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Element Concentrations in Urine of Patients Suffering from Chronic Arsenic Poisoning

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Xie, Y., Miyamoto, H., Kondo, M., Koga, H., Zhang, A., Ohmichi, M., Inaba, Y. and Chiba, M. *Element Concentrations in Urine of Patients Suffering from Chronic Arsenic Poisoning.* Tohoku J. Exp. Med., 2001, **193** (3), 229-235 —— In order to know the element levels in the urine of patients with chronic arsenic poisoning caused by arsenic assimilated from burning coal via air and food, we investigated various elements in the urine of 16 patients with this disease and 16 controls living in the same county in Guizhou Province of China. Concentrations of 25 elements (Al, As, Ba, Be, Bi, Ca, Cd, Cr, Cu, Fe, Ga, Mg, Mn, Mo, Ni, P, Pb, Rb, Sb, Se, Sn, Sr, Ti, V and Zn) were determined by an inductively coupled plasma mass spectrometer or an inductively coupled plasma atomic emission spectrometer. The average concentrations of Cu, Ga and Sn as well as As in the patients were significantly higher, and those of Cr, Rb, Sr and Ti in the patients were significantly lower than the control values. Al, Ba, Mn, Ni and Se were under detection limit in the patients, though they could be detected in the controls. There were no positive correlations between the concentration of As and the concentrations of other elements, including Cu, Ga and Sn in the patients. The results of this study suggest that As from burning coal might influence the urinary excretion of some elements. ——— arsenic poisoning; human trace and major elements; urine; burning coal; China

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Urinary elements are important indexes reflecting the changes in element metabolism due to body burden with xenobiotic substances, and are often used in medical studies, especially epidemiological surveys in preventive medicine.

In Guizhou Province in southwest China, about 200 000 local inhabitants are exposed to arsenic (As), because they use domestic coal for their daily life (Wang et al. 1996). The raw coal contains much As (usually about 600 ppm [Zhou et al. 1998], in some cases as high as 35 000 ppm [Finkelman et al. 1999]). They burn it with indoor stoves without chimneys in order to cook, to dry crops directly over the coal fires and to warm themselves in winter, so the indoor air and crops are polluted by As emitted from burning coal. Owing to assimilation of As from coal via air and food, more than 3000 local inhabitants suffer from endemic chronic arsenic poisoning (arsenism), of which the main clinical manifestations are hyperkeratoses on the palms and foot soles (Liu et al. 1992; Wang et al. 1996; Finkelman et al. 1999). In 85 villages in Guizhou, 2548 endemic arsenism patients were found, and the prevalence rate was 17.28% (Zhou et al. 1998). This cause of the endemic arsenism in Guizhou is unique and different from the causes of arsenism that have occurred in the other areas in the world, for example, some areas in north China (Huang et al. 1983; Sun 1994), Taiwan (Tseng 1977), south Asia (Nickson et al. 1998; Subramanian and Kosbett 1998) and Latin America (Cebrian et al. 1983; Grinspan and Biagini 1985). In those areas the endemic arsenism is caused by excessive As in natural drinking water.

The effect of As on organisms is complicated. As may interfere with the metabolisms of other elements (Diaz-Barriga et al. 1990; Ip and Ganther 1992; Berry and Galle 1994), and it may change the element contents in tissues (Gerhardsson et al. 1982; Hu et al. 1991; Ademuyiwa et al. 1996; Zhang et al. 1998; Hunder et al. 1999) and/or element excretion in urine (Cikrt et al. 1988). However, there has been no report about the urinary levels of various elements in arsenism patients or about the influence of arsenical exposure on the urinary excretion of other elements, though there have been some reports about the element contents in the scalp hair of the inhabitants in some endemic areas of water-caused arsenism in north China (Hu et al. 1991; Zhang et al. 1998).

In the present paper, in order to determine the element levels in the urine of the patients with the endemic arsenism caused by As from burning coal, we measured various elements in the urine of 16 patients and 16 controls living in the same county in Guizhou Province.

