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A metabolic profiling analysis of the nephrotoxicity of acyclovir in rats using ultra performance liquid chromatography/mass spectrometry

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ABSTRACT

Acyclovir (ACV) exposure is a common cause of acute kidney injury (AKI). The toxicity mechanism of ACV has always been a matter of debate. The present study investigated into the time-effect relationship and dose-effect relationship of ACV-induced nephrotoxicity in rats using metabonomics. Twenty-four rats were randomly divided into four groups: a 0.9% NaCl solution group, and 100, 300, and 600 mg/kg ACV-treated groups; the ACV or vehicle solution was administered with a single intravenous injection. Urine was collected at different time periods (12 h before administration, and 0–6 h, 7–12 h, and 13–24 h after administration). Routine urinalysis was conducted by a urine automatic analyzer. Renal markers, including urine urea nitrogen, urine creatinine, and urinary N-acetyl-β-D-glucosaminidase (NAG) activity, were determined using established protocols. Urinary metabolites were evaluated using ultra performance liquid chromatography/mass spectrometry (UPLC/MS). In the ACV-treated rats, increased levels of protein (PRO), occult blood (BLD), white blood cell (WBC), and NAG activity in urine were observed, while the urine creatinine and urea nitrogen levels showed a decrease compared with the control. Moreover, urine metabolites significantly changed after the treatment with ACV, and all the effects induced by ACV were dose–time dependent. Finally, 4 metabolites (guanine, 4-guanidinobutyric acid, creatinine, and urea) were identified, which can be used for further research on the mechanism of ACV-induced nephrotoxicity.

1. Introduction

Acyclovir [9-(2-hydroxyethoxymethyl) guanine] (ACV), which is an acyclic nucleoside, has been widely used for the treatment of viral infections, such as herpes simplex, herpes zoster, and hepatitis B. However, with the extensive clinical application of ACV, adverse drug reactions (ADRs), especially acute renal injury, have rapidly increased (Fleischer and Johnson, 2010; Obada et al., 2010). The Chinese State Food and Drug Administration has also issued a drug use warning for ACV (Monitoring, 2009). Therefore, evaluating its safety is imperative.

It has been widely believed that ACV-induced nephrotoxicity is secondary to crystalluria (Lyon et al., 2002; Sawyer et al., 1988). For example, as reported by Giustina, treatment with low-dose intravenous ACV (5 mg/kg/d for 2 days) caused acute renal failure (Giustina et al., 1988). In addition, there have also been several reports of ACV-induced nephrotoxicity with biopsy evidence of tubular damage in the absence of crystal formation (Ahmad et al., 1994; Vachvanichsanong et al., 1995; Vomiero et al., 2002). These data may suggest that ACV induces direct insult to tubular cells. In our previous work, we found that administration of ACV at a human clinical equivalent dosage (150 mg/kg) for 9 days led to renal dysfunction and an increase in both the serum creatinine and blood urea nitrogen levels and fibrous proliferation in the glomerulus and renal tubules(Lu et al., 2014). However, no crystal formation was found. Therefore, metabolomics is ideally suited to address this issue through overview the whole changes of the metabolism related to ACV-induced nephrotoxicity. This research results may support the viewpoint that ACV induces direct insult to renal tubular cells.

Metabolites considered to be relevant to kidney disease have been analyzed in a distinctly 'low-tech' manner by physicians since the Middle Ages (Weiss and Kim, 2012). Metabonomics is now defined as the quantitative measurement of the dynamic

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multi-parametric metabolic responses of living systems to pathophysiolo- gical stimuli or genetic modifications, which is widely used to characterize the biochemical patterns of the endogenous metabolites in cells, body fluids or tissues (Zhao, 2013). It found a series of molecules such as amino acids, lipids or nucleic acids, which provides an overview of metabolism related to disease or drug exposure. Recently, metabolomics has evolved into a valuable tool in nephrotoxicity, and play an important role in providing novel and specific biomarkers for renal injury (Zhao and Lint, 2014). Therefore, mang urinary and kidney metabolomics was performed in gentamicin, cisplatin, or tobramycin-induced nephrotoxicity (Boudonck et al., 2009). For example, in gentamicin-induced nephrotoxicity, amino acid and sugar excretion was increased prior to histopathologically evident kidney injury (Portilla et al., 2006; van de Poll et al., 2004).

In the present study, we studied the changes of renal damage indexes and urinary metabolic profiles in rats induced by ACV administration (in different concentrations and for different durations) used metabolomics technology to elucidate the time-effect relationship and dose-effect relationship of biomarkers to ACV-induced nephrotoxicity. These results may provide information for further study on the mechanism of ACV-induced nephrotoxicity.

