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Arsenic and Lipid Peroxidation in Patients with Blackfoot Disease

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Blackfoot disease is a peripheral vascular disorder endemic in a limited area on the southern coast of Taiwan. This disease results in gangrene of the lower extremities. It occurs in people using artesian well water containing variable concentrations of arsenic (Chen and Wu 1962). Arsenic contamination of drinking water is known to cause chronic poisoning. An association between the effects of chronic exposure to a high level of arsenic in drinking water and the occurrence of a variety of skin disorders, skin cancer, and Blackfoot disease was found in 1968 (Tseng et al. 1968). Though the discovery of fluorescent compounds in drinking water of endemic area has arisen the interest for reinvestigation of the cause of Blackfoot disease (Lu et al. 1982), the importance of arsenic in connection with the disease has not been doubted.

Lipid peroxidation, the oxidative deterioration of polyunsaturated fatty acids, is widely accepted as a general mechanism for cellular injury and death (Gutteridge and Quinlan 1983; Halliwell 1984). Numerous studies have shown increased indices of lipid peroxidation in laboratory animals exposed to the acute toxic effects of various transition metal ions, including Fe, Ni, Cd, Co, Cu, Pb, Sn and V (Sunderman Jr. 1986). In vitro studies also showed that the primary mechanism of the acute toxic effects of these metal ions is due, at least in part, to metal-catalyzed peroxidation of polyunsaturated fatty acids (Knight and Voorhees 1990).

The chemical form of most of the arsenic in the artesian well water is inorganic As (III). It is rather toxic. In this study, we therefore try to find evidence revealing whatever differences in arsenic and malondialdehyde (an end product of the lipid peroxidation) may occur in patients with Blackfoot disease in comparison to the normal population, and to attempt to elucidate the

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possible etiological connection of arsenic and malondialdehyde with the disease on the basis of analytical results.

MATERIALS AND METHODS

A Perkin-Elmer Model 5100PC atomic absorption spectrophotometer equipped with electrodeless discharge lamp, and a Mercury/Hydride System MHS-10 were used to determine the arsenic concentration in urine. The arsenic electrodeless discharge lamp was operated at 8 W, and determination was carried out at the most sensitive (193.7 nm) wavelength with a 0.7 nm slit.

High purity water (18.3 MΩ-cm) was used throughout this work. All the acids and bases used were of suprapure grade (E. Merck). The other reagents were of analytical reagent grade.

The urine specimens were collected from 35 normal controls and 35 patients with Blackfoot disease (inpatients from the Prevention and Treatment Center for Blackfoot Disease). The spot urine specimens were collected in the presence of 0.5 ml of 6 mol/l sulfuric acid, and they were either kept at 4 ℃ for no longer than 2 days or stored for a few weeks at -20 ℃. The blood samples were collected by venipuncture into 7-ml evacuated tubes containing EDTA solution as anticoagulant (Wong et al. 1987). Centrifuge the blood and remove the supernatant plasma, being careful to avoid contamination with platelets. The plasma was stored at -70 ℃ for no longer than 3 weeks prior to analysis (Uysal et al. 1986).

Determination of arsenic was made by adding 0.5 ml urine sample directly, into a reaction flask (of MHS-10 Mercury/Hydride System) containing 10 ml of 1.5% HCL and 1 ml of diluted Antiform 110 A Emulsion (1: 100), and subsequently adding 2 ml of reductant solution. The arsine evolved was measured following the instrumental procedures described (Lin et al. 1985). The standard urine sample (Seronorm Trace Elements Urine, batch no. 009024, Nycomed), which was used to check analytical reliability, was analyzed by the same procedures.

Duplicate aliquots of blood plasma (50 μl) were pipetted into polypropylene tubes that contained 0.75 ml of phosphoric acid (0.44 mmol/l). The samples were analyzed for malondialdehyde by the thiobarbituric acid (TBA) reaction, with HPLC separation of the MDA-TBA adduct, using tetraethoxypropane (TEP) as the standard (Wong et al. 1987). The malondialdehyde in urine was determine by similar method (Knight et al. 1988).

