Effect of emodin on small intestinal peristalsis of mice and relevant mechanism

Hong-Quan Zhang, Cheng-Hua Zhou, Yu-Qing Wu

AIM: To investigate the effect of emodin on small intestinal peristalsis of mice and to explore its relevant mechanisms.

METHODS: The effect of emodin on small intestinal peristalsis of mice was observed by charcoal powder propelling test of small intestine. The contents of motilin and somatostatin in small intestine of mice were determined by radioimmunoassay. The electrical potential difference (PD) related to Na\(^+\) and glucose transport was measured across the wall of reverted intestinal sacs. Na\(^+\)–K\(^+\)-ATPase activity of small intestinal mucosa was measured by spectroscopic analysis.

RESULTS: Different dosages of emodin can improve small intestinal peristalsis of mice. Emodin increased the content of motilin, while reduced the content of somatostatin in small intestine of mice significantly. Emodin 0.2, 0.4, 0.8, and 1.6 g/L decreased PD when there was glucose. However, emodin had little effect when glucose was free. The Na\(^+\)–K\(^+\)-ATPase activity of small intestinal mucosa of mice in emodin groups was inhibited obviously.

CONCLUSION: Emodin can enhance the function of small intestinal peristalsis of mice by mechanisms of promoting secretion of motilin, lowering the content of somatostatin and inhibiting Na\(^+\)–K\(^+\)-ATPase activity of small intestinal mucosa.

© 2005 The WJG Press and Elsevier Inc. All rights reserved.

Key words: Emodin; Small intestinal; Motilin; Somatostatin; Na\(^+\)–K\(^+\)-ATPase

Zhang HQ, Zhou CH, Wu YQ. Effect of emodin on small intestinal peristalsis of mice and relevant mechanism. *World J Gastroenterol* 2005; 11(20): 3147-3150

http://www.wjgnet.com/1007-9327/11/3147.asp

INTRODUCTION

Rhubarb has been used to treat gastrointestinal disorders for hundreds of years. The effective components of rhubarb are anthraquinone derivatives, emodin being the most important. Traditional viewpoints hold that emodin primarily acts on the large intestine and it can enhance the excitability of smooth muscles of the large intestine\(^1,2\). We have obtained more than 95% pure emodin from China Pharmaceutical University In this study, we evaluated whether emodin had any effects on small intestinal peristalsis of mice and explored its relevant mechanisms.

MATERIALS AND METHODS

**Animals and treatment**

Fifty healthy ICR mice (weighing 18-22 g) were randomly divided into five groups: normal control, model, low dose of emodin, medium dose of emodin and high dose of emodin. The animals in emodin treatment groups and model group were put through intragastric gavage (IG) with compound diphenoxylate 0.2 mL/10 g. Those in normal control were treated with NS. Thirty minutes later different medications were given: mice in normal control and model group were given NS, and those in emodin treatment groups were given emodin 0.1, 0.2, and 0.4 g/kg respectively. All of the animals were administered as above for 1 wk and were fasted for 12 h before the last administration.

**Charcoal powder propelling test of small intestine**

Thirty minutes after the last administration, all animals were IG charcoal powder suspended liquid 0.2 mL/10 g. Twenty minutes later the mice were killed by exarticulation. The small intestine from pylorus to the boundary of ileum and cecum was isolated and its length was “total length of small intestine”. The length from pylorus to the foreland of charcoal powder was “charcoal powder propelling length”.

Charcoal powder propelling ratio= [charcoal powder propelling length (cm)/total length of small intestine(cm)] ×100%.

**Determination of motilin and somatostatin**

Fifty mice were administered as above. Thirty minutes after the last administration, the small intestinal tissues were treated according to Sun *et al.*\(^3\). The contents of motilin and somatostatin in small intestine of mice were determined by radioimmunoassay according to the protocols of the kit.

**Determination of Na\(^+\)–K\(^+\)-ATPase activity**

After isolating small intestinal tissues, the mice above were intercepted between 5 cm intestinal segments below the Treitz ligament. The intestines were rinsed repeatedly by...
Potential difference (PD) of isolated small intestine

About 8 cm of jejunum was taken, and cleaned with K-H solution. The wall of intestinal sacs was reverted. We ligated one end, and the other end was tied with tubuliform glass-tube. The reverted intestinal sac full of K-H solution was put in a tube full of 25 mmol/L glucose K-H solution (glucose K-H) or glucose-free K-H (at 37°C). The mixed gas including 50 mL/L CO₂ and 95% O₂ was injected. The electrical potential difference (PD) was measured across the wall of reverted intestinal sacs. The drugs in experiment were dissolved by glucose-free KH or glucose KH, PH was regulated and the osmotic pressure was modulated with mannitol. PD was recorded every 2.5 min and observed 15 min continuously.

