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Acute biochemical effects of $La(NO_3)_3$ on liver and kidney tissues by magic-angle spinning ^1H nuclear magnetic resonance spectroscopy and pattern recognition

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Abstract

High-resolution magic-angle spinning (MAS) ^1H nuclear magnetic resonance (NMR) spectroscopic and pattern recognition (PR) based methods have been applied to studies on the acute biochemical effects of $La(NO_3)_3$ on rats. Male Wistar rats were treated with various doses of $La(NO_3)_3$ (2, 10, and 50 mg/kg body weight), and MAS ^1H NMR spectra of their intact liver and kidney tissues were analyzed using principal components analysis to extract metabolic information. The biochemical effects of $La(NO_3)_3$ were characterized by the increase of triglyceride and bile acid and the decrease of glycogen in liver tissue, together with a slight elevation of triglyceride level in kidney tissue. The target lesion of $La(NO_3)_3$ to liver was found by MAS NMR–PR methods. This study illustrated the power of the combination of MAS ^1H NMR and pattern recognition for the analysis of biochemical effects of rare earths.

Keywords: Magic-angle spinning NMR; $La(NO_3)_3$; Pattern recognition; Metabolism; Liver; Kidney; Tissue

High-resolution ^1H magic-angle spinning (MAS)[1]-NMR spectroscopy has become an increasingly powerful technique for investigating the metabolic state of various intact tissues in the areas of drug toxicity [1,2] and disease diagnosis [3,4]. Biological tissues are semi-solid-heterogeneous-complex-containing species with a wide range of molecular weights such as proteins, lipids, and low-molecular-weight metabolites [5]. ^1H NMR spectra of tissues suffer from major line-broadening contributions when acquired by conventional solution-state NMR spectroscopy because of the lack of isotropic molecular motion in biological tissues. There are several line-broadening factors, including dipole–dipole interactions, NMR chemical shift anisotropy, and magnetic field inhomogeneities. These factors could be overcome by rotating a sample at the magic angle $\theta = 54.7°$, where θ is the angle between the sample spinning axis and the external magnetic field [6]. MAS ^1H NMR spectroscopy has been successfully applied to studies on biological tissues, such as rat liver [5,7,8], mammalian kidney [1,9], human prostate [10], and rat testicular tissue [11].

The application of ^1H NMR spectroscopy to the study of metabolic composition of biofluids coupled with pattern recognition (PR) to classify the NMR-derived data has led to a "metabonomic" approach to biochemical assessment [12]. Pattern recognition analysis is performed in a multidimensional parameter space and displayed using dimension-reduction techniques [14]. Principal components analysis (PCA) is a widely applicable dimension-reduction method, and the combination of MAS ^1H NMR and PCA has become a well-established technique for studies on metabolic changes in intact tissues [5,7,8,13].

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[1] *Abbreviations used:* MAS, magic-angle spinning; PR, pattern recognition; PCA, principal components analysis.

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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 [15–17]. It is important to elucidate 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 hepatotoxicity of light rare earths, such as La and Changle (a kind of rare earth complex used as agriculture additive) analyzed by ^{1}H NMR spectroscopy of biofluids [18,19], and the acute kidney and liver lesions induced by heavy rare earth Lu the using NMR-PR method [20]. The aim of this current study was to investigate the acute toxicity of $La(NO_3)_3$ by the direct analysis of tissues using the MAS NMR spectra combined with pattern recognition method to further elucidate the biochemical effects of light rare earths.

Materials and methods

Animal handling and tissue preparation

Twenty Male Wistar rats (weight range 250–300 g) were separated into four groups ($n = 5$) and housed individually in metabolism cages which allowed free access to food and water under controlled condition (temperature, humidity, and a 12-h light–dark cycle). Each rat received either various doses of $La(NO_3)_3$ (i.p. 2, 10, and 50 mg/kg body weight, $n = 15$) or saline (i.p. 0.9%, $n = 5$). Animals were sacrificed by exsanguinations from the abdominal aorta under isoflurane anaesthesia at 48 h after dosing. The left lateral lobe of the liver and the whole kidney were excised, immediately snap-frozen in liquid nitrogen, and stored at −70 °C until NMR spectroscopic analysis.

MAS ^{1}H NMR measurement

Samples (15–23 mg) were placed in 4-mm-diameter zirconia rotors with a spherical insert and a Kel-F cap after soaking with D_2O (for locking signal). All MAS ^{1}H NMR spectra were recorded on a Bruker AV-600 spectrometer at a MAS spin rate of 5000 Hz [21]. MAS NMR spectra were acquired at 298 k, as measured by the thermocouple system, and maintained by the cooling of inlet gas pressures for sample spinning. For each sample, 16 free-induction decays were collected into 64 k data points using a one-dimensional standard solvent presaturation suppression pulse sequence (relaxation delay-90°-t_1-90°-t_m-90°-ACQ) in which a secondary radio frequency irradiation field is applied at the water resonance frequency during the relaxation delay of 3 s and during the mixing period ($t_m = 150$ ms), with t_1 of 6 μs. The FIDs were weighted by an exponential function with a 0.3-Hz line-broadening factor prior to Fourier transformation. Phase and baseline correction were made for each spectrum. A spectral width of 8992.806 Hz and an acquisition time of 0.911 s were used.

Data reduction of MAS ^{1}H NMR and pattern recognition

Whole spectrum δ 0.0–10.0 was segmented into regions of 0.04 ppm width using MestRe-c 2.3 (http://qobrue.usc.es/jsgroup/MestRe-c). The area for each segmented region was calculated and the integral values resulted in an intensity distribution description of the whole spectrum with 248 variables prior to PR analysis. The δ region between 4.8 and 5.4 was removed prior to statistical analysis to eliminate the variation in water suppression efficiency. The remaining 233 spectral segments were scaled to total integrated area of each spectrum [8,22]. Principal component analysis of the data was performed using the program written by ourselves.

Results and discussion

Conventional solution-state ^{1}H NMR spectral analysis of tissue extracts requires extensive sample preparation, involving the destruction of the subcellular and molecular organization within the tissue. High-resolution MAS ^{1}H NMR spectroscopy of intact tissue allows the measurement of cellular metabolites without destruction and preparation of the tissues; thus the direct detection of the tissues could provide much biochemical information from the cell using the MAS-NMR technique [8]. However, MAS ^{1}H NMR spectra of tissues can be described only with regard to relative signal intensities, since a reference compound of known concentration cannot be added into tissue samples for the purpose of quantitation. Therefore, the behavior of endogenous metabolites in response to $La(NO_3)_3$-induced toxicity was expressed as a ratio of the overall spectral profile [1].

MAS ^{1}H NMR analysis of liver tissue

Typical examples of MAS ^{1}H NMR spectra with signals from both small metabolites and large macromolecules of control and $La(NO_3)_3$ (2, 10, and 50 mg/kg body weight)-treated rats are shown in Fig. 1. The main resonances of the metabolites from liver tissue are assigned and listed in Table 1 [23].

Increases of lipid triglycerides and decreases of glycogen and glucose were observed in the MAS ^{1}H NMR spectra of liver tissues from the higher doses (10 and 50 mg/kg body weight) of $La(NO_3)_3$-treated groups. The increase of triglyceride in liver tissue represents drug-induced steatosis, which could be caused by