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RESEARCH ARTICLE

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

# Investigation on the acute biochemical effects of light rare earths on rat serum by NMR-based metabonomic approaches



**Abstract** $^1$H-NMR spectroscopy and pattern recognition (PR) method were used to assess the acute biochemical effects of light rare earths. Male Wistar rats were treated with both $La(NO_3)_3$ and $Ce(NO_3)_3$ at doses of 2, 10, and 50 mg/kg body weight. Serum samples from the rats with the two kinds of doses of light rare earths were obtained after 48 h and analyzed by a 600 MHz $^1$H-NMR spectrometer. Each NMR spectra was data-processed to provide 238 intensity-related descriptors as input coordinates in a multidimensional space and analyzed by PR method. Many low-molecular weight metabolites were identified by $^1$H-NMR spectra of the rat serum. An increase in ketone bodies, creatinine, lactate, succinate, and various amino acids (valine, leucine, and glutamine) were found from the higher doses (10 and 50 mg/kg body weight) of rare earths-treated groups, together with a decrease of glucose in the serum from $Ce(NO_3)_3$-dosed groups. These findings may mean that high-dosage of La and Ce impair a specific region of liver. The similar toxicities with various mechanisms for La and Ce are implicated by NMR-based metabonomic approach. $Ce(NO_3)_3$ exhibited a higher toxicity than $La(NO_3)_3$ at the same doses.

**Keywords** light rare earth, serum, nuclear magnetic resonance (NMR), pattern recognition, biochemical effects

## 1 Introduction

With their widespread application in agriculture, industry, culture, medicine, and daily life, rare earth compounds will enter the ecological environment and the human body through food chains. It is important to know the acute and long-term effects of rare earths on the environment, nature balance, and the human body [1–4]. In recent years, NMR has been used for the rapid multicomponent analysis of low molecular weight compounds in biofluids for clinical diagnosis of inborn diseases and investigation of biochemical effects of medicines with the advent of high field NMR spectrometers [5–8]. $^1$H-NMR spectroscopy of biofluids presents comprehensive biochemical profiles of low-molecular-weight metabolites reflecting the biochemical effects caused by xenobiotics, and these biochemical profiles can be obtained with minimal sample preparation and without destruction to the samples. Moreover, the improvements in the sensitivity at higher magnetic field strengths have led to an increase in the complexity of the biofluid $^1$H-NMR spectra [8]. As a result of the complexity of $^1$H-NMR spectra, many subtle changes in metabolite resonances might be overlooked within the natural biological variation. Pattern recognition (PR) analysis is performed in a multidimensional parameter space using dimension-reduction techniques to gain important biological information from complex $^1$H-NMR spectra of biofluids [9]. The combination of $^1$H-NMR and principal components analysis (PCA) has become a well-established technique for the studies on the metabolic changes in biofluids, intact tissues, and tissue extractions [3, 10–13].

Lanthanum and Cerium are the two main components of Changle (a kind of rare earth complex including La, Ce, Pr, and Nd used as an agriculture additive). The aim of the current study is to investigate the acute biochemical profiles of the metabolites in the serum from the $La(NO_3)_3$- and $Ce(NO_3)_3$-treated rats and compare the similarities and differences of the lesions induced by the two rare earth compounds by NMR-PR method.

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Li Zhongfeng, Wu Huifeng, Zhang Xiaoyu, Li Xiaojing, Liao Peiqiu, Li Weisheng, Pei Fengkui (✉)
Changchun Institute of Applied Chemistry，Chinese Academy of Sciences, Changchun 130022, China
E-mail: peifk@ciac.jl.cn

## 2 Experimental

### 2.1 Samples collection and storage

Thirty-five male Wistar rats (weighing ranging form 250 to 300 g) were divided into seven groups ($n$ = 5) at random and housed individually in metabolism cages with 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)、$Ce(NO_3)_3$ (i.p. 2, 10, and 50 mg/kg body weight, $n$ = 15) or saline (i.p. 0.9%, $n$ = 5). At 48 h after dosing, serum samples of the blood from the sacrificed rats were separated by ultrafiltration and centrifugation and were stored frozen at −70°C until NMR spectroscopic analysis.

