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Relieving visceral hyperalgesia effect of Kangtai capsule and its potential mechanisms via modulating the 5-HT and NO level *in vivo*

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ABSTRACT

Kangtai capsule (KT) is one type of traditional Chinese medicine preparation derived from the proved recipe, which was frequently applied as an effective clinical treatment of IBS. However, there still lack the reasonable and all-round analytical approach and the scientific studies on its underlying mechanisms. Therefore, our study aimed to develop the novel method for evaluating its quality as well as to interpret the potential mechanisms. In our study, high performance liquid chromatography (HPLC) fingerprint was applied to provide a chemical profile of KT. The neonatal maternal separation (NMS) on Sprague-Dawley pups was employed to evaluate the therapeutic effect of KT by virtue of various parameters including visceral hyperalgesia, serum nitric oxide (NO) level, and tissue 5-hydroxytryptamine (5-HT) level. Consequently, a chromatographic condition, which was carried at 30 °C with a flow rate of 0.5 ml/min on AQUA 3μ C18 column with mobile phase of acetonitrile and water–phosphoric acid (100:0.1, v/v), was established to give a common fingerprint chromatography under 254 nm with a similarity index of 0.963 within ten batches of KT samples. On the NMS model, KT markedly elevated the pain threshold of NMS rats. Furthermore, KT at three doses significantly decreased 5-HT content from distal colon of visceral hyperalgesia rats induced by NMS, while the significant decrease of 5-HT content in serum was only observed in the group with KT at high dose. However, compared with that in NMS rats without KT, there was no apparent difference of 5-HT level from brain issue in the rats with various doses. Besides, KT could substantially elevate the concentration of NO in the serum. The results showed our study developed the simple, rapid, accurate, reproducible qualitative and quantitative analysis by HPLC fingerprint for the quality control for KT. Data from the pharmacological investigation suggested that the curative effect of KT to the visceral hypersensitivity may be concerned with the level of 5-HT and NO *in vivo*, promising its potential in irritable bowel syndrome treatment.

Introduction

The irritable bowel syndrome (IBS) is prevalent functional bowel disorder described firstly by Powell (1818), associated with a combination of chronic abdominal pain, discomfort diarrhea and/or constipation (Rey and Talley 2009; Rivkin 2003) and affects around 3–15% of the world's population according to the definitions set by International Congress of Gastroenterology in Rome (Cremonini and Talley 2005). Then IBS occupies around 3% of all general practice consultation and soars to as high as 40% of gastrointestinal referrals (Thompson et al. 2000), which causes the extensive social expenditure. The organic cause and the relevance of the criteria in clinical practice, however, are still unclear (Rey and Talley 2009; Bommelaer et al. 2004; Gilkin 2005). Traditionally, IBS has been considered a disorder of brain–gut–axis, and the potential underlying mechanisms of IBS associated with functional gastrointestinal disorders are multi-factorial and some hypotheses have been proposed to illustrate them, including abnormal motility, visceral hypersensitivity, inflammation and infection, neurotransmitter imbalance, and psychosocial factors (e.g., anxiety, depression, somatisation) (Rivkin 2003; Gilkin 2005). Among these, visceral hypersensitivity is defined as low threshold of stimuli perception from GI and currently regarded as a critical pathophysiological mechanism, bringing about various symptoms of IBS (Greenwood-van 2007). It is reported that visceral hypersensitivity

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has been described in majority of patients with IBS, especially the rectal hypersensitivity, which could be detected in 95% of patients with IBS (Azpiroz et al. 2007; Mertz et al. 1995). Hence, rectal hypersensitivity could be thought a biological marker of the syndrome. Besides, both Serotonin (5-hydroxytryptamine, 5-HT) and Nitric oxide (NO) have been considered to contribute to onset of visceral hypersensitivity depending on the level of them *in vivo* (Rivkin 2003; Sikander et al. 2009; Kuiken et al. 2006; Tjong et al. 2011a,b).

Kangtai capsule (KT) is a Chinese herbal medicine compound preparation, based on a famous proved recipe proposed by Prof. Fusheng Zhou, including Bai Shao (Radix Paeoniae Alba, RPA), Mu Xiang (Radix Aucklandiae, RA), Fang Feng (Radix Saposhnikoviae, RS), Yan Hu Suo (Rhizoma Corydalis, RC), Bai Zhu (Rhizoma Atractylodis Macrocephalae, RAM), and Shou Wu Teng (Caulis Polygoni Multiflori, CPM). KT recipe had clinical efficacy of reinforcing spleen and stomach, tonifying spleen to resolve dampness, promoting qi circulation to relieve flatulence and was frequently applied as effective clinical treatment of IBS by Prof. Fusheng Zhou (Huang et al. 2007; Zhou et al. 2004). All these raw herbs were frequently used for composing prescriptions, which were applied for the treatment of GI and psychological disorder in the clinic, for example Tongxieyaofang including RAM, RPA and RS (Sun et al. 2004; Bian et al. 2006), Weichangan Wan including RAM and RA (Hu and Tang 2009; Hu et al. 2010), Shuganjieyu Decoction including RAM, RPA, RS, CPM, RC (Chen et al. 2010).

