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Short communication

Plasma metabolic profiling analysis of nephrotoxicity induced by acyclovir using metabonomics coupled with multivariate data analysis

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ABSTRACT

Acyclovir (ACV) is an antiviral agent. However, its use is limited by adverse side effect, particularly by its nephrotoxicity. Metabonomics technology can provide essential information on the metabolic profiles of biofluids and organs upon drug administration. Therefore, in this study, mass spectrometry-based metabonomics coupled with multivariate data analysis was used to identify the plasma metabolites and metabolic pathways related to nephrotoxicity caused by intraperitoneal injection of low (50 mg/kg) and high (100 mg/kg) doses of acyclovir. Sixteen biomarkers were identified by metabonomics and nephrotoxicity results revealed the dose-dependent effect of acyclovir on kidney tissues. The present study showed that the top four metabolic pathways interrupted by acyclovir included the metabolisms of arachidonic acid, tryptophan, arginine and proline, and glycerophospholipid. This research proves the established metabonomic approach can provide information on changes in metabolites and metabolic pathways, which can be applied to in-depth research on the mechanism of acyclovir-induced kidney injury.

1. Introduction

Acyclovir (ACV) is a synthetic nucleoside analog known for its therapeutic effects on viral infections, especially those caused by the herpes simplex and herpes zoster viruses [1,2]. Unfortunately, the clinical use of ACV may result in adverse side effect, of which nephrotoxicity is one of the most severe [3]. ACV-induced nephrotoxicity largely affects the quality of patients' lives. Thus, finding approaches to reveal characteristic of ACV-induced renal injury is meaningful objective.

Metabonomics, a branch of systems biology belonging to the "omics" field, provides global metabolic profile information on biological samples, including cell-, tissue-, and organism-level samples, under a specific condition, such as pathophysiological stimulation, genetic modification, or environmental conditions [4–7]. Metabonomics focuses on small and low-weight molecules that are the final product of biological metabolite pathways and, as such, play an important role in metabolism [8]. Metabonomics has become a powerful method in the study of many fields, such as toxicology, disease diagnosis, and therapeutic efficacy [7,9]. It has been used in finding new biomarkers related to drug-induced renal injury [10,11].

In the present study, we used mass spectrometry-based metabonomics to determine biomarkers of nephrotoxicity induced by acyclovir, trying to find disturbed pathways related to ACV-induced kidney injury. The results found in this study may provide information for clinical use of acyclovir, and lay the foundation for further research on mechanism of ACV-induced nephrotoxicity.

2. Material and methods

2.1. Reagents and materials

Acetonitrile (HPLC-grade) was purchased from Oceanpak (Goteborg, Sweden). Formic acid was purchased from ROE (USA). Distilled water was obtained from Wahaha Company (Hangzhou, China). ACV was obtained from Jiangsu Hansoh Pharmaceutical Co., Ltd. (Lianyungang, China). Assay kits for BUN (blood urea

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nitrogen) and Scr (serum creatinine) were bought from the Biosino Bio-technology and Science Inc. (Beijing, China).

2.2. Animal treatment

Male Wistar rats weighing 200 ± 20 g were raised in an SPF-level lab and were acclimatized in metabolism cages for one week before drug administration. The animals were randomly divided into three groups: the control group (NS), the low-dose ACV group (LA), and the high-dose ACV group (HA). ACV was first dissolved in normal saline (0.9%, w/v) before administration. ACV was administered intraperitoneally to the rats daily for three consecutive days, and the dosages for LA and HA groups were set as 50 and 100 mg/kg. The control group was intraperitoneally administered with an equivalent volume of normal (0.9% saline) for three consecutive days. On the last day, 800 μL of blood was drawn from the intraorbital angular vein after slight anesthetization. After blood collection, the rats were sacrificed, and kidney tissues were immediately removed and stored in 10% formalin solution. Blood samples (including serum and plasma) were centrifuged at 3500 rpm for 15 min. The obtained serum was used for biochemical assay. Plasma samples were stored at −80 °C prior to metabonomics analysis. Kidney slices were stained with hematoxylin and eosin to observe pathological features using a light microscope at 200× magnification.

2.3. Metabonomics data acquisition

Data acquisition was performed by rapid-resolution liquid chromatography coupled with quadruple-time-of-flight mass spectrometry (RRLC-Q-TOF-MS) (Agilent, USA). The preparation of plasma was as follows: 100 μL thawed plasma was mixed with 300 μL of acetonitrile, ultrasonicated for 10 min in cold water, vortexed for 1 min, and centrifuged at 15,000 rpm for 15 min. A 10 μL aliquot of the supernatant was injected onto an ACQUITY UPLC HSS C_{18} column (2.1 × 100 mm, 1.7 μm; Waters). The column temperature was 40 °C, and the flow rate was 0.3 mL/min. The RRLC binary solvent system consisted of mobile phases A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). Gradient elution was adopted to obtain the whole profile of plasma metabolites. The gradient started with 1% B, then, 0–3 min, B: 1–52%; 3–7 min, B: 52–74%; 7–9 min, B: 74–80%; 9–10 min, B: 80–90%; 10–12 min, B: 90–99%; 12–16 min, B: 99–99%; 16–17 min, B: 99–1%; 17–20 min, B: 1–1%.

