The protective effects of total phenols in *Magnolia officinalis* rehd. et wils on gastrointestinal tract dysmotility is mainly based on its influence on interstitial cells of cajal

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Abstract: *Magnolia officinalis* Rehd. et Wils is a kind of herb which is widely used for gastrointestinal tract mobility disorder in Asian countries. In this study, we investigated whether the total phenols of *Magnolia officinalis* Rehd. et Wils (TPM) treatment improves gastrointestinal tract dysmobility induced by intraperitoneal injection of atropine (5 mg/kg) in rats. Rats were randomly grouped into three units: TPM-pretreated/atropine-treated group, atropine-treated group and control group. TPM were administrated for 7 days. Gastric residual rate and intestinal transit were measured 20 min after atropine injected, and gastrointestinal hormones (including: gastrin (GAS), motilin (MTL), somatostatin (SS) and p substance (PS) levels in serum were also measured by ELISA kits. The number and distribution of interstitial cells of Cajal (ICCs) in stomach were detected by immunohistochemistry analysis, while c-kit and stem cell factor (SCF) expressions in stomach were also measured by western blotting. We found that TPM pretreatment significantly improved atropine-induced gastric residual rate increase, while had no significantly effects on intestinal transit; it also significantly normalized GAS, MTL and PS serum levels. Atropine-induced ICCs numbers decreased in both sinuses ventriculi and body of stomach, which is improved by TPM pretreatment. Western blotting results showed the expressions of c-kit and SCF were down-regulated after atropine injection, which can be reversed with TPM pretreatment. These results above indicates that TPM treatment can significantly protected atropine-induced gastric dysmobility, which may owed to its regulation on c-kit/SCF signing pathway.

Keywords: *Magnolia officinalis* Rehd. et Wils, gastrointestinal tract, mobility disorder, interstitial cells of Cajal (ICCs)

Introduction

*Magnolia officinalis* Rehd. et Wils is a herb with long tradition and a large variety of uses on treatment of dyspepsia, abdominal distention and constipation among Asian countries. Modern natural product chemical and pharmacological researches showed that the extracts (alcohol or aqueous) obtained from this plant’s dried bark had the activities on antioxidant and antiviral [1]. Our previous study indicated that the total phenols of *Magnolia officinalis* Rehd. et Wils (TPM), which is mainly including magnolol and honokiol, showed the significant protective effects on dyspepsia and gastrointestinal (GI) tract motility disorders. However, the cellular and molecular mechanisms underlying the effects of TPM on GI tract motility have not been explored to date.

Gastric motility plays a very important role in digestion, which is precisely regulated by neurotransmitters and hormones. The neuroregulation of gastric motility includes parasympathetic and sympathetic nerve regulation, and the enteric nervous system (ENS) which is distributed almost every area from stomach to anus. ENS’s vital functions have drawn researchers’ attention in recent years. Interstitial cells of Cajal (ICCs) is a kind of specialized cells in GI tract, which function as a pacemaker and generate spontaneous electrical activity (slow
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Dysfunctions of ICCs have been associated with several gastrointestinal mobility disorders, including irritable bowel syndrome [3], diabetic gastroenteropathy [4], and intestinal pseudoobstruction [5]. ICCs express c-kit, a tyrosine kinase receptor, which plays a critical role in the control of ICCs growth, differentiation, and apoptosis, and the maintenance of rhythmic activities in the gastrointestinal tract [6]. The natural ligand for c-kit has been identified as stem cell factor (SCF), which activates the c-kit receptor, inducing multiple downstream signaling pathways [7]. Disrupting SCF/c-kit function impairs the development of functional ICCs, and results in a defect in the enteric nervous system (ENS), which finally leads to the dysfunction of the gastrointestinal tract. In the other hand, gastrointestinal hormones also play an important role in regulation of gastrointestinal tract functions. For example, gastrin (GAS) promotes gastric acid secretion and peristalsis [8], motilin (MTL) regulates gastric emptying and the migrating motor complex of small intestine [9]. Thus, gastrointestinal hormones levels disorder can also cause dysfunction of the gastrointestinal tract.

In this study, we investigated the mechanism by which TPM protects against atropine-induced gastrointestinal tract dysmotility in rats. We found that TPM effectively prevents atropine-induced changes in ICCs distribution and gastrointestinal hormone levels, reverses atropine-induced inhibition of the SCF/c-kit signaling pathway in stomach, and improves atropine-induced gastrointestinal dysmotility. Our results indicated that TPM prevents atropine-induced gastrointestinal dysmotility by regulating ICCs' function.

