Comparative Study of Methods for Determining Lanthanide Elements in Biological Materials by Using NAA, HPLC Postcolumn Reaction, and ICP-MS

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ABSTRACT

Mice were injected intravenously with one of the following elements: Y, La, Ce, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, or Yb, at a dose of 25 mg/kg body wt. After 20 h, the various organs were taken out, and the element concentrations were determined by NAA, HPLC postcolumn reaction, or ICP-MS. The results by these three methods were within the acceptable range. About 85% of the amount administered of each element was found in liver, lung, and spleen.

Index Entries: Lanthanide elements; NAA; ICP-MS; HPLC postcolumn reaction; mouse; iv injection; distribution; Arsenazo-III.

INTRODUCTION

The use of lanthanide elements has been increasing in the fields of both traditional and ultramodern industries. There have been no reports on the health effects in relation to environmental contamination owing to disposal of used products containing such rare elements. The possibility that general residential areas may be influenced by rare elements cannot

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be denied, because the types and amounts of the products containing rare elements are increasing and old or used ones may be discharged into the common environment. There is a concern that leaching by rain, especially acid rain, may enlarge the contaminated area.

We succeeded in determining Tb, Eu, and Dy using 2,6-pyridinedicarboxylic acid as a fluorometric ligand *(1,2)*. In this article, we introduce a colorimetric method of the HPLC postcolumn reaction as a determination technique for the lanthanide elements. The results were comparable to the values determined by neutron activation analysis (NAA) and inductively coupled plasma-mass spectrometry (ICP-MS).

In addition, we describe the distribution of the lanthanide elements after 20 h of iv injection in mice.

MATERIALS AND METHODS

Chemicals Administered to Mice

The chloride compounds of yttrium (Y), lanthanum (La), cerium (Ce), neodymium (Nd), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), and ytterbium (Yb) were used. Each compound was dissolved in a 5% glucose solution.

Animal Treatment

Five-week-old mice were divided into 12 groups, which consisted of five mice each, and another group served as a control. Each mouse received a single iv injection with one of the elements at the dose of 25 mg element/kg body wt. At 20 h after the injection, the animals were sacrificed using ether anesthesia, and organ specimens were removed after perfusion by the 5% glucose solution.

Reagents

Nitric acid (HNO_3) and perchloric acid ($HClO_4$) of analytical grade or reagents for atomic absorption were used. Formic acid, 2-hydroxybutyric acid, and 2,7-*bis*-(2-arsenophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid (Arsenazo-III) were of the reagent grade commercially available.

Analytical Procedure

NAA

The lyophilized liver samples were powdered by grinding. Then samples of about 100 mg each were weighed in polyethylene bags and sealed by heating. Irradiations were carried out with appropriate standard samples in an IEA-RI nuclear research reactor. The detailed condi-

Table 1
Experimental Conditions of NAA

Element	Irradiation time	Neutron flux	Decay time	Radioisotope measured	half life	gamma ray energy(keV)
La	30 min	(a)	1d	^{140}La	40.27h	1597
Ce	30 min	(b)	4d	^{141}Ce	32.5d	145.4
Nd	8 h	(c)	6d	^{147}Nd	11.1d	91
Sm	30 min	(a)	1d	^{153}Sm	47.1h	69 and 103
Eu	3 min	(a)	1h	^{152m}Eu	9.35h	841
Gd	30 min	(a)	4h	^{159}Gd	18h	363
Tb	30 min	(b)	11d	^{160}Tb	73d	298
Dy	30 min	(a)	19h	^{165}Dy	2.36h	634
Ho	10 min	(a)	20h	^{166}Ho	26.9h	80 and 1378
Er	15 min	(a)	1d	^{171}Er	7.8h	308
Yb	30 min	(a)	15h	^{175}Yb	101h	396

(a) $2.67 \times 10^{11} n/cm^2/s$.
(b) $1.54 \times 10^{12} n/cm^2/s$.
(c) $10^{12} n/cm^2/s$.

tions are listed in Table 1. The radioactivities were counted by an ENERTEC hyperpure Ge detector coupled to an EG & G Ortec 4096 channel pulse height analyzer connected to a Monydata PC 200 Plus microcomputer.

