anesthetized by subcutaneous injection of 2% pentobarbital sodium (3.5 mL/kg). Blood were obtained immediately from the inferior vena cava, and then the small bowel was obtained from pylorus to cecum. The distance travelled by the marker in the small bowel was measured in centimeter and reported as a percentage of total length of the small bowel. Subsequently, a segment of the complete ileum tissue (2 cm from the ileocecal valve) was harvested for further analyses of cytokines, gene expression, and myeloperoxidase (MPO) activity.

Determination of Concentrations of Cytokines in Serum and the Ileum Tissue The serum was prepared by centrifugation at a speed of 800×g, at 4°C for 15 min. The ileum tissue was homogenized, centrifuged at a speed of 2000×g, at 4°C for 15 min, and then the supernatant was obtained. The protein concentration in the supernatant was determined using the method of Bradford.³¹ The concentrations of tumour necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6 and IL-10 in serum and the ileum tissue were determined with an ELISA kit for rats (R&D Systems, Germany) according to the manufacturer's instructions. Values were expressed as pg/mL in serum or pg/g in the ileum tissue.

Analysis of Expression of Cytokine Gene in the Ileum Tissue The separate total RNA from ileum tissue was extracted using Trizol reagent (Invitrogen, U.S.A.) according to the manufacturer's instructions. Target mRNAs of TNF- α , IL-1 β , IL-6 and IL-10 were separately reverse-transcribed to complementary DNA and measured using real-time polymerase chain reaction (RT-PCR) as described in our previ-

Table 1. The Primer Sequences Used for RT-PCR

Gene		Primer sequence
TNF- α	Forward	5'-ACTCCCAGAAAAGCAAGCAA-3'
	Reverse	5'-CGAGCAGGAATGAGAAGAGG-3'
IL-1 β	Forward	5'-AGGCTTCCTTGTGCAAGTGT-3'
	Reverse	5'-TGAGTGACACTGCCTTCCTG-3'
IL-6	Forward	5'-CTGCTCTGGTCTTCTGGAGT-3'
	Reverse	5'-GGTCTGGTCCCTTAGCCACT-3'
IL-10	Forward	5'-ATAACTGCACCCACTTCCCA-3'
	Reverse	5'-TTTCTGGCCATGGTTCTCT-3'
GAPDH	Forward	5'-GGCATTGCTCTCAATGACAA-3'
	Reverse	5'-TGTGAGGGAGATGCTCAGTG-3'

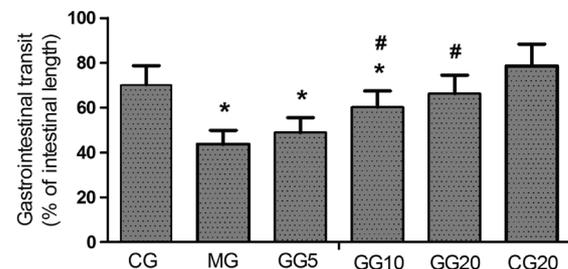


Fig. 1. Effects of GRb1 on Gastrointestinal Motility

CG (control group), MG (POI model group), GG5, GG10 and GG20 (ginsenoside Rb1-treated POI model group at a dose of 5, 10 and 20 mg/kg, respectively), CG20 (control group treated with GRb1 at a dose of 20 mg/kg). Values are expressed as the mean±S.D. * $p<0.05$ means the significance of MG, GG and CG20 compared with CG, and # $p<0.05$ means the significance of GG compared with MG, respectively.

ous study.^{23,32} Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal standard to normalize the target mRNAs, and relative quantifications and calculations were performed by the $2^{-\Delta\Delta CT}$ method to analyze gene expression.³³ The primer sequences were shown in Table 1.

Assessment of MPO Activity in the Ileum Tissue The ileum tissue was homogenized, centrifuged at a speed of 2000×g, at 4°C for 15 min, and then the supernatants were obtained. The protein concentration in the supernatant was determined using the method of Bradford.³¹ MPO activity was quantitatively measured by spectrophotometry at 460 nm as described in our previous study.³² Values were expressed as units/g in the ileum tissue.

