Differences in lead isotopic fingerprints between blood, hair and organs in lead-poisoned rats

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Lead isotopic ratios of biosamples such as blood, hair and teeth are used as “fingerprints” to trace and identify sources of lead pollution in the environment. However, the reliability of this technique has not been confirmed. In previous studies, we investigated the differences in lead isotopic ratios between blood, hair, and organs from the same human body. To explore the relationship between isotopic ratios in environmental lead and biosamples, and to investigate the influence of exposure dose, rats were exposed to 0, 30, 60, and 120 mg/kg body weight of lead acetate every day for 4 weeks by intragastric administration. Lead isotopic ratios of blood, hair, liver, kidney and femur in rats, as well as that of the test substance lead acetate, were measured using inductively coupled plasma mass spectrometry. There were significant differences ($P < 0.05$) in lead isotopic fingerprints between blood, hair, and tissues. Lead fingerprints of blood were influenced by lead exposure dose, while those of other tissues and organs were generally not. Its reliability and availability indicated that blood, but not hair, was thus a suitable biomarker for environmental lead exposure. The results of this study provide the scientific rationale for the use of lead isotopic ratios for the identification of human lead pollution sources.

lead fingerprint, isotopic ratio, blood, hair, tissues, organs, rats

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Despite decades of study and regulation, lead poisoning remains a significant public health problem, especially in developing countries such as China. Identification of potential lead sources and prevention of lead pollution thus remain critically urgent tasks. Lead has four isotopes; ²⁰⁴Pb, ²⁰⁶Pb, ²⁰⁷Pb, and ²⁰⁸Pb. The composition of lead is largely unaffected by physical or chemical fractionation processes, and lead sources can therefore be identified by their isotopic “fingerprints” made up of characteristic ratios of the four isotopes. Compared with traditional epidemiologic methods, identification of lead sources based on isotopic ratios is faster, more accurate and less costly, and is therefore becoming a powerful complementary tool for determining potential lead sources. Many studies have investigated lead isotopic ratios in biological samples such as blood and/or

hair to identify potential environmental lead sources [1–8], but the reliabilities of these different biological biomarkers have not yet been reported.

We previously demonstrated differences in isotopic fingerprints between blood, hair, and organs from the same individual [9,10]. In this study we aimed to explore the relationship between isotopic ratios in environmental lead and in different biosamples. Rats were subjected to lead poisoning and the isotopic ratios in blood, hair, and organs, together with those of the lead solution, were measured using inductively coupled plasma mass spectrometry (ICP-MS). This preliminary study aimed to confirm that differences in lead isotopic ratios exist among blood, hair, and other organs; to determine if isotopic ratios in biosamples are affected by lead exposure dose; and to identify the most reliable and suitable source of biosamples for tracing environmental lead sources.

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1 Materials and methods

1.1 Reagents

The soluble lead salt, lead acetate ($\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$) (AR grade) was used in all experiments. The certified lead isotopic reference material NIST SRM 981 was used for correction of isotopic ratio measurements. Nitric acid (E-Pure grade, 71%), hydrogen peroxide (E-Pure grade, 30%) and perchloric acid (GR grade, 70%–72%), were used for digestion of biological samples. Ultra-pure water $18 \text{ M}\Omega \text{ cm}^{-1}$ was used as a blank and to prepare the solutions.

1.2 Animal experiments

Physically healthy, male Sprague-Dawley rats, approximately 4 weeks old and weighing 80–100 g, were obtained from the Department of Laboratory Animal Science, Peking University Health Science Center. All the animals were maintained on a standard SPF laboratory low-lead diet, under the same standard barrier conditions.

After adaptive feeding for 1 week, the rats were randomly separated into one control and three experimental groups, with eight rats in each group. The experimental groups consisted of low-, median-, and high-dose groups that received 30, 60, 120 mg/kg body weight of lead acetate every day for 4 weeks, respectively. Aqueous lead acetate solution was administered orally once a day at 1 mL/100 g body weight, using a plastic syringe with a catheter. The control group received ultra-pure water. The dorsal hair was shaved from the rats prior to the start of the experiment.

After 4 weeks of lead exposure, the rats were anesthetized by ether inhalation and killed by exsanguinations. The dorsal hair was shaved again and blood was collected into low-lead lithium heparin tubes. Liver, kidney, and femur were collected and weighed after elimination of connective and adipose tissue. All samples were stored at -20°C .

All animal procedures were carried out by licensed investigators and in accordance with the regulations of the Animals Act.

