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Journal of Inorganic Biochemistry 99 (2005) 2151–2160
Journal of Inorganic Biochemistry
www.elsevier.com/locate/jinorgbio

NMR spectroscopic-based metabonomic investigation on the acute biochemical effects induced by $Ce(NO_3)_3$ in rats

Huifeng Wu, Xiaoyu Zhang, Peiqiu Liao, Zhongfeng Li, Weisheng Li, Xiaojing Li, Yijie Wu, Fengkui Pei *

Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, 5625 Renmin Street, Changchun 130022, PR China

Received 15 May 2005; received in revised form 15 July 2005; accepted 26 July 2005
Available online 6 September 2005

Abstract

An integrated metabonomic approach based on high-resolution ^1H NMR spectroscopy has been applied to the investigation of the acute biochemical effects caused by $Ce(NO_3)_3$ in rats. Male Wistar rats were separated into 8 groups and each was treated with one of following compounds, mercury II chloride ($HgCl_2$), 2-bromoethanamine hydrobromide (BEA), carbon tetrachloride (CCl_4), α-naphthylisothiocyanate (ANIT), and three doses of $Ce(NO_3)_3$ (i.p. 2, 10 and 50 mg/kg body weight). Urine was collected over a 48-h time course, and serum and tissue samples (liver and kidney) were gained after exposure to $Ce(NO_3)_3$ for 48 h. Histopathology and plasma clinical chemistry were also performed for all the tissue and plasma samples. Urine and serum samples were analyzed by 600 MHz ^1H NMR spectroscopy. All the ^1H NMR spectra were data-processed and analyzed using principal components analysis or hierarchical clustering analysis to show the time- and dose-dependent biochemical variations induced by $Ce(NO_3)_3$. Metabolic profiles of urinary ^1H NMR spectra from animals treated with $Ce(NO_3)_3$ exhibited an increase in trimethylamine *N*-oxide (TMAO), dimethylamine (DMA), dimethylglycine (DMG), taurine (Tau) and amino acids (valine, leucine and isoleucine), together with a decrease in citrate. The ^1H NMR spectral analysis of serum presented the elevation of acetone, acetoacetate, lactate and creatinine levels. These findings indicated the impairment of fatty acid β-oxidation in liver mitochondria and renal lesions. This work illustrates the high reliability of NMR-based metabonomic approach on the study of the biochemical effects induced by rare earths.

Keywords: NMR spectroscopy; Biofluids; $Ce(NO_3)_3$; Metabonomics

1. Introduction

NMR spectroscopy-based approach has been successfully applied to the studies on the biochemical effects of drugs and inborn diseases [1]. ^1H NMR spectroscopy of biofluids or tissues presents comprehensive biochemical profiles of endogenous metabolites reflecting the biochemical effects caused by xenobiotics. Biofluids from rat subjects under similar physiological conditions are highly reproducible, which is favorable in the diagnosis of metabolic and diseased states. Numerous endogenous metabolites in human and animal biological fluids have been assigned by Nicholson et al. [2] using high resolution NMR spectroscopy. However, recent developments in hardware have led to an increase in the complexity of ^1H NMR spectra from biological samples due to the improvements in the sensitivity and relative resolution of NMR spectrometers at higher magnetic field strengths [3]. As a result of the complexity of ^1H NMR spectra, many subtle changes in metabolite resonances might be overlooked within the natural biological variation. It is necessary to use powerful methods of data reduction

* Corresponding author. Tel.: +86 431 5262219/2251; fax: +86 431 5685653.
E-mail address: peifk@ciac.jl.cn (F. Pei).

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doi:10.1016/j.jinorgbio.2005.07.014

and analysis to gain the maximum amount of biochemical information from ^{1}H NMR spectra.

