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Research Paper

Bidirectional effects of methanol extract of Wei-Chang-An pill on gastrointestinal transit and the spasmolytic activity on isolated rat jejunum

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ABSTRACT

Ethnopharmacological relevance: Wei-Chang-An pill (WCA pill), a traditional Chinese medicine, has been used for treating various gastrointestinal diseases for several decades. Despite the popular medicinal use of WCA pill, less data was available to its activity and mechanism in gastrointestinal disorders. To examine the effects of the methanol extract of WCA pill (ME) on gastrointestinal tract so as to assess some of the possible mechanisms involved in the clinical treatment.
Materials and methods: ME was studied on gastrointestinal transit *in vivo* including gastric emptying and small intestinal motility in normal and neostigmine-induced mice, as well as on the isolated tissue preparations of rat jejunum *in vitro*.
Results: *In vivo*, the gastric emptying decreased and intestinal transit increased after administration of ME in normal mice. However, administration of ME accelerated the intestinal transit ranging from 0.01 to 0.8 mg/mL and reduced it at the concentration of 1.6 and 3.2 mg/mL, while the gastric emptying was inhibited throughout the concentrations in neostigmine-induced mice. *in vitro*, ME caused inhibitory effect on the spontaneous contraction of rat-isolated jejunum in dose-dependent manner ranging from 0.01 to 6 mg/mL and also relaxed the acetylcholine chloride (Ach, 10^{-6} M)-induced and K^+ (60 mM)-induced contractions. ME shifted the Ca^{2+} concentration–response curves to right, similar to that caused by verapamil (0.025 mM).
Conclusions: These results indicated that ME might play a bidirectional role in gastrointestinal transit modulation and the effects on isolated tissue are probably mediated through calcium influx and muscarinic receptors, which provides pharmacological basis for the clinical use of WCA pill in gastrointestinal tract disorders.

1. Introduction

Traditional Chinese medicine (TCM) formulas have been widely used in China since ancient times to treat different diseases. They are commonly prescribed as complex prescriptions to achieve sufficient treatment under complex conditions and generally exert their curative effects through possible mechanisms known as multiple components to multiple pathophysiological targets rather than single component as synthetic drugs, which are vulnerable to quality of its raw herbs depending on the cultivation areas and climatic conditions and so on (Peter, 2002; Chen et al., 2010). Thus, chemical composition analysis is quite imperative to be introduced as a more effective and reliable method so as to synthetically evaluate the quality of TCM.

Functional gastrointestinal diseases such as gastroesophageal reflux, functional dyspepsia and irritable bowel syndrome, as well as other symptoms (e.g. abdominal pain, nausea, vomiting, diarrhea, constipation, *etc.*) are highly common and varied, the physiological mechanisms of which are critically involved in gastrointestinal motility disorders (Kimura and Sumiyoshi, 2012). Gut function is controlled by both the enteric (intestinal) nervous system and the central nervous system. The autacoids (serotonin, acetylcholine and prostaglandins) play an important role in the control of intestinal movements and secretions (Afroz et al., 2006). Gastrointestinal tract is vital to food digestion and nutrient absorption as well as normal salt and water homeostasis, for example, diarrhea is a disorder characterized by discharge of semisolid or watery fecal matter from the bowel three or more times in a day (Mbagwu and Adeyemi, 2008; Suleiman et al., 2008), which has a substantial effect on

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quality of life and health-care costs. Given the complexity of the symptoms, most experienced clinicians expect to use a holistic remedy for these diseases, so it is important to open up new ways in which a broader range of symptoms can be remission or improvement.

WCA pill, a traditional Chinese pharmaceutical preparation, consists of 10 Chinese medicinal herbs, which possess the properties of eliminating damp pathogen, regulating vital energy to alleviate pain, and removing food in the stomach and intestine due to indigestion (Committee of National Pharmacopoeia, 2010). It has been used to treat various gastrointestinal diseases with the dosage of 4 mg/kg by humans, such as diarrhea, abdominal pain, enteritis, dysentery and vomiting for several decades (Ling et al., 2005). The previous studies that had been carried out in our laboratory including investigation of antinociceptive, antidiarrhoeal and gastrointestinal motility activities of ME (Hu et al., 2009; Liu et al., 2013; Wang et al., 2012) and the medicinal materials in the formula of pharmacokinetic were studied (Zhang et al., 2011, 2012). In addition, the chemical composition of ME was analyzed. The previous studies had been carried out in our laboratory including analyzing and identification of the chemical composition of the ME by HPLC-MS and HPLC-DAD-ESI-MS/MS. Twelve active components were analyzed, including costunolide and dehydrodehydrocostus lactone from the principal herb *Radix aucklandiae*; naringin, hesperidin and neohesperidin from *Fructus Aurantii*; magnolol and honokiol from the ministerial herbs *Cortex Magnoliae officinalis*; aloe-emodin, rhein, emodin, chrysophanol and physcion from adjunctive and messenger herb *Radix et Rhizoma Rhei*. There were totally 68 compounds detected in ME and 41 compounds were identified. (Liu et al., 2013; Zhang et al., 2013), which provides a data basis for assessment of the quality of WCA pill. However, the explicit mechanisms are unclear, which should be for further research.