1.3 Sample preparation

Hair, blood and organ samples were wet digested in an open quartz system using a mixture of HNO_3 and HClO_4 (10:1 by volume). After 12 h cold digestion, the quartz vials then were progressively heated on a hot plate to 100, 120, 140, 160 and 180°C , until white vapor was given off. Ultrapure water was then added to drive off residual acid. The residue was diluted before analysis.

1.4 Measurements

Lead isotopic ratios were estimated using ICP-MS with Elan DRCII (PerkinElmer Instruments(Shanghai) Co. Ltd). Four lead isotopes ^{204}Pb , ^{206}Pb , ^{207}Pb , and ^{208}Pb were

measured using the optimized parameters for the method: radio frequency power 1100 W, plasma gas flow rate 15 L/min, auxiliary gas flow rate 1.85 L/min, nebulizer gas flow rate 0.95 L/min, lens voltage 6.1 V, plus stage voltage 1100 V, peak hoper transient scanning mode at one point scans, sweep 150/reading, dwell time 30 ms, and nine replicates. The relative standard deviations ($n=7$) of 10 ng/mL lead standard solution for $^{208}\text{Pb}/^{206}\text{Pb}$, $^{207}\text{Pb}/^{206}\text{Pb}$, and $^{204}\text{Pb}/^{206}\text{Pb}$ were 0.12%, 0.13%, and 0.27% under these optimization conditions.

The lead signal was corrected for mercury using the ^{204}Hg signal. Before the measurements the instrument had undergone a routine conditioning and testing procedure for 1–2 h. Lead isotopic certified reference material NIST SRM 981 was used for isotope signal normalization. The common analyte internal standardization method [11,12] was applied for non-spectroscopic matrix effects and instrumental drift.

1.5 Statistical analysis

Statistical analyses were performed using SPSS version 14.0. One-way ANOVA was used to compare isotopic ratios between different dose groups, and paired t tests were used to compare isotopic ratios between blood, hair, and different organs.

2 Results and discussion

2.1 Comparison of lead fingerprints among different dose groups

The lead isotope ratios in the different dose groups are compared in Tables 1–3. One-way ANOVA was used with a significance level of $P = 0.05$.

Significant differences in $^{208}\text{Pb}/^{206}\text{Pb}$ ratios were found between the low- and median-dose groups for blood ($P < 0.01$), the low- and high-dose groups for blood ($P < 0.05$); the low- and median-dose groups for liver ($P < 0.05$), and the median- and high-dose groups for liver ($P < 0.01$). There were no significant differences ($P > 0.05$) between any dose groups for kidney, femur or hair (Table 1).

There were no significant differences in $^{207}\text{Pb}/^{206}\text{Pb}$ ratios between any of the dose groups for all the measured biosamples (Table 2).

There were significant differences in $^{204}\text{Pb}/^{206}\text{Pb}$ ratios only in kidneys between the low- and medium dose groups (Table 3).

Of the total of 45 comparisons from three dose groups, five different organs, and three isotopic ratios, only five demonstrated significant differences ($P > 0.05$), indicating that lead fingerprints in the body remain relatively stable. The $^{207}\text{Pb}/^{206}\text{Pb}$ ratio was the most stable, and showed no differences between dose groups or biosamples ($P < 0.05$). The femur provided the most stable biosample in terms of isotopic ratios, with no significant differences between dose

Table 1 Comparison of $^{208}\text{Pb}/^{206}\text{Pb}$ ratios among dose groups ($n=8$)^{a)}

Sample	Dose group	Pb isotopic ratios		P of multiple comparisons		P of total comparisons
		Mean	Std. error	Median-dose	High-dose	
Blood	low	2.1032	0.0039	0.001**	0.026*	0.005**
	median	2.0955	0.0051		0.229	
	high	2.0981	0.0022			
Liver	low	2.1038	0.0028	0.019*	0.079	0.001**
	median	2.0970	0.0075		0.000**	
	high	2.1089	0.0046			
Kidney	low	2.1051	0.0032	0.400	0.266	0.158
	median	2.1069	0.0053		0.058	
	high	2.1027	0.0037			
Femur	low	2.1112	0.0052	0.364	0.149	0.156
	median	2.1080	0.0027		0.734	
	high	2.1065	0.0034			
Hair	low	2.0926	0.0298	0.732	0.330	0.606
	median	2.0958	0.0095		0.522	
	high	2.1018	0.0053			

a) ** indicates $P<0.01$, * indicates $P<0.05$.**Table 2** Comparison of $^{207}\text{Pb}/^{206}\text{Pb}$ ratios among dose groups ($n=8$)^{a)}