The application of ^{1}H NMR spectroscopy to the study of the metabolic composition of biofluids combined with pattern recognition (PR) to classify the NMR-derived data has led to a 'metabonomic' approach to biochemical assessment [4]. One widely used pattern recognition method is principal components analysis (PCA), in which the NMR spectra are reduced to a set of peak intensity descriptors and analyzed to identify similarities and differentiations of biochemical effects between control and drug-induced animals. PCA is a technique of dimension reduction [5], with each principal component (PC) being a linear combination of the original variables with appropriate weighting coefficients. All PCs are calculated such that they are orthogonal with all other PCs. The first PC contains the largest proportion of variance in the data set, with subsequent PCs involving progressively smaller proportion of total variance. Thus, a plot of the first and second PCs may contain a significant proportion of the information content of the original data set [5]. The combination of ^{1}H NMR and principal components analysis has become a well-established technique for the studies on the metabolic changes in biofluids, intact tissues and tissue extractions [6–9]. Hierarchical clustering analysis (HCA) is widely used in pattern recognition to classify original samples into clusters with various features due to its conceptual simplicity, general applicability and efficiency. HCA comprises two separate methods, agglomerative and divisive. When using hierarchical agglomerative clustering, each individual sample is initially assigned to a separate cluster. In a stepwise performance, the most similar clusters are combined into larger units, ending when there exists one super-cluster containing all observations. In contrast, the divisive technique begins with the single super-cluster, and proceeds stepwise by dividing the cluster into its most dissimilar two parts. Hierarchical clustering can be used in standard setting to define a set of n cluster solutions and each solution can then be evaluated for its respective fit of the data [5].

With their widespread application in agriculture, industry, culture, medicine and daily life, rare earth compounds will enter the ecological environment and human body through food chains [10–12]. It is very important to know the acute and chronic effects of rare earths on the environment, nature balance, and the human body after its entry into bodies and the environment. We have previously reported the chronic nephro- and hepato-toxicity of light rare earths, such as La and Changle (a kind of rare earth complex including La, Ce, Pr and Nd used as an agriculture additive) analyzed by ^{1}H NMR spectroscopy of biofluids [13–15], and the acute kidney and liver lesions induced by heavy rare earth Lu using NMR-PR method [16]. Cerium is widely used in chemistry and medicine, especially in agriculture (main compound of Changle). The aim of current study was to investigate the acute biochemical profiles of cerium (Ce) in biofluids (urine and serum) to further understanding the biochemical effects of light rare earths using NMR-based metabonomic approaches.

2. Materials and methods

2.1. Drug administration and samples

Forty male Wistar rats (weight range 250 ± 20 g) were acclimatized for 48 h in plastic cages prior to group allocation and treatment. They were then housed individually in metabolism cages in a well-ventilated room under controlled conditions (temperature, humidity and a 12 h light–dark cycle). Food and tap water were provided ad libitum. The animals were treated with a single intraperitoneal dose either $HgCl_2$ (1.5 mg/kg body weight), 2-bromoethanamine hydrobromide (BEA) (250 mg/kg body weight), $Ce(NO_3)_3$ (2, 10 and 50 mg/kg body weight) or saline (0.9%) and the others were administrated with an oral dose of CCl_4 (1.5 ml/kg body weight) and α-naphthylisothiocyanate (ANIT) (150 mg/kg body weight). Urine was collected at the following time intervals, −8 to 0 h (predose), 0–8, 8–16, 16–24, 24–32, 32–40 and 40–48 h. Urinary volume and pH were measured and the urine was centrifuged at 3000 rpm for 5 min at 4 °C to remove the particulate contaminants. Animals were sacrificed by exsanguinations from the abdominal aorta under isoflurane anaesthesia at 48 h after dosing. Serum samples of blood were collected in heparin containers from abdominal aortal exsanguinations, and the serum was separated by ultrafiltration (10-kDa cutoff) and centrifugation (at 3000 rpm for 10 min at 4 °C). All the samples were stored at −70 °C until NMR spectroscopic analysis.

2.2. ^{1}H NMR spectroscopic measurement of urine and serum

Fifty microlitres buffer solution (0.2 M Na_2HPO_4 and 0.2 M NaH_2PO_4, pH 7.0) was mixed with either 400 μL urine or serum to minimize variations in the pH of the urine samples. Proton NMR measurements of urine and serum (50 μL D_2O was added for locking signal) were recorded on a Bruker-Av600 MHz spectrometer at 298 K. For each urine sample, 16 free induction decays (FIDs) were collected into 64 K data points using a water presaturation pulse sequence (D–90°–t_1–90°–t_m–90°–acquired FID) with relaxation delay 5 s and flip angle 90°. Water signals and the broad protein resonances were suppressed by a combination of presaturation and the Carr–Purcell–Meiboom–Gill (90°–(τ–180°–τ)$_n$–acquisition)