Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2018, **16**(4): 0302–0312
Chinese Journal of Natural Medicines

Screening and analysis of key active constituents in Guanxin-shutong capsule using mass spectrum and integrative network pharmacology

LIU Feng[1, 2, 3], DU Xia[4], LIU Pei-Rong[5], SUN Yu-Hong[2], ZHANG Yan-Min[1*]

[1] *School of Pharmacy, Health Science Center, Xi'an Jiaotong University, Xi'an 710061, China;*
[2] *Shaanxi Institute of International Trade & Commence, Xianyang 712046, China;*
[3] *Shaanxi Buchang Pharmaceutical Co. Ltd, Xi'an 710075, China;*
[4] *Shannxi Academy of Traditionnal Chinese Medicine, Xi'an 710003, China;*
[5] *School of Life Sciences, Northwest University, Xi'an 710069, China*

Available online 20 Apr., 2018

[ABSTRACT] Guanxinshutong capsule (GXSTC) is an effective and safe traditional Chinese medicine used in the treatment of cardiovascular diseases (CVDs) for many years. However, the targets of this herbal formula and the underlying molecular mechanisms of action involved in the treatment of CVDs are still unclear. In the present study, we used a systems pharmacology approach to identify the active ingredients of GXSTC and their corresponding targets in the calcium signaling pathway with respect to the treatment of CVDs. This method integrated chromatographic techniques, prediction of absorption, distribution, metabolism, and excretion, analysis using Kyoto Encyclopedia of Genes and Genomes, network construction, and pharmacological experiments. 12 active compounds and 33 targets were found to have a role in the treatment of CVDs, and four main active ingredients, including protocatechuic acid, cryptotanshinone, eugenol, and borneol were selected to verify the effect of (GXSTC) on calcium signaling system in cardiomyocyte injury induced by hypoxia and reoxygenation. The results from the present study revealed the active components and targets of GXSTC in the treatment of CVDs, providing a new perspective to enhance the understanding of the role of the calcium signaling pathway in the therapeutic effect of GXSTC.

[KEY WORDS] Mass spectrum; Systems pharmacology; Guanxinshutong capsule; Cardiovascular diseases; Calcium signaling pathway

[CLC Number] R917 **[Document code]** A **[Article ID]** 2095-6975(2018)04-0302-11

Introduction

Cardiovascular diseases (CVDs) are the largest cause of mortality and morbidity in the world, accounting for about 20 million deaths a year worldwide [1]. Recently, the Guanxinshutong capsule (GXSTC) has attracted public attention because of its effectiveness in the prevention and treatment of CVDs as well as its safety profile [2].

[Received on] 16-Jul.-2017
[Research funding] The work was supported by Ministry of National Science and Technology (No. 2011ZX09401-308-6), National Natural Science Foundation of China (Nos. 81773772 and 81704088) and Shaanxi Department of Science and Technology (No. 2016KTTSSF01-04-01).
[*Corresponding author] Tel: 86-29-82656264, Fax: 86-29-82655451, E-mail: zhang2008@mail.xjtu.edu.cn
These authors have no conflict of interest to declare.

GXSTC is a combination of the traditional herbs and Mongolian medicines, and composed of *Choerospondiatis fructus*, *Salviae miltiorrhizae*, *Caryophylliflos*, *Borneolum* and *Concretio silicea bambusae*. Pharmacodynamic studies have explored the mechanism of action for GXSTC in the treatment of CVDs. Liang *et al*. have investigated the protective effects of GXSTC against myocardial ischemia/reperfusion (MI/R) injury, and examined its role in controlling important factors that are involved in aggravating I/R injury [3, 4]. Previous reports have shown that the calcium signaling pathway plays a crucial role in the induction of cell death during the treatment of CVDs [5-7]. However, the targets and underlying molecular mechanisms of action for GXSTC in the treatment of CVDs are still unclear. It is necessary to carry out a systematic investigation and identify the mechanisms involved in the treatment of CVDs by GXSTC.

A traditional Chinese medicine (TCM) formula usually contains multiple components and has many targets involved

in various pathways, which makes the process of delineating the molecular mechanism of action of the formula extremely difficult. Wang and coworkers have proposed systems pharmacology as a powerful new tool to overcome these challenges [8]. Systems pharmacology provides a platform for determining the mechanisms of a TCM formula at various levels, from molecular and cellular levels to tissue and organism levels, by integrating pharmacokinetic data with targets, pathways, and network analyses. This method has been successfully developed and applied to identify the rules of drug combinations in TCM, understand the mechanisms of action at molecular/system levels based on the TCM formula, predict potential new drugs and targets, and explore new drug combinations and so on [9-13].