2. Materials and methods

2.1. Animals and treatment

Twenty-four male SD rats (180–220 g) bred in the animal house of Zhejiang Experimental Animal Center were used for the experiment. All animals were housed in standard animal enclosures with a regulated temperature (22–24 °C), relative humidity (60–80%), and 12 h light/dark cycle. Food and tap water were provided ad libitum. All experiments were carried out according to the guidelines of China for the care and use of laboratory animals. Each rat was randomly assigned to 1 of 4 experimental groups (each $n = 6$): high-dose ACV (600 mg/kg, slightly higher than the clinical highest dosage for humans), medium-dose ACV (300 mg/kg), low-dose ACV (100 mg/kg, the human clinical equivalent dosage), and a control group. ACV was administered intravenously to the rats once. ACV (Wuhan Humanwell Pharmaceutical Co., Ltd., China) was diluted in a 0.9% sodium chloride injection solution (Huadong Pharmaceutical Co., Ltd., Hangzhou, China). The control group received an equal volume of 0.9% sodium chloride injection solution.

2.2. Collection and preparation of urine samples

The urine samples were collected at different time points (12 h pre-dose, and 0–6 h, 7–12 h, and 13–24 h after administration). Some samples were used for analyzing renal function, while the other samples were stored at −80 °C for the purpose of UPLC/MS analysis. Prior to the UPLC/MS analysis, 200 μl of urine samples was thawed and then added to 500 μl of double-distilled water. Urine samples were then centrifuged at 12 000 rpm for 8 min at 4 °C. Supernatant was transferred to a new tube and then filtered through 0.22 μm nylon filters for UPLC/MS analysis.

2.3. UPLC/MS analysis of urine samples

UPLC/MS analysis was performed using an Agilent 1260 infinity ultra performance liquid chromatograph coupled to an Agilent 6520 Q-TOF/MS spectrometer with a dual ESI source (Agilent Technologies, Santa Clara, CA, USA). The system used an Agilent Eclipse Plus C_{18} column (2.1 mm × 50 mm, 1.8 μm). The column was maintained at 35 °C, and a 5-μl aliquot of the sample was introduced to the column. The UPLC mobile phase consisted of 0.1% formic acid (Tedia, Fairfield, OH, USA) in water purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA) (solution A) or acetonitrile (Tedia, Fairfield, OH, USA) (solution B). The linear gradient increased from 2% to 10% B in 2 min, and increased to 45% B in another 9.5 min. Then solution B was continuously increased from 45% to 60% in 1.5 min, and increased to 90% B in 4 min. Finally, the mobile phase was kept at 90% B for 2 min. Mass spectra were obtained on full-scan operation in positive ion mode with an m/z range of 50–1000. The capillary voltage was set at 4000 V. The skimmer voltage was set at 60 V and the fragmentor voltage at 150 V. Nitrogen flow was 10 l/min, and the gas temperature was 350 °C.

2.4. Measurement of renal function

Blood was collected 24 h after administration from the abdominal aorta; the kidneys were extracted and weighed immediately after urine collection. The renal index was calculated as kidney weight/body weight × 100 (%). Blood samples were then centrifuged at 3000 rpm for 10 min at 4 °C, and urine samples were centrifuged at 5000 rpm for 6 min at 4 °C. The concentration of creatinine and urea nitrogen in urine and blood and the activity of NAG in urine were measured using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. Qualitative examination of urine was performed by a urine analyzer (AU-4290, Arkray, Japan), including urine specific gravity (SG), pH, WBC, nitrite (NIT), PRO, glucose (GLU), ketones (KET), urobilinogen (URO), bilirubin (BIL), and BLD.

2.5. Data analysis

Data were expressed as mean ± SD. Statistical analysis was performed using a t-test with SPSS data analysis software version 13.0. P-values ≤0.05 were considered significant.

For UPLC/MS, XCMS software (Fraga et al., 2010) was used for raw peaks extraction, data baseline filtering, and peak alignment, and a standard normalization method was used in this data analysis. The resulting three-dimensional data involving the peak number, sample name, and normalized peak area were fed into SIMCA-P (version 11.0, Sweden) for principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). For the identification of potential markers, once potential m/z was obtained, the biomarkers were first derived by searching comparison with free online databases, such as ChemSpider (http://www.chemspider.com), the Human Metabolome Database (HMDB) (http://www.hmdb.ca), Metlin (http://metlin.scripps.edu/) and Pubchem (http://pubchem.ncbi.nlm.nih.gov/). Finally, the biomarkers were further confirmed by retention times and MS/MS spectra.

3. Results

3.1. Renal biomarker changes in the ACV-treated rats

As shown in Fig. 1, ACV significantly decreased urine creatinine concentration as well as urine urea nitrogen concentration compared with the control ($P < 0.01$, $P < 0.05$). ACV-treated rats exhibited a significant increase in NAG activity compared with the control rats (Fig. 2A) ($P < 0.01$, $P < 0.05$). The most obvious changes were observed 0–6 h after administration. Moreover, all the effects induced by ACV were dose dependent. In addition, obvious changes in serum creatinine levels and blood urea nitrogen levels were also observed 24 h after ACV administration (Fig. 2C, D) ($P < 0.01$, $P < 0.05$). The renal index significantly increased in the ACV-exposed groups compared with the control (Fig. 2B, $P < 0.01$). These data suggested that ACV treatment induced renal damage in SD rats.