### 2.2 $^1$H-NMR measurement

An aliquot of 50 μL of buffer solution (0.2 M $Na_2HPO_4$/ 0.2 M $NaH_2PO_4$, pH = 7.0) was mixed with 400 μL of serum to minimize variations in the pH of the serum samples.

Proton NMR measurements of serum samples (50 μL $D_2O$ was added for locking signal) were recorded on a Bruker-Av 600 MHz spectrometer at 298 K. Water signals and the broad protein resonances were suppressed by a combination of presaturation and the Carr-Purcell-Meiboom-Gill pulse sequence. Thirty-two free induction decays (FIDs) were colleted into 32 k data points with relaxation delay of 6 s and flip angle of 90°. All spectra were referenced to the $CH_3$ resonance of creatinine at $\delta$ = 3.06.

### 2.3 Data reduction and PR analysis of $^1$H-NMR spectra

Each spectrum $\delta$ = 0.0–10.0 was segmented into the region of $\delta$ = 0.04 width using MestRe-c 2.3. The area for each segmented region was calculated and the integral values were used for the intensity distribution description of the spectrum. The region ($\delta$ = 4.6–5.0) was deleted prior to statistical analysis to remove the variation in water suppression efficiency. The remaining 238 spectral segments were scaled to the total integrated area of the spectrum [8, 14]. Principal component analysis of the data was performed using an in-house software.

## 3 Results and discussion

### 3.1 $^1$H-NMR spectral analysis of serum samples from 48 h post dosed-rats with various doses of $La(NO_3)_3$ and $Ce(NO_3)_3$

Serum contains almost all of the low-molecular-weight compounds in the whole blood and a few high-molecular-weight compounds, and $^1$H-NMR spectra of serum from animal under similar physiological conditions are highly reproducible, which is useful in the diagnosis of metabolic and diseased states. The assignments [7, 15, 16] and the alterations of important metabolites in serum samples compared with that of the control are listed in Table 1. Figure 1 illustrates the typical 600 MHz $^1$H-NMR spectra of serum samples from control, $La(NO_3)_3$ (10 mg/kg body weight) and $Ce(NO_3)_3$ (10 mg/kg body weight)-treated rats at 48 h after dosing.

**Table 1** Assessment of metabolites in serum from $La^{3+}$ and $Ce^{3+}$ dosed rats after 48 h

| Metabolite | Chemical shift ($\delta$) and multiplicity | 2 (mg/kg body weight) | | 10 (mg/kg body weight) | | 50 (mg/kg body weight) | |
|---|---|---|---|---|---|---|---|
| | | La | Ce | La | Ce | La | Ce |
| Lipoprotein $CH_3$ | 0.84 | − | − | ↑ | − | ↑ | − |
| Valine | 0.97 (d) | − | − | ↑ | − | ↑ | − |
| 3-*D*-hydroxybutyrate | 1.20 (d) | ↑ | − | ↑ | − | ↑↑ | ↑ |
| Lactate | 1.33 (d) | ↑ | ↑ | ↑ | ↑ | ↑↑ | ↑↑ |
| Alanine | 1.48 (d) | − | − | ↑ | − | ↑ | − |
| Acetate | 1.93 (s) | − | − | − | ↑ | − | ↑ |
| Glycoprotein | 2.06 | − | − | − | ↑ | − | ↑ |
| Acetone | 2.24 (s) | − | − | − | ↑ | − | ↑ |
| Succinate | 2.40 (s) | − | − | − | ↑ | − | ↑ |
| Glutamine | 2.48 (m) | − | − | − | − | − | − |
| Lipid: $=CHCH_2^*CH=$ | 2.72 | ↑ | | ↑ | | ↑ | |
| Creatinine | 3.06 (s) | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ |
| TMAO | 3.27 (s) | − | ↑ | ↑ | ↑ | ↑ | ↑ |
| $\alpha$-Glucose | 5.22 (d) | − | − | | ↓ | | ↓ |

Abbreviations and keys: s, singlet; d, double; t, triplet; m, complex multiplet; −, not detectably different from control level; ↑, a detectable but minor elevation in concentration; ↑↑, an obvious elevation in concentration compared with control levels; ↓, a minor decrease (20%–50%) from control levels.

