Journal of Chromatography B, 1026 (2016) 272–278

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Effect of a traditional Chinese medicine prescription Quzhuotongbi decoction on hyperuricemia model rats studied by using serum metabolomics based on gas chromatography–mass spectrometry

Jiao Chen[a,1], Jia Zhou[a,1], Shuangshuang Wei[a], Zhijun Xie[a], Chengping Wen[a,**], Guowang Xu[b,*]

[a] College of Basic Medical, Zhejiang Chinese Medical University, 548 Binwen Road, Hangzhou 310053, China
[b] Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian 116023, China

ARTICLE INFO

Article history:
Received 30 July 2015
Received in revised form 19 October 2015
Accepted 22 October 2015
Available online 21 November 2015

Keywords:
Hyperuricemia
TCM
Metabolomics
Serum
GC–MS
Uric acid

ABSTRACT

Morbidity of hyperuricemia has constantly increased in population in decades, and hyperuricemia has proved to be an important risk factor for gout, cardiovascular diseases and others. Many urate-lowering drugs have unfavorable side effects and drug interactions. Quzhuotongbi decoction (QZTBD) is an empirical traditional Chinese medicine prescription for clinical therapy of hyperuricemia without serious adverse effects. In the study, we investigated the effects of QZTBD on urate and other metabolites in the sera of hyperuricemia model rats. Hyperuricemia model was established by orally administering yeast extract paste, and allopurinol served as a positive control drug. Serum metabolomics was performed by using a gas chromatography–mass spectrometry (GC–MS) method. Student's *t*-test and the principal component analysis (PCA) were employed to find the metabolic perturbations in hyperuricemia model rats. The levels of urate, lactate, pyruvate and ornithine were significantly increased, and xanthine, glyconic acids (ribonate, galactonate), amino acids (aspartate, proline, glutamine, serine, pyroglutamate, glutamate) and glucose were down-regulated greatly in the model rats. It demonstrated that nucleotide metabolism, amino acid metabolism and glycolytic pathway were disturbed by yeast administration. An orthogonal signal correction-partial least-squares discriminant analysis (OSC-PLS DA) was performed to assess the effects of yeast administering and drug treatment. 11 significantly distinctive metabolites among four groups were defined according to the variable importance for project values (VIP > 1) and univariate ANOVA (p value < 0.05). As compared to the model rats, the serum uric acid levels were lowered markedly under the treatment of allopurinol or QZTBD. Aspartate and glutamine involved in purine metabolism, were raised to normal level as well. The different influences on xanthine, glutamate pyroglutamate and galactonate suggested there were different mechanisms of two drugs in urate-lowering therapy. Our finding proved that QZTBD can efficiently lower the level of serum uric acid in a different way from allopurinol, which suggested that QZTBD based on the theory of TCM could be an effective therapeutic option for hyperuricemia.

1. Introduction

Hyperuricemia is defined as the serum urate concentration exceeding saturation point in extracellular fluid [1]. It has become more and more common due to its high and increasing morbidity among different populations [2,3]. The frequency of hyperuricemia has gradually increased over the past decades [4]. It is significantly correlated with sex and age, and occurs mainly in men and postmenopausal women.

Centuries ago, hyperuricemia was found to be the most significant risk factor of gout which is one of the oldest-known diseases [5], and it was the serological index of gout in the American Rheumatic Association (ARA) 1977 revised criteria for gout.

* Corresponding author. Fax: +86 411 84379559.
** Corresponding author. Fax: +86 571 86613131.
E-mail addresses: wengcp@163.com (C. Wen), xugw@dicp.ac.cn (G. Xu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jchromb.2015.10.031
1570-0232/

Besides, elevated urate concentration would cause kidney dysfunction [6]. Numerous studies found that a large number of patients with hypertriglyceridemia, hypertension, type 2 diabetes, obesity (especially visceral) and insulin resistance often acompanied with hyperuricemia [7–12].

In humans, urate is the metabolic end-product of purine. The accelerated urate formation and decreased renal uric acid excretion result in hyperuricemia [13]. The high incidence of hyperuricemia is partly attributed to environmental factors such as modern-day diet rich in purines and fructose [14]. The mechanism of anti-hyperuricemia relies on dietary and lifestyle management and pharmacological treatment. Drug treatment mainly includes: (i) inhibition of urate production, such as allopurinol or febuxostat; (ii) promotion of the excretion of uric acid; (iii) pegloticase for uricase replacement. However, only a few drugs are effective for hyperuricemia and many urate-lowering treatments have unfavorable side effects and drug interactions especially in the patients complicated with other diseases [15]. Allopurinol is recommended as the standard agent for lowering urate level in most countries [16–18], but what is clear is that allopurinol has the adverse reactions such as gastrointestinal symptoms and rash. In addition, allopurinol occasionally induced allergic responses called as allopurinol hypersensitivity syndrome (AHS) which could be life-threatening [19]. There is a growing need to find more effective and safe alternate therapies in lowering the serum urate level to manage hyperuricemia.

