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Novel chinmedomics strategy for discovering effective constituents from ShenQiWan acting on ShenYangXu syndrome

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[ABSTRACT] Elucidation of the efficacy of traditional Chinese medicine (TCM) is of importance for scientists of modern medicine to understand the value of TCM clinical experience, and it is necessary to have a biological language to scientifically describe the efficacy of TCM. With this background，*Chinmedomics* has been proposed by our team, which includes integrating serum pharmacochemistry and metabolomics technology, defining theory and research methods for expressing the efficacy of TCMs based on the biomarkers discovery of TCM syndrome and elucidating the efficacy of TCM formulae, discovering effective constituents, and finally elucidating the scientific value of TCM. In the present study, the innovative chinmedomics strategy was conducted to evaluate the therapeutic effects of ShenQiWan (SQW) acting on ShenYangXu (kidney-yang deficiency syndrome, KYDS). We analyzed the urine metabolic trajectory between the model and control groups, and identified the biomarkers by the multivariate analysis. We found that SQW caused significant restoration of abnormal metabolism of KYDs. Using the method of metabolomics, 17 potential urine biomarkers were analyzed through 4 repeated tests in our serial studies on SQW and KYDS. Under the premise of therapeutic efficacy, a total of 56 peaks were tentatively characterized *in vivo* by the use of serum pharmacochemistry. Correlation analysis between marker metabolites and *in vivo* constituents of SQW showed that 28 compositions had a close relationship with urine biomarkers of therapeutic effects, whichmight play a key role in the therapeutic effect of SQW. These compounds were imported into an online database to predict their targets. Twenty-three important potential targets were identified, which were related to the metabolism of steroid hormone, tryptophan utilization, and thyroid hormone. In conclusion, chinmedomics is a useful strategy for discovery of potentially effective constituents from complex TCM formulae.

[KEY WORDS] Chinmedomics; ShenQiWan; ShenYangXu syndrome; Metabolomics; Serum pharmacochemistry

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Introduction

The efficacy of traditional Chinese medicine (TCM) is directly related to patient's syndrome and formulae of TCM; the vagueness of syndrome and complexity of formulae, however, make the diagnosis and evaluation of efficacy of TCM difficult, greatly limiting the discovery of effective constituents of TCM. Therefore, the chemical biological characters of syndrome and efficacy of formulae will be the key scientific problems for discovery of effective material basis for TCM. Our team has long been engaging in the developing and evaluating innovative research methods for TCMs, aiming at addressing key scientific issues, such as chemical biological essence of syndrome and efficacy of TCM formulae. Several approaches are involved in this process: using metabolomics technology to identify biomarkers of syndromeevaluate the efficacy of TCM formulae; discovering the active form of constituents *in vivo* originated

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from TCM formula under the effective conditions using serum pharmacochemistry of TCM; and further analyzing the correlation between the exogenous constituents of formulae *in vivo* and endogenous biomarkers of syndrome to identify the constituents that are highly associated with clinical efficacy of formulae as the effective constituents [1-2].

Metabolomics, as a final response of '-omics', is a powerful technology which provides a unique perspective in studying of the biochemical processes of organism. It is a promising tool for investigating pathogenesis and diseases specific biomarkers of living systems by measuring alterations at the metabolic level, and exploring the underlying biological mechanisms [3]. TCM includes a complex set of chemical components with multiple targets. The untargeted metabolomics provides information on global metabolites which help evaluate the disease progression and drug efficacy. A lot of attention has been attracted by herbal medicine because of its unique clinical effectiveness. TCM has been applied for thousands of years in China with its unique advantage in diseases prevention and treatment [4]. Therefore, it is necessary to develop an effective approach to identifying the bioactive components of TCM. Generally, the potential bioactive components of a prescription could be the members of chemical constituents that can be absorbed into blood and their metabolites [5-6].

