Available online at SciVarse ScienceDirect

Chinese Herbal Medicines (CHM)

ISSN 1674-6384

Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

Original article

Investigation on Endogenous Metabolites in Pancreas of Diabetic Rats after Treatment by Genipin through ^{1}H-NMR-based Metabolomic Profiles

Ting Ling-hu[1, 2], Xiao-li Shen[1, 2], Jun-sheng Tian[1*], Xue-mei Qin[1*]

1. *Modern Research Center for Traditional Chinese Medicine of Shanxi University, Taiyuan 030006, China*
2. *College of Chemistry and Chemical Engineering of Shanxi University, Taiyuan 030006, China*

ARTICLE INFO

Article history

Received: January 30, 2016

Revised: March 15, 2016

Accepted: March 30, 2016

Available online: April 14, 2016

DOI: 10.1016/S1674-6384(16)60022-9

ABSTRACT

Objective To investigate the change rules of endogenous metabolites in pancreas of the diabetic rats, and to explore the mechanism of genipin treatment for diabetes rats. **Methods** Metabolomic method based on ^{1}H-NMR was applied, the diabetic rat model was prepared by ip injecting alloxan, and the high-, mid-, and low-dose genipin or metformin hydrochloride was ig injected as well as the rats in control and model groups were given the same volume of normal saline for 2 weeks. The pancreases of rats were collected and ^{1}H-NMR test was conducted, the metabolomic technology was adopted to analyze the endogenous metabolite changes in pancreas. **Results** The high-dose genipin possessed a better hypoglycemic effect, which could increase the contents of isoleucine, phosphatidylcholine, and phosphatidylethanolamine, and significantly reduce the contents of lactic acid, alanine, glutamine, aspartic acid, and creatine in pancreas of diabetic rats. **Conclusion** This study provided a theoretical basis for further exploration on the pathogenesis of diabetes and the mechanism of genipin for treatment of diabetes.

Key words

diabetes; genipin; metabolomics; nuclear magnetic resonance; pancreas

© 2016 published by TIPR Press. All rights reserved.

1. Introduction

Gardenia has been recorded in Chinese ancient book *Compendium of Materia Medica*. Ethnopharmacologically, gardenia has the effects of purging pathogenic fire and eliminating vexation, clearing heat and improving diuresis, cooling blood, and detoxifying (Liu et al, 2006), and used to treat five types of jaundice, smooth five shower, pass urine, quench thirst, and improve eyesight.

Genipin is the main component of the gardenia. Some scholars began to explore the efficacy of genipin in the treatment of diabetes and its complications. Our research group has studied the metabolomics of rat plasma by ^{1}H-NMR method, made the hypoglycemic effect of genipin clear and explored its mechanism (Tian et al, 2009). On the basis, this experiment studied the ^{1}H-NMR metabolomics of pancreas intervened by genipin (Jiang et al, 2012), attempted to figure out the metabolic pathways of genipin alleviating the symptoms of diabetes, so as to provide the scientific experimental basis for further study on the mechanism of genipin.

* **Corresponding author:** Tian JS Tel: +86-351-7019297 Fax: +86-351-7018379 E-mail: jstian@sxu.edu.cn
Qin XM E-mail: qinxm@sxu.edu.cn

Metabolomics is a fashionable systems biology method for study on the changes in body metabolites, this method comprehensively understand the process of pathological changes and the metabolic pathways of body material by revealing the changes of metabolites in the metabolism dynamic process, it shows its characteristics and advantages in the evaluation of experimental animal model, research of pathological mechanism and evaluation of drug efficacy (Tian et al, 2013). The technology has been widely used in the diagnosis and evaluation of diabetics, it can make a systemic response to subtle metabolic changes, identify the potential biomarkers, provide the basis for the pathogenesis of diseases (Liu et al, 2012; Zheng et al, 2013).

In this article, we applied the metabolomics method based on ^{1}H-NMR data to investigate the change of endogenous metabolites in pancreas of diabetic rats, and to explore the mechanism of genipin in the treatment of diabetic rats.