Traditional Chinese medicine (TCM) has a good effect on the treatment of hyperuricemia and inhibition of serious adverse effects. More and more Chinese herbs and extracts have been proven effective in hyperuricemic control and gout treatment [20–22]. Quzhuotongbi(QZTB) treatment is an important method in hyperuricemia therapy based on the principle of TCM. Quzhuotongbi decoction (QZTBD) in our study is an experience prescription, and previous studies have demonstrated its definite clinical effects on urate lowering therapy [23].

Animal model is enormously valuable in hyperuricemia study. Injection with uric acid, high purine food, uric acid precursors or urate oxidase inhibitor can increase the level of urate. The high purine diet is a primary cause of hyperuricemia. Yeast is rich in protein, vitamin B, nucleic acid and so on. Previous study found oral administration with yeast could successfully establish a hyperuricemia model [1], which was similar to the high-protein diets induced hyperuricemia in human beings. High yeast feed caused obvious elevated serum urate and maintained for a relatively long time which is suitable for the pharmacodynamics study of TCM.

Metabolomics is a newly arisen branch of systems biology. It focuses on the changes of small molecule metabolites induced by environmental stimuli or perturbation [24]. It pays great attention to the comprehensive analysis of metabolites in an organism which coincides with the holism concept of TCM. Metabolomics is playing an important role in studying the effect of TCM on many diseases [21,25,26]. As a commonly applied metabolomics method, gas chromatography–mass spectrometry (GC–MS) has high-resolution and high-sensitivity. Compared with other methods, GC–MS has a distinct advantage in qualitative analysis of metabolites which is attributed to its more comprehensive database of standard mass spectra.

To investigate the effect of QZTBD on hyperuricemia, animal model with hyperuricemia was established by yeast feed, and allopurinol was served as a positive control drug. A GC–MS method was employed to obtain the serum metabolic signatures of experimental rats. The discriminatory metabolites in model rats were defined by statistical analysis. The effects of QZTBD and allopurinol on metabolic profiles were further evaluated to explore the different mechanism in urate-lowering therapy.

2. Materials and methods

2.1. Reagents

Chromatographic grade methanol was provided by Tedia (Fairfield, OH, USA). Methoxyamine, methyl-trimethyl-silyl-trifluoroacetamide (MSTFA), pyridine and standard compounds for metabolites of identification were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ultra-pure water was provided by a Milli-Q system (Millipore Corp, Billerica, MA, USA).

All the herbs were purchased from Medical Pieces Co., Ltd., of Zhejiang Chinese Medical University (Hangzhou, Zhejiang, China). Allopurinol was brought from Chongqing Kerui Pharmaceutical Co., Ltd., (Chongqing, China). Yeast extract paste was obtained from Shanghai Juyuan Biotech Co., Ltd., (Shanghai, China). Uric acid test kit was acquired from the Ningbo Meikang Biotech Co., Ltd. (Ningbo, Zhejiang, China).

2.2. TCM preparation

QZTBD was composed of *Glabrous Greenbrier Rhizome* (60 g), *Dioscorea septemloba Thunb* (30 g), *Maydis stigma* (15 g), *coix seed* (30 g), *Alismatis rhizome* (15 g), *Humulus scandens* (15 g), *Parasitic loranthus* (15 g), *Herba Siegesbeckiae* (18 g), *turmeric* (12 g), *Corydalis Rhizoma* (18 g) and *Citrus medica* (12 g). Based on the traditional decoction method, all herbs were firstly soaked in 10 times distilled waters of their total weight for 1 h, then heated to boiling and kept for another 1 h. The filtered extract was concentrated to the required concentration (2.5 g/ml) using a rotary evaporator at 50 °C.

2.3. Animal model establishment and drug treatment

Twenty adult male SD rats weighing 240 ± 30 g, provided by Zhejiang Academy of Medical Sciences (Hangzhou, Zhejiang, China), were randomly divided into four groups: the blank control group (control), hyperuricemia model group (model), allopurinol treated group (XY) and QZTBD treated group (TCM). After a week of adaptive feeding, three groups were fed with 10% yeast extract paste (7.5 g/kg/d) diet, while the blank control group was given a control diet.

In order to evaluate the therapeutic efficacy, the administration of allopurinol and QZTBD was initiated on the 3rd day and continued for another 5 weeks. Unlike human, rodents can express the urate oxidase which induces the oxidation of urate [27]. Thus, rats in model and drug groups were fed by yeast extract paste diet in the whole process. Per day, rats in XY group were treated with 10 mg/kg of allopurinol, TCM group received QZTBD extract (2.5 g/mL) at the dose of 1 mL/100 g intragastrically, the control and model groups were given distilled water accordingly (1 mL/100 g). The dosages of drugs were calculated based on the weight of rats which were measured every three days.

Blood was collected from the eye socket vein in each rat on day 0 and 24 h after the last drug administration. After centrifugation at 1300× *g* for 10 min, the serum was isolated and stored at −80 °C for detection.

2.4. Serum uric acid assay

Serum uric acid (sUA) levels were measured based on an enzymatic-colorimetric method by using a standard test kit on a TBA-40FR automatic biochemical analyzer (Toshiba Medical Systems Co., Ltd., Tokyo, Japan).