Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2017, **15**(8): 0631–0640
Chinese Journal of Natural Medicines

Metabolomics coupled with similarity analysis advances the elucidation of the cold/hot properties of traditional Chinese medicines

JIA Yan[1, 2], ZHANG Zheng-Zheng[1, 2], WEI Yu-Hai[3], Qin Xue-Mei[1], LI Zhen-Yu[1*]

[1] *Modern Research Center for Traditional Chinese Medicine of Shanxi University, Taiyuan 030006, China;*
[2] *College of Chemistry and Chemical Engineering of Shanxi University, Taiyuan 030006, China;*
[3] *Qinghai Entry-Exit Inspection and Quarantine Bureau, Xining 810000, China*

Available online 20 Aug., 2017

[ABSTRACT] It recently becomes an important and urgent mission for modern scientific research to identify and explain the theory of traditional Chinese medicine (TCM), which has been utilized in China for more than four millennia. Since few works have been contributed to understanding the TCM theory, the mechanism of actions of drugs with cold/hot properties remains unclear. In the present study, six kinds of typical herbs with cold or hot properties were orally administered into mice, and serum and liver samples were analyzed using an untargeted nuclear magnetic resonance (NMR) based metabolomics approach coupled with similarity analysis. This approach was performed to identify and quantify changes in metabolic pathways to elucidate drug actions on the treated mice. Our results showed that those drugs with same property exerted similar effects on the metabolic alterations in mouse serum and liver samples, while drugs with different property showed different effects. The effects of herbal medicines with cold/hot properties were exerted by regulating the pathways linked to glycometabolism, lipid metabolism, amino acids metabolism and other metabolic pathways. The results elucidated the differences and similarities of drugs with cold/hot properties, providing useful information on the explanation of medicinal properties of these TCMs.

[KEY WORDS] Cold/hot property; Metabolomics; Traditional Chinese medicine; NMR; Similarity analysis

[CLC Number] R917 **[Document code]** A **[Article ID]** 2095-6975(2017)08-0631-10

Introduction

The theory of medicinal properties is always pivotal to elucidate the characteristics of traditional Chinese medicine (TCM), which is in a range of medical practices utilized in China for more than four millennia. It is recorded in Chinese medical classics "Shennong's Herbal" that there are four properties of TCMs-- cold, hot, warm and cool, which can be summed up as cold and hot. The medicinal properties also correspond to philosophical frameworks such as the theory of Yin-Yang and Five Elements, the human body meridian systems, and the Zang Fu theory [1].

[Received on] 19-Aug.-2016
[Research funding] This study was supported by the Science and Technology Innovation Team of Shanxi Province (No. 2013131015) and Program for the Outstanding Innovative Teams of Higher Learning Institutions of Shanxi (OIT).
[*Corresponding author] Tel/Fax: 86-351-7018379, E-mail: lizhenyu@sxu.edu.cn
These authors have no conflict of interest to declare.

The cold or hot property, regarded as the own specific characteristics of each drug, is believed to mainly originate from the reactions of the body to a specific herbal medicine. Generally speaking, herbs with the cold property are deemed to clear away heat, eliminate toxic substances, nourish yin and remedy hot syndromes; in contrast, herbs with the hot property usually dispel cold, warm up the interior, support yang, and thus treat cold syndromes [2]. Many modern scientific researches have been undertaken to elucidate the cold/hot properties of TCMs. One finding has been documented that chemical materials underlie the changes that are correlated with cold/hot properties of herbal drugs, such as the predictive system of the cold/hot property of herbal medicines [3]. Liang *et al*. have characterized Chinese medicine with cold and hot properties based on molecular network and chemical fragments [1]. Liu *et al.* have pointed out that the investigation of the distinct abilities of Chinese herbs to regulate neural cell functions appear to be associated with their cold or hot properties [2]. Study of the impact of cold- and hot-natured herbs on organs and tissues have also been performed by testing the central nervous system,

prostaglandin, and endocrine system [4-5].

Metabolomics, a technique to study metabolic responses of complex organisms to drug or other stimuli, provides complementary information for exploring whole-organism function by identification of potential biomarkers, which is consistent with holistic view of TCM theory [6]. Nuclear magnetic resonance (NMR) spectroscopy, with the nondestructive nature of the analysis, the robust and reproducible measurements and the minimal preparation requirement, is one of most commonly used means of metabolomics [7]. The analysis of metabolomics based on NMR has been successfully applied in diverse research fields, encompassing disease diagnosis and evaluation [8], pharmacology and toxicity [9], nutritional intervention [10], and environment biology [11].

In the present work, an untargeted NMR-based metabolomics approach was used to map the metabolic profiles in serum and liver of mice treated by six kinds of herbal drugs with cold/hot properties. Differential endogenous metabolites were identified and the changed metabolic pathways were used to explain cold/hot properties of drugs. By combination of similarity analysis, it was expected to provide a basic view on the theory of TCM.