HPLC Postcolumn Reaction

An aliquot of the liver samples, 20–100 mg, was wet-ashed using HNO_3 and $HClO_4$. After heating at 100–120°C for 1–2 h and then at 200°C for about 1 h, the residue was dissolved in 0.5% HNO_3. This solution was passed through an ion-exchange column (Shim-pack IC-CI) installed with HPLC (Shimadzu LC 6A); 0.05–0.2 mol/L 2-hydroxybutyric acid (pH 4.4) was used as a mobile phase. The eluents were reacted with Arsenazo-III to develop color. Optical density at 655 nm was measured for all lanthanide elements, with 2.5 ng as the detection limit.

ICP-MS

0.5% HNO_3 solution after digesting liver samples was applied to ICP-MS (Yokogawa PMS-200). The detection limits were 0.02 ng element/mL.

RESULTS

The Element Concentrations in Liver

Table 2 shows the element concentrations in liver, determined by HPLC-colorimetric and ICP-MS methods as a mean value and SD (in μg/g, wet wt). Table 3 shows the results obtained by HPLC-colorimetric and NAA methods. In order to compare the results obtained by these three methods, element ratios were calculated as shown in Table 4. The ratios for HPLC/ICP-MS ranged from 0.91–1.17, whereas the ratios for NAA/ICP-MS were from 0.76–1.33. As for the CV(%), values were relatively small for HPLC/ICP-MS, below 10%, whereas for NAA/ICP-MS, they were relatively large.

The Element Concentrations Determined in Various Organs of Mice

Figure 1A shows the Tb or Yb concentrations in various organs of mice. In control mice, all lanthanide elements were under the detection limit. Figure 1B shows the distribution ratios of the total amounts administered. More than 85% of the dose administered was found in liver, lung, and spleen. Figure 2 shows the element concentrations in spleen, lung, and liver.

DISCUSSION

In the papers reported previously, analytical methods applied to investigate the distribution of the lanthanide elements administered, used mainly radioisotopes *(3–5)*. Since analytical instruments and techniques have been markedly improved recently, we tried to determine the lanthanide elements in biological materials using HPLC equipped with fluorometric and colorimetric detectors, and ICP-MS. In addition, some of the samples were analyzed by NAA in order to check results of the previously mentioned methods.

The injection dose chosen of 25 mg element/kg was extremely high. The reason was that neither the distribution ratios in individual organs nor the detection limits of the methods applied were known. It was assumed that the element concentrations in organs, such as thymus, submandibular gland, or sexual organs, could be very low and close to the detection limits. Therefore, such a relatively high dose was chosen.

The detection limit of the HPLC postcolumn colorimetric determination using Arsenazo-III was 2.5 ng which allowed determination of all lanthanide element included in this study. In the case of using the gradient mode for the mobile phase, each element could be separated, although it took a little longer and the detection limits were somewhat higher.

Table 2
Concentrations of Lanthanide Elements Determined in the Liver of Mice by HPLC Postcolumn Reaction and ICP-MS Methods

Element	Concentrations (μg/g wet wt)	
	HPLC (Arsenazo-III)	ICP-MS
Y	138.7 ± 15.5	141.5 ± 12.8
La	151.5 ± 17.7	164.1 ± 19.6
Ce	155.8 ± 15.1	161.3 ± 12.3
Nd	129.3 ± 16.9	141.6 ± 19.3
Sm	129.4 ± 28.4	134.6 ± 31.5
Eu	149.8 ± 25.4	147.7 ± 21.7
Gd	126.8 ± 7.4	108.4 ± 7.5
Tb	158.2 ± 11.8	156.8 ± 10.9
Dy	149.3 ± 19.9	141.2 ± 19.1
Ho	186.4 ± 15.8	187.1 ± 13.0
Er	191.7 ± 31.5	190.0 ± 31.0
Yb	154.3 ± 18.9	163.4 ± 21.8

Mean ± SD, n = 4 ~ 5, 20 h after iv injection of 25 mg element/kg.

