Pharmacological Research 114 (2016) 196–208

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Perspective

Systems-biology dissection of mechanisms and chemical basis of herbal formula in treating chronic myocardial ischemia

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ARTICLE INFO

Article history:
Received 1 August 2016
Received in revised form 13 October 2016
Accepted 28 October 2016
Available online 3 November 2016

Keywords:
Herbal medicine
Systems biology
Transcriptome sequencing
Chronic myocardial ischemia

ABSTRACT

Herbal medicine is a mixture of multiple compounds, and is intended to exhibit therapeutic effects by attacking multiple disease-causing modules simultaneously. However, it is still a challenge for scientists to untangle the complex biological mechanisms and underlying material basis of herbal medicine. Here, this study was designed to build a systems-biology platform for exploring the molecular mechanisms and corresponding active compounds, with a typical example applied to an herbal formula Qishenkel (QSKL) in the treatment of chronic myocardial ischemia. We have applied an approach integrating transcriptome sequencing, bioactivity profiling inference, computational ligand-receptor evaluation and experimental validation to study the effects on pig myocardial ischemia treated with QSKL. Numerous biological modules were revealed and indicated the coordinated regulation of molecular networks from various aspects of cardiac function. In addition, gene expression profiles were utilized to identify a number of key therapeutic targets of herbal formula, such as angiotensin-converting enzyme and calcium channels. Then, these therapeutic targets were used to fish the potential active ingredients based on a combination of target structure-based and chemical ligand-based methods. Some active compounds, including luteolin, cryptotanshinone, licochalcone A, glycyrrhetinic acid, salsolinol, isoacid chlorogenic C, salvianolic acid A and salvianolic acid B, have been validated by direct biochemical methods. This strategy integrating different types of technologies is expected to provide not only a detailed understanding about the combined therapeutic effects of herbal mixture but also a new opportunity for discovering novel natural molecules with pharmacological activities.

Abbreviations: QSKL, qishenkeli; TCM, traditional Chinese medicine; MI, Myocardial ischemia; HF, heart failure; ACE, angiotensin-converting enzyme; CHD, coronary heart disease; CHF, chronic heart failure; APS, *Astragalus propinquus* Schischkin., root and rhizome; SMB, *Salvia miltiorrhiza* Bunge., root and rhizome; LGT, *Lonicera japonica* Thunb., flower; ACD, *Aconitum carmichaelii* Debeaux., lateral root; GGL, *Glycyrrhiza glabra* L., root and rhizome; SNH, *Scrophularia ningpoensis* Hemsl., root; PCA, Principal component analyses; LTCCs, L-type Ca^{2+} channels; SERCA2a, sarcoplasmic/endoplasmic reticulum calcium ATPase 2a; RyR, Ryanodine receptors; DRM, Desmin-related myopathy; ECM, extracellular matrix; FFAs, Free fatty acids; CMap, Connectivity Map; ACEI, Angiotensin-converting enzyme inhibitor; β-AR, β-adrenergic receptor; SEA, Similarity ensemble approach; RAAS, Renin–angiotensin–aldosterone system; LCx, left circumflex coronary artery; LVIDd, Left ventricular internal dimension diastole; LVIDs, Left ventricular internal dimension systole; LEDV, Left ventricular end-diastolic volume; LESV, left ventricular end-systolic volume; EF, left ventricular ejection fraction; SV, stroke volume; FS, fractional shortening; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; LVESP, left ventricular systolic pressure; LVEDP, left ventricular diastolic pressure; HR, heart rate; LVET, left ventricular ejection time; BNP, brain natriuretic peptide; cTnT, troponin T; TTD, Therapeutic target database; OMIM, online mendelian inheritance in man; MM, morbid map; TCMSP, traditional Chinese medicine systems pharmacology database.

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http://dx.doi.org/10.1016/j.phrs.2016.10.026
1043-6618/

1. Introduction

Combination therapy, modulating the activities of multiple targets to achieve therapeutic efficacies, has been a promising choice for treatment of disease, especially complex diseases, such as HIV, cancer and diabetes disease [1–3]. The main reason is that complex diseases normally involve networked physiological systems featured as redundancy and multifunctionality, which limits the therapeutic opportunity of single-agent applications [4]. In comparison, combinations of drugs are thought to be more effective to counter this complex situation due to the synergistic action or negative regulation of resistance [5,6].

Indeed, the concept of combinatorial therapy has been advocated and practiced in traditional medical treatments for thousands of years, such as traditional Chinese medicine (TCM), which often use botanical mixtures (or named formulae) characterized as multi-component and multi-function to treat disease [7–9]. In development, considerable knowledge has been accumulated concerning clinical efficacy and safety of herbal formulae in targeting complex diseases. However, essential chemical ingredients have not been identified in most formulae, and especially precise mechanisms of formulae remain to be addressed, which hamper the modernization of traditional medical systems.