Materials and Methods

Plant Materials

Three kinds of cold medicine (CMs), Phellodendri Chinensis Cortex, Coptidis Rhizoma, and Scutellariae Radix, and three kinds of hot medicine (HMs), Aconiti Lateralis Radix Praeparata, Zingiberris Rhizoma, and Cinnamomi Cortex, were purchased from Beijing Tong Ren Tang Group Co., Ltd. (Beijing, China). All the herbal drugs were authenticated by Prof. QIN Xue-Mei and the voucher specimens were deposited in the herbarium of the Modern Research Center for Traditional Chinese Medicine of Shanxi University, Taiyuan, China. For TCMs with typical cold or hot properties, the hot and cold properties were defined as previously described [1].

Analytical grade $K_2HPO_4{\cdot}3H_2O$ and $NaH_2PO_4{\cdot}2H_2O$ were obtained from Guangfu Fine Chemical Research Institute (Tianjin, China) and Zhiyuan Chemical Reagent Co., Ltd. (Tianjin, China), respectively. HPLC-grade acetonitrile was obtained from Fisher Scientific Worldwide Co., Ltd. (NJ, USA). Sodium 3-trimethylsilyl [2,2,3,3-d_4] propionate (TSP) was procured from Cambridge Isotope Laboratories Inc (Andover, MA, USA). Deuterium Oxide (D_2O, 99.9%) was purchased from Norell (Landisville, PA, USA). Phosphate buffer was prepared with K_2HPO_4 and NaH_2PO_4 (0.1 mol·L^{-1}, pH 7.4), containing 10% D_2O and 0.01% TSP.

Sample preparation of herbal drugs

All the herbal drugs were smashed and soaked in distilled water at room temperature and extracted by boiled hot water(1 : 10, *W*/*V*) firstly for 3 h, and then (1 : 8, *W*/*V*) 2 h, respectively. The afforded decoction were combined, concentrated in a rotary evaporator, freeze-dried to powder under vacuum, and then stored at −20 ºC until use.

Chemical analysis of the decoctions of herbal drugs

The freeze-dried decoction powder (about 30 mg) was redissolved in KH_2PO_4 buffer in D_2O (adjusted to pH 6.0 by 1 mol·L^{-1} NaOD) containing 0.05% TSP. The sample was then centrifuged for 10 min at 13 000 r·min^{-1}. The supernatants (600 μL) of all the samples were transferred into 5-mm NMR tubes for NMR analysis. Six replicates were prepared for each drug. Then the ^{1}H NMR spectrum was acquired using the noesygppr1d pulse sequence, which consisted of 64 scans requiring a 2.654s acquisition time with the following parameters: spectral width = 12 345.7 Hz, spectral size = 65 536 points, and a relaxation delay = 1.0 s.

Experimental animals and treatment protocol

Fifty six male Institute of Cancer Research (ICR) mice weighing 18 to 22 g were commercially obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and housed in an environmentally controlled breeding room (temperature: 20 ± 2 ºC, humidity: 60% ± 5%) a week before the start of the experiments. The mice were starved for 12 h before scarifying. Eyeball blood samples were separated using refrigerated centrifugation at 3 500 r·min^{-1} for 15 min to afford the serum samples. The serum samples were snap-frozen in liquid nitrogen and stored at −80°C for further analysis. All animal experiments [license number SCKX-2012-0001] were carried out in accordance with the National Guidelines for Experimental Animal Welfare (MOST, China, 2006) at the Center for Animal Experiments, Shanxi University, which has full accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care International. Maximum effort was exerted to minimize animal suffering and the number of animals necessary for the attainment of reliable data.

The mice were weighed and randomly divided into seven groups (n = 8/group): normal control group (NC); three CM groups, including Phellodendri Chinensis Cortex group (C1, 2.5 g·kg^{-1}), Coptidis Rhizoma group (C2, 2.5 g·kg^{-1}), Scutellariae Radix group (C3, 2.5 g·kg^{-1}); and three HM groups, including Aconitilateralis Radix group (H1, 3.75 g·kg^{-1}), Praeparata Zingiberris Rhizoma group (H2, 2.5 g·kg^{-1}), and Cinnamomi Cortex group (H3, 1.25 g·kg^{-1}). The administration doses used in the present study (equivalent to the raw drug) were calculated according to recommended therapeutic doses [12]. The drugs were orally administered (8:00 am) into mice and same volume of distilled water was given to mice in NC group using a feeding atraumatic needle once per day for 7 days. Their body weights were recorded every three days. On the day 7, the animals were sacrificed. Orbital blood and livers were collected for further study. The blood was centrifuged at 4 ºC and 13 000 r·min^{-1} for 15 min, and the supernatants were stored at –80 ºC prior to use. The liver was quickly dissected and washed with ice-cold saline solution, and then frozen in liquid nitrogen before use.

NMR measurements of the serum and liver samples

Serum samples were thawed and then prepared as