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Metabolomics and its application to the evaluation of the efficacy and toxicity of traditional Chinese herb medicines

Jian Shi[a,b], Bei Cao[a,b], Xin-Wen Wang[a], Ji-Ye Aa[a,c,*], Jin-Ao Duan[d], Xuan-Xuan Zhu[d], Guang-Ji Wang[a], Chang-Xiao Liu[e]

[a] State Key Laboratory of Natural Medicines, Key Laboratory of Drug Metabolism and Pharmacokinetics, Jiangsu Key laboratory of Drug Design and Optimization, China Pharmaceutical University, Nanjing, China
[b] Pharmacy Department, Drum Tower Hospital Affiliated to Medical School of Nanjing University, Nanjing, China
[c] Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai, China
[d] Key Lab of Chinese Medicine, Nanjing University of Chinese Medicine, Nanjing, China
[e] Research Center of New Drug Evaluation, The National Laboratory of Pharmacodynamics and Pharmacokinetics, Tianjin Institute of Pharmaceutical Research, Tianjin, China

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ABSTRACT

Traditional Chinese herb medicines (TCHMs) have been used in the treatment of a variety of diseases for thousands of years in Asian countries. The active components of TCHMs usually exert combined synergistic therapeutic effects on multiple targets, but with less potential therapeutic effect based on routine indices than Western drugs. These complex effects make the assessment of the efficacy of TCHMs and the clarification of their underlying mechanisms very challenging, and therefore hinder their wider application and acceptance. Metabolomics is a crucial part of systems biology. It allows the quantitative measurement of large numbers of the low-molecular endogenous metabolites involved in metabolic pathways, and thus reflects the fundamental metabolism status of the body. Recently, dozens of metabolomic studies have been devoted to prove the efficacy/safety, explore the underlying mechanisms, and identify the potential biomarkers to access the action targets of TCHMs, with fruitful results. This article presents an overview of these studies, focusing on the progress made in exploring the pharmacology and toxicology of various herbal medicines.

1. Introduction

Traditional Chinese herb medicines (TCHMs) have been used for the treatment of a variety of diseases for thousands of years in Asian countries. The major theories of TCHMs include yin–yang, the five phases, Zang Fu organ theory, six-channel pattern identification, four-aspect pattern identification, etc. Among them, the principle of Yin and Yang is one of the most fundamental concept in Traditional Chinese Medicine (TCM). An imbalance of yin and yang in the body, i.e., hyperactivity or hypoactivity of yin and yang, could lead to disease. TCM aims to rectify these imbalances in body using various strategies, emphasizing the holistic and personalized concepts. In general, the practitioner diagnoses patients using various methods, such as observation, smell, interrogation, and palpation, to define a unique characteristic of each individual patient. Based on these information, the TCM practitioner formulates a personalized therapeutic strategy, including the combinations of multicomponent herbal medicines, acupuncture, exercise, diet, and lifestyle factors [1].

In contrast, Western science (including Western medicine) accustomed to understanding the nature of complex things by reducing them to simpler or more fundamental elements, and has therefore been called "reductionism" [2]. Reductionism has been the principal paradigm of science for at least the past 200 years.

Abbreviations: COMET, Consortium for Metabonomic Toxicology; GC–MS, gas chromatography–mass spectrometry; HPLC/DAD/ESI–MS, high performance liquid chromatography/diode-array detection/electrospray ionization–mass spectrometry; HPLC-MS, high performance liquid chromatography mass spectrometry; NMR, nuclear magnetic resonance; PCA, principal component analysis; PLS-DA, partial least squares discriminate analysis; SD, Sprague-Dawley; TCA, tricarboxylic acid; TCHMs, Traditional Chinese herb medicines; TCM, traditional Chinese medicine; TSG, tanshinone IIA (T), salvianolic acid B (S) and ginsenoside Rb1 (G); UPLC–Q-TOF-MS, ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry.

* Corresponding author at: Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing 210009, China. Tel.: +86 25 83271081; fax: +86 25 83271060.
E-mail addresses: jiyea@cpu.edu.cn, jiye_2005@aliyun.com (J.-Y. Aa).

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It pervades the medical and pharmaceutical sciences and deeply affects the way we diagnose, treat, and prevent diseases. In fact, reductionism has achieved dramatic successes in modern medicine. However, it also has major drawbacks, such as its oversimplification of complex diseases, universal side effects, and astonishing R&D expenditure [2].

Currently, there is a new focus on traditional medicine, particularly TCHMs, which interact with multiple targets with fewer adverse effects and lower toxicity, such as Dan-shen extracts in the management of myocardial ischemia [3] and the Chinese medicinal formulation Realgar-Indigo naturalis as an effective treatment for promyelocytic leukemia [4]. Because two or more chemicals in these herbal medicines interact with multiple targets simultaneously, they are considered to be rational and efficient forms of therapy designed to control complex diseases [5,6]. One of the fundamental advantages of multicomponent therapeutic agents is the production of "synergy", whereby the combined effects of the components are greater than the sum of their individual effects. This makes multicomponent therapeutics a systematic approach, rather than a reductionist approach, which produces an additive effect. It has been observed that the combinations of agents can effectively reduce the adverse effects and ameliorate the adaptive resistance to a drug in a synergistic manner, thereby increasing the likelihood of conquering chronic complex diseases such as cancer [7].

