Original Article

Urine metabolic changes in rats after tripterygium wilfordii poisoning

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Abstract: Tripterygium wilfordii (Hook. f.) is a woody vine of the Celastraceae family, and native to China (south of the Yangtze River), Korea, and Japan, is used in the clinic for the treatment of inflammatory and autoimmune diseases, especially rheumatoid arthritis. In this study, we developed a urine metabolomic method by gas chromatography-mass spectrometry (GC-MS) to evaluate the effect of Tripterygium wilfordii poisoning on rats. The Tripterygium wilfordii group rats were given 0.5 and 1.0 g/kg (Low, High) of Tripterygium wilfordii by continuous intragastric administration each day for 7 days. Partial least squares-discriminate analysis (PLS-DA) revealed that Tripterygium wilfordii induced metabolic perturbations. Compared to the control group, increased L-glutamine, arabinitol, L-ornithine, arabinofuranose, xylonic acid, benzoic acid, pentitol, cinnamic acid, galactonic acid of Low group; while increased D-gluconic acid, arabinofuranose, glucaric acid, benzoic acid, galactonic acid of High group. The results indicate that metabolomic method by GC-MS may be useful to elucidate Tripterygium wilfordii poisoning.

Keywords: Metabolomics, GC-MS, Tripterygium wilfordii, poisoning, urine, rat

Introduction

Tripterygium wilfordii Hook F, that is core to traditional Chinese herbal medicine, was praised for its possible anti-inflammatory properties in ancient traditional scripts that date back thousands of years. Tripterygium Wilfordii is a typically traditional Chinese medicine with complex chemical constituents and a variety of pharmacological effects [1-3]. The diterpenoid epoxide triptolide and the quinone triterpene celastrol are two important bioactive ingredients extracted from Tripterygium wilfordii that show a divergent therapeutic profile and can perturb multiple signal pathways. Both compounds promise to turn traditional medicines into modern drugs [4, 5].

With tremendous potentiality, Tripterygium Wilfordii has been used in inflammation or overactivity of the immune system, including rheumatoid arthritis, multiple sclerosis, and lupus. Although Tripterygium Wilfordii contains various pharmaceutical components and obvious effects, its underlying liver and kidneys side effects have largely restricted its wide application [6-8]. According to statistics, Tripterygium Wilfordii ranks third in the list of toxicity of Chinese herbal medicine [9]. However, its toxic components are also considered as effective components.

Currently, metabonomics is widely used in researches of the toxicology. Metabonomics refers to a holistic and dynamic analytical approach to all the low relative molecular mass metabolites in an organism or cells [10-15]. Nowadays, the vigorous advance on modernization of Chinese materia medica (CMM) has achieved great success. In the modernization of Chinese materia medica research, the application of metabonomics methods has a broad potential for future development. The technique of gas chromatography-mass spectrometry (GC-MS) is a selective, sensitive and reliable, and is therefore considered a “gold standard”
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The aim of the study is to examine and document fluctuations in urine metabolites in response to drug toxicity, to develop and validate a method that allows the detection and quantification of *Tripterygium wilfordii* in urine specimens by GC-MS. Not only that, research on toxic mechanism of *Tripterygium wilfordii* is helpful to develop preparations of high efficiency and low toxicity, and provide a guidance for rational drug use in clinical practice.

Material and methods

**Chemicals and animals**

Trimethylchlorosilane (TMCS) and N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) were purchased from Sigma-Aldrich (Shanghai, China). HPLC-grade acetonitrile and n-heptane were purchased from Tedia Reagent Company (Shanghai, China). Methylhydroxylamine hydrochloride and pyridine were purchased from Aladdin Industrial, Inc. (Shanghai, China). Sprague-Dawley rats (male, 220±20 g) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd.

**Tripterygium wilfordii decoction**

These raw materials (*Tripterygium wilfordii Hook. F*) were obtained from the First Affiliated Hospital of Wenzhou Medical University, China, and stored in an environment of normal atmospheric pressure and decoction at 100°C for 30 minutes, and then the residues were discarded, the final decoction concentration was fixed at 2.0 g/mL. The decoction was stored at 4°C.