SUBJECTS AND METHODS

Subjects

The subjects were 16 endemic arsenism patients (7 males and 9 females with the average age of 37.1 years) and 16 unexposed healthy controls (8 males and 8 females with the average age of 40.1 years) residing in the same county in southwest Guizhou, China. The 16 patients, who lived in a severe endemic area of the coal-caused arsenism, had been exposed to As for 8 to 25 years and had typical clinical manifestations of dermal injuries, which were diagnosed according to the Diagnostic Criteria of Endemic Arsenism (Wang 1994), but they had no other diagnosed serious diseases. The healthy controls had similar life style including food custom as the patients, but they did not use coal containing high amounts of As. They were subjected to the clinical hepatic and renal tests, and all were found to be normal. All the subjects gave their informed consent to be studied.

Determination of element concentrations in urine

Urine was collected from the subjects in the early morning on one day in late March, 2000. Urine samples were stored in frozen state until the analysis was performed. After 1 ml urine was digested with 0.4 ml concentrated HNO_3 and 0.2 ml H_2O_2 in a microwave oven (MLS-1200 MEGA, Milestone, Italy), the residue was filled up to 10 ml with mil-Q water. The concentrations of 22 elements (Al, As, Ba, Be, Bi, Cd, Cr, Cu, Fe, Ga, Mn, Mo, Ni, Pb, Rb, Sb, Se, Sn, Sr, Ti, V and Zn) were determined by an inductively coupled plasma mass spectrometer (ICP-MS, Yokogawa Co., Tokyo), and the concentrations of 3 other elements (Ca, Mg, P) were determined by an inductively coupled plasma atomic emission spectrometer (ICP-AES, Seiko Instruments Inc., Tokyo). The apparatus and analytical conditions of the determination are shown in Table 1. Much attention was paid to avoidance of contamination from laboratory tools, environment and reagents. All glassware was soaked in nitric acid solution for at least one day.

Urinary creatinine was determined with a commercially available kit (Eiken Kagaku, Tokyo), and then the urinary element concentrations were adjusted by creatinine concentration.

Statistical analysis

The average values and degrees of variation were represented by arithmetic mean±S.D. for the data with a symmetric distribution, and by geometric mean and range for the data with a positively skewed distribution, or by median and range for the data with other shapes of distributions. Analyses of significant differences between the groups were performed with Student's t-test. When necessary, logarithmic transformation was made for the data with a positively skewed distribution before the t-test was made.

TABLE 1. *Instrumental operating conditions for urinary element determination*

Condition	ICP-MS	ICP-AES
Plasma gas flow	14±0.5 liter/min	16 liter/min
Auxiliary gas flow	0.8±0.2 liter/min	0.6 liter/min
Carrier gas pressure	2.4 kg/cm^2	2.2 kg/cm^2
RF Power	1.2 kw	1.1 kw

ICP-MS, Inductively coupled plasma mass spectrometer (Yokogawa Analytical Systems, PMS-200 [Yokogawa Co., Tokyo]). ICP-AES, Inductively coupled plasma atomic emission spectrometer (SPS 1500 V [Seiko Instruments Inc., Tokyo]).