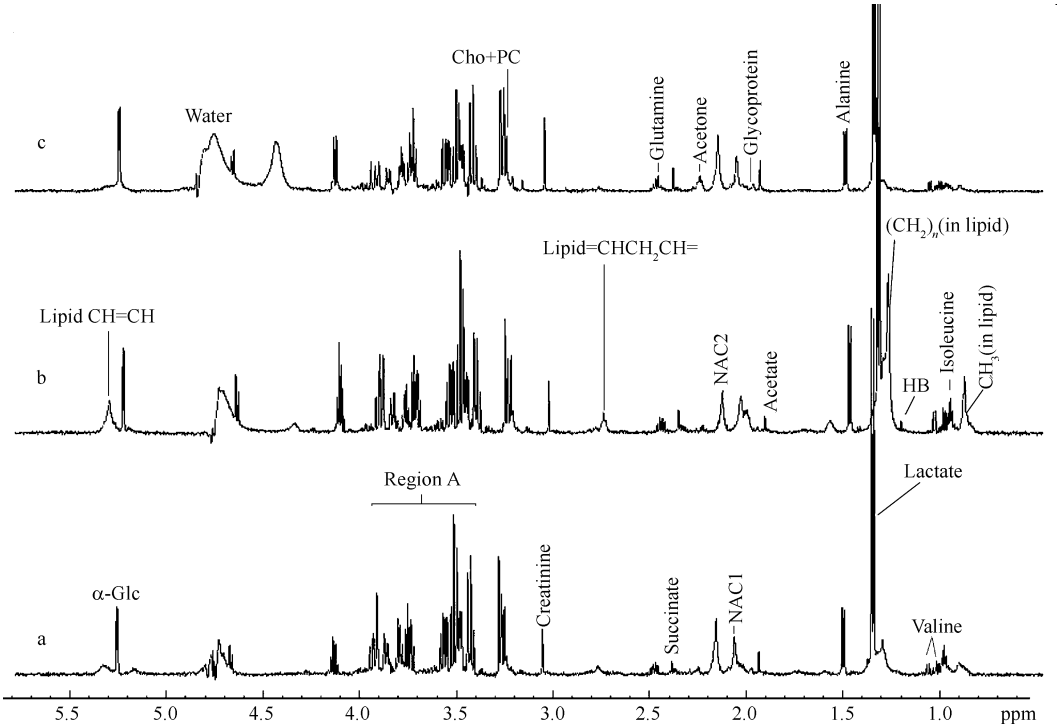


**Fig. 1** 600 MHz high-resolution $^1$H CPMG NMR spectra of serum from control and two rare earth-treated rats: a. the control, b. 10 mg/kg body weight $La(NO_3)_3$, c. 10 mg/kg body weight $Ce(NO_3)_3$

