Phytomedicine 23 (2016) 621–631

Contents lists available at ScienceDirect

Phytomedicine

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

Targeted metabolomic profiling of cardioprotective effect of *Ginkgo biloba* L. extract on myocardial ischemia in rats

Zhe Wang, Jinlan Zhang*, Tiankun Ren, Zhen Dong

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, PR China

ARTICLE INFO

Article history:
Received 13 September 2015
Revised 16 February 2016
Accepted 7 March 2016

Keywords:
Metabolomic
Ginkgo biloba L. extract
High-performance liquid chromatography–mass spectrometry
Myocardial ischemia
Cardioprotective effect

ABSTRACT

Background: Myocardial ischemia (MI) is one of the highest mortality diseases in the world. It is closely associated with metabolism disorders of endogenous substances. *Ginkgo biloba* L. extract (GBE) is a popular herbal medicine used for prevention and therapy of MI. But its regulation effect on the metabolism disorders caused by MI remains currently unknown.
Purpose: Our metabolomic profiling study provided insight into endogenous metabolic disorders of MI and cardioprotective mechanisms of GBE.
Study design: The rats were preventive administrated of GBE (200 mg/kg, i.g.) for 4 weeks and then subcutaneous injected of isoproterenol to establish MI model. Heart marker enzymes and histopathological examination were adopted to evaluate MI model and effect of GBE. On this base, endogenous metabolites in rat plasma and heart were well profiled using the developed targeted metabolomic profiling platform to comprehensively analyze metabolic pathways and find biomarkers.
Methods: A targeted metabolomic profiling platform was developed and only 100 μl biological sample was used to quantify 808 metabolites covering the core network of lipid, energy, amino acid and nucleotide metabolism. Then using this platform, endogenous metabolites of rats undergoing MI model and GBE pre-treatment were well profiled. Orthogonal partial least squares discriminant analysis (OPLS-DA) was used to discriminate between groups and find biomarkers.
Results: The metabolomic profiles of MI model rats pre-protected by GBE were significantly different from those of unprotected. 47 metabolites were found as potential biomarkers and indicated MI would lead to disturbed metabolism due to inflammation, oxidative stress and structural damage; while GBE could effectively restore fatty acid, sphingolipid, phosphoglyceride, glyceride, amino acid and energy metabolism, closely related to its antioxidant, PAF antagonist and hypolipidemic properties.
Conclusion: The cardioprotective effect of GBE can be achieved through the comprehensive regulation of multiple metabolic pathways.

Introduction

Myocardial ischemia (MI) is characterized by an imbalance between the supply and demand of myocardial oxygen, causing cardiac dysfunction, arrhythmias, myocardial infarction, and sudden death. Due to its high morbidity, high mortality and high disability; it has become a major threat to human health. Thus researches of its pathology and treatment are always hot areas (Shimokawa et al. 2008). In recent years, herbal medicines have been widely used in prevention and therapy of ischemic heart disease due to its good curative effect, multi-targets and small side effects. Extract from *Ginkgo biloba* L. (Ginkgoaceae) leaves (GBE) is one of the most popular herbal medicines with multiple pharmacological activities. It contains two groups of active components: flavonol glycosides mainly derived from the aglycones of quercetin, kaempferol and isorhamnet, and terpene lactones including ginkgolide A, B, C and bilobalide (Liebgott et al. 2000). Previous researches have reported that GBE has antioxidant properties, to modify vasomotor function, to reduce adhesion of blood cells on endothelium, to inhibit activation of platelets, to affect ion channels, to alter signal transduction and to reduce blood lipids (Zhou et al. 2004). Although many

Abbreviations: AST, aspartate aminotransferase; Cer, ceramide; Cer-1P, phosphoceramide; CK, ceating kinase; dhCer, dihydroceramide; dhCer-1P, phosphodihydroceramide; dhSph, dihydrosphingosine; DG, diglyceride; FA, fatty acid; GBE, *Ginkgo biloba* L. extract; HexCer, glycoceramide; LDH, lactate dehydrogenase; LPA, lyso-phosphatidic acid; LPC, lyso-phosphatidylcholine; LPE, lyso-phosphatidylethanolamine; MDA, malondialdehyde; MI, myocardial ischemia; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; SM, sphingomyelin; SOD, superoxide dismutase; Sph, sphingosine; TCA, tricarboxylic acid; TG, triglyceride.

* Corresponding author. Tel.: +86 10 83154880; fax: +86 10 63017757.
E-mail address: zhjl@imm.ac.cn (J. Zhang).

http://dx.doi.org/10.1016/j.phymed.2016.03.005
0944-7113/

researchers studied GBE in prevention and therapy of ischemic heart disease (Kamel et al. 2010; Panda et al. 2008, 2009; Liebgott et al. 2000), there is no report about its regulation and influence on the metabolic pathways. Especially due to MI is closely related to disorder of lipid metabolism (Rasmiena et al. 2013), to study its effect on lipid metabolic pathways has an important significance.

Metabolomics can comprehensively characterize small molecule metabolites in biological systems and provide an overview of the metabolic status and global biochemical events after external stimulation, such as disease model and drug treatment. Thus it becomes a novel method in pharmacology and pharmacodynamics study (Rasmiena et al. 2013). Currently, there were a lot of metabonomic researches on cardiovascular diseases. Griffin et al. reviewed the role of metabolomics in gaining mechanistic insight into cardiac disease processes, and in the search for novel biomarkers. They showed a series of valuable results, such as MI could be characterized by increased lactate levels, changes in TCA cycle intermediates and the breakdown products of AMP; profile of eicosanoids might provide novel therapeutic strategies for myocardial infarction, etc. (Griffin et al. 2011). Rasmiena et al. also summarized evidence derived exclusively by metabolomic studies in the context of ischemic heart disease. They found various metabolic pathways related inflammation, oxidative stress, plaque composition and lipid metabolism changed after ischemic heart disease, and listed several potential biomarkers for diagnosis and therapy (Rasmiena et al. 2013). These researches suggested metabolomics is a powerful tool for the study of pathology, prevention, diagnosis and therapy of cardiovascular diseases.