TABLE 2. *Element concentrations in urine*

	Patients (n=16)	Controls (n=16)
Al	ND	419 (ND-7.96×10^3)[a]
As	184.4±200.0*	86.8±64.2
Ba	ND	3.5 (ND-1.09×10^3)[a]
Be	ND	ND
Bi	ND	ND
Ca	(1.25±0.57)×10^5	(1.62±0.94)×10^5
Cd	5.2±6.6	3.3±2.3
Cr	35.2±32.9*	81.0±64.7
Cu	94.7±67.8**	42.3±19.2
Fe	(1.10±0.44)×10^3	(1.45±0.69)×10^3
Ga	4.2±3.3**	1.6±1.1
Mg	(0.90±0.48)×10^5	(1.27±0.63)×10^5
Mn	ND	12 (ND-295)[a]
Mo	255±158	332±328
Ni	ND	29.5 (0.6-805.6)[b]
P	(5.49±2.18)×10^5	(4.60±2.35)×10^5
Pb	31.0±29.4	19.7±35.5
Rb	945±382**	1,916±838
Sb	4.8 (1.2-97.3)[b]	2.3 (0.5-6.4)[b]
Se	ND	26.3±16.4
Sn	43.7±39.8**	12.9± 9.6
Sr	131±67*	448±216
Ti	7.5±8.4**	23.2±14.8
V	14.9±9.7	15.7±10.7
Zn	(5.85±6.54)×10^3	(4.33±4.41)×10^3

ND, not detectable.

All values are expressed in μg/g creatinine; the data are mean±S.D., except for the ones marked with "a" or "b."

a, median (range).

b, geometric mean (range).

*$p<0.05$, **$p<0.01$ as compared with the controls.

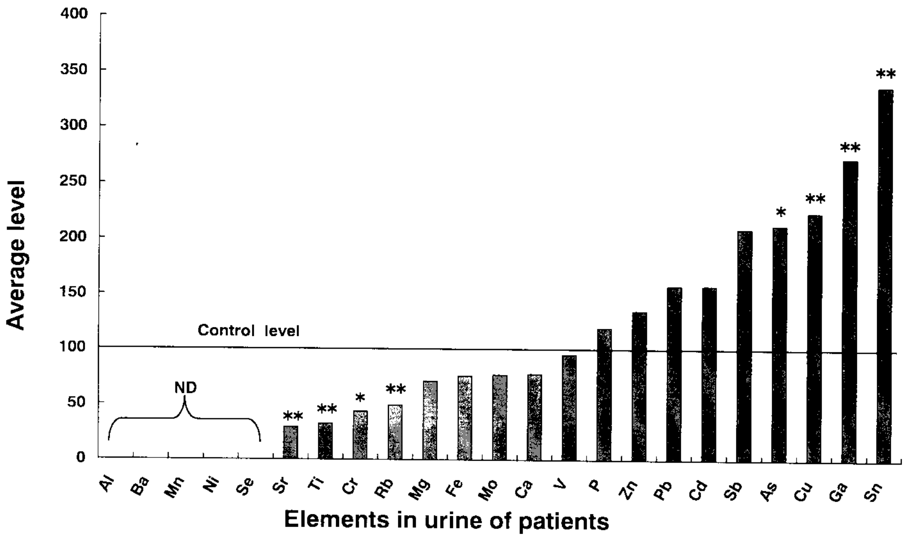

Fig. 1. Relative levels of the elements in the patient's urine. The average values of the controls were fixed at 100 (units), and then relative values of the average concentrations of the patients were calculated. $^{*}p<0.05$, $^{**}p<0.01$ as compared with the controls. ND, not detectable.

RESULTS

As concentration in urine

The average As concentration of the patients (184.4 μg/g creatinine or 163.6 μg/liter) was significantly higher than that of the controls (86.8 μg/g creatinine or 64.3 μg/liter) as shown in Table 2.

Other element concentrations

The concentrations of various elements are shown in Table 2. Be and Bi in all samples as well as Al, Ba, Mn, Ni and Se in the patients' urine were under the detection limit, which was 10 ng/ml with a small range of variations for various elements. The average concentrations of Cu, Ga and Sn were significantly higher, and those of Cr, Rb, Sr and Ti were significantly lower in the patients than in the controls ($p<$ 0.01 or 0.05).

Fig. 1 shows the relative levels of the average element concentrations of the patients, with the corresponding control values of the elements fixed at 100 (units).

No significant correlations were found between the concentration of As and the concentrations of the other elements, including Cu, Ga and Sn in the patients (data not shown).