Sample	Dose-group	Pb isotopic ratios		P of multiple comparisons		P of total comparisons
		Mean	Std. error	Median-dose	High-dose	
Blood	low	0.8593	0.0020	0.135	0.044*	0.112
	median	0.8581	0.0018		0.513	
	high	0.8575	0.0005			
Liver	low	0.8571	0.0016	0.041*	0.305	0.117
	median	0.8555	0.0010		0.301	
	high	0.8563	0.0015			
Kidney	low	0.8564	0.0018	0.934	0.542	0.501
	median	0.8568	0.0009		0.677	
	high	0.8577	0.0023			
Femur	low	0.8571	0.0013	0.515	0.692	0.374
	median	0.8581	0.0018		0.989	
	high	0.8585	0.0038			
Hair	low	0.8860	0.0430	0.460	0.304	0.146
	median	0.8647	0.0025		0.002**	
	high	0.8596	0.0019			

a) ** indicates $P<0.01$, * indicates $P<0.05$.**Table 3** Comparison of $^{204}\text{Pb}/^{206}\text{Pb}$ ratios among dose groups ($n=8$)^{a)}

Sample	Dose group	Pb isotopic ratios		P of multiple comparisons		P of total comparisons
		Mean	Std. error	Median-dose	High-dose	
Blood	low	0.0548	0.0004	0.999	0.997	0.872
	median	0.0548	0.0002		0.930	
	high	0.0548	0.0001			
Liver	low	0.0548	0.0002	0.461	0.470	0.693
	median	0.0548	0.0001		0.991	
	high	0.0548	0.0001			
Kidney	low	0.0544	0.0002	0.006**	0.109	0.0203*
	median	0.0546	0.0002		0.177	
	high	0.0545	0.0001			
Femur	low	0.0545	0.0001	0.299	0.203	0.394
	median	0.0545	0.0001		0.806	
	high	0.0545	0.0002			
Hair	low	0.0541	0.0014	0.425	0.455	0.665
	median	0.0544	0.0002		0.959	
	high	0.0544	0.0002			

a) ** indicates $P<0.01$, * indicates $P<0.05$.

groups for any of the lead isotope ratios.

Statistical analysis demonstrated that blood lead isotopic ratios varied with exposure dose, while the lead fingerprints of other organs generally did not. The blood lead isotopic ratio in the low-dose group differed from that of the test substance, but the difference decreased as the dose increased, and disappeared in the high-dose group.

The results of this study suggest that: (a) Blood may act as the first barrier to lead exposure in the body, and is thus likely to play a role in the selective accumulation or fractionation of lead isotopes, whilst possessing a threshold for selective accumulation or fractionation. As concentration of the test substance increases, the threshold is exceeded, and the difference in lead isotope ratios between the blood and

the test substance declines. (b) Blood transports the lead to various tissues and organs. When the lead, which has already been buffered by the blood, arrives at the tissues and organs it accumulates there and becomes relatively stable, with its isotopic ratios relatively unaffected by the test substance dose. Further studies are needed to verify these assumptions.

2.2 Comparison of lead isotopic fingerprints between blood, hair and organs

Comparisons of lead isotope ratios between blood, hair, and organs are presented in Tables 4–6. Where lead fingerprints were not significantly different between dose groups, data

Table 4 Comparison of $^{208}\text{Pb}/^{206}\text{Pb}$ ratios between blood, hair and organs ^{a)}

Sample pair	<i>P</i> value of paired <i>t</i> tests			
	Total groups	Low-dose group	Median-dose group	High-dose group
Blood-liver	0.050*	0.609	0.721	0.002**
Blood- kidney	0.001**	0.384	0.011*	0.031*
Blood-femur	<0.001**	0.037*	<0.001**	0.004**
Blood-hair	0.440	0.274	0.939	0.211
Liver-kidney	0.349	0.303	0.003**	0.019*
Liver-femur	0.007**	0.011*	0.007**	0.374
Liver-hair	0.112	0.313	0.720	0.072
Kidney-femur	0.005**	—	—	—
Kidney-hair	0.033*	—	—	—
Femur-hair	0.007**	—	—	—

a) ** indicates $P < 0.01$, * indicates $P < 0.05$, “—” indicates lead isotope ratios in this organ were not influenced by dose, and dose groups were therefore combined.

Table 5 Comparison of $^{207}\text{Pb}/^{206}\text{Pb}$ ratios between blood, hair and organs ^{a)}

Sample pair	<i>P</i> value of paired <i>t</i> tests			
	Total groups	Low-dose group	Median-dose group	High-dose group
Blood-liver	<0.001**	—	—	—
Blood- kidney	0.009**	—	—	—
Blood-femur	0.490	—	—	—
Blood-hair	0.050	—	—	—
Liver-kidney	0.279	—	—	—
Liver-femur	0.034*	—	—	—
Liver-hair	0.019*	—	—	—
Kidney-femur	0.048*	—	—	—
Kidney-hair	0.025*	—	—	—
Femur-hair	0.038*	—	—	—

a) ** indicates $P < 0.01$, * indicates $P < 0.05$, “—” indicates that lead isotope ratios in this organ were not influenced by dose, and dose groups were therefore combined.