Chronic myocardial ischemia (MI) is defined by the result of a partial or complete blockage of coronary arteries. Cardiac dysfunction caused by MI and its long-term sequence such as heart failure (HF) profoundly impact patient prognosis and quality of life, and have been a major cause of death and disability worldwide [10,11]. Till now, a series of classical agents, such as angiotensin-converting enzyme (ACE) inhibitors, calcium channel blockers, angiotensin II receptor antagonists, vasodilators and beta-blockers have been proved to be cardioprotective in both preclinical and clinical studies [12]. However, many of these drugs play functional roles in biological processes outside the scope of the drug's intended effects. For example, vasodilators improve the clinical symptoms at an early stage, but the long-term treatments lead to increased mortality [13]. ACE inhibitors and beta-blockers improve myocardial function in the long-term use but do not produce beneficial effects in the treatment of early-stage hemodynamic disorders [14]. Alternatively, there is a growing interest in the use of traditional medicine for long-term prevention of heart attack in high risk patients. For example, the study of TCM has led to design of some formulae for prevention and treatment of heart failure in the basic and clinical research, such as Qili Qiangxin Capsules and Qishenkeli (QSKL) [15,16].

QSKL is a traditional Chinese medicine that has long been used for the routine treatment of coronary heart disease (CHD) and chronic heart failure (CHF) in China. It consists of six Chinese herbs *Astragalus propinquus* Schischkin., root and rhizome (APS), *Salvia miltiorrhiza* Bunge., root and rhizome (SMB), *Lonicera japonica* Thunb., flower (LGT), *Aconitum carmichaelii* Debeaux., lateral root (ACD), *Glycyrrhiza glabra* L., root and rhizome (GGL), and *Scrophularia ningpoensis* Hemsl., root (SNH), and is widely produced in China in accordance with the Chinese Pharmacopoeia standard of quality control. Previously, our preclinical and clinical studies have proved the therapeutic effects of QSKL on HF and partially explored its molecular mechanism characterized by multi-targets and multi-functions [17–19]. However, the chemical basis of QSKL is still unknown that link the therapeutic effects and explicit molecular targets of QSKL in its effects on myocardial function.

In this work, based on an array of computational and experimental approaches, we propose a systems-biology strategy for identification of key active ingredients of QSKL and dissection of underlying mechanisms (Fig. 1). The proposed methodology mainly includes three categories: (1) transcriptome-based understanding of the drug actions and exploration of potential therapeutic targets; (2) systematical ligand-target interaction prediction methods to build relationships between therapeutic targets and effective herbal molecules; and (3) combined biological network analysis and experimental validation to discover the chemical basis and their molecular actions. This strategy in dissecting the complexity of clinically effective formulae at the chemical, molecular, cellular, and organism levels may be an effective path to explore the value of traditional medicine.

2. Results

2.1. QSKL promotes cardiac repair and improves myocardial function

After 8 weeks of treatment, echocardiographic, hemodynamic and physicochemical evaluation were conducted to assess the cardiac function in the three groups to evaluate the role of QSKL in preventing heart from myocardial ischemia. In comparison with the sham group, myocardial ischemia caused a significant decline in LVEF and FS ($P < 0.05$ and $P < 0.001$, for LVEF and FS, respectively; Fig. 2A), indicating an impairment of heart function. When treating with QSKL at 0.33 g/kg, LVEF and FS were significantly increased compared to the MI group ($P < 0.01$ and $P < 0.05$, for LVEF and FS, respectively; Fig. 2A). Hemodynamic analysis showed similar results that QSKL significantly increased blood pressure related indices SBP, DBP and MAP, and LV +dP/dt max and LV–dP/dt max compared to the MI group ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.01$ and $P < 0.01$ for SBP, DBP and MAP, and hemodynamic indexes LV + dP/dt max and LV–dP/dt max, respectively; Fig. 2B). Furthermore, the analysis of biomarkers BNP and cTnT showed that MI caused the increase of serum cTnT and BNP ($P < 0.001$ and $P < 0.001$ for cTnT and BNP, respectively). This increase was significantly suppressed by QSKL treatment ($P < 0.001$ and $P < 0.001$, for cTnT and BNP, respectively; Fig. 2C). In addition, the coronary angiography and HE staining were also involved to further determine the model. The examination of coronary angiography displayed the coronary artery luminal occlusion and confirmed that blood flow at the site of occlusion was almost completely abolished by the Ameroid constrictor, indicating introduction of the ischemia in the animal model (Supplementary Fig. S1A and B). The results of HE staining also showed that the cardiac myocytes exhibited an irregular shape and arrangement, with myocardial fibrosis in the model group compared with that in the sham group (Supplementary Fig. S1C and D). These results collectively demonstrated the success of the MI model and confirm the cardiac protective effect of QSKL on the MI model.

2.2. Quantifying analysis of expression profiles in pig left ventricular tissues reveals the cardiac regulating effects of QSKL

To gain insights into the molecular profiles of QSKL in treating MI, a systematic transcriptomic analysis was performed in MI pigs treated with QSKL for two months. A total of nine barcoded mRNA libraries were prepared from pig LV samples, including LV samples from animals with Sham operation ($n = 3$), MI ($n = 3$) and QSKL treated ($n = 3$). From these samples, a total of 110,463,375 read pairs were generated from RNASeq experiments (Supplementary Table S1). More than 98 million read pairs (88.8%) were aligned to the pig genome (Sscrofa10.2), where ~35 million (32.2%) mapped within exons, ~54 million (49.3%) mapped to other regions. To validate the reliability of the data, six representative genes CACNB2, CACNG6, RyR2, TNXB, SERCA2a and ITGB8 were analyzed by real-time RT-PCR, and the results were correlated with those of the RNAseq data (Supplementary Fig. S2).