2.4. Metabonomics data processing

Multivariate data analysis, including principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA), was used to identify the plasma metabolites responsible for the differentiation of the treatment groups (NS, LA, HA). Multivariate data analysis was performed with SIMCA-P+ 11.5 software (Umetrics AB, Umea, Sweden). In our present study, metabolites that were far from the cluster in the s-plot and loading plots and with a VIP value >1.0 were chosen as biomarkers. An independent sample *t*-test was performed using SPSS 17.0 software to determine whether or not the chosen biomarkers were significantly changed.

2.5. Characterization of biomarkers

Candidates of metabolites were searched from human metabolome database (HMDB, http://www.hmdb.ca/) utilizing the detected m/z value. The biomarkers were unambiguously characterized by comparison with reference standards or MS/MS fragment information. The perturbation was interrogated using MetPA (http://metpa.metabolomics.ca./MetPA/faces/Home.jsp), which was an important tool in metabonomics pathway analysis.

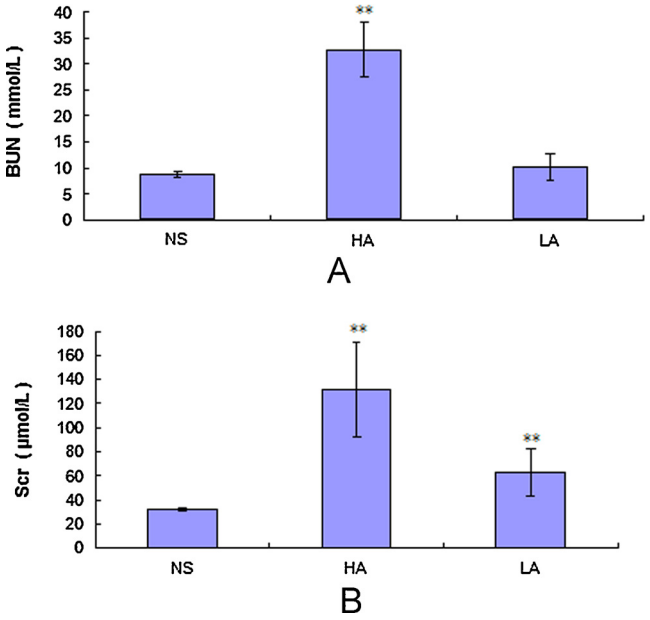

Fig. 1. Effect of ACV on BUN and Scr levels. (A) Changes in BUN levels. (B) Changes in Scr levels. Data represents mean ± SD (**$p < 0.01$, compared with the control group).

3. Results and discussion

3.1. Clinical chemistry and histopathological examination

The blood urea nitrogen (BUN) level significantly increased in rats exposed to the high dose of ACV but showed no obvious change in rats administered the low dose of ACV (Fig. 1A). Serum creatinine (Scr) activity significantly increased by both high and low doses of ACV (Fig. 1B). The detailed data of clinical chemistry was shown in Table S1. BUN and Scr are generally conventional monitor of nephrotoxicity and have been used as standard determiner of kidney injury for many years. When BUN and Scr level significantly elevate, it shows the kidney has been injured.

As shown in Fig. S1, the high dose of ACV caused apparent tubular dilation, irregular arrangement of cells, and development of interstitial inflammatory cells. Compared with the HA group, the LA group showed milder kidney damage, such as apparent tubular dilation, tubular epithelial edema, and irregular arrangement of cells.

Coupled with the results from the biochemical analysis, ACV-induced nephrotoxicity could be confirmed in rats of the HA and LA groups.

3.2. Metabolic profiling analysis

Typical total ion current (TIC) chromatograms of plasma samples were obtained from rats in the control and ACV-dosed groups in positive mode. Some discrimination was found in the TIC chromatograms in positive mode between NS, HA and LA groups (Fig. S2). PCA and PLS-DA was further applied to better visualize the discrimination among the gained complex data. Fig. 2A demonstrated distinctions between the different groups by PCA. The samples in the low-dose ACV group were located near those of the control group, whereas samples from the high-dose ACV group were found far from those of the control group. These data indicated that the nephrotoxicity induced by ACV was dose-dependent. The differentiation by PLS-DA between the LA and NS and HA and NS groups