Table 6 Comparison of $^{204}\text{Pb}/^{206}\text{Pb}$ ratios between blood, hair and organs ^{a)}

Sample pair	<i>P</i> value of paired <i>t</i> tests			
	Total groups	Low-dose group	Median-dose group	High-dose group
Blood-liver	0.774	—	—	—
Blood- kidney	<0.001**	0.041*	0.137	0.002**
Blood-femur	<0.001**	—	—	—
Blood-hair	0.023*	—	—	—
Liver-kidney	<0.001**	0.014*	0.002**	0.004**
Liver-femur	<0.001**	—	—	—
Liver-hair	0.005**	—	—	—
Kidney-femur	0.853	0.298	0.264	0.642
Kidney-hair	0.289	0.618	0.026*	0.180
Femur-hair	0.242	—	—	—

a) ** indicates $P < 0.01$, * indicates $P < 0.05$, “—” indicates that lead isotope ratios in this organ were not influenced by dose, and dose groups were therefore combined.

for all dose groups were combined.

There were significant differences in $^{208}\text{Pb}/^{206}\text{Pb}$ ratios between blood and liver, blood and kidney, and blood and bone, and between any two of kidney, bone and hair. There were no significant differences between hair and blood, or hair and liver (Table 4).

There were significant differences ($P < 0.05$) in $^{207}\text{Pb}/^{206}\text{Pb}$ ratios between any two biosamples, apart from blood and bone, blood and hair, and liver and kidney (Table 5).

There were significant differences in $^{204}\text{Pb}/^{206}\text{Pb}$ ratios between any two biosamples, apart from blood and liver, kidney and femur, kidney and hair, and femur and hair (Table 6).

Thus among the 10 biosample comparisons, there were significant differences in all three isotopic ratios for two (blood-kidney, and liver-femur), in two isotopic ratios for six pairs, and in one isotopic ratio for two pairs (blood-hair, and liver-kidney). These results indicate that general differences in lead isotopic ratios exist among biosamples in the

body, and that the samples can be distinguished by at least one lead isotopic ratio. The presence of differences among biosamples in rats is in accord with the results from humans observed in our earlier research.

The current study provides the first evidence of differences among biosamples in terms of lead isotope ratios in animals. However, the mechanism responsible for this difference remains unclear, and suitable reference materials are currently extremely rare. Further studies are therefore needed to confirm the above assumptions.

2.3 Comparison of lead isotopic fingerprints between biosamples and test substance

Comparisons of lead isotopic ratios between biosamples and the test substance for all experimental groups and for separate dose groups are presented in Figures 1 and 2, respectively.

It is interesting to note that the lead isotopic ratios of

Figure 1 Distribution of $^{204}\text{Pb}/^{206}\text{Pb}$ vs $^{207}\text{Pb}/^{206}\text{Pb}$ in blood, hair and tissues of total groups.

Figure 2 Distribution of $^{204}\text{Pb}/^{206}\text{Pb}$ vs $^{207}\text{Pb}/^{206}\text{Pb}$ in blood, hair and tissues of low-, median-, high-dose groups.

blood, hair and organs are distributed within different elliptical areas around the test substance (Figure 1). Relative to the test substance, liver ratios are distributed in the upper left region, kidney ratios in the lower left region, and femur ratios in the lower right region. In contrast, hair ratios are distributed in the lower right region, furthest from the test substance, while blood ratios are distributed within an elliptical area with the test substance at the center.

The scattered points for blood and liver become more centralized with increasing dose (Figure 2). In the high-dose group, blood lead isotopic ratios are distributed more densely and closely around the test substance, compared with other tissues.

It is likely that some bio-fractionation occurs in tissues and organs after the lead is taken up by the rats, and the lead isotopic ratio of some samples differs from that of the isotopic ratio of the test substance. Hair is thus unsuitable as a biomarker, while the reliability and availability of blood make it better suited as a biomarker for tracing environmental lead exposure, especially under high-dose conditions.

3 Conclusions

(1) There are differences in lead isotopic ratios between blood, hair and other organs in lead-poisoned rats.

(2) Blood lead isotopic ratios are influenced by lead exposure dose, while those of other tissues are generally not.

(3) Blood is more suitable than hair as a biomarker for identifying environmental lead sources.

(4) This represents the first report of differences in lead isotopic ratios between blood, hair and organs in rats, but further research is needed to confirm these results.

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