Both of the rare earths could cause remarkable changes to the biochemical composition in the serum sample. An increase of 3-hydroxybutyrate (HB) was observed in the $^1$H-NMR spectra of serum samples from various doses of $La(NO_3)_3$-treated groups. However, acetone is only obviously existent in the serum of rats receiving higher doses of $Ce(NO_3)_3$. HB and acetone are the ketone bodies which are metabolic products of fatty acid metabolism in liver mitochondria. The presence of ketonemia shows that a high level of fatty acids has been mobilized by fatty tissues. Acetoacetate is formed from acetyl-CoA in the citric acid cycle, and its conversion into 3-hydroxybutyrate is catalyzed by 3-hydroxybutyrate dehydrogenase in the mitochondria and into acetone under the catalysis of acetoacetate decarboxylase [17]. It is likely that the rare earth causes enzymatic metabolic disorders, resulting in the accumulation of large amounts of the ketone bodies. In this study, the high level of HB in the serum indicated that $La(NO_3)_3$ affected the activity of the key enzymes related to the metabolism of HB in liver mitochondria, and the elevated levels of acetone indicated the disturbance of fatty acid metabolism caused by $Ce(NO_3)_3$. All of them illuminate that the two rare earths produced different decompensation to rats.

No obvious change was observed for the 2 mg/kg body weight $La(NO_3)_3$-and $Ce(NO_3)_3$-dosed groups. After administration of different doses, the intensity of the peaks for alanine and valine gradually increased with increase in dose of $La(NO_3)_3$. This is a common phenomenon observed in patients with hepatic failure, regarded as distinctive features of hepatic coma [18]. Alanine is an important amino acid in the plasma, and the increase in alanine aminotransferase is a known marker of liver damage [14]. It may mean that $La(NO_3)_3$ impairs the enzymes related to the catabolism of amino acids, and liver cells cannot effectively intake the amino acids, causing an increase in free amino acids in the serum.

An increase in acetate, succinate, and glycoproteins and a decrease in glucose were found in the higher doses (50 mg/kg body weight) of $Ce(NO_3)_3$-treated groups. Acetate is an end product of fatty acid oxidation (degradation of short-chain fatty acid: (acetoacetic acid) + acetyl-CoA → acetoacetyl-CoA + (acetic acid)). The elevated level of acetate in the serum illustrated the energy metabolism disorders, which were related to the increase in ketone bodies. Succinate is an excretion of the tricarboxylic acid cycle (TCA). The liver is responsible for the synthesis of many proteins, such as LDL and glycoproteins. The high level of glycoprotein could imply that $Ce^{3+}$ affected the protein metabolism as fatty acid metabolism in liver. Thus the amounts of glycoprotein exceeding normal levels in the serum indicated the hepatic insufficiency induced by $Ce(NO_3)_3$. It is possible that the higher doses (50 mg/kg body weight) of $Ce(NO_3)_3$ influenced the activity of mitochondrial enzymes related to the TCA cycle. The higher $Ce^{3+}$ doses caused serious functional damage to the liver and enzyme defects, leading to a sharp decline in plasma glucose concentration, and this is a demonstration of typical pathological hypoglycemia [19].

Compared with the $^1$H-NMR spectra of serum from control rats, the amount of lactate are obviously increased for all experimental groups. It is possible that rare earths can affect the fat metabolism which mainly occurred in liver as carbohydrate metabolism [20]. In the higher doses (10 and

50 mg/kg body weight) of rare earths-treated groups, the amount of creatinine was increased. Creatinine (Cn) is a by-product of creatine and filtrated by animal kidneys. The higher level of Cn in blood exceeding normal levels is a sign of renal maladjustment. The result is that lower rates of glomeruler filtration, which might be expected due to perturbation of the rennin-angiotension system, could cause a reduction in renal cortical blood flow [3]. Normally, the hepatotoxicities produced by $La^{3+}$ and $Ce^{3+}$ could induce hepatic insufficiency, which often leads to hepatorenal syndrome (HRS). From these findings, it is likely that two kinds of rare earths could cause similar biochemical effects of disturbance to fat metabolism in liver mitochondria and hepatorenal syndrome indicated by the increase of HB, acetate, and Cn.

### 3.2 PCA analysis of serum samples

Principal component analysis (PCA) is a technique of dimension reduction [7], with each principal component (PC) being a linear combination of the original variables with appropriate weighting coefficients and 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 [9, 21]. PCA was chosen as a preliminary method of analyzing the $^1$H-NMR spectra for the reason that PCA produced a tighter clustering of points and hence good classification [10].