**Instrumentation and conditions**

Agilent 6890N-5975B GC/MS, HP-5MS (0.25 mm×30 m×0.25 μm), were from Agilent Company (Santa Clara, California, USA). Ma-
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Sample preparation

The 250 µL of acetonitrile was added to 100 µL of urine, kept in an ice-bath for 15 min, and then were centrifuged at 10000 g for 10 minutes at 4°C. The 150 µL of the supernatant was transferred to a GC vial and evaporated to dryness. Methoximation was carried out at 70°C for 24 h after 50 µL of methylhydroxylamine hydrochloride (15 mg/mL in pyridine) was added. The 50 µL MSTFA (with 1% TMCS as the catalyst) was added and kept at 70°C for another hour, and then vortexed after adding 150 µL n-heptane [21].

Metabolomics study

Rats were housed under a natural light-dark cycle conditions with controlled temperature (22°C). All rats were housed at Laboratory Animal Research Center of Wenzhou Medical University. All experimental procedures were approved ethically by the Administration Committee of Experimental Animals of Wenzhou Medical University.

Thirty rats (220±20 g) were randomly divided to Tripterygium wilfordii group (Low, High) and control group. Tripterygium wilfordii group were given Tripterygium wilfordii (0.5 g/kg for Low dosage group, 1.0 g/kg for High dosage group, each dosage was 10 rats) by continuous intragastric administration for 7 days. Control group were given saline by continuous intragastric administration for 7 days.

Urine samples were collected from the rats from the Tripterygium wilfordii group and con-
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Data analysis

The GC-MS data was exported into Microsoft Excel, with the peaks normalized to the total sum of spectrum prior to multivariate analyses. The resulting data was processed through principal component analysis (PCA) and partial least squares discriminate analysis (PLS-DA) using SIMCA-P 11.5 software (Umetrics, Umea, Sweden).

Statistical analysis

Statistical analysis was carried out using SPSS software (Version 18.0, SPSS). Independent samples T-test was applied in order to detect significant differences in all metabolites between two groups. A P value of < 0.05 was considered statistically significant.

Table 1. Summary of the changes in relative levels of metabolites in rat urine after Tripterygium wilfordii poisoning

<table>
<thead>
<tr>
<th>NO</th>
<th>Renten/m</th>
<th>Metabolite</th>
<th>VIP</th>
<th>Dosage group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>time/min</td>
<td></td>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>1</td>
<td>17.2933</td>
<td>Tetradecanoic acid</td>
<td>4.47074</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>17.9651</td>
<td>L-Glutamine</td>
<td>4.13882</td>
<td>↑,**</td>
</tr>
<tr>
<td>3</td>
<td>19.9924</td>
<td>Uric acid</td>
<td>3.74723</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>18.855</td>
<td>D-Gluconic acid</td>
<td>2.61717</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>17.2343</td>
<td>Pentaric acid</td>
<td>2.48823</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>14.421</td>
<td>Pentanedioic acid</td>
<td>1.936</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>16.1011</td>
<td>Arabinitol</td>
<td>1.7693</td>
<td>↑,*</td>
</tr>
<tr>
<td>8</td>
<td>14.929</td>
<td>L-Omithine</td>
<td>1.51666</td>
<td>↑,*</td>
</tr>
<tr>
<td>9</td>
<td>22.127</td>
<td>Arabinofuranose</td>
<td>1.45091</td>
<td>↑,*</td>
</tr>
<tr>
<td>10</td>
<td>18.5581</td>
<td>Glucaric acid</td>
<td>1.23818</td>
<td>↑,*</td>
</tr>
<tr>
<td>11</td>
<td>18.1071</td>
<td>Xylonic acid</td>
<td>1.21924</td>
<td>↑,*</td>
</tr>
<tr>
<td>12</td>
<td>14.1191</td>
<td>Benzoic acid</td>
<td>1.1791</td>
<td>↑,*</td>
</tr>
<tr>
<td>13</td>
<td>14.017</td>
<td>Pentitol</td>
<td>1.14513</td>
<td>↑,**</td>
</tr>
<tr>
<td>14</td>
<td>15.764</td>
<td>D-Fructose</td>
<td>1.10604</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>13.912</td>
<td>Cinnamic acid</td>
<td>1.07213</td>
<td>↑,**</td>
</tr>
<tr>
<td>16</td>
<td>14.8</td>
<td>Myristic acid</td>
<td>1.03675</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>17.784</td>
<td>Heptanedioic acid</td>
<td>1.02776</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>19.148</td>
<td>Galactonic acid</td>
<td>1.02757</td>
<td>↑,*</td>
</tr>
</tbody>
</table>

