Journal of Chromatography B, 870 (2008) 178–185

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

A metabonomic characterization of CCl_4-induced acute liver failure using partial least square regression based on the GC/MS metabolic profiles of plasma in mice

Xin Huang[a], Li Shao[a], Yifei Gong[a], Yong Mao[a], Changxiao Liu[b], Haibin Qu[a,*], Yiyu Cheng[a,*]

[a] Pharmaceutical Informatics Institute, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China
[b] Tianjin Key Laboratory of Pharmacokinetics and Pharmacodynamics, Tianjin Institute of Pharmaceutical Research, Tianjin 300193, China

ARTICLE INFO

Article history:
Received 29 January 2008
Accepted 5 May 2008
Available online 6 June 2008

Keywords:
Metabonomics
PLS-RA
GC/MS
Carbon tetrachloride
Reactive oxygen species
Acute liver failure

ABSTRACT

This work characterized the metabolism disorders of acute liver failure (ALF) induced by carbon tetrachloride (CCl_4) in a mouse model with different dosage of intoxication (100, 500 and 1000 mg/kg). Metabolic profiles of mice plasma were detected by gas chromatography/mass spectrometry (GC/MS) after chemical derivatization. Here an effective information-extracting approach was implemented on the basis of partial least square regression analysis (PLS-RA). PLS modeling was achieved with two kinds of *Y*-vectors for the acquired metabonomics data and eight metabolites with different changing behaviors were selected. ALF of mice induced by CCl_4 was characterized by the elevation of glutamate, citrate, serine and threonine, as well as the decrease of α-glycerophosphate, docosahexaenoic acid, palmitic acid and oleic acid in plasma. The difference in the concentrations of serine, threonine, palmitic acid and oleic acid remained insignificant between the control and 100 mg/kg groups, while significant distinction appeared when comparing the control and two higher dosed groups. The underlying regulation of CCl_4-perturbed metabolic pathways was discussed according to the selected metabolites. The present study demonstrated a great potential of PLS-RA in exploiting a comprehensive metabolic effects of CCl_4 intoxication and its efficient capability to reveal the hepatotoxic mechanism of ALF induced by reactive oxygen species (ROS).

1. Introduction

Acute liver failure (ALF) is a rare condition consisting of rapid-onset severe liver injury accompanied by coagulopathy and encephalopathy, with a mortality rate of approximately 90% in patients [1,2]. Approximately 2000 cases per year occur in the US resulting in liver transplantation or death in more than 35% of these cases, frequently due to multiorgan failure (MOF). Nevertheless, etiology of ALF is mysterious in approximately 20% of adult patients in the US [3]. In addition, clinic trials of ALF are still difficult to perform since the scantiness of available clinic samples with both poor diagnosis and prognosis [2].

So far, specific metabolic disorders and metabolic syndromes of ALF have been investigated in literature [4–6]. Integrated approaches delineating the comprehensive metabolic perturbation of ALF are still necessary on the basis of clinic diagnostic and therapeutic researches. Metabonomics, defined as "the quantitative measurement of the dynamic multiparametric responses of a living system to pathophysiological stimuli or genetic modification" [7], aims to this destination and is gaining increasing appreciation in recent years [7–12]. Until now, metabonomic approach has proved profitable in providing valuable site- and pathway-related information of metabolic profiles from a variety of biofluids such as urine, plasma and cerebrospinal fluid, etc. [13–15]. Since metabonomics depends on the ability to describe the changes of low molecular-weight metabolites in various biofluids, analytical methods such as NMR and MS based platforms, should produce comprehensive profiles of metabolites from the biological samples. Among these analytical techniques, the GC/MS method has been proved both robust [16,17] and effective [11,12], and gained increased implementation recently in performing the global metabolic profiles.