Statistical analysis
All data were expressed as mean±SD. Statistical analysis was performed using unpaired t test. Differences were considered significant when P≤0.05.

RESULTS

Effect of emodin on charcoal powder propelling movement of small intestine in mice

The charcoal powder propelling ratio in normal control was 56.4±4.9%, while it greatly decreased to 44.7±10.3% in model group. Comparing with model group, emodin 0.1, 0.2, and 0.4 g/kg induced obvious increase of the charcoal powder propelling ratio of small intestine, and reached 54.6±6.6%, 67.2±18.4%, and 70.6±10.1%, respectively (Table 1).

Table 1 Effect of emodin on charcoal powder propelling movement of small intestine in mice (mean±SD, n = 10)

| Group             | Dose (g/kg) | Charcoal powder propelling ratio (%) |
|-------------------|-------------|-------------------------------------|
| Normal control    | —           | 56.4±4.9                            |
| Model             | —           | 44.7±10.3                           |
| Emodin low dose   | 0.1         | 54.6±6.6                            |
| Emodin medium dose| 0.2         | 67.2±18.4                           |
| Emodin high dose  | 0.4         | 70.6±10.1                           |

*P<0.05 vs model; **P<0.01 vs model; ***P<0.01 vs normal control.

Effect of emodin on the contents of motilin and somatostatin in small intestine of mice

Comparing with normal control, the content of motilin was lower while the content of somatostatin was higher in model group (P<0.01). After treatment with different doses of emodin, the content of motilin increased (P<0.05, P<0.01), while that of somatostatin decreased significantly (P<0.05, P<0.01) (Figure 1).
Effect of glucose-free K-H and different concentration of emodin on PD of isolated small intestine There are no significant differences in comparison between the same group before administration and 15 min after administration. The results indicated that emodin dissolved by glucose-free K-H had no evident inhibitory effect on PD of isolated small intestine (Table 2).

Table 2 Effect of emodin (glucose-free K-H) on transmural potential difference (PD) of mice in vitro (mean±SD, n = 10)

| Group       | Concentration (g/L) | Before administration | 15 min after administration |
|-------------|---------------------|-----------------------|-----------------------------|
| K-H solution| 2.43±0.43           | 2.25±0.39             |
| Emodin      | 0.2                 | 2.15±0.58             |
|             | 0.4                 | 2.42±0.40             |
|             | 0.8                 | 2.55±0.34             |
|             | 1.6                 | 2.38±0.21             |

Effect of emodin on the activity of Na⁺−K⁺-ATPase in small intestinal mucosa of mice The activity of Na⁺−K⁺-ATPase in small intestinal mucosa of mice was higher significantly in model group than that in normal control (P<0.01). In emodin treatment groups the activity of Na⁺−K⁺-ATPase in small intestinal mucosa of mice decreased prominently (P<0.05, P<0.01) (Table 3).

Table 3 Effect of emodin on the activity of Na⁺−K⁺-ATPase in small intestinal mucosa of mice (mean±SD, n = 10)

| Group            | Dose (mg/kg) | Activity of Na⁺−K⁺-ATPase (μmol p1/μg Pro/h) |
|------------------|--------------|---------------------------------------------|
| Normal control   | —            | 15.82±3.29                                  |
| Model            | —            | 20.84±4.29                                  |
| Emodin low dose  | 0.1          | 17.41±2.56                                  |
| Emodin medium dose| 0.2         | 16.57±1.74                                  |
| Emodin high dose | 0.4          | 16.20±2.09                                  |

DISCUSSION
Rhubarb is one of the most frequently used evacuants in clinic. Emodin is one of the primary components of rhubarb. Emodin is thought to be acting mainly on the large intestine [8-11]. In this study, we have attempted to investigate the effect of emodin on small intestinal peristalsis of mice and to explore its relevant mechanisms.

We observed the effect of emodin on charcoal powder propelling movement of small intestine in mice. The result showed that emodin increased the charcoal powder propelling ratio of small intestine, which indicates that emodin can enhance the function of small intestinal peristalsis of mice.