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 the least significant difference test for multigroup comparisons. Differences were considered significant if the p value was lower than 0.05.

RESULTS

Effects of GRb1 on Gastrointestinal Motility The results were shown in Fig. 1. There were no significant differences in gastrointestinal transit between the CG and the CG20 groups ($p>0.05$). The gastrointestinal transit of the MG group was significantly decreased ($p<0.05$) when compared with that of the CG group. However, GRb1 induced a progressive increase in the gastrointestinal transit in a dose-dependent

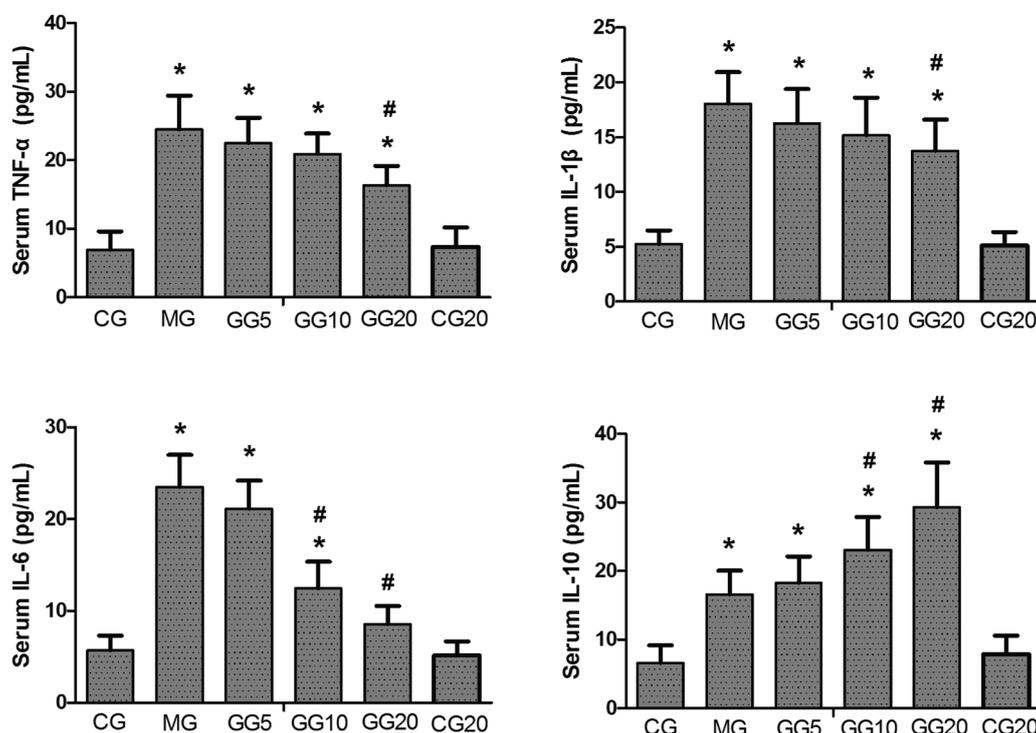


Fig. 2. Effects of GRb1 on Concentrations of TNF- α , IL-1 β , IL-6 and IL-10 in Serum

CG (control group), MG (POI model group), GG5, GG10 and GG20 (ginsenoside Rb1-treated POI model group at a dose of 5, 10 and 20 mg/kg, respectively), CG20 (control group treated with GRb1 at a dose of 20 mg/kg). Values are expressed as the mean \pm S.D. * p <0.05 means the significance of MG, GG and CG20 compared with CG, and # p <0.05 means the significance of GG compared with MG, respectively.

manner, differed significantly in the GG10 and the GG20 groups when compared with that in the MG group (p <0.05). In addition, while the gastrointestinal transit of the GG5 and the GG10 groups was still significantly slower than that of the CG group (p <0.05), that of the GG20 group returned to the level of the CG group.