As a new member in system biology, metabonomics has the obligation to demonstrate a temporal but comprehensive interpretation of metabolic changes to various interventions [18]. In general,

Abbreviations: ALF, acute liver failure; GC/MS, gas chromatography/mass spectrometry; ROS, reactive oxygen species; MOF, multiorgan failure; PCA, principle component analysis; PLS-RA, partial least square regression analysis; LV, latent variable; TMCS, trimethylchlorosilane; MSTFA, *N*-methyl-*N*-trimethylsilyltrifluoroacetamide; RSD, relative standard deviation; TCA, tricarboxylic acid; AA, amino acid or arachidonic acid; β-HB, β-hydroxybutyrate; DHB, 2,4-dihydroxybutyrate; AMA, aminomalonic acid; AGP, α-glycerophosphate; POA, palmitoleic acid; PA, palmitic acid; LOA, linoleic acid; OA, oleic acid; IOA, isooleic acid; SA, steric acid; DHA, docosahexaenoic acid; PAG, palmitic acid glyceride; SAG, steric acid glyceride; RP, ribose phosphate; PUSFA, polyunsatured fatty acids; VLDL, very low density lipoprotein, amino acids in this work are abbreviated using the standard three-letter convention.

* Corresponding authors. Tel.: +86 571 87951138; fax: +86 571 87951138.
E-mail addresses: quhb@zju.edu.cn (H. Qu), chengyy@zju.edu.cn (Y. Cheng).

1570-0232/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2008.05.049

multivariate statistical methods such as principal component analysis (PCA) [11–14] and partial least square (PLS) method [19] are popular in handling the acquired metabonomics data. PLS method can afford not only good classifications of observations, but also extract information-rich latent structures by regression during the modeling [20]. Regression analysis (RA) with PLS hence becomes a good choice and the regression coefficient of each variable is capable to illustrate both the correlation and changing direction of corresponding metabolite. So PLS-RA provides a rapid visualization of the perturbed metabolites and leads to a comprehension of regulation for the relevant pathways.

Reactive oxygen species (ROS) are one causative factor in the etiology of multiorgan degenerative diseases, including ALF and other hepatopathies [21]. The oxygen free radicals and oxidative stress enhanced by ROS are considered as a critical incentive of massive hepatocyte necrosis accompanied with fulminant impairments of the whole living system [22]. Threaten of ROS-induced ALF can be caused by a variety of factors, including environmental contaminants and exposure to some industrial chemicals (acetone, trichloromethane and carbon tetrachloride, etc.) [23,24]. Moreover, it is reported that the hepatotoxic mechanism of some drug-induced ALF, such as acetaminophen, is related to ROS [25]. In this study, CCl_4-induced acute liver injure in mice was introduced to investigate ROS-induced ALF using metabonomics methodology. Through the detection of metabolic difference of mice plasma analyzed by GC/MS, we aimed (i) to demonstrate the great potential of the proposed PLS-RA based data-processing method in metabonomics; (ii) to investigate the overall metabolic disorders and relevant disturbed pathways of ALF induced by ROS and (iii) to provide a complementary approach consisted of several metabolites in monitoring ALF, whether the liver damage can be auto-regulated or irreversible fulminate.

2. Experimental

2.1. Chemical and reagents

N-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA), trimethylchlorosilane (TMCS), docosane, methylhydroxylamine hydrochloride, and pyridine were of analytical grade and purchased from Fluka (Buchs, Switzerland). Carbon tetrachloride, natural amino acids, oxalate, malic acid, citric acid, D-ribose, galactose, glucose, palmitic acid, and steric acid were of analytical grade from China National Pharmaceutical Group Co. (Shanghai, China). Unsaturated fatty acids (oleic acid, linoleic acid, arachidonic acid, and docosahexaenoic acid) were of GC grade (purity >98%) and purchased from Sigma Co. (St. Louis, MO, USA).