Motilin, which is made up of 22 amino acids, has long been recognized as an important endogenous peptide regulator of gastrointestinal motor function. Motilin is secreted by M cells distributing in the recess of mucosa in duodenum and jejunum [12-14]. In the interphase of digestion, motilin is secreted with periodicity. Motilin can touch off the occurrence of three phases of migrating motor complex (MMC) through acting on the motilin nerve cells in the nervous system of intestinal tract, which arouses the intense constriction of stomach and segmentation contraction of small intestine. The punchy contractive wave of three phases of MMC extended to a distance along the small intestine at the rate of 5-10 cm/min. And when it passes through the gastrointestinal tract, it can clean up the contents of gastrointestinal including the alimentary residue of last diet, cellular fragment falling off and bacteria. So motilin is a street sweeper [15]. Motilin plays an important role in regulation of gastrointestinal movement by touching off the occurrence of three phases of MMC. In our study we found that the content of motilin in small intestine of mice in model group was lower than that in normal control, and after treatment with emodin, the content of motilin was increased at different degree. So emodin can enhance the function of small intestinal peristalsis of mice by promoting secretion of motilin.

Somatostatin distributed in the gastrointestinal tract diffusely. Somatostatin can inhibit gastrointestinal function by two ways: one is by inhibiting the activity of adenyl cyclase through inhibitory G protein; the other is by inhibiting the release of cholinergic neurotransmitter [16-19]. The result of this study showed that the content of somatostatin in small intestine of mice was increased significantly comparing with normal control. Different dosages of emodin could inhibit the secretion of the somatostatin prominently. This may be another mechanism of emodin to promote the movement of small intestine.

Some electrolyte can get across the epithelium of small intestine by active transport or passive transport, which leads to electrical potential difference (PD) between mucous membrane and chorion of small intestine. When it was adjusted to zero, passive transport of electrolyte was inhibited, while active transport continued. Then the change of PD between mucous membrane and chorion of small intestine reflected the change of active transport [20]. Glucose absorption in small intestine mucous membrane was completed by active transport. Active transport needs to consume energy, the main substance supplying energy is Na⁺−K⁺-ATPase [21,22]. So the decrease of active transport of ion indicated that Na⁺−K⁺-ATPase may be inhibited.

In this study we used high glucose K-H solution in order to study the role of glucose accompanied Na⁺-transportation, which leads to PD maintaining a steady high level, contributing to eliminate interference of organic component in drugs to PD. In order to study glucose-non-dependent Na⁺-transportation, we used glucose-free K-H to observe the change of simple Na⁺-transportation PD. We found that when glucose existed, different doses of emodin could lower PD significantly. On the contrary, when glucose was absent, emodin had no evident effect on PD. The above results indicated that emodin had no significant effect on simple Na⁺-transportation PD, it mainly inhibited the active transportation of Na⁺, especially inhibited glucose-dependent Na⁺-transportation.

Sodium pump is Na⁺−K⁺-ATPase in substance. One ATP molecule breaking down, there are three sodium ions moving out of the cell membrane and two potassium ions...
moving into the cell membrane at the same time. By this way a potential energy reservoir is established, which is in favor of the transmembrane transportation. The absorption of glucose and amino acid by intestinal mucosa is just through this secondary active transport[23-25]. When the activity of Na+–K+–ATPase in intestinal mucosa is reduced, the potential energy reservoir is lessened. And the absorption of glucose, amino acid and sodium ions by intestinal tract is decreased too, which results in the enhancement of the osmotic pressure in enteric cavity and the peristalsis of intestinal tract which is fortified and quickened subsequently[22]. Our study indicated that the the activity of Na+–K+–ATPase in intestinal mucosa in emodin treatment groups was decreased prominently comparing with that in model group. The decrease of the activity of Na+–K+–ATPase in intestinal mucosa may be also one of the mechanisms for emodin to accelerate the movement of small intestine.

In conclusion, emodin can enhance the function of small intestinal peristalsis of mice and its mechanisms may be relevant to promoting secretion of motilin, lowering the content of somatostatin and inhibiting Na+–K+–ATPase activity of small intestinal mucosa.