However, on the other hand, the multiple components and their effects on multiple targets make the assessment of TCHMs efficacy and the investigation of the underlying mechanisms very challenging. The key issue that the value of TCM has not been fully recognized worldwide is mainly due to the lack of scientific approaches.

In recent decades, rapid progress in genomics, proteomics, and metabolomics (or metabonomics) has greatly facilitated the study of systems biology, which is an exciting development [8]. Generally, systems biology accommodates the holistic and composite characteristics of a problem and evaluates the problem using computational and mathematical tools, rather than reducing a complex thing to its component parts [2]. The holistic properties of systems biology correspond well to those of TCM, which is also characterized by holistic concepts, such as the use of the multiple components of herbal medicines, and making diagnoses and evaluating drug efficacy based on the output of the whole body, including the information from the patient's pulse, smell, face, and tongue.

2. Metabolomics in TCHMs research: opportunities and challenges

Metabolomics is defined as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification" by Nicholson et al. [9]. It allows the quantitative measurement of large numbers of low-molecular-weight endogenous metabolites, including lipids, amino acids, peptides, nucleic acids, organic acids, vitamins, thiols and carbohydrates, which have an important role in biological systems and represent attractive candidates to understand phenotypes. Therefore, it is a crucial component of systems biology. Instead of assessing a few compounds in a targeted analysis, metabolomics is a non-targeted method that analyzes as many low-molecular-weight compounds as possible, with sufficiently high sensitivity [10].

Metabolomics focuses on the analysis of global metabolites and their functions in the biological system, thus any factor affecting this system, can be reflected by the metabolome. The integral and systematic study of metabolomics is in agreement with TCM theory in nature; consequently, the metabolomics may be the best approach to fit the holistic concept of TCM. Therefore, the introduction of the concept of metabolomics offers great and novel opportunities to reinvestigate TCM [11]. In recent years, metabolomics research in various fields of TCHMs has increased dramatically as shown in Fig. 1, including efficacy/safety studies, mechanistic studies, TCHM products fingerprinting studies, active/toxic components discovery studies, and so on.

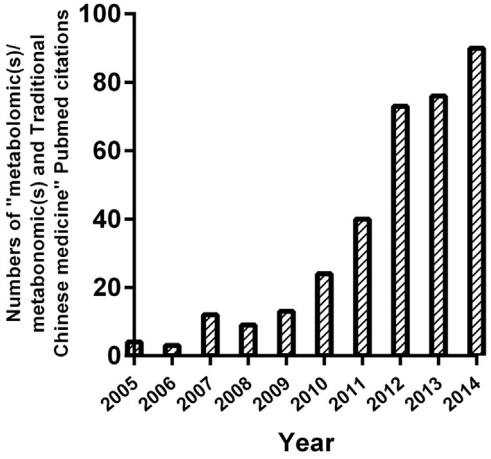

Fig. 1. Numbers of publications involved in metabolomics and Traditional Chinese medicine in recent years.

Despite its potential and advantages, there are still great challenges for the metabolomics, especially for studies on TCHMs [12,13]. One of these challenges is the high sensitivity of the metabolome to various genetic and environmental stimuli, which might make data analysis and interpretation very difficult. Generally, the variation in clinical studies is greater than animal and cell studies because it is harder to control the genetics, nutritional status, disease, environmental factors. So it is extremely important to have an adequate sample size to get confident conclusions. It is also helpful to minimize confounding variables by utilizing certain strategies in the design of a study such as restriction, matching and randomization.

Another challenge maybe the choice of analytical platform. The main analytical methods have been discussed in several excellent reviews of metabolomics [12,14,15]. In brief, nuclear magnetic resonance (NMR) and mass spectrometry (MS)-based techniques are the most widely used platforms in metabolomics research [9]. The key advantages of NMR are its high reproduciblility and minimal sample preparation requirement [12,16]. And the analysis process of NMR is non-destructive to samples, making it possible to facilitate other related studies especially when the samples are scarce and precious. Furthermore, NMR could provide structural information which is quite useful in the identification of unknown metabolites. However, the major limitations of NMR include lower sensitivity (usually 1–10 μmol/L) and resolution compared to MS-based techniques [17]. MS, usually coupled with separation techniques, particularly liquid chromatography (LC) and gas chromatography (GC), have much higher resolution and sensitivity than NMR, although it has lower reproducibility. Therefore, MS-based techniques have the ability to detect a huge number of metabolites with a broad range of concentrations in a biological sample. Generally, GC/MS is often simpler, faster and give higher resolution than LC/MS, but it could not deal with the metabolites which cannot be readily derivatized and volatilized. For the LC/MS, the many different separation modes, such as normal phase liquid chromatography, reversed phase liquid chromatography and hydrophilic interaction chromatography, make it a more flexible method than GC/MS. Normally, the choice depends on the purpose of the study and the nature of the samples, as each method has its own advantages and disadvantages. In fact, due to the large