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Chinese patent medicine Xin-Ke-Shu inhibits Ca^{2+} overload and dysfunction of fatty acid β-oxidation in rats with myocardial infarction induced by LAD ligation

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ABSTRACT

Myocardial infarction (MI) occurs during a sustained insufficient blood supply to the heart, eventually leading to myocardial necrosis. Xin-Ke-Shu tablet (XKS) is a prescription herbal compound and a patented medicine extensively used in the clinical treatment of coronary heart disease (CHD). To understand the molecular mechanism of the XKS action against MI in detail, it is necessary to investigate the altered metabolome and related pathways coincident with clinical features. In this study, tissue-targeted metabonomics based on ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS) were developed to explore the metabolic changes associated with XKS treatment in the heart tissue of rats with MI induced by a left anterior descending coronary artery ligation (LAD). The metabolic disorder induced by LAD was alleviated after low-dose XKS (LD) and intermediate-dose XKS (MD) treatment. XKS modulated six perturbed metabolic pathways. Among them, inhibition of Ca^{2+} overload and dysfunction of fatty acid β-oxidation-related metabolic pathways likely underlie the therapeutic effects of XKS against MI. In agreement with its observed effect on metabolite perturbation, XKS reversed the over-expression of the four key proteins, long-chain acyl-CoA synthetase 1 (ACSL1), carnitine palmitoyl transferase-1 (CPT1B), calcium/calmodulin-dependent kinase II (CaMKII), and phospholipase A2IIA (PLA2IIA). Both metabolite and protein changes suggested that XKS exerts its therapeutic effect on metabolic perturbations in LAD-induced MI mainly by inhibiting the Ca^{2+} overload and fatty acid β-oxidation dysfunction.

1. Introduction

Myocardial infarction (MI) remains a leading cause of morbidity and mortality in developing and developed countries. It occurs in the presence of a sustained insufficient blood supply to the heart, eventually resulting in myocardial necrosis [1]. Several mechanisms, including oxidative stress, lipid peroxidation, Ca^{2+} overload, dysfunction of energy metabolism and mitochondrial oxidation, inflammatory response, and irreversible DNA damage, are responsible for MI [2,3]. Because of the complex etiology of MI, single-target drugs usually fail as a cure for this multi-factorial disease [4]. Furthermore, the effectiveness of current anti-ischemic medicines (e.g., angiotensin converting enzyme inhibitors and β-blockers) is limited by their side effects, including bradycardia and hypotension [5]. Recently, multi-component medicines have become a promising approach for treating MI.

As multi-component medicines, TCM formulae exhibit few side effects and high therapeutic efficacy, which is characteristic of a holistic therapeutic effect [6]. Xin-Ke-Shu tablet (XKS) is a prescription herbal compound containing the roots of *Aucklandia lappa* Decne (Mu-Xiang), roots of *Panax notoginseng* (Burk.) F.H. Chen. (San-Qi), fruits of *Crataegus pinnatifida* Bge. (Shan-Zha), roots of *Pueraria lobata* (Willd.) Ohwi. (Ge-Gen), and roots of *Salvia miltiorrhiza* Bge. (Dan-Shen). It is a patented medicine extensively used in the clinical treatment of coronary heart disease (CHD) and its quality is strictly controlled [7]. In a previous study, we demonstrated therapeutic efficacy of XKS against atherosclerotic myocardial ischemia through rescue of endothelium-

Abbreviations: CHD, coronary heart disease; MI, myocardial infarction; LAD, left anterior descending coronary artery ligation; ISO, isoprenaline; XKS, Xin-Ke-Shu; UPLC-Q-TOF/MS, ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry; PCA, principal component analysis; OPLS-DA, orthogonal partial least squares discriminant analysis; VIP, variable importance of project; LPCs, lysophosphatidylcholines; LCFAs, long-chain fatty acids; PLA2IIA, phospholipase A2 IIA; CaMKII, calcium/calmodulin-dependent kinase II; ACSL1, long-chain acyl-CoA synthetase 1; CPT1, carnitine palmitoyl transterase-1; CPT2, carnitine palmitoyl transterase-2; CAT, carnitine-acylcarnitine translocase

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dependent vessel relaxation, affecting vascular cell adhesion molecule and endothelial nitric oxide synthase expression [8]. In addition, XKS treatment is also effective in rats with isoproterenol (ISO)-induced myocardial ischemia via down-regulation of calcium/calmodulin-dependent kinase II (CaMKII) [9]. These observations hint that inhibition of Ca^{2+} overload in heart tissue may be the critical mechanism of action of XKS against CHD.

Compared with rats with ISO-induced MI, the model of left anterior descending coronary artery ligation (LAD)-induced MI better mimics the alteration of hemodynamics and myocardium metabolism of CHD patients [10]. In addition, LAD-induced MI is characterized by a specific location of the infarction in the heart and defined pathological changes. In over 90% of patients, MI is caused by a coronary artery stenosis and blockage [11]. Intracellular Ca^{2+} overload of the cardiomyocytes is a crucial factor activating the myocardium from reversible to irreversible injury [12]. Many factors can elicit Ca^{2+} overload, including non-specific injury of the cell membrane, intracellular adenosine triphosphate (ATP) depletion, functional change of calmodulin, and stress associated with reactive oxygen species; all of these are involved in mitochondrial dysfunction [13] and in the activation of phospholipase, calpain, and other enzymes [14].

