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Short communication

Metabonomic study on the plasma of streptozotocin-induced diabetic rats treated with Ge Gen Qin Lian Decoction by ultra high performance liquid chromatography–mass spectrometry

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ABSTRACT

Changes in endogenous metabolites in the plasma of streptozotocin (STZ)-induced diabetic rats treated with Ge Gen Qin Lian Decoction (GGQLD) were studied. The endogenous compounds in plasma were detected using ultra high performance liquid chromatography coupled with quadrupole-time-of-flight tandem mass spectrometry (UHPLC-Q-TOF-MS). Rats were divided into three groups: control, model, and administration (4.95 g crude drug/kg body weight). After the final administration, plasma samples from the three groups were analyzed using metabonomics. The three sample groups could be clearly distinguished. The administration group exhibited a distinct return to the levels of phytosphingosine and dihydrosphingosine of the control group according to the principal component analysis score, and the corresponding biomarkers were defined. Significant changes in endogenous metabolites, such as dihydrosphingosine, phytosphingosine, cholylglycine, and pantothenic acid, were identified in STZ-induced diabetic rats. These biochemical changes are associated with the metabolism of sphingolipids, fats, and acetyl coenzyme-A, which could be useful to further investigate the characteristics of STZ-induced diabetes mellitus and the therapeutic mechanism of action of GGQLD. This metabonomic analysis could provide a useful starting point toelucidate the therapeutic effects and mechanism of action of GGQLD in diabetes mellitus.

1. Introduction

Diabetes mellitus (DM) is one of the most common chronic metabolic diseases. DM can be caused by hereditary and environmental factors. More than 90% of cases are type 2 (T2DM) [1].

Ge Gen Qin Lian Decoction (GGQLD) is composed of four herbs: *Pueraria lobata* (Willd.) Ohwi (Ge-Gen), *Scutellaria baicalensis* Georgi (Huang-Qin), *Coptis Chinensis* Franch (Huang-Lian), and *Glycyrrhiza uralensis* Fisch (Gan-Cao). *Pueraria* isoflavones and the compatibility of wine-steamed *Rhizoma coptidis* alkaloids of GGQLD have been employed to improve glucolipid metabolic disorders and insulin resistance (IR) [2]. In recent years, studies have shown that GGQLD has significant hypoglycemic effects during T2DM treatment. The therapeutic activities of GGQLD for DM have been investigated *in vivo* and *in vitro* [3,4]. In animal experiments, GGQLD exhibited antilipidemic effects in a high-fat diet-induced model of hyperlipidemia. The hypoglycemic effects of GGQLD were confirmed in a dexamethasone-induced model of IR and a streptozotocin (STZ)-induced model of T2DM in rats in which it improved glucose tolerance. GGQLD additionally improves high-fat diet-induced IR and reduces the expression of inflammatory cytokines. Hence, GGQLD promotes the reduction/delay of DM [5,6]. Additionally, a recent report demonstrated structural alterations in gut microbiota with the alleviation of type 2 diabetes with GGQLD [7].

As a part of systems biology, metabonomics has been successfully applied to DM research [8–10] and has been demonstrated as a feasible and powerful tool for the identification and quantitation of biomarkers. In recent years, metabonomics has been widely used in many fields [11–13], such as studying the mechanism of "hot symptoms" in TCM [14]. In particular, metabonomics has been used to

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evaluate the curative effects and mechanism of action of Chinese herbs [15].

The metabonomic study of GGQLD as a T2DM treatment in rats based on nuclear magnetic resonance (NMR) has been reported [16], and the related metabolic pathway has been described. However, the mechanism of action remains unclear. The present metabonomic study employed ultra high performance liquid chromatography coupled with quadrupole-time-of-flight tandem mass spectrometry (UHPLC-Q-TOF-MS) to investigate the GGQLD treatment of STZ-induced diabetic rats. We aimed to identify the biomarkers of STZ-induced diabetic rats treated with GGQLD and to determine the affected pathways. Our data may provide new insights into the anti-DM activity of GGQLD.

2. Materials and methods

2.1. Reagents

HPLC-grade methanol and acetonitrile were purchased from Tedia (Fairfield, OH, USA). HPLC-grade formic acid was obtained from Dikma (Richmond Hill, NY, USA). Deionized water was used. Our research team prepared GGQLD and performed quality control as described by Zhang et al. [17]. The fingerprint of Gegen Qinlian Decoction for this study can be found in Supplementary materials.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.jpba.2015.12.031.

