Journal of Ethnopharmacology 214 (2018) 124–133

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Metabolic profiles revealed anti-ischemia-reperfusion injury of *Yangxinshi tablet* in Rats

Hai Zhang[a,1], Yahong Zhao[b,1], Zhengxiang Xia[a], Hongli Du[c], Yue Gao[d], Dan Xue[b], Zhenyu Zhu[d,*], Yifeng Chai[d,*]

[a] Department of Pharmacy, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai 201204, China
[b] Department of Chinese Materia Medica, Central Research Institute, Shanghai Pharmaceuticals Holding Co.Ltd., Shanghai 201203, China
[c] Department of Pharmacy, Shanghai Eastern Hepatobiliary Surgery Hospital, Shanghai 200433, China
[d] School of Pharmacy, Second Military Medical University, Shanghai 200433, China

ARTICLE INFO

Keywords:
Ischemia-reperfusion
Yangxinshitablet
Metabolomics
Biomarker
UHPLC-QTOF/MS

ABSTRACT

Ethnopharmacological relevance: Myocardial ischemia-reperfusion (I/R) injury is a serious injury that is resulted from the recovery of blood supply after myocardial ischemia. *Yangxinshi tablet* is a compound Chinese herbal preparation and often used to alleviate the myocardial ischemia in clinical, but its protective mechanism of anti-myocardial ischemia reperfusion injury remains unclear. The objective of this study was to evaluate the anti-I/R injury effect of *Yangxinshi tablet* on a myocardial I/R rat model and to identify serum biomarker metabolites associated with I/R based on ultra-high performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (UHPLC-QTOF/MS) metabolomic method, and explore the metabolic mechanism of anti-I/R injury of *Yangxinshi tablet*.

Materials and methods: Unsupervised principle component analysis highlighted significant differences in the metabolome of the myocardial I/R, healthy control and drug-treated rats. Partial least squares-discriminant analysis revealed 25 metabolites as the most potential biomarker metabolites discriminating the myocardial I/R rats and control rats. Most of the metabolites were primarily involved in oxidative stress, energy metabolism, fatty acid metabolism, amino acid metabolism. These metabolites were validated by assessing the efficacy after intragastric administration of *Yangxinshit ablet* to the myocardial I/R rat model.

Results: Based on metabolomic results, the action mechanism of anti-I/R injury of *Yangxinshi tablet* was concluded as follows: (1) enhance the ability of scavenging free radicals and reactive oxygen species in vivo; (2) provide energy for myocardium via accelerating the intracellular carnitine transportion to accelerate the oxidation of fatty acid and (3) attenuate ceramide to reduce cardiomyocyte apoptosis.

Conclusions: Yangxinshi tablet has cardio-protection effects on I/R rats via regulation of multiple metabolic pathways involving in oxidative stress, energy metabolism, fatty acid, and amino acid metabolisms. This study will be meaningful for its clinical application and valuable for further exploring the action mechanism of *Yangxinshi tablet*.

1. Introduction

Nowadays, coronary heart disease is a major cause of death worldwide (Dalen et al., 2014; Hausenloy and Yellon, 2013). Ischemia reperfusion injury (I/R) has become a major factor in the treatment of coronary heart disease, although arterial bypass and other surgical procedures have been widely used (Ong et al., 2015). Ischemia reperfusion injury is complex and paradoxical (Li et al., 2016), which can cause rhythm problems and lead to heart failure. Many scholars have studied the molecular mechanism of the injury, which include oxygen free radical damage (Yin et al., 2015), calcium overload (Meng et al., 2016), abnormal myocardial energy metabolism (Zhan et al., 2015) and

Abbreviations: ANOVA, analysis of variance; CK, creatine kinase; EIC, extracted ion chromatogram; ESI, electrospray ionization; I/R, ischemia reperfusion injury; IPA, Ingenuity pathway analysis; LDH, lactate dehydrogenase; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; QC, quality control; ROS, reactive oxygen species; RSD, relative standard deviation; SOD, superoxide dismutase; TCA, Tricarboxylic acid cycle; TIC, total ion chromatogram; UHPLC-QTOF/MS, ultra-high performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry

