Determination of Reference Concentrations of Strontium in Urine by Inductively Coupled Plasma Atomic Emission Spectrometry

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Abstract

Objective: The aim of this study was to establish reference concentrations of urinary strontium by inductively coupled plasma atomic emission spectrometry (ICP-AES).

Methods: For the determination of strontium, urine samples were collected from healthy Japanese (n=146; 115 males, 31 females; mean age, 33±9 years; age range, 18 to 58 years). The urine samples stored at or below –20°C were thawed with incubation at 40°C for 30 min and sediments were dissolved by vigorous shakings. Then, the samples were centrifuged at 3000 g for 5 min, and the supernatant was directly aspirated into a P-5200-3600/1200 ICP-AES system from Hitachi Ltd., Tokyo, Japan.

Results: A steeper increase in the S/N ratio and a good effective linearity of the calibration line was obtained at 407.771 nm in the range of 0–300 µg/L strontium standard solution. Urine samples having the same background signal as that of 18 MΩ cm ultrapure blank water, a good correspondence of the single peak pattern of the spectra, accuracy and precision of spike recovery were also confirmed. Urinary strontium concentrations showed a log-normal distribution and a geometric mean concentration of 143.9 µg/L, with 5–95% confidential interval of 40.9–505.8 µg/L.

Conclusion: The results of this study will be useful as guidelines for the biological monitoring of strontium in normal subjects and in individuals therapeutically or environmentally exposed to strontium.

Key words: strontium, trace element, ICP-AES, reference value, log-normal distribution

Introduction

Strontium ranks 15th in abundance among the elements. It is widely distributed in the geosphere, natural environment and human tissues. Strontium compounds have many industrial and commercial applications such as in glass coating of color television picture tubes, in ferrite ceramic magnets and, because of its brilliant crimson color in fire, in pyrotechnics and signaling devices.

Strontium belongs to the alkaline earth elements of group 2A of the periodic table and it shows the same biological behavior as that of calcium and can actually displace calcium

in hard tissue metabolic processes (1). Although there is still no evidence that supports strontium as an essential trace or ultratrace element, there are reports of its involvement in anabolic activity in the bone (2). Because of its bone-seeking activity, strontium emerged as a drug for the management of osteoporosis in the 1950s. The approach was soon abandoned because it appeared to interfere with vitamin D synthesis (3). This adverse effect is now thought to be caused by calcium-poor diets and dosing.

In recent years, there has been renewed interest in strontium ranelate, which is used in novel pharmaceutical preparations such as PROTELOS® (*Laboratories Servier*) authorized in 27 European countries as a new antiosteoporotic treatment since September 21, 2004 (4–7). In Japan, Astellas Pharma Inc. is developing the drug and is now in phase-II trials. This new approach to osteoporosis treatment offers doctors and patients alike new hope in the fight against osteoporosis. On the other hand, recent research also suggests possible health hazards of strontium overload and its toxicity from endemic soil pollution

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(8), high dietary strontium (9) and chronic renal failure (10, 11). Because 17.5% of dietary strontium is excreted via urine (12), patients with renal failure or undergoing hemodialysis are likely to show strontium retention and overload (13, 14).

For years strontium has been considered to be a nonessential, largely innocuous element that did not pose any specific or significant health threat, therefore there is limited information related to its determination in human samples. Under such circumstances, there is now a need for a reliable analytical method for trace strontium and for reference concentrations in biological fluids. Inductively coupled plasma atomic emission spectrometry (ICP-AES) is a powerful method for trace element analysis in various types of biological fluid sample. Urine is one of the predominant excretion routes of elements and it is frequently utilized for biological monitoring of exposure to chemical substances. In the present study, a rapid, sensitive and reliable method for the determination of urinary strontium by ICP-AES is described. Reference concentrations of urinary strontium are established for healthy Japanese population. We suggest that the method and concentrations reported in this study will be useful as a practical guideline for the biological monitoring of strontium in various cases, including those of exposure to occupational, environmental and next-generation pharmaceuticals.