Effects of GRb1 on Concentrations of TNF- α , IL-1 β , IL-6 and IL-10 in Serum The results were shown in Fig. 2. There were no significant differences in the serum concentrations of TNF- α , IL-1 β , IL-6 and IL-10 between the CG and the CG20 groups (p >0.05). The serum concentrations of TNF- α , IL-1 β , IL-6 and IL-10 of the MG group were significantly increased (p <0.05) when compared with those of the CG group. However, GRb1 induced a progressive decrease in the serum concentrations of pro-inflammatory cytokine TNF- α , IL-1 β and IL-6 in a dose-dependent manner. The serum concentrations of TNF- α and IL-1 β of the GG20 group were significantly decreased when compared with those of the MG group (p <0.05), although the serum concentrations of TNF- α and IL-1 β in all three GG groups were still significantly higher than those in the CG group (p <0.05). The serum concentration of IL-6 of the GG10 and the GG20 groups was significantly decreased when compared with that in the MG group (p <0.05), and the serum concentration of IL-6 of the GG20 group returned to the level of the CG group, although the serum concentration of IL-6 of the GG5 and the GG10 groups was still significantly higher than that of the CG group (p <0.05). In addition, GRb1 also induced a progressive increase in the serum concentration of anti-inflammatory cytokine IL-10 in a dose-dependent manner, differed significantly in the GG10 and the GG20 groups when compared with that in the MG group (p <0.05).

Effects of GRb1 on Concentrations of TNF- α , IL-1 β , IL-6 and IL-10 in the Ileum Tissue The results were shown in Fig. 3. There were no significant differences in the concentrations of TNF- α , IL-1 β , IL-6 and IL-10 in the ileum tissue between the CG and the CG20 groups (p >0.05). The tissue concentrations of TNF- α , IL-1 β , IL-6 and IL-10 of the MG group were significantly increased (p <0.05) when compared with those of the CG group. However, GRb1 induced a progressive decrease in the tissue concentrations of pro-inflammatory cytokine TNF- α , IL-1 β and IL-6 in a dose-dependent manner. The tissue concentrations of TNF- α and IL-1 β of the GG10 and the GG20 groups were significantly decreased when compared with those of the MG group (p <0.05), and the tissue concentrations of TNF- α and IL-1 β of the GG20 group returned to the level of the CG group, although the tissue concentrations of TNF- α and IL-1 β in the GG5 and the GG10 groups were still significantly higher than those in the CG group (p <0.05). The tissue concentration of IL-6 of the GG10 and the GG20 groups was significantly decreased when compared with that in the MG group (p <0.05), and returned to the level of the CG group, although the tissue concentration of IL-6 in the GG5 group was still significantly higher than that in the CG group (p <0.05). In addition, GRb1 also induced a progressive increase in the tissue concentration of anti-inflammatory cytokine IL-10 in a dose-dependent manner, differed significantly in the GG10 and the GG20 groups when compared with that in the MG group (p <0.05).

Effects of GRb1 on Gene Expression of TNF- α , IL-1 β , IL-6 and IL-10 in the Ileum Tissue The results were shown in Fig. 4. There were no significant differences in the gene expression of TNF- α , IL-1 β , IL-6 and IL-10 in the ileum tissue between the CG and the CG20 groups (p >0.05). The