Materials and methods

Plant materials

The bark of Magnolia officinalis Rehd. et Wils was purchased from Hehuachi herbal medicine market (Chengdu, China), and identified by Prof. Xianming Lu, a veteran botanist from Chengdu University of Traditional Chinese Medicine. The extract of total phenols of Magnolia officinalis Rehd. et Wils (TPM) was yielded by 90% ethanol heating reflux of the barks for 60 min, then vacuum dried. The high performance liquid chromatography (HPLC) (Agilent 1200) of the extracts was performed with a monitoring wave, timing the phase contractions of the tunica muscularis in gastrointestinal tract [2]. Dysfunctions of ICCs have been associated with several gastrointestinal mobility disorders, including irritable bowel syndrome [3], diabetic gastroenteropathy [4], and intestinal pseudoobstruction [5]. ICCs express c-kit, a tyrosine kinase receptor, which plays a critical role in the control of ICCs growth, differentiation, and apoptosis, and the maintenance of rhythmic activities in the gastrointestinal tract [6]. The natural ligand for c-kit has been identified as stem cell factor (SCF), which activates the c-kit receptor, inducing multiple downstream signaling pathways [7]. Disrupting SCF/c-kit function impairs the development of functional ICCs, and results in a defect in the ENS, which finally lead to the dysfunction of the gastrointestinal tract. In the other hand, gastrointestinal hormones also play an important role in regulation of gastrointestinal tract functions. For example, gastrin (GAS) promotes gastric acid secretion and peristalsis [8], motilin (MTL) regulates gastric emptying and the migrating motor complex of small intestine [9]. Thus, gastrointestinal hormones levels disorder can also cause dysfunction of the gastrointestinal tract.

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Figure 1. The HPLC profile of total phenols of Magnolia officinalis Rehd. et Wils (TPM). A. HPLC chromatogram for the standard substances of magnolol and honokiol; B. HPLC chromatogram for total phenols of Magnolia officinalis Rehd. et Wils (TPM).
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wavelength of 203 nm, Inertsil R ODS-2 C18 (250 mm×4.6 mm, 5 μm), a column temperature of 35 °C, a mobile phase of acetonitrile/water (11:9, v/v), and a flow rate of 1.0 mL/min. The chromatographic profiles of the extract are shown in Figure 1. Magnolol and honokiol were regarded as markers (Sigma, USA).

Animals and experimental protocols

The study was conducted in accordance to the Laboratory Animal Care Committee at Chengdu University of Traditional Chinese Medicine. 24 adult male Sprague-Dawley rats (10-week old and weighing 250-300 g, supplied by Sichuan Chengdu Dashuo Biotechnology Ltd) were housed at room temperature (25°C) with 60% humidity and a 12-h light/dark cycle. Rats were fed standard rat chow and water ad libitum.

The rats were assigned to three groups (8 rats for each group): TPM-pretreated/atropine-treated group, atropine-treated group, and control group. Rats in the TPM-pretreated/atropine-treated group received a gavage of TPM (14.00×10^{-2} g/kg body weight). Rats in the atropine-treated or control group were received a gavage of normal saline (placebo control), respectively. Drug pretreatment lasts for 7 days. At end of experiment, rats were deprived of food (free to take water) for 18 h, and gastrointestinal dysmotility was induced by an intraperitoneal injection of atropine sulphate [10] (5 mg/kg body weight, Sigma Aldrich, USA) 60min after the last time TPM administration; Meanwhile, animals in the control group were injected with the same volume.

**Figure 2.** The protective effects of TPM on atropine-induced gastric and intestinal dysmobility in rats. Gastric residual rate (A) and intestinal transit (B) were measured in control (Ctrl), atropine-treated (Atropine) and TPM-pretreated/atropine-treated (TPM+atropine) rats via the method described above. Data are shown as mean ± SD (n = 8). *: P < 0.05, **: P < 0.01.

**Measurement of gastrointestinal (GI) tract transit**

Method for GI tract transit measurement was improved on the basis of method described previously [11]. 20 min after atropine or saline injection, a 12% carbon powder suspension (0.2 mL/10 g body weight) was administered to the rats intragastrically. The animals were then euthanized and dissected after another 20 min. Blood samples were collected via aorta abdominalis and centrifuged for obtaining serum. Then, stomach was removed, washed with cold PBS, dried with filter paper immediately after cardia and pylorus were ligated, and then weighted with/without removing gastric contents. The gastric emptying was evaluated by gastric residual rate, which was calculated as: weight (with gastric contents removed)/weight (without gastric contents removed) ×100%. Meanwhile, the small intestine was also removed. The distance of carbon powder transmission as measured from the gastric pylorus to the distal small intestine. Intestinal transit was calculated as: (distance of carbon powder transmission/total length of small intestine) ×100%.