Figure 2 shows the PC plots (PC1 versus PC2) of data from $^1$H-NMR spectra of serum samples from control, various $La(NO_3)_3$- and $Ce(NO_3)_3$-dosed rats after removal of the abnormal samples. Except for the 2 mg/kg body weight $La(NO_3)_3$-dosed groups, the clear separation among $La(NO_3)_3$-treated, $Ce(NO_3)_3$-treated, and control samples was exhibited in the PC plots (PC1 versus PC2) of $^1$H-NMR spectral data from serum samples. In the 10 mg/kg body weight $La(NO_3)_3$- and $Ce(NO_3)_3$-dosed groups (Fig. 2(B)), $La(NO_3)_3$ is only devoted to PC2 orientation, however, $Ce(NO_3)_3$ has an important contribution in PC1 and PC2 orientations. These indicate that both rare earths have definite effect to rats, though the toxicities induced by them are different.

In order to compare the regions of the spectra contributing to the classifications, the scatter loadings plots of PC1 versus PC2 based on $^1$H-NMR spectral descriptor of serum samples were made (Fig. 3). The regions responsible for the PCA distinction between the lowest (2 mg/kg body weight) $La(NO_3)_3$-, $Ce(NO_3)_3$-dosed and control rats were assigned at $\delta$ = 2.24 (acetone), $\delta$ = 2.40 (succinate), and $\delta$ = 3.04 (creatinine) in the PC1 loading orientation (Fig. 3(A)), and

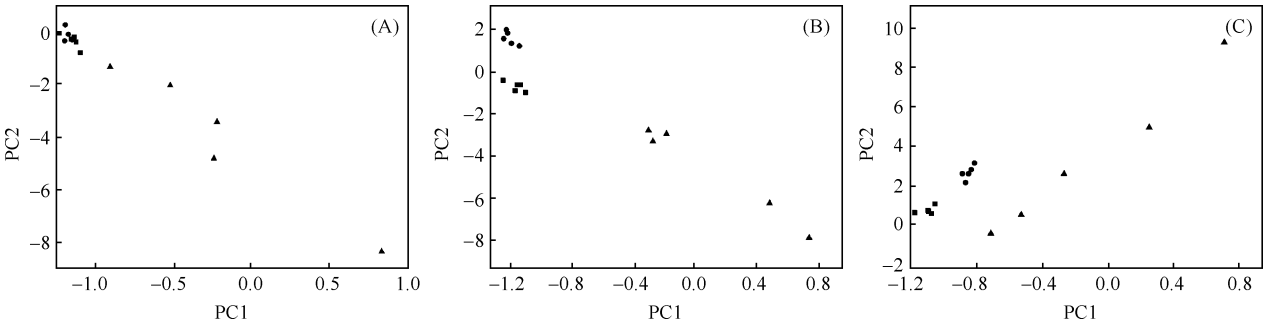


**Fig. 2** Plots of PC1 versus PC2 based on the $^1$H-NMR spectra descriptors of the serum samples from control、$La(NO_3)_3$-and $Ce(NO_3)_3$ 3-treated rats
(A) $La(NO_3)_3$ and $Ce(NO_3)_3$ (2 mg/kg body weight) and control; (B) $La(NO_3)_3$ and $Ce(NO_3)_3$ (10 mg/kg body weight) and control;
(C) $La(NO_3)_3$ and $Ce(NO_3)_3$ (50 mg/kg body weight) and control