Materials and Methods

Subjects

Taking advantage of the periodical medical examination of companies, spot urine samples were collected from 146 healthy workers (115 male, 31 female) from a Japanese electronic firm. Their mean age was 33 ± 9 y. o., ranging from 18 to 58 years. Informed consent of all participants and their legally authorized representatives was obtained. They agreed to the purpose of this study and were informed that their samples will never be used other than the purpose of this study. None of the subjects was occupationally, environmentally or therapeutically exposed to strontium. The samples were collected and stored in plastic containers at or below -20°C until analysis.

Strontium standard solution

Ultrapure water of 18 M Ω cm obtained using a WG240/260 water purifier (Water Still System, Yamato scientific Co., Ltd., Tokyo, Japan) was used to prepare standards and samples in the study. A calibration curve was constructed using solutions containing strontium at 0, 50, 100, 200 and 300 $\mu\text{g/L}$. The standards were prepared by dilution of a commercial 1000-ppm strontium stock solution (code 193-06061, Lot YPG8206) from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Apparatus

An ICP-AES system (P-5200-3600/1200, Hitachi Ltd., Tokyo, Japan) was used for the determination of strontium. The operating conditions were as follows: RF power at torch, 1.0 kW; voltage, 700 V; frequency, 27.12 MHz; slit width, 30 μm ; slit height, 10 mm; observation height, 15 mm; auxiliary plasma gas flow rate 1.0 L/min; plasma gas flow rate, 16.0 L/min, and nebulizer plasma gas flow rate, 0.4 L/min. The

characteristic wavelengths of strontium are 407.771 nm and 421.552 nm. To select the most sensitive method, the signal-to-background ratio (S/B ratio) was examined in the strontium concentration range of 0–300 $\mu\text{g/L}$.

Urine analysis

The specific gravity of the urine samples was determined using a clinical refractometer from Erma (Tokyo, Japan). The urine samples were thawed by incubating at 40°C for 30 min and sediments were dissolved by vigorous shaking. Then, the samples were centrifuged at 3000 g for 5 min, and the supernatant was directly introduced into the ICP-AES system. When the peak height exceeded the linear calibration range, the sample was appropriately diluted. The strontium concentration was calculated from a standard curve.

Since Levine and Fahy (15) found that urinary lead content is proportional to $24/\text{SG}$, where SG is the last two digits of a specific gravity of the urine sample, quantitative urinary concentrations of trace elements and chemical metabolites adjusted for urine specific gravity have been employed in the fields of environmental and industrial medicine for more than 50 years. This adjustment procedure normalizes all results to a specific gravity of 1.024 by multiplying the analytical result by $24/\text{SG}$.

Strontium concentration was adjusted to normal urine density using Eq. 1 based on the suggestion of Levine and Fahy (15):

$$\{\text{Sr}\} = [\text{Sr}] \frac{24}{\text{SG}} \quad (1)$$

where $\{\text{Sr}\}$ is the specific gravity-corrected strontium concentration and $[\text{Sr}]$ is the observed strontium concentration. Specific gravity-adjusted strontium levels were used in statistical analysis.

Quality assurance

The spectra of urine samples were examined to confirm that there were no interfering lines near the selected wavelengths of strontium by comparing them with those of standard strontium solutions at 100, 150, 200 and 300 $\mu\text{g/L}$. Matrix spike samples were analyzed to determine the effect of sample matrix on analytical accuracy. For this purpose 1-ml aliquots of strontium were added to 1-ml urine samples and increase in emission intensity was measured. Accuracy was evaluated using % recovery, obtained by dividing spiked sample concentration by certificate value and then multiplying by 100. Reproducibility was evaluated using % coefficient of variation (% CV), obtained by dividing the standard deviation by the arithmetic mean concentration and then multiplying by 100.

Statistical analysis

The statistical analysis software StatView for Windows v. 5.0 from SAS Institute Inc. Cary, NC, USA and MS Excel 2000 for Windows were used for data entry and analysis.

Results

Figure 1 shows the signal-to-noise ratios of strontium at

Fig. 1 Signal-to-background ratio of standard solutions at the characteristic wavelengths of strontium.

two wavelengths. In the 0–300 µg/L concentration range, there is a steeper increase in the S/N ratio at 407.771 nm than at 421.552 nm. A good effective linearity of the calibration line at 407.771 nm in the same concentration range is shown in Figure 2. The regression analysis of strontium concentration and signal intensity in this range gave the equation:

$$y = 3805x + 17047 \quad (r = 0.999) \quad (2)$$

where x = Sr concentration (µg/L) and y = signal intensity.