Furthermore, in order to elucidate the pharmacological action and its underlying mechanisms of ME on the smooth muscle tissues and gastrointestinal motility, we examined the effects of ME on non-treated, Ach-treated, KCl-treated and $CaCl_2$-treated of rat isolated jejunum and gastrointestinal motility including gastric emptying and small intestinal motility on non-treated and neostigmine-treated mice.

2. Materials and methods

2.1. Plant materials and preparation of ME

WCA pill (Lot no. 20070507) was prepared with the following ten herbs: 25% the dried root of *Aucklandia lappa* Decne., 10% the resinous wood of *Aquilaria sinensis* (Lour.) Gilg., 15% the immature fruit of *Citrus aurantium* L., 15% the dried bark of *Magnolia officinalis* Rehder & E.H. Wilson, 10% the duramen of *Santalum album* L., 7% the dried rhizoma of *Rheum officinale* Baill., 5% the mature fruit of *Croton tiglium* L., 0.5% *Moschus*, 5% the dried rhizoma of *Ligusticum chuanxiong* S.H. Qiu, Y.Q. Zeng, K.Y. Pan, Y.C. Tang & J.M. Xu, and 7.5% the fruit of *Ziziphus jujuba* Mill. in Tianjin Lerentang Pharmaceutical Factory. These herbs were purchased from Medicinal Material Company (Hebei Province, China) and identified by Professor Wen-Yuan Gao, and all the voucher specimens (No. WCA pill-111001) were deposited at the School of Pharmaceutical Science and Technology at Tianjin University.

WCA pill (100 g) was powdered and extracted thrice with 1 L of methanol, 2 h for each time. The filtrate was collected and then the solvent was removed under reduced pressure in a rotary evaporator (Buchi B-480) and the ME was obtained, with a yield of 40.07% (w/w).

2.2. Chemicals

Neostigmine methylsulfate injection (1 mg/2 mL) was donated by Affiliated Hospital of Logistics College of Chinese People's Armed Police Forces. Verapamil and acetylcholine (Ach) were purchased from National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Calcium chloride, glucose, magnesium chloride, potassium chloride, sodium bicarbonate, sodium dihydrogen phosphate, sodium chloride, sodium hydroxide and trichloroacetic acid were produced by Tianjin Fengchuan Chemical Reagent Science And Technology Co., Ltd. (Tianjin, China). HPLC grade acetonitrile and methanol were purchased from Fisher (USA). Water was purified by a Milli-Q water purification system (Millipore, USA). All chemicals used were of analytical grade and solubilized in distilled H_2O/saline. Vehicles used had no effect in control experiments.

Standards including costunolide, dehydrodehydrocostus lactone, narirutin, naringin, hesperidin, neohesperidin, magnolol, honokiol, aloe-emodin, rhein, emodin, chrysophanol and physcion were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All the 13 reference compounds have over 98% purity (see their chemical structures in Fig. 1).

2.3. Animals

Adult male and female KM mice weighing about 18–22 g were purchased from Tianjin Experimental Animal Center (License no. SCXK (Jin) 2009-0002). Male Wistar rats, weighing about 280–330 g used in the experiments were purchased from Experimental Animal Center, Chinese Academy of Medical Sciences, Peking, SCXK -2007-004. All animals were housed at the Animal Breeding Laboratory of Affiliated College of Chinese People's Armed Police Forces (Tianjin, China). The Animal Ethics Committees of the Faculty of Medicine approved all experimental protocols in accordance with Principles of Laboratory Animal Care and Use in Research (Ministry of Health, Beijing, China).

2.4. Analysis of the chemical compounds of ME by HPLC

All analyses were performed on an Agilent 1100 liquid chromatography system (Agilent Technologies, USA), equipped with a quaternary pump, an online degasser, and a column temperature controller, coupled with an DAD (Alltech Associates, USA) as the detector. The analytical column was a Kromasil C18 (250 mm × 4.6 mm i.d., 5 μm particle size) and the column temperature was kept at 35 °C. The mobile phase was a linear gradient prepared from acetonitrile (A), methanol (B), and water (containing 1% acetic acid) (C). The composition of the gradient was A–B–C, 4.3:0.7:95 at 0 min, 20:2.5:77.5 at 15 min, 22:3.5:74.5 at 40 min, 50:8:42 at 70 min, 69:11:20 at 100 min and then the system was returned to initial conditions. The flow rate was 0.8 mL/min, and the injection volume was 20 μL. The reference (narirutin 62.5 μg/mL, naringin 160.80 μg/mL, hesperidin 11.76 μg/mL, neohesperidin 161.68 μg/mL, aloe-emodin 3.29 μg/mL, rhein 9.08 μg/mL, emodin 8.36 μg/mL, honokiol 140.16 μg/mL, costunolide 28.74 μg/mL, dehydrodehydrocostus lactone 27.16 μg/mL, magnolol 173.28 μg/mL, chrysophanol 24.78 μg/mL, physcion 2.92 μg/mL) were prepared with methanol. 0.4 g of ME was accurately weighed and prepared with 25 mL of methanol in a conical flask. The solution was filtered through a 0.45 μm syringe filter before analysis.

2.5. Gastric emptying and small intestinal motility in normal mice

The method was described by previous studies to examine the gastric emptying (Wang et al., 2012). Mice were fasted for 24 h