Table 3
Concentrations of Lanthanide Elements Determined in the Liver of Mice by HPLC Postcolumn Reaction and INAA

Element	Concentation (μg/g dry wt)	
	HPLC (Arsenazo-III)	INAA
La	950 ± 162	1039 ± 154
Ce	848 ± 131	753 ± 131
Nd	764 ± 255	598 ± 211
Sm	718 ± 146	556 ± 102
Eu*	212 ± 47	247 ± 55
Gd	757 ± 68	676 ± 75
Tb	583 ± 23	695 ± 66
Dy*	243 ± 30	254 ± 31
Ho	1087 ± 380	790 ± 126
Er	986 ± 106	1049 ± 197
Yb	937 ± 19	1170 ± 105

Mean ± SD, n = 4 ~ 5, 20 h after iv injection of 25 mg element/kg (*10 mg element/kg)

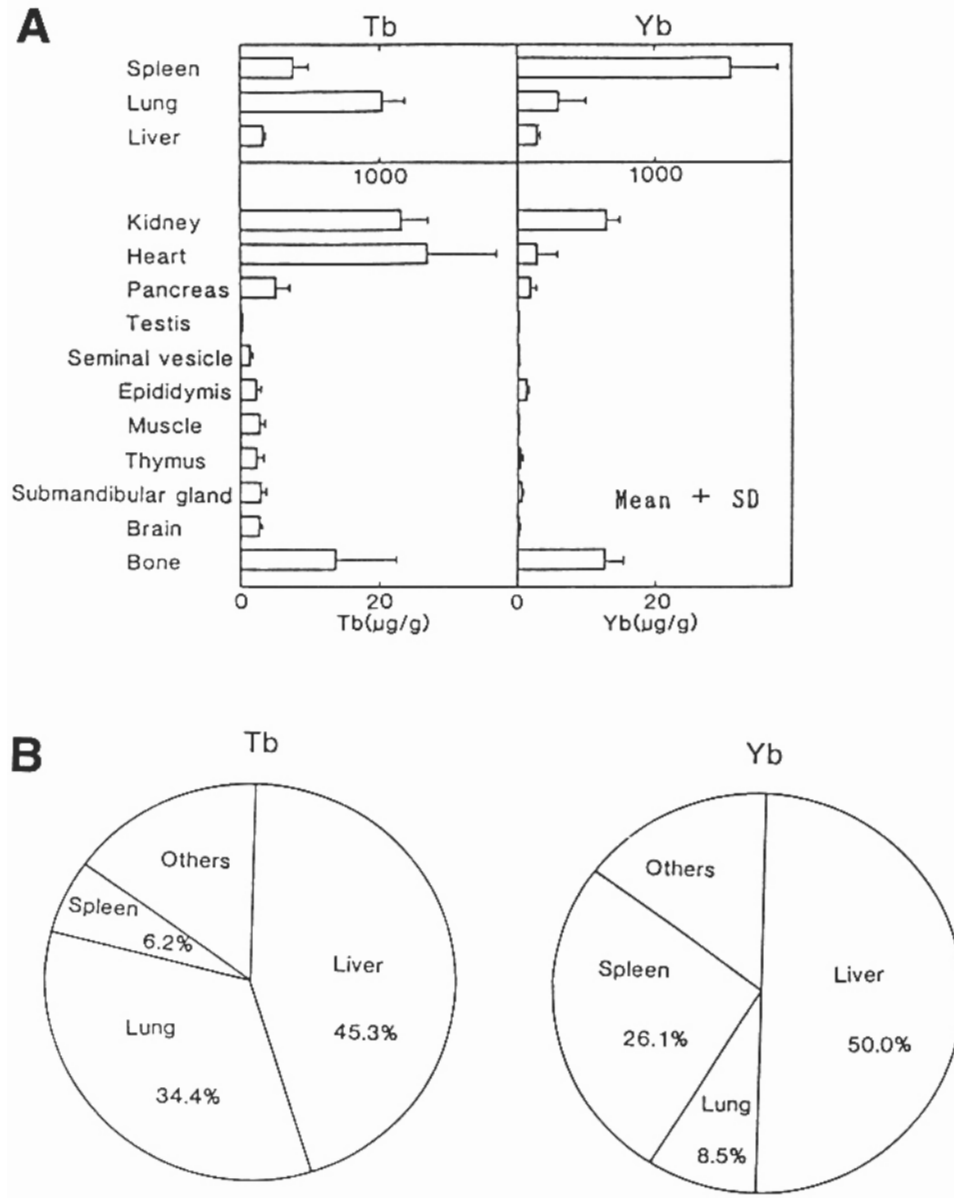

Fig. 1. The concentrations of Tb and Yb in various organs of mice injected with Tb or Yb (A), and the distribution ratios of the amounts administered (B).