Fig. 2 Calibration line used for strontium determination by ICP-AES at $\lambda = 407.771$ nm.

Figure 3 (A) shows the fluctuations of the blank background signal at 407.771 nm when 18 MΩ cm ultrapure water was introduced to the ICP-AES system. Figure 3 (B–F) shows the spectra for strontium standards at 50, 100, 200, 300 µg/L and urine at 407.771 nm. A good correspondence of the single peak pattern among spectra shown in Figures 3 (B–E) and (F) was observed. Table 1 shows the background signal of 18 MΩ cm ultrapure water used as the blank sample, the strontium standard solution, and urine. As the percent difference (range: –0.70–1.57%) of the background signal of the blank sample in

Fig. 3 (A) Fluctuations of blank background signal from 18 MΩ cm ultrapure water. (B–F) Emission spectra from strontium standard solutions and from a blank urine sample: (B) 100 µg/L standard; (C) 150 µg/L standard; (D) 200 µg/L standard; (E) 300 µg/L standard; (F) Blank urine sample.

Table 1 Background signal differences between strontium standard solutions and urine samples with blank sample (n=3)

Sample	Background Level	Difference from blank (%)
Blank*	153015±525	
Strontium Standard (100 µg/L)	151950±116	-0.70
Strontium Standard (200 µg/L)	153983±637	0.63
Strontium Standard (300 µg/L)	155088±816	1.36
Urine	155418±276	1.57

* The blank was 18 MΩ cm ultrapure water.

Table 2 Recovery and reproducibility of spiked strontium standard solutions (n=5)

Strontium concentration (µg/L)	Recovery (%)	CV (%)
50	100.7	1.0
100	101.2	2.4
200	95.1	1.7

Fig. 4 Frequency percent distribution histogram for strontium concentrations corrected for specific gravity of urine (n=146).

this table shows, both strontium standard solution and urine showed the same background signal as that of the blank. The sample detection limit was set at the threefold the background signal noise deviation of the blank sample. The positive y-intercept value of 17047 in Eq. 2 was ignored and the threefold background signal noise deviation of the blank sample (Table 1) was substituted for y in Eq. 1, then the detection limit was confirmed to be 0.414 µg/L. Table 2 shows the spike recovery from urine matrix.

Logarithmic urinary strontium concentration showed a significant relationship with urine specific gravity ($r=0.476$, $p<0.001$).

Figure 4 shows a histogram of the distribution of corrected urinary strontium concentrations. The frequency distribution in this figure shows a pronounced right-shift and increased skewness. High positive values of skewness (1.46) and kurtosis (3.78) were obtained, indicating a long tail of high urinary strontium concentrations (positive skewness) and a tail longer than normality (positive kurtosis). The shape of the histogram shown in Figure 4 suggests not a Gaussian, but a log-normal distribution. The linearity of the observed distribution plotted

on log-normal probability paper confirmed the log-normal distribution in Figure 4. A histogram obtained after a logarithmic transformation of corrected urinary strontium concentrations became close to a normal or a Gaussian distribution (skewness, -0.39; kurtosis, -0.10).

From the analysis of 146 samples in this study, the geometric mean urinary strontium concentration was determined to be 143.9 µg/L, with a 5–95% confidential interval of 40.9–505.8 µg/L.

Discussion

In trace element analysis by ICP-AES, matrix-matched calibration is often carried out for various materials (16). The method often requires acid (17) and microwave digestion (18). Although pretreatments are effective to dissolve a sample and compensate for the matrix effects, they often cause contamination or a decrease in recovery rate. In the present study the background signal of urine samples was the same as that of the blank sample, proving that urine has an insignificant matrix effect; therefore, no matrix-matching procedure was necessary. The linearity of our calibration line in the range of 0–300 µg/L, the good correspondence of the single peak pattern of spectra, accuracy and precision confirm that the method is reliable and adequate for measuring trace strontium in urine samples.