Note: Variable importance in the projection (VIP) was acquired from the PLS-DA model with a threshold of 1.0. Marks indicate the direction of the change, i.e. ↑ for increase, - for no change. Compared control group with Tripterygium wilfordii group (0.5, 1.0 g/kg, Low, High), *P < 0.05 and **P < 0.01, as indicated by the statistical analysis T-test.

Results and discussion

Metabolomics study

Figure 1 provides the typical metabolic profiles of urine acquired through GC-MS technique. Metabolic profile data pretreatment resulted in a final dataset consisting of seventy-two metabolic features from GC-MS analyses. The six quality control samples first investigated the reproducibility of the metabolic features. The GC-MS analysis showed that more than 70% of the seventy-two metabolic features had a CV% (coefficient of variance) of no more than 30%. The endogenous metabolites in the urine were identified using the NIST 2005 mass spectrometry database.

In order to explore the metabolic profile changes of Tripterygium wilfordii in rats after different dosage (0.5, 1.0 g/kg, Low, High), we compared the GC-MS spectrum of PCA of the Tripterygium wilfordii group (Low, High) with the rats in the control group (Figure 2A), the corresponding load diagram was shown in Figure 2B. We compared the GC-MS spectrum of PLS-DA of the Tripterygium wilfordii group (Low, High) with the rats in the control group (Figure 3A, Figure 3B), and PLS-3D result was shown in Figure 3C. Figure 3A PLS-DA and Figure 3C score chart showed that the first principal components of the rats in the Tripterygium wilfordii group (Low, High) were distinguished from the rats in the control group; the results of PLS-DA were better than PCA (Figure 2A).

Changes in metabolite

Tripterygium wilfordii is a widely used traditional Chinese medicine that exhibits anti-inflammatory and anti-rheumatoid arthritis activity [22-24]. However, the clinical application of Tripterygium wilfordii is limited by its narrow therapeutic window and severe toxicity on several organs including liver and kidney. For patients with inflammation and autoimmune diseases, Tripterygium wilfordii is recommended for oral administration under the guidance of a doctor. Metabolomics is a newly emerging field.
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omics approach to the investigation of metabolic phenotype changes induced by environmental or endogenous factors [25-31].

The identification of endogenous compounds that can be used as metabolic biomarkers of Tripterygium wilfordii poisoning would represent an alternative approach of significant importance to detect hidden effects. In this study, the changes of metabolites between Tripterygium wilfordii groups and their control group were shown in Table 1. Compared to the control group, increased L-glutamine, arabinitol, L-ornithine, arabinofuranose, xylonic acid, benzoic acid, pentitol, cinnamic acid, galactonic acid in rat urine of Low group; and increased D-gluconic acid, arabinofuranose, glucaric acid, benzoic acid, galactonic acid in rat urine of High group. These findings may be useful for new evidences in Tripterygium wilfordii poisoning study. Additional prospective studies will be required to better understand these observations.

Conclusion

In this article, these biomarkers (L-glutamine, arabinitol, L-ornithine, arabinofuranose, xylonic acid, benzoic acid, pentitol, cinnamic acid, galactonic acid, D-gluconic acid, glucaric acid) were the additional evidence for the Tripterygium wilfordii poisoning. We demonstrated that metabolomic methods based on GC-MS could provide a useful tool for exploring biomarkers to elucidate Tripterygium wilfordii poisoning.

Disclosure of conflict of interest

None.

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