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ORIGINAL ARTICLE

UPLC-Q-TOF/MS based metabolomic profiling of serum and urine of hyperlipidemic rats induced by high fat diet

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Abstract Hyperlipidemia is considered to be a high lipid level in blood, can induce metabolic disorders and dysfunctions of the body, and results in some severe complications. Therefore, hunting for some metabolite markers and clarifying the metabolic pathways in vivo will be an important strategy in the treatment and prevention of hyperlipidemia. In this study, a rat model of hyperlipidemia was constructed according to histopathological data and biochemical parameters, and the metabolites of serum and urine were analyzed by UPLC-Q-TOF/MS. Combining pattern recognition and statistical analysis, 19 candidate biomarkers were screened and identified. These changed metabolites indicated that during the development and progression of hyperlipidemia, energy metabolism, lipid metabolism, amino acid metabolism and nucleotide metabolism were mainly disturbed, which are reported to be closely related to diabetes, cardiovascular diseases, etc. This study demonstrated that a UPLC-Q-TOF/MS based metabolomic approach is useful to profile the alternation of endogenous metabolites of hyperlipidemia.

1. Introduction

Hyperlipidemia is characterized by an increase in plasma total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and/or a decrease in high-density lipoprotein cholesterol (HDL-C) [1]. It was found that hyperlipidemia is a major risk factor for atherosclerosis and cardiovascular diseases, such as hypercholesterolemia and hypertriglyceridemia, which are the leading causes of death in the United States and other western countries. Emerging data establish dyslipidemia as a significant contributor to the development of diabetic neuropathy, and accumulated data from several large-scale trials of patients with type 2 diabetes also point to early dyslipidemia as a major independent risk factor for the development of diabetic neuropathy [2]. In addition, one study suggests that hyperlipidemia and thereby atherosclerosis are the leading causes of cardiac illness and deaths, in

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which circumstance heart attack and stroke are responsible for more deaths than all other causes combined [3]. Therefore, it is imperative for us to diagnose these diseases at an early stage in order to improve the clinical outcome and reduce mortality. The progression of diseases is mainly due to genetic determinants, environmental factors, and their interactions [4]. Diet, as an important environmental factor, plays a pivotal role in the development of this metabolic syndrome [5]. In order to improve the clinical outcomes of hyperlipidemia and the related chronic diseases mentioned above, it is necessary for us to deeply investigate the pathophysiology of hyperlipidemia. As hyperlipidemia is a systemic progressive disorder, alterations of endogenous metabolites may provide a prognostic index at the initial stage of this disorder compared with those of the putative lipid levels at the symptomatic stage.

Serum- or plasma-based biochemical assays and histopathological evaluation are commonly used in the study of hyperlipidemia [6]. However, these standard approaches may be inadequate. Metabolomic, as a sensitive and unbiased analytical method that assesses all metabolites in biological samples, has been widely used in the biomedical sciences [7]. This approach shows a significant potential for the diagnosis of human diseases [8], specification of genetic modifications [9], physiological evaluations [10], biomarkers screening [11], and drug toxicity/safety assessments [12]. Metabolomics focuses on the comprehensive measurement of small molecular weight compounds and visualizes abnormal, disease-related physiological states in whole samples. Moreover, it not only determines the relationships between phenotype and metabolisms, but also identifies the key metabolites associated with a particular phenotype and investigates the biological function and metabolic changes in the organism [13]. Up to now, the major two analytical platforms used in the metabolomic analysis have been nuclear magnetic resonance (NMR) [14] and mass spectrometry (MS), which are usually coupled to gas chromatography (GC) [4], liquid chromatography (LC), and capillary electrophoresis (CE) [15].

In the present study, we constructed a Sprague-Dawley (SD) rat hyperlipidemia model and applied an ultra-performance liquid chromatography and Q-TOF mass spectrometry (UPLC-Q-TOF/MS) based metabolomic approach together with histopathological examination and a biochemical assay to investigate the biochemical abnormalities associated with hyperlipidemia in both serum and urine. The present work, as a compensation for the results reported previously, will be more beneficial to profile the alternation of endogenous metabolites of hyperlipidemia, to dissect the underlying mechanisms related to hyperlipidemia and the strong association with atherogenesis progression. The findings of the metabolic pathways and potential biomarkers should be further explored for clinical prevention and treatment of hyperlipidemia and the related chronic diseases in order to advance the progression of amelioration in subjects with hyperlipidemia-associated conditions and to improve the clinical outcome for these hyperlipidemia-related conditions.

2. Materials and methods

2.1. Ethics statement

All animal treatments were strictly in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Animal experiments were approved by the Administrative Committee of the Experimental Animal Care and Use of Second Military Medical University (SMMU, License no. 2011023).

2.2. Reagents and materials

HPLC-grade methanol and acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany). Formic acid was obtained from Fluka (Buchs, Switzerland). Ultrapure water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). The following compounds were obtained from Shanghai Jingchun Reagent Co.: linoleic acid, succinic acid and taurine. Hippuric acid was purchased from Larodan AB (Malmoö, Sweden). Sphinganine and C16 Sphinganine were purchased from Acros Organics (New Jersey, USA).

2.3. Animal study

A total of 12 adult male Sprague-Dawley rats (180 ± 20 g) obtained from the SLAC Laboratory Animal Co., Ltd. (Shanghai, China) were maintained under standard laboratory conditions (temperature of 21–23 °C, relative humidity of 45–65%, and 12 h/12 h light/dark cycle) with aseptic food and tap water ad libitum. After one week of habituation, 6 rats, selected randomly, were fed a basic diet as the normal control group (NC), while the others were fed a high-fat diet (HFD) for 4 weeks during the experimental period. Before the end of the experiment, the rats were housed in metabolic cages and fasted overnight (allowed free access to water). Rat urine was collected in tubes. Then the rats were weighed and anesthetized with an intraperitoneal injection of 3% sodium pentobarbital (2 mL/kg). An abdominal incision was made to expose the liver and inferior vena cava. Blood (3–4 mL) was withdrawn from the abdominal aorta and collected in tubes. Serum was extracted by centrifugation at 3500 rpm for 15 min. The serum was divided into two parts, one for the lipid levels assay and the other for metabolomic analysis. The serum and urine were both stored at −80 °C before UPLC-Q-TOF/MS analysis.

2.4. Histopathological examination

After being weighed, the livers were washed with saline and put in a buffer solution of 10% formalin. Fixed tissues were processed routinely for paraffin embedding, and 4 μm sections were prepared and dyed with hematoxylin-eosin; the stained areas were viewed under an optical microscope at 200 ×. The hepatic index (HI) was estimated from the ratio of total liver weight to body weight.

2.5. Biochemical analysis

Serum was thawed and incubated at room temperature. Lipid levels, including total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C), were determined using an automatic biochemical analyzer. The arteriosclerosis index (AI) was calculated as follows:

$$\mathrm{AI} = \frac{\mathrm{TC} - \mathrm{HDL} - \mathrm{C}}{\mathrm{HDL} - \mathrm{C}} \quad (1)$$

2.6. Metabolomic study

2.6.1. Extraction of the metabolites in serum and urine

The serum samples were thawed at room temperature prior to analysis. Methanol (400 μL) was added to every 100 μL of serum. After vigorous shaking for 1 min and incubation on ice for 10 min, the mixture was centrifuged at 14,000 g for 15 min at 4 °C to precipitate the protein. The urine samples were thawed at room temperature prior to analysis. To reduce the effect of the solvent and get a good peak