Original Article

Metabolic changes in rats after intragastric administration of MGCD0103 (Mocetinostat), a HDAC class I inhibitor

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Abstract: MGCD0103, an isotype-selective HDACi, has been clinically evaluated for the treatment of hematologic malignancies and advanced solid tumors, alone and in combination with standard-of-care agents. In this study, we developed a serum metabolomic method based on gas chromatography-mass spectrometry (GC-MS) to evaluate the effect of intragastric administration of MGCD0103 on rats. The MGCD0103 group rats were given 20, 40, 80 mg/kg of MGCD0103 by intragastric administration each day for 7 days. Pattern recognition analysis, including both principal component analysis (PCA) and partial least squares-discriminate analysis (PLS-DA) revealed that intragastric administration of MGCD0103 induced metabolic perturbations. As compared to the control group, the levels of L-alanine, L-isoleucine, and L-leucine of MGCD0103 group decreased. The results indicate that metabolomic methods based on GC-MS may be useful to elucidate side effect of MGCD0103 through the exploration of biomarkers (L-alanine, L-isoleucine, and L-leucine). According to the pathological changes of liver at difference dosage, MGCD0103 is hepatotoxic and its toxity is dose-dependent.

Keywords: Metabolomics, GC/MS, MGCD0103, rat

Introduction

Histone deacetylation is an important epigenetic event implicated in the development and progression of cancer, by regulating the accessibility of DNA for gene expression and transcription. The basic repeating unit of chromatin is the nucleosome, composed of DNA wrapped around a core of histone proteins [1]. Histones of the nucleosome core can be acetylated and deacetylated depending on the opposing activities of enzyme families, histone deacetylases (HDACs), and histone acetyltransferases [2].

MGCD0103, an isotype-selective HDACi, has been clinically evaluated for the treatment of hematologic malignancies and advanced solid tumors, alone and in combination with standard-of-care agents. MGCD0103, a compound with favorable pharmacokinetic/pharmacodynamic profiles, showed mechanism-based antileukemia activity in a recent phase I trial and was also deemed tolerable in a subsequent advanced solid tumor trial [2-4]. Unlike SAHA, MGCD0103 is a nonhydroxamate isotypeselective HDAC inhibitor that targets HDAC isotypes 1 to 3 and 11 [5]. Preclinical studies showed that MGCD0103 has significant in vivo antitumor activity with low toxicity. Induction of histone acetylation in tumors by MGCD0103 has been observed to correlate with antitumor activity in mouse models with human tumor xenografts [6].

Metabolic profiling is a useful tool to study toxicity as it provides a unique mechanistic perspective on responses to toxic insult [7]. In recent years, metabolomics has been widely applied to uncover biomarkers [8] and metabolic fingerprint in drug discovery and clinical toxicology [9], especially to investigating systematic metabolic responses to toxins [10] and the associat-
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**Metabolomics study**

Rats were housed under a natural light-dark cycle conditions with controlled temperature (22°C). All forty 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.

Forty rats (220±20 g) were randomly divided to MGCD0103 group (Low, Medium, High) and control group. MGCD0103 group were give MGCD0103 (20, 40, 80 mg/kg, Low, Medium, High, each dosage was 10 rats) by continuous intragastric administration for 7 days. Control group were give saline by continuous intragastric administration for 7 days.

Blood samples were collected from the rats from the control group and intragastric administration of MGCD0103 group at 8:00 am after 2 days, respectively. The blood samples were collected and then centrifuged at 8000 g for 10 min at 4°C. The serum was stored at -80°C until measurement.

**Histopathology**

After metabolomics experiment, rats were deeply anesthetized with 10% chloral hydrate (i.p., 20 mg/kg). The liver were rapidly isolated and immersed in freshly prepared 4% w/v form-
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aldehyde (0.1 M phosphate buffers, pH 7.2) for 48 h and then embedded in paraffin. Then 4-µm-thick histologic sections were prepared and stained with hematoxylin and eosin by routine HE method. The morphological changes were observed under light microscope.

**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 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.

**Results and discussion**

**Metabolomics study**

Metabolomics, focusing on the low molecular weight endogenous metabolites in

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Figure 2. PLS-DA score results of rat serum samples (A), PLS-DA 3D score results of rat serum samples (B), after intragastric administration of MGCD0103 (20, 40, 80 mg/kg, Low, Medium, High), Control (Class 1), Low (Class 2), Medium (Class 3), High (Class 4); the corresponding load diagram (C).
biological samples, is one of the newest ‘omics’ [14]. Metabolomics is a newly emerging omics approach to the investigation of metabolic phenotype changes induced by environmental or endogenous factors [15-19]. It has shown promising results in healthcare fields, especially in disease diagnosis and drug-toxicity assessment, as reviewed recently [20, 21]. In drug-toxicity assessment, metabolomics is often concerned with finding toxicity-related biomarkers by investigating the changes in metabolic signatures induced by drug exposure [12, 22].