Previously, we demonstrated the appropriateness and legitimacy of procedures to determine the reference concentrations of urinary trace elements (19, 20). In these studies, the obtained urinary trace element concentrations were corrected for specific gravity, the frequency distribution of the corrected urinary concentrations was confirmed to be log normal, and the geometric mean concentration and confidence interval were calculated by logarithmic transformations of the corrected urinary concentrations and the exponentiation of the log-transformed concentrations.

Creatinine level and urine specific gravity are two common clinical parameters used for the adjustment of urinary trace element concentrations. It is not appropriate to adjust for creatinine level when an element is excreted primarily by tubular secretion (21). Although the mechanism of strontium excretion is as yet unknown, creatinine was not used for adjustment because our present study included subjects of both sexes with a wide range of ages, so it may be influenced by muscularity, physical activity, urinary flow, time of day, diet, and other states (21, 22).

For these reasons, specific gravity was used to normalize urinary strontium level in this study. As a log-normal distribution is quite common for both biological and non biological parameters (23), it was not surprising that urinary strontium showed this type of distribution pattern. Log transformation of trace element data prior to analysis is becoming the standard in determining reference concentrations (24, 25).

Although raw data may be useful for detecting correlations to other parameters, for identifying significant changes in case of exposure or massive intake, log-transformed data are more reliable and useful because they approximate a normal distribution, which is necessary for statistical analyses. The same data treatment can be used to obtain reference concentrations of

other trace elements that are excreted via urine.

In recent years, many reports have dealt with the analysis of trace elements in human biological specimens from various countries. However, reference urinary strontium concentrations are scarce (26–28). Komaromy-Hiller et al. (29) determined urinary strontium in 1439 subjects from the U.S. by inductively coupled plasma mass spectrometry, yielding 139.4 ± 76.1 $\mu\text{g/L}$ (mean $\pm$ SD) and 19.0–256.1 $\mu\text{g/L}$ for central 95%. By flameless atomic absorption spectrometry, Leeuwenkamp et al. (30) obtained mean urinary strontium concentration of 158 $\mu\text{g/L}$. Iyengar et al. (31) reported a reference concentration of 110–390.1 $\mu\text{g/L}$. Judging from these reports, it can be assumed that the mean urinary strontium concentration is ≈ 100 $\mu\text{g/L}$ and has a wide distribution range of 20–400 $\mu\text{g/L}$. In this study, we determined urinary strontium concentration in Japanese workers, and obtained the geometric mean concentration of 143.9 $\mu\text{g/L}$, with a 5–95% confidential interval of 40.9–505.8 $\mu\text{g/L}$, which compares well with those reported in the literature.

Strontium might not be under strict homeostatic control besides bone affinity, so the rate of elimination via urine is directly proportional to its concentration. This elimination phase can be described by the negative exponential function of time. The log-normal distribution of urinary strontium concentration in the histogram reflects this nonlinear process of strontium elimination from the body with time. A wide confidential interval and a high volatility of urinary strontium observed in this study may suggest the fluctuation of strontium

concentration per certain unit of its excretion time.

We insist on the necessity of trace element monitoring because the number of people with chronic organ failure or functional disorders would increase as the percentage of elderly population increases, leading to potential problems related to trace element disturbances (32). We previously reported abnormally high concentrations of serum fluoride and boron in patients undergoing hemodialysis treatment (33–35). Although fluoride and boron are also known as antiosteoporotic agents (36–39), an enhanced retention of these elements in the skeletal system more frequently results in osteomalacia, or in the possible disruption of bone metabolism (40, 41). Trace element imbalance in patients with chronic renal failure is a common phenomenon for which proper management is not yet available (42, 43).

Awareness and understanding of monitoring of urinary trace elements should be a priority for health care providers. Urine sampling is simple, non-invasive and easy to perform repeatedly. It is well suited for reliable strontium analysis by ICP-AES with minimal sample preparation. The reference concentrations and analytical method for the determination of urinary strontium concentration described in this study can be used as a biological benchmark for the prevention of excessive retention of strontium arising from its therapeutic use or renal function disorders and the protection of individuals exposed to strontium in their working or living environment.

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