Element recoveries by HPLC postcolumn colorimetric method were also studied, and values from 98.2% (Nd) to 105.0% (Ce) were found.

Lyophilized samples for NAA and wet-ashed samples for HPLC and ICP-MS were prepared separately from the same liver specimens. The sample solutions after wet-ashing were divided into two portions, which were then applied to HPLC and to ICP-MS. This way of sample prepa-

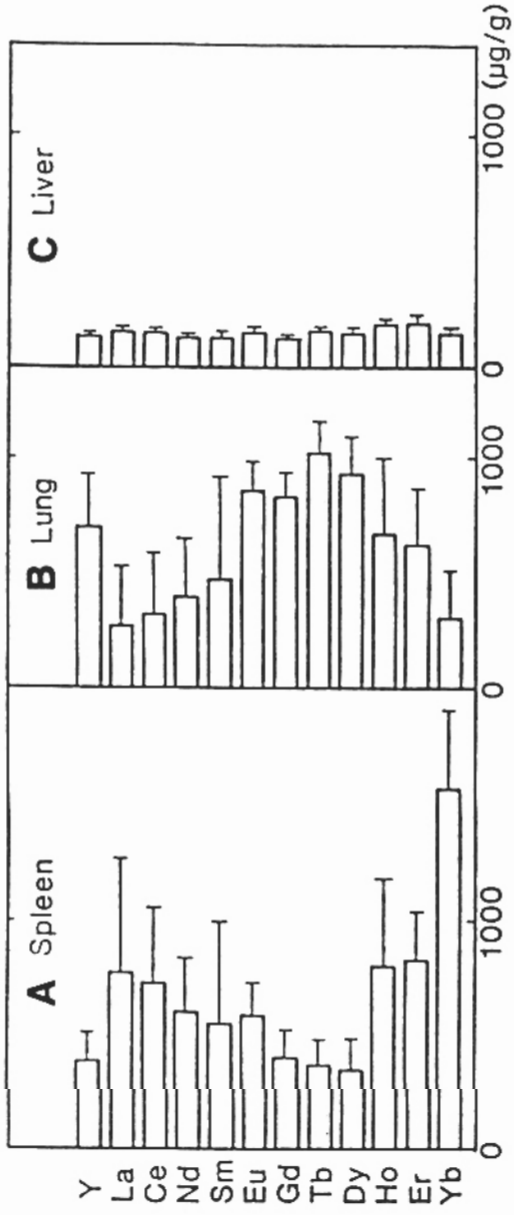

Fig. 2. The concentrations of the elements determined in the spleen, lung, and liver of mice injected with respective lanthanide elements.

Table 4
Comparison of the Results Obtained by the Three Methods Studied

Element	HPLC/ICP-MS		INAA/ICP-MS	
	MEAN	CV(%)	MEAN	CV(%)
Y	0.98	5.1	-	-
La	0.93	9.1	1.17	6.9
Ce	0.97	7.3	0.86	13.8
Nd	0.91	3.5	0.78	2.9
Sm	0.97	4.5	0.76	13.5
Eu	1.01	2.6	0.77*	14.3
Gd	1.17	2.0	1.15	4.3
Tb	1.01	2.2	0.82	3.9
Dy	1.06	2.6	0.83*	12.8
Ho	1.00	2.5	0.78	11.1
Er	1.01	1.6	1.02	20.4
Yb	0.94	3.7	1.33	3.7

*Estimated value.
$n = 4 \sim 5$.

ration may be considered as a reason why better agreement was found between the results by HPLC postcolumn reaction and ICP-MS than agreement between the results of either of these methods and the NAA results, because differences in the element distribution inside the liver may occur.

SUMMARY

In order to be able to investigate health effects of lanthanide elements, three methods of their determination were examined. Twelve of the lanthanide elements, Y, La, Ce, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, and Yb, were determined in various organ samples of mice by NAA, HPLC postcolumn reaction, and ICP-MS. The results obtained by the three methods agreed well within acceptable limits. The lanthanide elements injected intravenously were found mainly in the liver, lung, and spleen, whereas much lower concentrations were detected in other organs of mice.

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