* Corresponding authors.
E-mail addresses: zzyzyfzhu@163.com (Z. Zhu), yfchai@smmu.edu.cn (Y. Chai).
[1] Both authors equally contributed to this work.

https://doi.org/10.1016/j.jep.2017.09.003
Received 7 January 2017; Received in revised form 5 September 2017; Accepted 6 September 2017
Available online 08 September 2017
0378-8741/

endogenous protective effect of heart (Zhong and Wang, 2008). However, its metabolic mechanism is rarely studied. Although many anti-heart failure drugs can reduce the injury, their effect is not significant yet. Therefore, it is urgent to explore the novel effective and reliable anti-I/R injury drugs. Traditional Chinese medicine (TCM) has a long history and the patients have widely accepted TCM as an alternative or complementary therapy in China and many other Asian countries (Chen et al., 2016b; Tian et al., 2014; J. Zhang et al., 2009; Zhang et al., 2008; Zhao, 2013b). Accumulated evidence showed that TCM has been widely used for the treatment of various disease such as hyperlipidema (Chen et al., 2015a; Miao et al., 2017), cardiovascular disease (Liu et al., 2014; Meng et al., 2016; Yu et al., 2016) and kidney disease (L. Chen et al., 2017; Zhang et al., 2016b, 2015). *Yangxinshi tablet* is a Chinese patent medicine developed by Shanghai Pharmaceuticals Holding Co. Ltd., including *Astragalus membranaceus* (Fisch.) Bge. var. mongholicus (Bge.) Hsiao, root; *Codonopsis pilosula* (Franch.) Nannf root; *Salvia miltiorrhiza* Bge., root; *Pueraria lobata* (Willd.) ohwi, root; *Epimedium brevicornu* Maxim, root and rhizome; *Crataegus pinnatifida* Bunge, fuit; *Rehmannia glutinosa* (Gaetn.) Libosch. ex Fisch. et Mey., root; *Angelicae sinensis*, root; *Coptis chinensis* Franch, root; *Corydalis yanhusuo* W. T. Wang ex Z. Y. Su et C. Y. Wu, root; *Ganoderma lucidum* Karst, body; *Panax ginseng* C. A. Mey, root and rhizome; *Glycyrrhiza uralensis* Fisch, root and rhizome. It is frequently used in supplementing qi, activating blood circulation, removing blood stasis and relieving pain (Zhang et al., 2016a, 2016b). In clinical, it is for treatment of chest tightness, precordial tingling, coronary heart disease, angina pectoris, and its annual sale practically approaches 20 million dollars. What's more, *Yangxinshi tablet* also has been reported to have protective effects on myocardial ischemia induced by ligation of the coronary artery in rats. Myocardial I/R injury is a primary cause of heat disability and its serious impact contributes substantially to the burden of metabolic disorders.

Metabolomics was a new platform of systems biology. Metabolomics enables the simultaneous quantitative measurement of numerous low molecular weight molecules in complex biological samples. As a powerful analytical platform, recently, metabolomic application has rapidly increased in the fields of novel potential disease biomarker discovery (D.Q. Chen et al., 2017a; Zhao, 2013a; Zhao et al., 2012c), disease action mechanism (D.Q. Chen et al., 2017b; H. Chen et al., 2017; Miao et al., 2015a; Zhang et al., 2016a; Zhao et al., 2012b) and evaluation of drug efficacy and toxicity (D.Q. Chen et al., 2016; Wang et al., 2017). ^{1}H NMR and mass spectrometry (MS) techniques were two popular analytical techniques applied to the analysis of metabolic profile. In the MS-based metabolomic application, ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QTOF/MS) is suitable for the analysis of targeted and untargeted metabolomics because of its rapidly separation and high reproducibility (Zhao et al., 2014b, 2013a; Zhao and Lin, 2014). In agreement with the holistic opinion of TCM, metabolomics approach based on UHPLC-QTOF/MS used to identify metabolic biomarkers associated with myocardial I/R injury, evaluate the anti-I/Rinjury effects of *Yangxinshi tablet* in an I/R rat model, and elucidate the underlying metabolic mechanism of anti-I/R injury of *Yangxinshi tablet*.