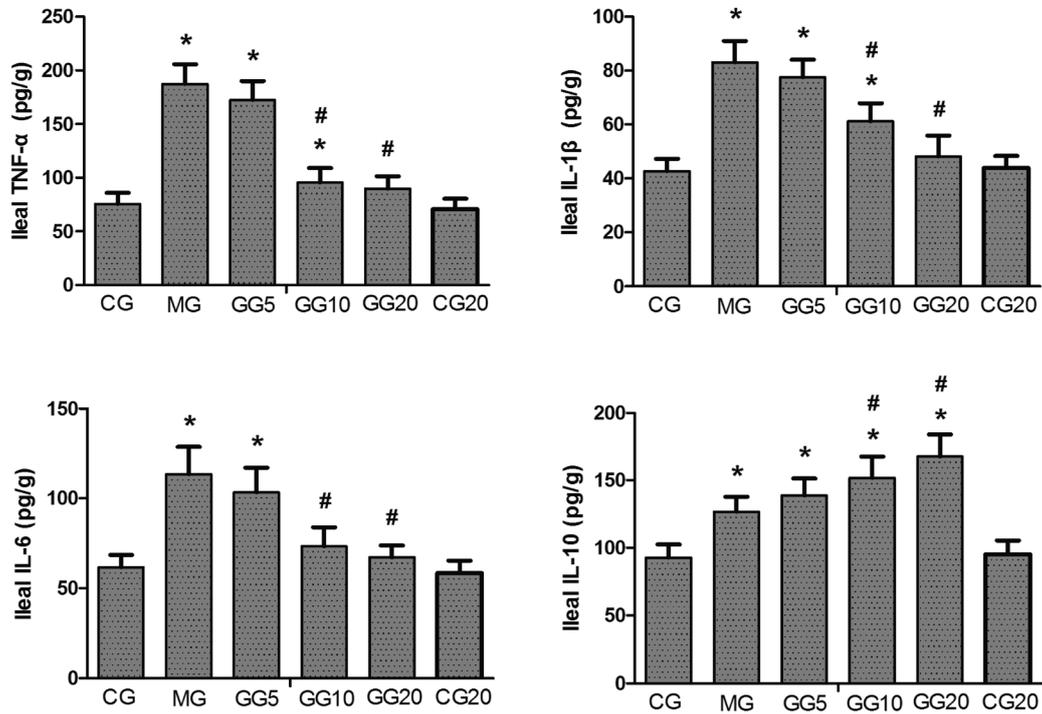


Fig. 3. Effects of GRb1 on Concentrations of TNF- α , IL-1 β , IL-6 and IL-10 in the Ileum Tissue

CG (control group), MG (POI model group), GG5, GG10 and GG20 (ginsenoside Rb1-treated POI model group at a dose of 5, 10 and 20mg/kg, respectively), CG20 (control group treated with GRb1 at a dose of 20mg/kg). Values are expressed as the mean \pm S.D. * p <0.05 means the significance of MG, GG and CG20 compared with CG, and # p <0.05 means the significance of GG compared with MG, respectively.

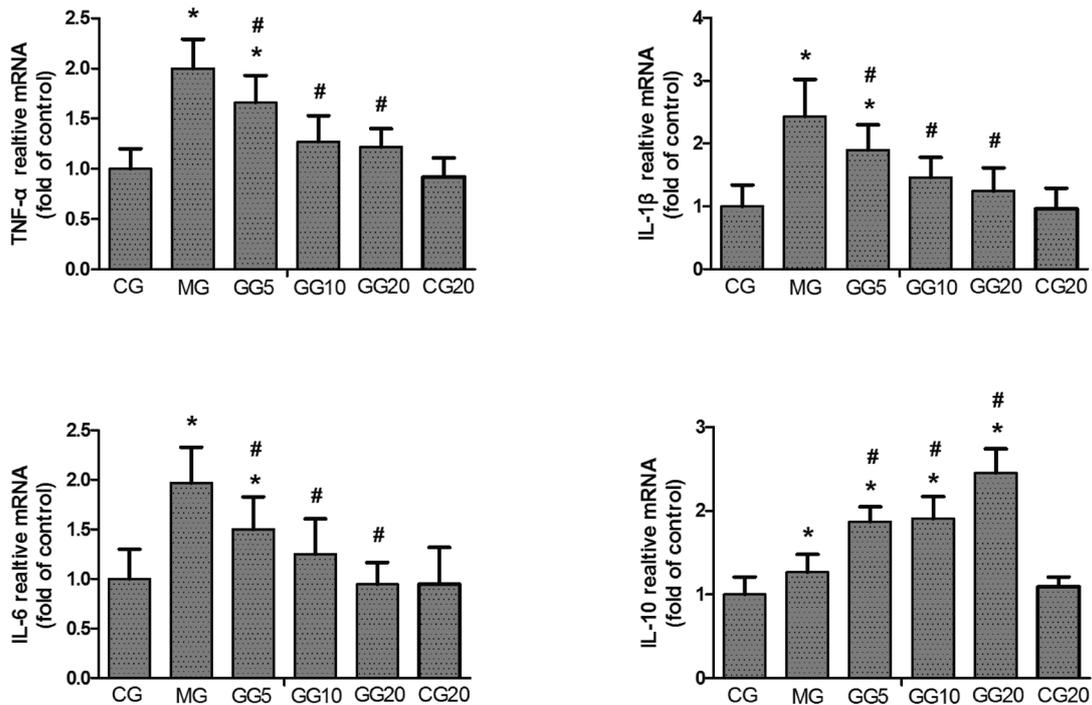


Fig. 4. Effects of GRb1 on Gene Expression of TNF- α , IL-1 β , IL-6 and IL-10 in the Ileum Tissue