2. Materials and methods

2.1 Drugs, reagents, and instruments

Genipin (> 98%) was supplied by Linchuanzhixin Biotechnology Co., Ltd. (China). Metformin Hydrochloride Capsules (0.25 g) were purchased from Tianheng Pharmaceutical Co., Ltd. (China). Alloxan (5 g) was purchased from Sigma (USA). D_2O was purchased from Merck Company in USA. Bruker 600-MHz Avance III NMR Spectrometer was purchased from Bruker Company (Germany).

2.2 Animals

SPF-grade male Sprague-Dawley rats (180–200 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (SCXK (Jing) 2011–2012). All animals were fed under the controlled conditions of 12 h-light/dark cycles (lights on at 8:00 a.m.), temperature of (24 ± 1) ^{o}C, and humidity of (45 ± 15)%. The animals were acclimated to the new environment for 1 week before experiments, with free access to food and water.

2.3 Treatment

Thirty rats were randomly selected and ip injected with alloxan to prepare the diabetic model, others (n = 6) were injected with the same volume of physiological saline as the control. After injected continuously for 3 d and fasted for 10 h, the blood-glucose was measured using the tail vein blood and the rats with fasting blood glucose (FBG) level higher than 16.70 mmol/L were considered as diabetic rats.

Diabetic rats were weighed and randomly divided into five groups: positive control (diabetic but not treated, DM), metformin hydrochloride (125 mg/kg body weight, positive group, YV), genipin high-, mid-, and low-dose (100, 50, and 25 mg/kg body weight, GH, GM, and GL) groups, and the control rats were served as negative control group (NC). The rats in medication groups were ig injectd with drugs every day, rats in DM and NC groups were injected with the same volume of physiological saline as control. The food and water were monitored daily to quantify the intake of food and water. In addition, all rats were weighed once a week.

2.4 Samples collection and preparation

Rats were ip injected with 20% urethane anesthesia on day 14, and dissected after the blood samples were collected. Then the pancreas of rats were collected and their appendiculate fat and other organizations were removed, it needed to clean the surface with physiological saline water and weight the viscera, finally were cryopreserved at −80 ^{o}C refrigerator for further analysis.

The pancreas were weighed 200 mg and cut up in tin foil paper, then put in 5 mL centrifugal tube and added in 600 μL of methanol and 300 μL of distilled water. The mixture was transferred to 1.5 mL centrifugal tube after homogenizing for 4 min and then centrifuged in the high-speed refrigerated centrifuge at 4 ^{o}C and 13 000 r/min for 15 min. The supernatant was transferred to 1.5 mL centrifugal tube and blowed dry by nitrogen, then added in 650 μL phosphate buffer containing TSP (prepared with D_2O, pH 7.4) and centrifuged in the high-speed refrigerated centrifuge at 4 ^{o}C and 13 000 r/min for 20 min. A 600 μL aliquot of supernatant was added into a 5 mm NMR tube for ^{1}H-NMR analysis (Zhao et al, 2011; Shin et al, 2011). Data collected in the Bruker 600-MHz Avance III NMR spectrometer, using NOESYGPPR1D sequence, 1.0 s relaxation delay, 64 scans.

2.5 ^{1}H-NMR spectra data processing and analysis

The ^{1}H-NMR spectra were processed using MestReNova software (Mestrelab Research, Spain), the chemical shift of TSP 0 was considered as a standard to calibrate the chemical shift and adjust the baseline and the phase (Gao et al, 2007; Ghosh et al, 2012; Peng et al, 2014). In reference to literatures and to combine with Chenomx NMR Suite (Chenomx Inc., Canada) software the signal peak was affiliated, the spectra were divided and to the signal integral computed in 0.01 PPm across the region 0.50−9.00 PPm. The total sum of data were normalized prior to analysis, and then imported to SIMCA-P+13.0 software package (Sweden) for multivariate statistical analysis. R^2X and R^2Y describe the fitting condition of PLS-DA model, Q^2 described the predictable degree of the model, their size directly reflected the reliable degree of model, its value was bigger and more reliable. Biomarker data with differences were conducted by SPSS 17.0 software (USA), independent samples t test was used for statistics and comparison, and $P < 0.05$ was considered to have a significant difference.

3. Results

3.1 General description on biological traits

After ip injected alloxan, rats in GM group appeared

Download English Version:

https://daneshyari.com/en/article/3097860

Download Persian Version:

https://daneshyari.com/article/3097860

Daneshyari.com