Metabonomics, a global metabolic profiling strategy for tissues and biological fluids [15], provides information on the entire metabolic state of an organism in response to drug therapy or a disease [16,17]. The integrity and systemic features of metabonomics highly coincide with the holistic basis for TCM [18]. Our previous metabonomics studies on plasma revealed that XKS pretreatment alters a series of metabolites to alleviate ISO-induced MI metabolic disorder [19]. Considering the advantages of the LAD-induced MI model, and the complexity of the Ca^{2+} overload mechanism and altered metabolome in the MI rat, detailed investigation of molecular mechanisms of XKS activity against LAD-induced MI is needed.

Therefore, in the current study, heart tissue-targeted metabonomics based on ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS) were used to evaluate the efficacy of XKS against LAD-induced MI in a rat model and reveal its underlying molecular mechanisms. Further, the disturbance of the metabolome associated with MI and the therapeutic targets of XKS were elucidated by Western blotting.

2. Materials and methods

2.1. Reagents and materials

Standard XKS tablets containing Mu-Xiang, San-Qi, Shan-Zha, Ge-Gen, and Dan-Shen (1:1:15:15:15, w/w) were provided by Wo Hua Pharmaceutical Co., China (batch no. 090629). Liquid chromatography coupled with electrospray ionization hybrid linear trap quadrupole orbitrap (LC-LTQ-Orbitrap) was used for quality control evaluation of the XKS tablets [7]. Captopril (P) was purchased from Chang Zhou Pharmaceutical Co., China. Chromatographic-grade formic acid and acetonitrile were obtained from Fisher Scientific (Waltham, MA, USA) and J.T. Baker (Phillipsburg, NJ, USA), respectively. Pure water (18.2 MΩ cm) used for LC-MS was generated by a Milli-Q system (Millipore, France). All other chemicals were of analytical grade. Assay kits for the determination of aspartate transaminase (AST), creatine kinase isoenzyme-MB (CK-MB), lactate dehydrogenase (LDH), and creatine kinase (CK) activities were from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Standard compounds used for biomarker validation were from Bei Na Biotechnology Co., China. Antibodies against carnitine palmitoyl transferase-1 (CPT1B), CaMKII, long-chain acyl-CoA synthetase 1 (ACSL1), and phospholipase A2IIA (PLA2IIA) were purchased from Abcam (Cambridge, Britain; ab134988), Cell Signalling (Boston, USA; 11945S), Cell Signalling (Boston, USA; 9189S), and Sigma-Aldrich (St. Louis, USA; SAB4200129), respectively.

2.2. Experimental animals

Eighty-nine male Sprague-Dawley rats (weighing 200 ± 15 g) were obtained from the Institute of the Chinese Academy of Medical Science (CAMS, China). All animal experiments were conducted under the control and approval of the Ethics Committee of the Institute of Medicinal Plant Development, CAMS (Beijing, China). The animals were housed in cages (5 per cage) and maintained (20–25 °C and 40%–60% relative humidity) under controlled conditions of 12 h light-12 h dark cycles with water and food freely available. The rats were acclimatized for a week prior to the experiment. Throughout the experiment, all animals were handled humanely.

2.3. MI model and drug administration

MI was induced by LAD as described previously [17]. Animals were fixed onto a pad after anesthesia with pentobarbital sodium (intraperitoneal injection, 30 mg kg^{-1}). Middle thoracotomy was used to open the chest between the 4th and 5th ribs under sterile conditions, the heart was rapidly exteriorized, and a 6–0 silk suture was looped under the left anterior descending coronary artery to form MI. The sham surgery group underwent the same procedure except for the ligation. In total, 35 rats died after surgery. Rats that survived in the MI procedure were randomly assigned into five groups: captopril (P, 10 mg kg^{-1} d^{-1}), low-dose XKS (LD, 1.4 g kg^{-1} d^{-1}), intermediate-dose XKS (MD, 2.8 g kg^{-1} d^{-1}), high-dose XKS (HD, 5.6 g kg^{-1} d^{-1}), and model (M) (n = 9 in each group). Drug administration was initiated 24 h after surgery and was continued for 3 weeks. Rats in the sham group (S, n = 9) and model group (M) received an equivalent volume of water (10 mL kg^{-1} d^{-1}) by oral gavage.

2.4. Sample collection

Rats (n = 54) were anesthetized with pentobarbital sodium after the final drug administration. Blood samples were collected from the aorta ventralis into sodium heparin tubes. The blood was centrifuged at 3600 × g for 10 min at 4 °C, and 1000 μL of the supernatant was stored at −80 °C before determination of AST, CK-MB, LDH, and CK levels. After the blood was drawn, thoracotomy was conducted, and the hearts were collected. Each heart was sliced transversely into three portions (ca. 3 mm per portion) from the apex to the basal part of the LV; the first portion (the middle part) was immediately fixed in 10% neutral buffered formalin (w/v) for histopathological evaluation; the second portion (the apex part) was stored at −80 °C for metabonomics; and the third portion (the basal part) was stored at −80 °C before Western blotting analysis.

2.5. Plasma biochemistry assays and histopathology

The degree of cardiac injury was evaluated by determining the AST, CK-MB, LDH, and CK activities in the plasma using standard kits (Nanjing Jiancheng Bioengineering Institute) and a clinical automatic biochemical analyzer system (Beckman, AU480, USA) following the manufacturer's instructions.

The first portion of the heart was embedded in paraffin wax; 5-μm histological sections were stained with hematoxylin and eosin (HE). The sections were analyzed under a light microscope and photomicrographs were generated (Olypus, BX53, Japan). Infarct size (%) was expressed as infarct circumference/left ventricular (LV) circumference × 100%.

2.6. Preparation of heart samples for UPLC-Q-TOF/MS analysis

Metabolites were extracted from the prepared hearts as described in our previous study [9]. Briefly, 2000 μL of a chilled water, methanol, and chloroform mixture (1:2:1, v/v/v) was added to a 100-mg heart (LV