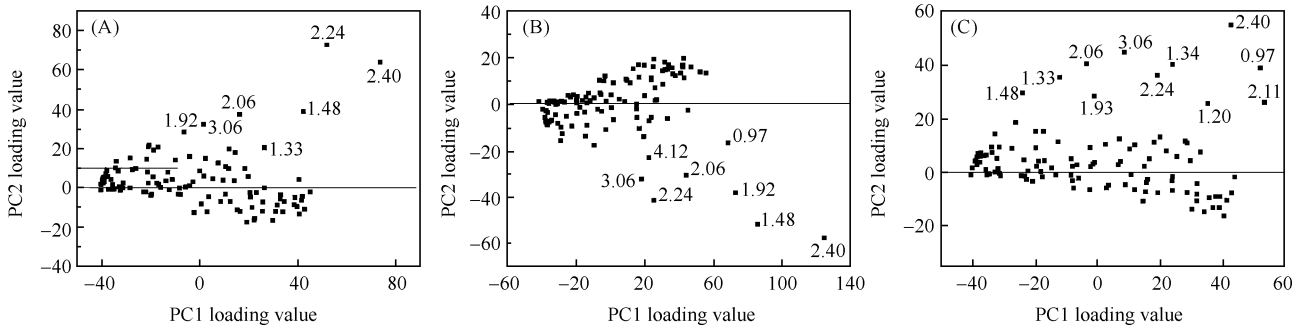


**Fig. 3** Scatter loading plots of PC1 versus PC2 based on the $^1$H-NMR spectra descriptors of the serum samples showing the contribution of integral regions of the NMR spectrum to the separation
(A) $La(NO_3)_3$ and $Ce(NO_3)_3$ (2 mg/kg body weight) and control; (B) $La(NO_3)_3$ and $Ce(NO_3)_3$ (10 mg/kg body weight) and control;
(C) $La(NO_3)_3$ and $Ce(NO_3)_3$ (50 mg/kg body weight) and control

the changes of endogenous metabolites in Table 1 show that these dedications were devoted to the inducement of $Ce(NO_3)_3$. In the loading plots (Fig. 3(B) and 3(C)) of the control, $La(NO_3)_3$-and $Ce(NO_3)_3$-dosed (10 and 50 mg/kg body weight) samples, the separation was attributed to the NMR signal at $\delta$ = 0.97 (valine), $\delta$ = 1.20 (HB), $\delta$ = 1.33 (lactate), $\delta$ = 1.48 (alanine), $\delta$ = 1.93 (acetate), $\delta$ = 2.24 (acetone), $\delta$ = 2.40 (succinate), and $\delta$ = 3.06 (creatinine), which was consistent with the visual comparison of the NMR spectra from rare earths-treated and control rats and contributes to both the PC1 and PC2 loading orientations. However, the alternations in the amounts of valine ($\delta$ = 0.97), HB ($\delta$ = 1.20), and alanine ($\delta$ = 1.48) were only found in the $La(NO_3)_3$-dosed sample, and changes in acetone ($\delta$ = 2.24) and succinate ($\delta$ = 2.40) level were presented in $Ce(NO_3)_3$-treated groups. The changes of these metabolites displayed the disorder of the organism under higher doses of $La(NO_3)_3$ and $Ce(NO_3)_3$ (10 and 50 mg/kg body weight), and the differences of the metabolic profiles from the $^1$H-NMR spectra could explain the detailed variations of the two kinds of rare earths.

In conclusion, these results clearly show that the combination of the NMR technique with pattern recognition method is an efficient approach to investigate the acute biochemical effects of $La(NO_3)_3$ and $Ce(NO_3)_3$. Our results from NMR-PR analyses of rat serum suggest that the two kinds of rare earths caused similar effects of fatty acid metabolism disorders and hepatic lesions, together with a possible hepatorenal syndrome. An increase in ketone bodies, creatinine, lactate, succinate, and various amino acids (valine, leucine and glutamine) were found from the higher doses (10 and 50 mg/kg body weigh) of rare earths-treated groups, together with a decrease of glucose in the serum from $Ce(NO_3)_3$-dosed groups. Similar toxicities with various mechanisms for La and Ce are implicated by NMR-based metabonomic approach. $Ce(NO_3)_3$ exhibited the higher toxicity than that of $La(NO_3)_3$ at the same doses.

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