ShenQiWan (SQW), a well-known TCM formula for nourishing ShenYangXu (kidney-yang deficiency syndrome, KYDs), has been used for thousands of years in China. SQW is consisted of *Radix Rehmanniae Preparata, Fructus Macrocarpii, Rhizoma Dioscoreae Oppositae, Poria, Rhizoma Alismatis, Cortex Moutan Radicis, Achyranthes bidentate,* and *Plantago asiatica.* However, the global components of this TCM that are responsible for its therapeutic effects remain unknown. Given the chemical complexity of SQW, UPLC and sub-2-μm particle size columns were used in the present study to increase chromatographic resolution and to shorten analysis time. In addition, the Metabolynx^{TM} software, UNIFI workstation, and MarkerLynx software were also used in the data analysis with the mode of principal component analysis (PCA) and orthogonal partial least-squared discriminant (OPLS-DA).

KYDS is a common syndrome in clinical practice which mainly involves harms and dysfunction of hypothalamic-pituitary-adrenocortical (HPA) and hypothalamic-pituitary-thyroid (HPT) axis [7-10]. The earliest animal model of Kidney-Yang Deficiency in TCM syndrome system was developed by KUANG An-Kun [11]. After that, several animal models have been successively established to mimic KYDS The classical one is based on subcutaneous injection of corticosterone and developed by SHEN Zi-Yin [12], which induces adrenocortical deficiency after withdrawing administration of corticosterone. This animal model mimics the pathological state of immune suppression and renal damage, reflecting functional disorders of the hypothalamic–pituitary–target gland (adrenal, thyroid and gonad) axis. After the animal model is established，ddrenal gland and thymus show an obvious atrophy and decrease in organ index in the HPA axis. The HPT axis show a decrease in the serum level of T3 and T4 and thyroid shows significant atrophy. The model is consistent with the KYDS iagnosis standard in the clinical practice of TCM, which has been widely applied to evaluate therapeutic effect and mechanism of Kidney-Yang tonifying herbs [13]. Thus, it is necessary to pay more attention to the research of TCM formulae. Chinmedomics theory, in which metabolomics is used as a tool to reveal the biological essence of diseases and serum pharmacochemistry is used as another tool to identify the bioactive components, is considered to be an integrated analytical technique which can be used to explain the synergistic properties and the metabolites of prescription and the mechanism of action for the treatment of the related diseases [13-14]. Thus, the chinmedomics approach will be helpful for us to bridge active ingredients and biomarkers of diseases [15-16]. In the present study, we utilized the technology of metabolomics to explore the metabolic changes in KYDS through 4 repeated tests experiments and reveal the therapeutic effect of SQW. Based on the generated information, potential pharmacodynamic targets and the constituents absorbed into blood after administration were identified. Finally, we also explored the real-time operating system for metabolomics and serum pharmacochemistry to probe potentially effective constituents from SQW acting on KYDS.

Materials and Methods

Materials and reagents

Acetonitrile (HPLC grade) was purchased from Merck (Darmstadt, Germany); methanol (HPLC grade) was obtained by Fisher Scientific Corporation (Loughborough, UK). Milli-Q Ultra-pure water system (Millipore Corporation, MA, USA) was used to ultrapure water and mobile phase. ShenQiWan was purchased from Beijing Tong Ren Tang Pharmaceutical Co., Ltd. (Beijing, China). Corticosterone was obtained from Sigma-Aldrich (St. Louis, MO, USA). Corticotropin releasing hormone (CRH), corticosterone (CORT), and adrenocorticotropic hormone (ACTH) kits were provided by Beijing Huaying biological Ltd. (Beijing, China) ; testosterone (T) kit was provided by Beijing North Biotechnology Institute (Beijing, China); cAMP kit and cGMP kits were provided by R&D (Minnesota, USA).

Animals and treatment

Male Wistar rats (weighing 220 ± 20 g) were supplied by GLP Center of Heilongjiang University of Chinese Medicine (Harbin, China). They were housed under standard conditions with controlled humidity (50% ± 5%), temperature (25 ± 1 °C) and light-dark cycle (12 h/12 h) and had free access to food pellets and tap water. All the animals were allowed to acclimatize in metabolism cages for 1 week prior to treatment. All the rats were randomly divided into 12 groups, including three control groups [CON (n = 5), CON-14-day (n = 5) and CON-21-day (n = 5)], three model groups [MOD (n = 5),