However, it is still a bottleneck for the MI metabolomic study to develop an appropriate analytical platform using micro amount of sample, which can simultaneously cover enough endogenous metabolites related to multiple metabolic pathways. Firstly, the polarity of endogenous metabolites are significantly different and thus sample preparation is not the same; Secondly, it is difficult to analyze all metabolites by one method due to the great difference of their abundance; Thirdly, sample amount of animal and clinical experiments is always little and precious, and it needs to use a micro sample to obtain as much information as possible. So it has a high requirement for the establishment of analytical platform.

In this paper, an integrated targeted metabolomic method was developed. Lipids and polar metabolites could be simultaneously extracted and separated from only 100 μl biosample. Then it was applied to study the metabolic profiles of MI and cardioprotective effect of GBE. To the best of our knowledge, we reported the first metabolic profiles of MI model rats pre-protected by GBE. The potential biomarkers found in rat plasma and myocardium were well analyzed to explain the cardioprotective mechanism of GBE on MI.

Materials and methods

Chemicals and reagents

Quercetin, kaempferol, isorhamnetin, bilobalide, ginkgolide A, ginkgolide B and ginkgolide C standards used for quality control were supplied by National Institutes for Food and Drug Control (Beijing, China). Diltiazem was purchased from Tanabe Seiyaku Co., Ltd. (Tianjin, China). All lipid standards were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Isoproterenol and metabolomic standards were purchased from Sigma-Aldrich (St. Louis, MO, USA) and J&K Scientific (Beijing, China).Isotope labeled internal standards were purchased from Cambridge Isotope, Inc. (Tewksbury, MA, USA). All solvents and other chemicals used were of the highest grade available.

Herbal extract

Ginkgo biloba L. (Ginkgoaceae) extract was purchased from Zixi Co., Ltd. (Nanjing, China; Batch number: No-022). The herbal extract was complying with the monograph in Chinese Pharmacopoeia Commission (2010). It was the 100% genuine dry extract from *Ginkgo biloba* L. leaf with 70% ethanol (v/v) as extraction solvent. The "drug-extract" ratio (DER) is within 50–56:1. The extract was characterized using the methods in Chinese Pharmacopoeia Commission (2010). The result showed that the contents of main components were 28.39% (w/w) of total flavonol glycosides, calculated as quercetin (acid hydrolysis, 4.67%, w/w), kaempferol (acid hydrolysis, 5.78%, w/w) and isorhamnetin (acid hydrolysis, 0.86%, w/w); and 8.34% (w/w) of total terpene lactones, calculated as bilobalide (2.59%, w/w), ginkgolide A (2.28%, w/w), ginkgolide B (1.47%, w/w) and ginkgolide C (2.00%, w/w). The extract was also checked by the supplementary test method issued by China Food and Drug Administration (2015) (CFDA), and found the contents of three free flavonol aglycones were trace with 0.47 mg/g of free quercetin, 0.57 mg/g of free kaempferol and 0.096 mg/g of free isorhamnetin respectively, which means the purchased GBE was not adulterated. GBE was suspended in 0.5% carboxymethylcellulose sodium for oral administration. 1 ml oral liquid contained 20 mg 100% genuine dry extract.

Animal experiment

24 male Sprague–Dawley rats (200 ± 15 g) were purchased from Vital River Laboratories Co., Ltd. (Beijing, China). The animals were housed under specific pathogen-free conditions (12 h light/12 h dark photoperiod, 25 ± 2 °C, 50 ± 5% relative humidity). All rats were allowed to acclimate for 2 weeks before experiments. Research was conducted in accordance with all institutional guidelines and ethics and approved by the Laboratories Institutional Animal Care and Use Committee of the Chinese Academy of Medical Sciences and Peking Union Medical College.

Rats were divided randomly into four groups of six rats: group A as control rats, group B as MI model rats, group C as MI model rats pre-treated with diltiazem (50 mg/kg, i.g.), and group D as MI model rats pre-treated with GBE (200 mg/kg, i.g.). Pre-treated rats were consecutively administrated for 28 days. Except group A received saline, other groups received two injections of isoproterenol (85 mg/kg, s.c.) on the 27th and 28th days at an interval of 24 h. The rats were dissected after euthanization and blood samples were collected using heparin as an anticoagulant to obtain plasma by centrifugation (3000 rpm, 15 min) on the 29th day and then kept at −80 °C. Hearts were removed, washed immediately with saline and cut in half. The apical half of heart was fixed in 10% buffered formalin for histoarchitectural examination. The other section was grinded into powder in liquid nitrogen and kept at −80 °C. Tissue samples were homogenized in saline at the ratio of tissue to solution was 1:10.

Biochemical analysis and oxidative stress related parameters

The heart marker enzymes CK, AST and LDH in plasma and heart homogenates were determined using standard kits supplied from BioSino Bio-technology and Science, Inc. (Beijing, China). Antioxidant enzyme SOD and lipid peroxidation product MDA were determined using standard kits purchased from Jiancheng Tech Co., Ltd. (Nanjing, China).The protein content of heart homogenates was determined with BCA protein assay kit purchased from Boster Co., Ltd. (Wuhan, China).