DISCUSSION

Some local inhabitants living in the area where our 16 arsenism patients resided are still exposed to excessive As from burning coal (Yang et al. 1998; Zhou et al. 1998; Xie et al. 1999), though various countermeasures have been tried to prevent arsenic poisoning, and the domestic contamination by As has been alleviated. According to a recent report, 32 out of 219 villagers in a village in this area got arsenic poisoning from 1991 to 1997 (Zhou et al. 1998).

Element concentrations in urine reflect the present situations of these elements inside the body. The average urinary As concentration was significantly higher in the patients than in the controls (Table 2), suggesting that these patients were still exposed to As when the urine samples were collected. The average As value of the 16 patients (184.4 μg/g creatinine or 163.6 μg/liter) was lower than the average value (400 μg/liter) of the endemic arsenism patients in the same area measured in 1991

(Zhou et al. 1993), indicating that the extent of arsenical exposure of the patients in this area had reduced. This value of the 16 patients (184.4 μg/g creatinine) was comparable to the average values (129.0 μg/g creatinine for the low exposure group, and 257.6 μg/g creatinine for the high exposure group) of urinary As in some French smelter workers exposed to As (Telolahy et al. 1993). The average As value (86.8 μg/g creatinine or 64.3 μg /liter) of the 16 controls in this study was similar to the control value (70 μg/liter) measured in this area in 1991 (Zhou et al. 1993).

Because As has extensive and complicated influences on many tissues and physiological functions, the results of this study showed notable differences in urinary concentrations of many elements between the patients and controls, which may suggest that As from burning coal might influence the metabolisms, including urinary excretion, of these elements in the arsenism patients.

As shown in Table 2 and Fig. 1, Cu, Ga and Sn concentrations in urine were significantly higher in the patients than in the controls. It was conjectured that these elements might come from coal, along with As. However, there was no positive correlation between the concentration of As and the concentrations of these elements in urine.

The Cu concentration in the patients was significantly higher than in the controls. This finding might be related to the As-enhanced Cu accumulation in kidneys, as reported in previous papers (Maehashi et al. 1983; Ademuyiwa et al. 1996; Hunder et al. 1999).

It is well known that Se interacts with some kinds of elements, especially toxic metals such as Hg, Cd, and Pb, and that it reduces the toxicity of these metals (Himeno 1996). It was reported that there were antagonistic interactions between As and Se in organisms (Ferm 1977; Howell and Hill 1978; Babich et al. 1989; Kraus and Ganther 1989), and it was also found that As and Se were concentrated and precipitated in the lysosomes of the renal cells in the form of insoluble selenide (As_2Se) (Berry and Galle 1994). Therefore, the reason for very low Se levels in the patients (Table 2 and Fig. 1) suggests that Se in the body was consumed to reduce the toxicity of As.

The average Ni, Mn and Ba concentrations were significantly lower in the patients than in the controls. This might have some relation with the fact that lower-than-control contents of Ni, Mn and Ba in scalp hair were found in the patients with endemic combined poisoning of arsenic and fluoride or in the inhabitants living in the endemic area of this disease in China (Hu et al. 1991).

It is not clear why Sr, Ti, Cr, and Rb levels in urine of the patients were significantly lower than in the controls. These elements may play some roles and be used inside the body. The reason for the low Al concentration in the patients is not clear, either.

The urinary excretion of elements in the subjects can be influenced by many factors such as diet, health conditions, especially renal function, and even other elements emitted from burning coal. In this study, though these factors were not under strict surveillance, there were similarity and comparability in customs of life, including diet between the patients and controls, and none of the subjects had any serious diseases diagnosed except for the arsenic poisoning in the individual patients. Therefore it is difficult to explain the reason for the differences in element concentrations.

In conclusion, the results of this study not only provide information concerning the various element levels in urine from healthy Chinese adults, but also may suggest the possibility that As from burning coal might influence the urinary excretion of some elements in the patients.

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