2. Materials and methods

2.1. Drugs and reagents

Methanol, acetonitrile and formic acid were chromatographic grade (Merck, Darmstadt, Germany). The drugs of *Yangxinshi tablet* (Batch No. 150406, Shanghai, China) were from Shanghai Pharmaceuticals Holding Co. Ltd. The compound 3-hexenedioic acid (St Louis, MO, USA) were purchased from Aladdin Reagent Co., Ltd. *Qishenyiqi Pills* (Tasly Pharmaceutical Group Co., Ltd., Batch number: 150310, Tianjin, China) were purchased from Changhai hospital (Shanghai, China). All other chemicals were analytical grade. The 0.9% sodium chloride injection (Shandong Hualu Pharmaceutical Co., Ltd., Batch number, D15101402, Jinan, Shangdong province, China); Isoflurane (Shandong Keyuan Pharmaceutical Co., Ltd., Batch number, 20151027 Liaocheng, Shangdong province, China); Chloral hydmicee (Sinopharm Group Chemical Reagent Co., Ltd., Batch number, 20130426, Beijing, China); Formalin solution and 4% paraformaldehyde (Wuhan Google Biotechnology Co., Ltd., Batch number, G-1002, Wuhan, Hubei province, China); Distilled water was prepared in our laboratory.

The quantitative determination of 10 compounds in *Yangxinshi tablet* was completed using high performance liquid chromatography including puerarin, daidzin, tanshinol, rutin, tetrahydropalmatine, luteolin, icariin, apigenin, ginsenoside RD and ferulic acid (Supplementary material). The concentrations of the ingredients in *Yangxinshi tablet* were 1628.424, 347.508, 716.610, 384.330, 1631.058, 2387.658, 1854.096, 254.604, 2973.480, 581.826 μg/tablet, respectively.

2.2. Animals

Male Sprague-Dawley rats weighing 180–200 g (Shanghai SLAC Laboratory Animal Co Ltd) were under the conditions of room temperature (20–23 °C), humidity (50 ± 10%), light (12-h light/dark cycle) and were free access to diet. They acclimatized for 1 week prior to the start of the experiment. All experiments and procedures performed according to the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of China and the protocol approved by the Committee on the Ethics of Animal Experiments of Second Military Medical University.

2.3. Groups and drug administration

After acclimatization, rats were randomly divided into 6 groups (N = 8/group). The groups were assigned as follows: group 1, healthy controls; group 2, I/R injury model (I/R group); group 3, I/R model treated with *Yangxinshi tablet* at low dose (250 mg/kg); group 4, I/R model treated with *Yangxinshi tablet* at medium dose (500 mg/kg); group 5, I/R model treated with *Yangxinshi tablet* at high dose (1000 mg/kg); group 6, I/R model treated with *Qishenyiqi Capsule* (48 mg/kg). All drugs were dissolved in the distilled water and given by gastric gavage once daily for 7 days before developing animal models.

2.4. I/R injury procedure and samples collection

Before the experiment, the rats placed in the normal experimental environment adaptability, the normal provision of food and water, to ensure 12 h light cycle. Following adaptive feeding in rats, the rats were anesthetized with isoflurane, a longitudinal incision was made at 0.5 cm of the left sternal border of the mouse, and the third to fifth ribs were cut to fully expose the heart, to the anterior descending coronary artery to ligation, the ligature line tension, coronary occlusion, and then induced acute myocardial ischemia. After 40 min, the ligature loosened, and reperfusion performed for 2 h.

Blood samples were collected after completion of tests. Rats were anesthetized using chloral hydmicee with dose of 300 mg/kg and blood samples collected by abdominal artery sampling. After clotting at 4 °C for 2 h, the blood centrifuged at 4000 rpm for 10 min. The supernatant samples were transferred to Eppendorf tubes and immediately stored at −80 °C until analysis.

2.5. Measurement of biological parameters in the serum

The concentrations of creatine kinase (CK), lactate dehydrogenase (LDH) and superoxide dismutase (SOD) in serum were determined by using rat CK, LDH and SOD Elisa assay kits (Nanjing Jiancheng Bioengineering Institute), strictly following manufacturer's instructions.