2.2. Animal study and sample collection

Twenty-four male ICR mice (20 ± 2 g) were purchased from the Animal Center of Zhejiang Province (Hangzhou, China). These mice were acclimated for one week at a temperature around 20 °C and a relative humidity around 40%, with a 12 h light/12 h dark cycle. Food and tap water were provided ad libitum. After a fast of 12 h before treatment, they were divided into four groups randomly ($n = 6$), including the control group (bean oil only), low dosed group (CCl_4, 100 mg/kg, i.p., dissolved in bean oil), middle dosed group (500 mg/kg) and high dosed group (1000 mg/kg). Animals were sacrificed 48 h post-administration and plasma was quickly collected. A left lobe of the liver tissue was collected and fixed in 10% formalin for 12 h, embedded in paraffin wax and stained with hematoxylin–eosin (H–E). Stained samples were examined using an inversed optical microscopy (Leica Microsystems wetzlar GmbH, Germany) and photomicrographs of liver tissue were achieved through an equipped CCD camera.

2.3. Samples preprocessing and GC/MS analysis

In this study, processing of the plasma samples followed a protocol described in ref. [26] but with minor modifications. Briefly, 250 μL of acetonitrile was added into 100 μL of plasma for protein-precipitation (kept on ice for 15 min), then followed by centrifugation (10 000 g, 10 min, 4 °C). 150 μL of the supernatant was transferred to a GC vial and evaporated under a stream of nitrogen gas to dryness. Methoxymation was carried out at 70 °C for 1 h after methylhydroxylamine hydrochloride (50 μL, 15 mg/mL in pyridine) was added. MSTFA (50 μL, with 1% TMCS as the catalyst) was added, and the trimethylsilylation was performed at 70 °C for 1 h. After adding 150 μL *n*-heptane (containing 0.10 mg/mL of docosane, reference compound) and vortex mixing, the derivatized sample was transferred to a GC microvial after filtration.

The samples were analyzed on an Agilent 6890 gas chromatograph equipped with a 5973 mass selective detector and a 7683 injector (Agilent Technologies, Palo Alto, CA). A ZB-5MS fused-silica capillary column (0.25 mm × 30 m × 0.25 μm, Phenomenex, USA) was used for the separation with a helium carrier gas flow of 1.0 mL/min. 2.0 μL of derivatized samples was injected into the GC/MS instrument. To acquire a well separation, the GC oven was initially held at 85 °C for 5 min, and increased to 300 °C at a rate of 10 °C/min, where it was held for 5 min. Splitless mode was used and the electron energy was set at 70 ev. MS detection was implemented with electron ionization mode and full scan mode (m/z 60–600).

2.4. Identification of the endogenous metabolites

All collected plasma samples were analyzed and low molecular-weight metabolites were represented as the chromatographic peaks in the GC total ion current (TIC) chromatograms. EI-MS spectra of these peaks were interpreted using AMDIS (version 2.1, DTRA/NIST, USA) software and a majority of them can be well identified based on the NIST library 2002. Furthermore, identification of metabolites was performed using the commercial available standards by comparing their MS spectra and retention time, including twenty kinds of natural amino acids, the major organic acids, saccharides, fatty acids in biofluids, and cholesterol.

2.5. Data deconvolution and pattern recognition

Useful information was involved in these GC/MS data such as the retention characteristics, intensities (peak areas) and the MS spectra of the endogenous metabolites. In order to handle the complex metabonomics data, a matrix of peaks present in the collected samples must be generated. For this purpose, data denoising and peak detection were performed firstly based on the TIC chromatogram, using Agilent Enhanced ChemStation software (Agilent Technologies). The commensal peaks were aligned to generate a two-dimensional data table, in which rows and columns represent the samples and the relative peak areas of commensal metabolites, respectively. The resulting data table (CSV file) was imported into the SIMCA-P (version 11, Umetrics AB, Sweden) software for the multivariate analysis. Principal component analysis was applied with auto-scaling pretreated before. Partial least square method was performed with two different *Y*-vectors. Some metabolites were highlighted through their regression coefficients in PLS regression. Finally, significance of the between-group difference for these metabolites was examined by the students *T*-test using customer Matlab (version 6.5, Math Works, USA) scripts.