2.2. Animals

Animal studies were performed according to the *Principles of Laboratory Animal Care* (World Health Organization, Geneva, 1985). Eighteen male Sprague–Dawley rats (120–160 g) were purchased from the Hunan Slac Jynda Laboratory Animal Company (Hunan, China). The rats were housed in a specific pathogen-free breeding room (temperature: 20 ± 2 °C; humidity: 60 ± 5%; 12 h light–dark cycle). All of the rats were provided with free access to tap water.

2.3. Animal model and drug administration

The rat grouping and drug administration have been described by Zhang et al. [17]. The control and diabetic rats were randomly divided into 3 groups: the control group (6 rats treated with saline in a matched volume), diabetic model group (6 diabetic rats treated with saline in a matched volume), and diabetic Gegen Qinlian Decoction group (6 diabetic rats treated with 4.95 g of GGQLD crude drug/kg body weight). After the final administration, the rats fasted for 12 h with free access to water and then were anesthetized with ether. Blood samples from the three groups were obtained from the cardiac apex, placed in Eppendorf (EP) tubes with 0.15 mg of heparin sodium, and immediately refrigerated (4 °C). After 2 h, the tubes were centrifuged (3,000 rpm, 10 min, 4 °C), and the supernatants were quickly collected and stored at −80 °C until analysis.

2.4. Sample preparation

Frozen plasma samples were thawed at room temperature. Then, 50 μL of sample was placed in EP tubes, and 150 μL of methanol was added. The tubes were vortexed for 1 min, incubated for 3 h at 4 °C, and then centrifuged (15,000 rpm, 10 min, 4 °C). The supernatants were collected and dried with nitrogen, and the residues were reconstituted in 100 μL of methanol:water (15:85). Then, the samples were vortexed for 1 min and centrifuged (18,000 rpm, 15 min, 4 °C). The supernatants were collected for UHPLC-Q-TOF-MS analysis.

2.5. UHPLC-Q-TOF-MS conditions

UHPLC analysis was performed on a 1290 LC system (Agilent Technologies, Santa Clara, CA, USA), and chromatographic separation was performed on an Agilent Zorbax Extend-C_{18} column (2.1 × 100 mm; 3.5 μm). A mobile phase of 0.1% formic acid (solvent A) and acetonitrile (solvent B) was used. The initial mobile phase was 98% B and was then changed to 92% B in 2 min,

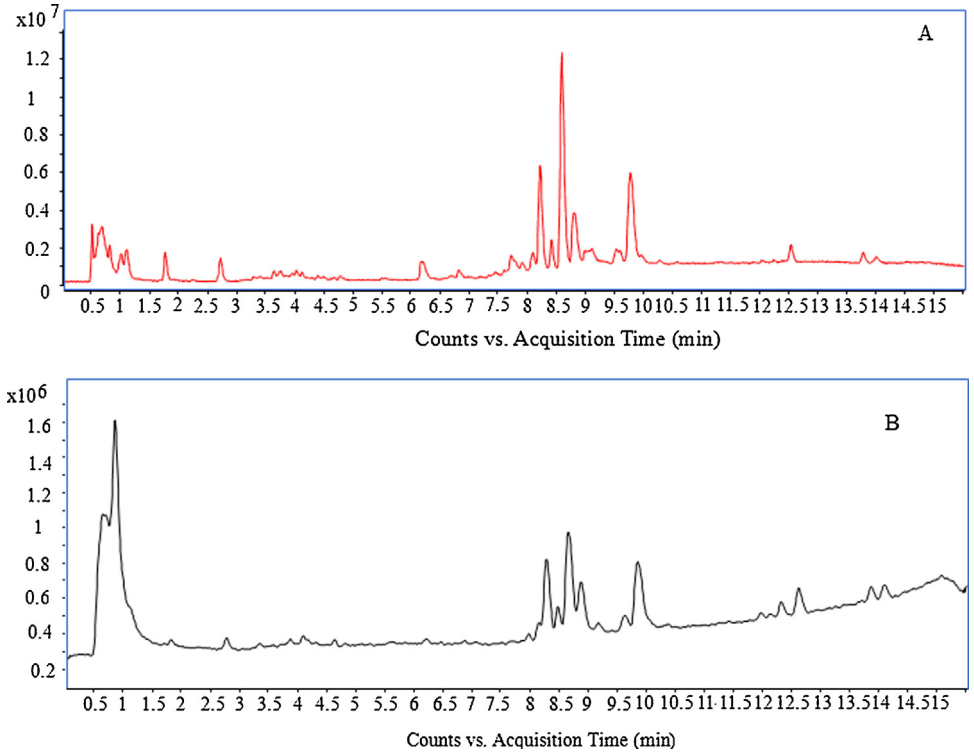

Fig. 1. Typical TIC chromatogram obtained from the same plasma of a rat with positive (A) and negative (B) mode.