It is well known that the traditional Chinese medicines (TCM) generally exert their curative effects through the multiple bioactive constituents rather than single specie as synthetic drugs, which are vulnerable to quality of its raw herbs, depending on the cultivation areas and climatic conditions and so on. Thus, chromatographic fingerprint is quite imperative to be introduced as a more effective and reliable method so as to synthetically evaluate the quality of TCM. The major bioactive constituents of KT preparations are glucosides, lactones and alkaloids. Therefore, in our study, eight relevant compounds: paeoniflorin in RPA, dehydrocostus lactone in RA, prim-O-glucosylcimifugin and 5-O-methylvisammioside in RS, tetrahydropalmatine in RC, atractylenolide II and atractylenolide III in RAM, 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside in CPM were selected for analysis through the HPLC technology. Meanwhile, HPLC was also adopted as an analytical tool of fingerprint. Since then, we have successfully established the HPLC fingerprint profile for the assessment of the quality of KT by HPLC equipped with PDA.

Furthermore, in order to elucidate possible mechanisms of action of KT, visceral hypersensitivity was chosen as a biological marker to evaluate the rat model processed by neonatal maternal separation (NMS), a well-established early-life stress model that mimic human IBS symptoms, and then examined the content of 5-HT and NO. After that, the data analysis was adopted to confirm whether KT exerts its efficacy of IBS symptoms by modulating the 5-HT and NO level.

Materials and methods

Animals

For the neonatal maternal separation model, all maternal and neonatal Sprague-Dawley rats were provided by Guangdong Medical Experimental Animal Center. All the animals were maintained under environmentally controlled conditions of 22 °C and 12 h light/12 h dark cycle. The animals received humane care in accordance with the guide for the care and use of laboratory animals, published by the US National Institution of Health (NIH Publication, revised in 1985). All animal experimental procedures were approved by our institutional animal research ethics committee in reference to the European Community guidelines for the use of experimental animals.

Table 1
Recipe of Kangtai Capsule (KT) formulation.

Components	Ratio
1. Bai Shao (*Paeonia lactiflora* Pall., root)	3
2. Mu Xiang (*Aucklandia lappa* Decne., root)	2
3. Fang Feng (*Saposhnikovia divaricate* (Turcz.) Schidchk., root)	2
4. Yan Hu Suo (*Corydalis yanhusuo* W. T. Wang, tuber)	3
5. Bai Zhu (*Atractylodes macrocephala* Koidz., rhizome)	3
6. Shou Wu Teng (*Polygonum Multiflorum* Thunb., lianoid stem)	6

Chemicals and reagents

Paeoniflorin, dehydrocostus lactone, prim-O-glucosylcimifugin, 5-O-methylvisammioside, tetrahydropalmatine, atractylenolide II, atractylenolide III and 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside were purchased from National Institutes for Food and Drug Control (Beijing, China). Atractylenolide II and Atractylenolide III were kindly provided by Shanghai R&D Center for Standardization of Traditional Chinese Medicines (Shanghai, China). 5-HT was provided by SIGMA Inc. (Lot29F0438). Cysteine (CYS) and Orthophthalaldehyde (OPT) were purchased from Mbchem Technology Group Co., Limited. The Acetonitrile (LC grade) was bought from Honeywell Inc. (U.S.A). Ultra-pure distilled water, prepared from special laboratory ultra-pure water machine, was used. Phosphoric acid (Shantou, China), methanol (Shantou, China), ethanol (Shantou, China) and other reagents were all of analytical grade.

KT consists of six medicinal plants as shown in Table 1. All raw herbs were purchased from Guangxi Yifang Chinese Herbal Medicine Department and authenticated by Professor Chen Jiannan, pharmacognosist of School of Chinese Materia Medica, Guangzhou University of Chinese Medicine. All of these accord with the demand in 2010 edition of China Pharmacopoeia.

KT was provided by our department and prepared as follow. In brief, RAM, RPA, RS, CPM and RC were mixed and refluxed three times (for 1 h each) with 4 l, 3.4 l and 3.4 l of 70% ethanol, successively. After cooling to room temperature, the combined extract was filtered and condensed by rotor evaporation under reduced pressure. And then, concentrated extract was dried to obtain powder by spray-drying before adding the fine power of RA. Finally, the mixture was mixed with an amount of medical starch and the loaded into capsules. And the extract of single herb was prepared according the procedure above without being filled into capsules.

Preparation of sample solution

The contents of KT were carefully taken out from KT. The contents or dried power of single herb were weighted accurately and extracted by ultrasonic extraction with 25 ml of 70% methanol for 30 min. Then, the extract solution was filtered through a 0.45 μm filter membrane before analysis.

Apparatus and chromatographic conditions

The HPLC analysis was performed on DIONEX SUMMIT HPLC system equipped with a PDA-100 detector, a P680 pump, an ASI-100 automatic sampler, and a STH585 thermostatic column compartment. The chromatographic separation was carried at 30 °C with a flow rate of 0.5 ml/min on an AQUA 3μ C18 125A (250 mm × 4.60 mm, 3μ, Phenomenex Inc., USA). The linear gradient elution, composed of solvent A (acetonitrile) and B (water–phosphoric acid, 100:0.1, v/v), was used for separation. The elution program was optimized and conducted as follow: a