**Measurement of gastrointestinal hormones in serum**

Gastrin (GAS), motilin (MTL), somatostatin (SS) and p substance (PS) levels in serum were measured by using enzyme-linked immunosorbent assay (ELISA) kits (Abcam, USA).

**Immunohistochemistry analysis**

The sinuses ventriculi and 1/3 body of stomach (0.5 cm×0.5 cm) was removed immediately after measurement of gastric residual rate. Affiliated tissues (such as adipose) were removed, and the tissues were fixed with 4% (W/V) paraformaldehyde (pH 7.4) for 8 h. After fixation, the tissues were dehydrated by gradient flushing with low concentration to high concentration of ethanol under 4°C for 30 min. Then, the tissue was sectioned with paraffin embedding after xylene transparent. The immu-
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n staining was performed as previously described [12]. Briefly, tissues were incubated in goat serum (99%) at room temperature for 20 min to reduce nonspecific antibody binding, and then incubated with primary antibodies against c-kit (rabbit anti-rat, 1:200 dilution, Abcam, USA) at 25°C for 60 min, followed by incubation at 4°C for 24 h. After primary antibody was removed by PBS washing, immunoreactivity was detected by incubation in HRP-coupled secondary antibody (goat anti-rabbit IgG H&L, 1:1000, Abcam, USA) at room temperature for 60 min. Nuclei were counterstained by using hematoxylin (Sigma, USA). Section was examined by using BA200 digital microscopic camera system (Motic, China), and optical density was analyzed by using Image-Pro Plus 6.0 system (Media Cybernetics, US).

Western blotting

Western blot was performed to check the SCF/c-kit signaling pathway. 1/3 upper parts of stomach tissues (gastric antrum and pace making area) were homogenized in RIPA lysis buffer, then sonicated and incubated on ice for 15 min. Protein was extracted by a total protein extraction kit (Chemgene, China). The proteins were separated by SDS-PAGE gel and electrophoretically transferred onto nitrocellulose membrane. After being blocked, the membranes were then incubated with primary antibodies against c-kit (dilution 1:1000, Abcam, USA) or SCF (dilution 1:1000, Abcam, USA) at 4°C, overnight. β-actin was used as an internal control. Bands were visualized using HRP-coupled secondary antibody (dilution 1:2000, Cell Signaling Technology, USA). Detection was performed by densitometry using the enhanced chemiluminescence detection system (Bio-RAD, USA).

Statistical analysis

Results are expresses as mean ± standard deviation (SD). Data differences between groups were tested for statistical significance by using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) method for multiple comparison tests. P values < 0.05 were considered statistically significant.

Results

**TPM improves atropine-induced dysmotility of gastrointestinal tract**

To examine the protective effect of TPM on GI tract dysmotility, we investigated the gastric emptying and intestinal transit of rats in control, atropine-treated, and TPM-pretreated/atropine-treated groups. Data were shown that, gastric residual rate was significantly higher in atropine-treated group than in control group (P < 0.01), while intestinal transit was significantly higher in atropine-treated group than in control group (P < 0.01), which indicated that atropine can reduced gastric emptying and small intestinal motility as expected. TPM treatment significantly reduced gastric residual rate (P < 0.01), while had no significant influences on intestinal transit in atropine-treated rats compared with
the placebo-treated group ($P < 0.05$), which indicated that TPM can improve gastric emptying reducing induced by atropine. (Data are presented in Figure 2).

TPM normalizes the gastrointestinal hormones levels which were disordered by atropine treatment

As the important role of gastrointestinal hormones in regulation of GI tract, we measured the serum levels of gastrointestinal hormones, which including, gastrin (GAS), motilin (MTL), somatostatin (SS) and p substance (PS) in each group's rats. Data were shown that, GAS, MTL, and PS levels were significantly down-regulated in atropine-treated group compared with control group ($P < 0.01$), while there was no significant changes in SS levels. TPM treatment significantly reversed atropine-induced GAS, MTL, and PS levels down-regulation ($P < 0.01$), while had no significant effects on SS level. Results indicated that atropine can induce gastrointes-
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TPM treatment up-regulates c-kit and SCF expressions in atropine-treated rats

As described in the Introduction section, impaired SCF/c-kit signaling pathway can lead to ICCs network defection, and finally cause GI tract dysmotility. We investigated whether TPM treatment improved the SCF/c-kit signaling pathway which impaired by atropine in stomach. Western blotting results showed that c-kit and SCF expressions were both significantly down-regulated in atropine-treated group compared with control group ($P < 0.05$), which suggesting that atropine impairs the SCF/c-kit signaling pathway. TPM pretreatment significantly turn over atropine-induced decrease of c-kit and SCF expressions ($P < 0.05$), promoting the SCF/c-kit signaling pathway in stomach. (Data are presented in Figure 5).