CG (control group), MG (POI model group), GG5, GG10 and GG20 (ginsenoside Rb1-treated POI model group at a dose of 5, 10 and 20mg/kg, respectively), CG20 (control group treated with GRb1 at a dose of 20mg/kg). Values are expressed as the mean \pm S.D. * p <0.05 means the significance of MG, GG and CG20 compared with CG, and # p <0.05 means the significance of GG compared with MG, respectively.

gene expression of TNF- α , IL-1 β , IL-6 and IL-10 of the MG group was significantly up-regulated (p <0.05) when compared with that of the CG group. However, GRb1 induced a progressive down-regulation in the gene expression of pro-inflammatory cytokine TNF- α , IL-1 β and IL-6 in a dose-de-

pendent manner, differed significantly in all three GG groups when compared with that in the MG group (p <0.05). In addition, while the gene expression of TNF- α , IL-1 β and IL-6 in the GG5 group was still significantly higher than that in the CG group (p <0.05), the gene expression of TNF- α , IL-1 β and

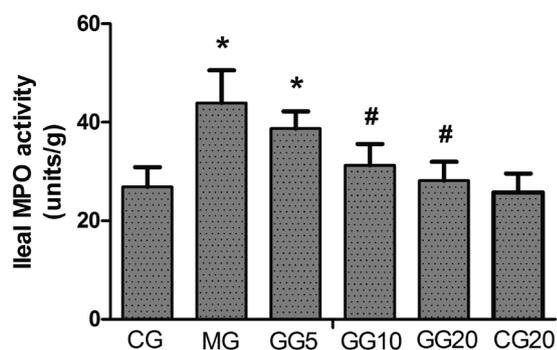


Fig. 5. Effects of GRb1 on MPO Activity in the Ileum Tissue

CG (control group), MG (POI model group), GG5, GG10 and GG20 (ginsenoside Rb1-treated POI model group at a dose of 5, 10 and 20 mg/kg, respectively), CG20 (control group treated with GRb1 at a dose of 20 mg/kg). Values are expressed as the mean \pm S.D. * $p < 0.05$ means the significance of MG, GG and CG20 compared with CG, and # $p < 0.05$ means the significance of GG compared with MG, respectively.

IL-6 of the GG10 and the GG20 groups returned to the level of the CG group. In addition, GRb1 also induced a progressive up-regulation in the gene expression of anti-inflammatory cytokine IL-10 in a dose-dependent manner, differed significantly in three GG groups when compared with that in the MG group ($p < 0.05$).

Effects of GRb1 on MPO Activity in the Ileum Tissue

The results were shown in Fig. 5. There were no significant differences in the MPO activity in the ileum tissue between the CG and the CG20 groups ($p > 0.05$). The MPO activity of the MG group was significantly increased ($p < 0.05$) when compared with that of the CG group. However, GRb1 induced a progressive decrease in the MPO activity in a dose-dependent manner, differed significantly in the GG10 and the GG20 groups when compared with that in the MG group ($p < 0.05$). In addition, while the MPO activity of the GG5 group was still significantly lower than that of the CG group ($p < 0.05$), that of the GG10 and the GG20 groups returned to the level of the CG group.

DISCUSSION

In the present study, to investigate whether GRb1 has an anti-inflammatory effect on POI and further contributes to the recovery of gastrointestinal motility, we used charcoal transport to assess gastrointestinal motility and some typical inflammatory parameters were determined. The results showed that GRb1 could increase gastrointestinal transit, reduce the level of systemic and intestinal inflammatory parameters in POI rats in a dose-dependent manner.