In the present work, a systems pharmacology method was utilized to investigate the active ingredients of GXSTC, their corresponding targets and their roles in modulating the calcium signaling pathway. The method included LC-MS, GC-MS, systems pharmacology and classical pharmacological studies. The workflow is shown in Fig. 1. This work would not only significantly improve our understanding of the active compounds of GXSTC and their corresponding targets, but also reveal the role of the calcium signaling pathway in the treatment of CVDs by GXSTC.

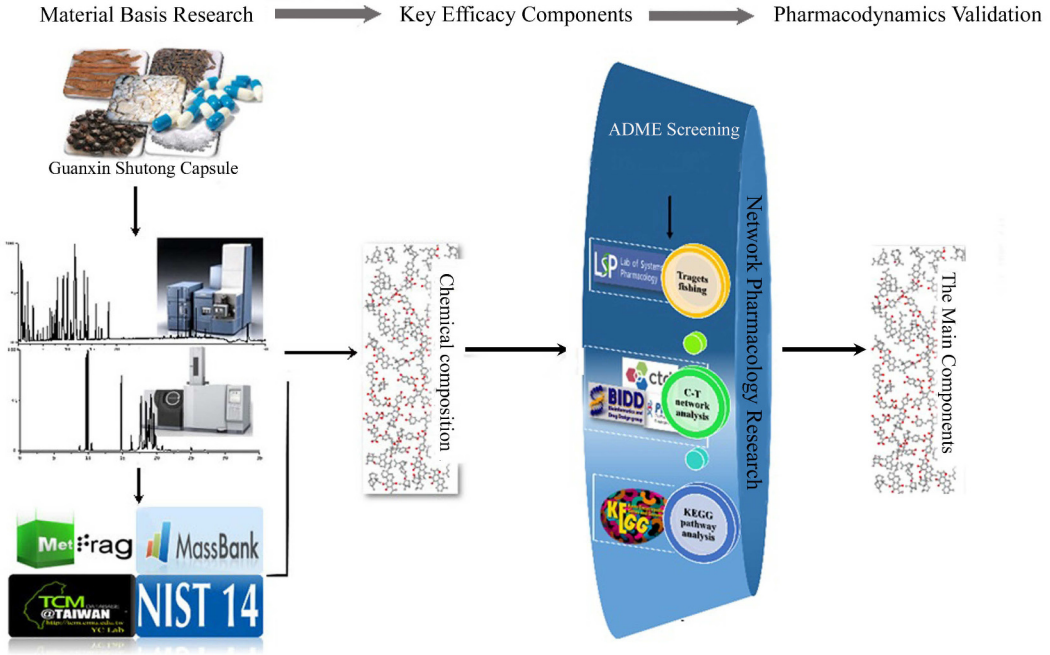

Fig. 1 The workflow of screening and analysis of key active constituents in Guanxinshutong capsule

Materials and Methods

Chemicals and materials

GXSTC (batch no. 150816) was provided by Buchang Pharmaceuticals (Xi'an, China). LC-MS-grade acetonitrile was obtained from Merck (Darmstadt, Germany). MS-grade sodium formate and analytical reagent grade ethanol were obtained from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). Protocatechuic acid, cryptotanshinone, borneol, and eugenol were purchased from China Pharmaceutical Biological Products Analysis Institute (Beijing, China). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco-BRL (Grand Island, NY, USA). Collagenase II was obtained from MP Corp (Santa Ana CA, USA), and trypsin was purchased from Amresco (Solon, OH USA). Fluo-3/AM was provided by Biotium (Hayward, CA, USA). Prime Script ^{TM} RT reagent Kit and SYBR Premix Ex Taq^{TM} II were purchased from Takara Bio, Inc. (Shiga, Japan). All primers used in the present study were obtained from AuGCT DNA-SYN Biotechnology (Beijing, China). Anti-F2R and NOS3 antibodies produced in rabbit were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Animals

Neonatal Sprague-Dawley (SD) rats (five-days-old) were obtained from the Animal Centre of Xi'an Jiaotong University School of Medicine (Xi'an, China, Production Certificate No. SYSK (Shan) 2007-003). The rats were housed under controlled temperature and humidity conditions (23 ± 2 °C, 55% humidity) with free access to tap water and standard rat diet. All the animals were handled according to the recommendations and regulations of the experimental animal affairs administration, and the surgical procedures and experimental protocols were approved by the Ethical Committee of Xi'an Jiaotong University. (XJTU-(Shan)-2011-0045)

UPLC-QTOF-MSE Analysis

The GXSTC extracts were analyzed on a Waters Xevo G2-XSQ-TOF system coupled with H-Class UPLC system. Separations were accomplished on an ACQUITY UPLC BEH