Discussion

In present study, we have shown that atropine induced inhibition of intestinal transit and gastric emptying, as a result of a decrease in the number of ICCs with impairing the c-kit/SCF signaling pathway in stomach. In another hand, atropine also induced the decrease of GAS, MTL, and PS levels in serum. TPM treatment effectively prevents atropine-induced gastric dysmotility, restores ICCs numbers, reverses the atropine-induced blockade of the c-kit/SCF signaling pathway, and normalizes GAS, MTL, and PS levels. These results suggest that the ICCs network and the SCF/c-kit signaling pathway play a very important role in the beneficial effects of TPM on atropine-induced GI tract dysmotility.
It is well documented that atropine suppresses GI transit in several animal models [10, 13-15]. Atropine-induced delay of gastric emptying which is caused by its effects on the proximal stomach [16]. Based on previous knowledge, we found that atropine-induced GI tract dysmotility by inhibiting gastric emptying and intestinal transit. TPM pretreatment improves atropine-induced gastric residual rate, suggesting that TPM has the effects on promoting in gastric mobility in rats. Our results provide an experimental evidence for the rationalization of tradition using of Magnolia officinalis Rehd. et Wils for curing GI tract dysfunction.

GAS, distributed almost in whole GI tract, can promote gastric emptying by relaxing pylorus and shrinking sinuses ventriculi [17]. MTL, a kind of brain-gut peptide, play a role of gastric contraction start, and strongly stimulate mechanical movement and electrophysiology activity in GI tract [18]. PS, also a kind of brain-gut peptide, is the strongest hormone on stimulate smooth muscle excitation in GI tract [19]. While, SS, another kind of brain-gut peptide, can inhibit GI tract mobility and secretion of gastrointestinal hormones [20]. In our research, we found that GAS, MTL and PS serum levels down-regulated by atropine treatment, which can be effectively reversed by TPM. However, there’s no change on SS level. These findings demonstrate that TPM can normalize the gastrointestinal hormones’ serum levels during GI tract dysfunction. However, the molecular mechanism of TPM’s effects on the gastrointestinal hormones secretion still remains unclear.

As ICCs play an important role in the regulation of GI tract smooth muscle contraction, damage to ICCs networks is likely responsible for atropine-induced GI tract dysmotility. Damage to ICC has been reported to be associated with abnormal GI tract electrical rhythmicity and contraction [21, 22]. Since almost all ICCs express c-kit, distribution of ICCs can be checked via immunohistochemical dye of c-kit [23]. We find that atropine impairs the ICC network by decreasing the total number of ICCs, which can impair generation and transmission of slow wave in stomach, and eventually lead to GI tract dysmotility. TPM pretreatment increases the number of ICCs, suggesting that the cellular and molecular targets of TPM are associated with ICCs networks. Since c-kit/SCF signaling pathway plays a critical role in the control of ICCs growth and differentiation, and maintenance of rhythmic activities in the GI tract [6]. Disrupting c-kit/SCF function results in a defect in the ICC network, GI mobility, and enteric motor neurotransmission. In this study, we find that atropine impairs the c-kit/SCF signaling transduction with reduction of c-kit and SCF expressions. TPM pretreatment effectively improves the expressions of c-kit and SCF in atropine-treated rats and prevents atropine-induced ICCs damage. These data suggest that TPM prevents atropine-induced gastric dysmotility by normalizing ICCs' function via regulation of the SCF/c-kit signaling pathway.

In summary, we studied the effects of TPM pretreatment on ICCs morphology, c-kit/SCF signaling pathway of stomach in a rat model of atropine-induced GI tract dysmotility. Our results suggest that the beneficial effects of TPM pretreatment on atropine-induced GI tract dysmotility is mediated by c-kit/SCF pathway activation in ICCs and normalized the secretion of gastrointestinal hormones.

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Disclosure of conflict of interest

None.

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References

[1] Marongiu B, Porcedda S, Piras A, Rosa A, Deiana M and Dessi MA. Antioxidant activity of supercritical extract of Melissa officinalis sub-
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