POI is commonly followed by abdominal surgery, leading to increased patient morbidity and prolonged rehabilitation.^{14,15} It is well known that POI is characterized by gastrointestinal dysmotility involved in response to surgical stress.¹⁶ Enhancement of gastrointestinal motility will significantly improve POI and enhance recovery after surgery.^{15,34} Therefore, to investigate the improvement effect of GRb1 on POI, it is important to assess gastrointestinal motility. In the previous study, charcoal transport is commonly used as an objective measurement to assess gastrointestinal motility in animal research.^{23,30,35,36} These studies demonstrate that POI is associated with decreased gastrointestinal transit assessed by charcoal transport. In the present study, we also employed

charcoal transport to determine the gastrointestinal motility, and further investigate the improvement effect of GRb1 on POI. The results showed that GRb1 had no direct effect on gastrointestinal transit rate with healthy animals, and therefore the possibility has been excluded that GRb1 can directly stimulate intestinal motility. However, gastrointestinal transit was decreased in POI rats, but GRb1 increased gastrointestinal transit of POI rats, and the effect is dose-dependent. These findings suggested that GRb1 has beneficial effects on the recovery of gastrointestinal motility in POI.

It is well accepted that intestinal inflammation plays a vital role in the development and progression of POI.¹⁶ A post-operative increase in intestinal inflammation has an positive correlation with the severity of POI,^{23,25,26} suggesting that decreased intestinal inflammation may improve POI. Therefore, to further study the mechanism of the contribution of GRb1 to the recovery of gastrointestinal motility, we investigate the anti-inflammatory effect of GRb1 on POI. In the previous study, pro-inflammatory cytokine TNF- α , IL-1 β and IL-6 are reported to be rapidly produced in the intestinal inflammatory process during induction of POI in animals.^{27,37,38} These cytokines act as inhibitors and contribute to the gastrointestinal dysmotility through their direct cytotoxic action or their effect on the production of nitric oxide and prostanoids.²⁴ In contrast to these pro-inflammatory cytokines, anti-inflammatory cytokine IL-10 actively down-regulates intestinal inflammatory process to prevent an excessive and prolonged inflammation.²⁴ In addition, it is reported that IL-10 deficiency could lead to a prolonged gastrointestinal dysmotility after surgery and abnormal high gene expression of pro-inflammatory cytokines in the animal model of POI.³⁹ In the present study, we found that POI rats showed significantly higher levels of pro-inflammatory cytokine TNF- α , IL-1 β and IL-6 in serum and the ileum tissue, while these elevated levels were significantly reduced by GRb1 in a dose-dependent manner, as well as their gene expression in the ileum tissue. In addition, although the level of anti-inflammatory cytokine IL-10 in serum and the ileum tissue was significantly increased in POI rats, GRb1 also continuously increased this elevated level in a dose-dependent manner, as well as this gene expression in the ileum tissue. These results suggested that GRb1 could reduce systemic and intestinal inflammation not only by decreasing the level of pro-inflammatory cytokine, but also increasing the level of anti-inflammatory cytokine in the animal model of POI.

In addition, MPO is a bioactive enzyme abundantly stored in azurophilic granules of neutrophils. It could be released into extracellular fluid in a state of inflammatory process. Therefore, the level of MPO activity has been commonly employed as a sensitive index to evaluate the extent of inflammatory response in various inflammatory disease.⁴⁰ In the present study, we found that POI rats showed significantly higher level of MPO activity in the ileum tissue, while this elevated level was significantly reduced by GRb1 in a dose-dependent manner. Combined with above cytokine results, we speculated that GRb1 may exhibit the effective anti-inflammatory activity by the inhibition of spleen tyrosine kinase,⁴¹ the stimulation of parasympathetic pathways,²¹ or the regulation of inducible nitric oxide synthase.⁴²

In conclusion, our study demonstrates that GRb1 has a potent anti-inflammatory effect on POI and further contributes

to the recovery of gastrointestinal motility. GRb1 may be a promising treatment for POI prophylaxis. However, GRb1 has multiple pharmacological effects, such as improvement of energy metabolism, oxidative stress and organ function.^{7,8)} Therefore, a further study is required to determine the exact mechanism of GRb1's contribution to the recovery of gastrointestinal motility in POI.

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