REFERENCES
1 Shen YJ. Pharmacology of traditional Chinese medicine.1th ed. Beijing: People's health publication 2002: 329-330
2 Kai M, Hayashi K, Kaida I, Aki H, Yamamoto M. Permeation-enhancing effect of aloe-emodin anthrone on water-soluble and poorly permeable compounds in rat colonic mucosa. Bio Pharm Bull 2002; 25: 1608-1613
3 Wan JZ. One kind of simple constipation model of mice. Chin Pharmocol Bull 1994; 10: 71-72
4 Chen Q. Methodology of pharmacology of traditional Chinese medicine.1th ed. Beijing: People's health publication 1993: 331-335
5 Sun WF, Xu W, Wang XC, Wei S, Luo XY, Xiao DY. Effect of Shengjiang Decoction on gastrointestinal peristalsis and gastrointestinal hormones of mice. J Anhui TCM College 2002; 21: 45-47
6 Jia B, Gu XL, Zhang SQ. The effect of Chinese traditional medicine additive on glucose absorption of isolated small intestine. Livestock And Poultry Industry 1998; 12: 15-16
7 Cui ZQ, Guo SD, Ye B, Zhang HR. The effect of taurine on transmural potential of mouse small intestine in vitro. Chinese Pharmocol Bulletin 1995; 11: 288-290
8 Iizuka A, Iijima OT, Kondo K, Itakura H, Yoshie F, Miyamoto H, Kudo M, Higuchi M, Takeda H, Matsumiya T. Evaluation of Rhabarb using antioxidative activity as an index of pharmacological usefulness. J Ethnopharmacol 2004; 91: 89-94
9 Ding M, Ma S, Liu D. Simultaneous determination of hydroxyanthraquinones in rhabarb and experimental animal bodies by high-performance liquid chromatography. Anal Sci 2003; 19: 1163-1165
10 Zhao J, Chang JM, Du NS. Studies on the chemical constituents in root of Rheum rhizastachyum. Zhongguo Zhongyao Zazhi 2002; 27: 281-282
11 Shang XY, Yuan ZB. Determination of six effective components in Rheum by cycloelectroded micellar electrokinetic chromatography. Yaoxue Xuebao 2002; 37: 798-801
12 Peeters TL. Central and peripheral mechanisms by which ghrelin regulates gut motility. J Physiol Pharmacol 2003; 54 Suppl 4: 95-103
13 Delinsky DC, Hill KT, White CA, Bartlett MG. Quantitative determination of the polypeptide motilin in rat plasma by externally calibrated liquid chromatography/electrospray ionization mass spectrometry. Rapid Commun Mass Spectrom 2004; 18: 293-298
14 Qi QH, Wang J, Hui JF. Effect of dachengqi granule on human gastrointestinal motility. Zhongguo Zhong Xi Jiehe Zazhi 2004; 24: 21-24
15 Yao T. Physiology. 5th ed. Beijing: People’s Health Publication 2002: 188-190
16 Peeters TL, Muls E, Janssens J, Urbaín JL, Bex M, Van Cutsem E, Depoortere I, De Roo M, Vantrappen G, Bouillon R. Effect of motilin on gastric emptying in patients with diabetic gastroparesis. Gastroenterology 1992; 102: 97-101
17 Leandros E, Antonakis PT, Albanopoulos K, Dervenis C, Konstadoulakis MM. Somatostatin versus octreotide in the treatment of patients with gastrointestinal and pancreatic fistulas, Can J Gastroenterol 2004; 18: 303-306
18 de Franchis R. Somatostatin, somatostatin analogues and other vasoactive drugs in the treatment of bleeding oesophageal varices. Dig Liver Dis 2004; 36 Suppl 1: S93-100
19 Konturek PC, Konturek SJ. The history of gastrointestinal hormones and the Polish contribution to elucidation of their biology and relation to nervous system. J Physiol Pharmacol 2003; 54 Suppl 3: 83-98
20 Kitaoka S, Hayashi H, Yokogoshi H, Suzuki Y. Transmural potential changes associated with the in vitro absorption of theamine in the guinea pig intestine. Biosci Biotechnol Biochem 1996; 60: 1768-1771
21 Cereijido M, Shoshani L, Contreras RG. The polarized distribution of Na+, K+-ATPase and active transport across epithelia. J Membr Biol 2001; 184: 299-304
22 Zhou XM, Chen QH. Biochemical study of Chinese rhabarb. XXI. Inhibitory effect of anthraquinone derivatives on Na+-K+-ATPase of the rabbit renal medulla and their diuretic action. Yaoxue Xuebao 1988; 23: 17-20
23 Laughery M, Todd M, Kaplan JH. Oligomerization of the Na+, K+-ATPase in cell membranes. J Biol Chem 2004; 279: 36339-36348
24 Ukkola O, Joannis DR, Tremblay A, Bouchard C. Na+K+-ATPase alpha 2-gene and skeletal muscle characteristics in response to long-term overfeeding. J Appl Physiol (1985) 2003; 94: 1870-1874
25 Horvath G, Agil A, Joo G, Dobos I, Benedek G, Baeyens JM. Evaluation of endomorphin-1 on the activity of Na(+)K(+)ATPase using in vitro and in vivo studies. Eur J Pharmacol 2003; 458: 293-297

Science Editor Guo SY  Language Editor Elsevier HK