Figure 1 provides the typical metabolic profiles of serum acquired through GC-MS technique. Metabolic profile data pretreatment resulted in a final dataset consisting of eighty-four metabolic features from GC-MS analyses. The endogenous metabolites in the serum were identified using the NIST 2005 mass spectrometry database.

In order to explore the metabolic profile changes of MGCD0103 in rats after different dose (20, 40, 80 mg/kg, Low, Medium, High), we compared the GC-MS spectrum of PLS-DA of the MGCD0103 group (Low, Medium, High) with the rats in the control group (Figure 2). Figure 2A PLS-DA score chart showed that the first principal components of the rats in the MGCD0103 group (Low, Medium, High) were distinguished from the rats in the control group. PLS-DA 3D (Figure 2B) score chart showed that the rats in MGCD0103 group were distinguished from the rats in the control group clearer than 2D Figure 2A.

Morphological changes of liver

The hepatic lobule, central veins and portal areas can be recognized, liver cells are arranged

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**Table 1.** Summary of the changes in relative levels of metabolites in rat serum after intragastric administration of MGCD0103

<table>
<thead>
<tr>
<th>NO.</th>
<th>Renten time/min</th>
<th>Metabolite</th>
<th>VIP</th>
<th>Dose group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>1</td>
<td>6.07094</td>
<td>Propanoic acid</td>
<td>2.60761</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>9.84831</td>
<td>Urea</td>
<td>2.11435</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>6.98521</td>
<td>L-Alanine</td>
<td>2.0555</td>
<td>↓*</td>
</tr>
<tr>
<td>4</td>
<td>9.2661</td>
<td>L-Norvaline</td>
<td>1.81848</td>
<td>↓**</td>
</tr>
<tr>
<td>5</td>
<td>18.3681</td>
<td>d-Mannose</td>
<td>1.60464</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>12.103</td>
<td>L-Threonine</td>
<td>1.39673</td>
<td>↓**</td>
</tr>
<tr>
<td>7</td>
<td>10.635</td>
<td>L-Isoleucine</td>
<td>1.36472</td>
<td>↓*</td>
</tr>
<tr>
<td>8</td>
<td>10.3924</td>
<td>Glycerol</td>
<td>1.35913</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>13.8671</td>
<td>L-Proline</td>
<td>1.25322</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>11.705</td>
<td>L-Serine</td>
<td>1.21188</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>7.38242</td>
<td>Glycine</td>
<td>1.18009</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>10.273</td>
<td>L-Leucine</td>
<td>1.17188</td>
<td>↓*</td>
</tr>
<tr>
<td>13</td>
<td>16.076</td>
<td>L-Lysine</td>
<td>1.14131</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>14.961</td>
<td>Butanoic acid</td>
<td>1.09059</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>20.157</td>
<td>Inositol</td>
<td>1.05507</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 decrease, ↑ for increase, - for no change. Compared control group with MGCD0103 group (20, 40, 80 mg/kg, Low, Medium, High), *P<0.05 and **P<0.01, as indicated by the statistical analysis T-test.
as funicular along with central veins, the liver cells become slightly edema, in low dosage group (Figure 3A). The structure of liver lobule can be recognized, in high dose group (Figure 3B). A plenty of steatosis of liver cells, and small, atrophy, hyperchromatic karyopyknosis with some dark blue fragment of nucleus in lobule are observed. According to the pathological changes of liver at difference dosage, MGCD-0103 is hepatotoxic and its toxity is dose-dependent.

**Changes in metabolite**

Metabolomics comprises the measurement of endogenous metabolites, including amino acids, nucleic acid precursors, lipids, and degradation products of chemical intermediates in catabolism and biosynthesis. The advantage of metabolomics is that it provides the most functional measure of cellular status and can help to describe an organism’s phenotype [23].

In this study, the changes of metabolites between MGCD0103 groups and their control group were shown in Table 1. Compared to the control group, the level of L-alanine, L-isoleucine, L-leucine of the MGCD0103 group decreased. Alanine plays a key role in glucose-alanine cycle between tissues and liver. In muscle and other tissues that degrade amino acids for fuel, amino groups are collected in the form of glutamate by transamination. Leucine is utilized in the liver, adipose tissue, and muscle tissue. In adipose and muscle tissue, leucine is used in the formation of sterols, and the combined usage of leucine in these two tissues is seven times greater than its use in the liver [24]. Leucine is the only dietary amino acid that has the capacity to stimulate muscle protein synthesis [25].

These finding may be useful for new evidences in MGCD0103 study. Additional prospective studies will be required to better understand these observations.

**Conclusion**

These biomarkers (L-alanine, L-isoleucine, L-leucine) were the additional evidence. According to the pathological changes of liver at difference dosage, MGCD0103 is hepatotoxic and its toxity is dose-dependent. We demonstrated that metabolomic methods based on GC/MS could provide a useful tool for exploring biomarkers to elucidate drug-toxicity.

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**Disclosure of conflict of interest**

None.

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