Creatinine concentrations, necessary for the correction of arsenic and malondialdehyde concentration for fluctuations in urinary volume, were routinely determined, using an automatic method based on the Jaffe reaction, by Hitachi Model 736-40 automatic analyzer.

RESULTS AND DISCUSSION

The analytical reproducibility of arsenic and malondialdehyde in Seronorm Trace Elements Urine (Bacth no. 009024, Nycomed, Norway) determined by HGAAS and HPLC is precise since the coefficients of the variation of both intra-assay and inter-assay are less than 10% (n = 7). From the seven determinations of the same control urine, a value of 98.4 ± 2.1 μg/l of arsenic was obtained, which agree well with the certified value of 100 μg/l. On the basis of verified analytical reliability of the established methods, various samples from the patients of Blackfoot disease and the control group were analyzed.

The analytical results for the concentration of arsenic in urine among 35 people without any particular exposure to arsenic are shown in Table 1. The subjects include 18 men and 17 women, and the age of the urine suppliers was from 54 to 85. The sex and age are matched with the patients. No variant values were seen between the different sexes ($P > 0.05$). The mean value of the controls is 39.4 ± 8.0 μg/g creatinine with a range of 25.6 - 60.0 μ g/g creatinine. The mean value of the arsenic in urine of 35 patients is 55.5 ± 13.1 μg/g creatinine with a range of 33.4 - 81.0 g/g creatinine. It is significantly higher than that of the controls as shown in Table 1 ($P < 0.01$). Urinary arsenic is therefore closely associated with Blackfoot disease.

Table 1. Arsenic in urine of Blackfoot disease and controls (μg/g creatinine)

	Blackfoot (n = 35)	Controls (n = 35)	P value
	Mean ± SD		
Arsenic	55.5 ± 13.1	39.4 ± 8.0	$P < 0.01$

The mean values of plasma malondialdehyde in patients and controls are 1.62 ± 1.37 μmol/l and 0.95 ± 0.50 μmol/l respectively. The malondialdehyde in plasma of patients is significantly higher than that of the controls ($P < 0.01$, Table 2). The mean value of urinary malondialdehyde of controls is 0.86 ± 0.58 μmol/g creatinine. The

Table 2. Lipoperoxide concentrations in urine and plasma of Blackfoot disease and controls

MDA	Blackfoot	Controls	P value
	Mean ± SD		
Plasma MDA (μmol/L)	1.62 ± 1.37 (n = 24)	0.95 ± 0.50 (n = 66)	P < 0.01
Urine MDA (μmol/g creatinine)	2.02 ± 0.86 (n = 35)	0.86 ± 0.58 (n = 35)	P < 0.01

mean value found for the 35 patients is 2.02 ± 0.86 μmol/g creatinine, which is obviously significantly higher than that of the controls ($P < 0.01$, Table 2).

In this study, both arsenic and malondialdehyde are found to be higher than those of the controls. Nevertheless, we can not distinguish whether the events are primary (arsenic/disease produces free radicals leading directly to lipid peroxidation and cellular injury) or secondary (arsenic/disease causes cells damage by an alternate mechanism which in turn leads to increased lipid peroxidation) (Halliwell 1984). In this latter regard, damaged cells and tissues are known to undergo increased lipid peroxidation, presumably secondary to membrane disruption by enzymes released from lysosomes, release of arsenic from their storage site, and failure of antioxidant mechanisms (Halliwell 1984).

Linear regression curve for urinary arsenic versus malondialdehyde concentration is shown in Fig. 1. Urinary malondialdehyde concentration is weakly correlated with urinary arsenic in patients ($r = 0.370$, $P < 0.05$). It may indicate that the mechanism of arsenic effects on the Blackfoot disease is due, at least in part, to the lipid peroxidation of polyunsaturated fatty acids.

Numerous studies have shown increased indices of lipid peroxidation in laboratory animals exposed to the acute toxic effects of various transition metal ions (Sunderman Jr. 1986). In addition, in vivo studies on the acute toxicities of Cu, Hg and Cd showed a further increased in the indices of lipid peroxidation in various rodent tissues by dietary deficiency of the antioxidants vitamin E or Se;

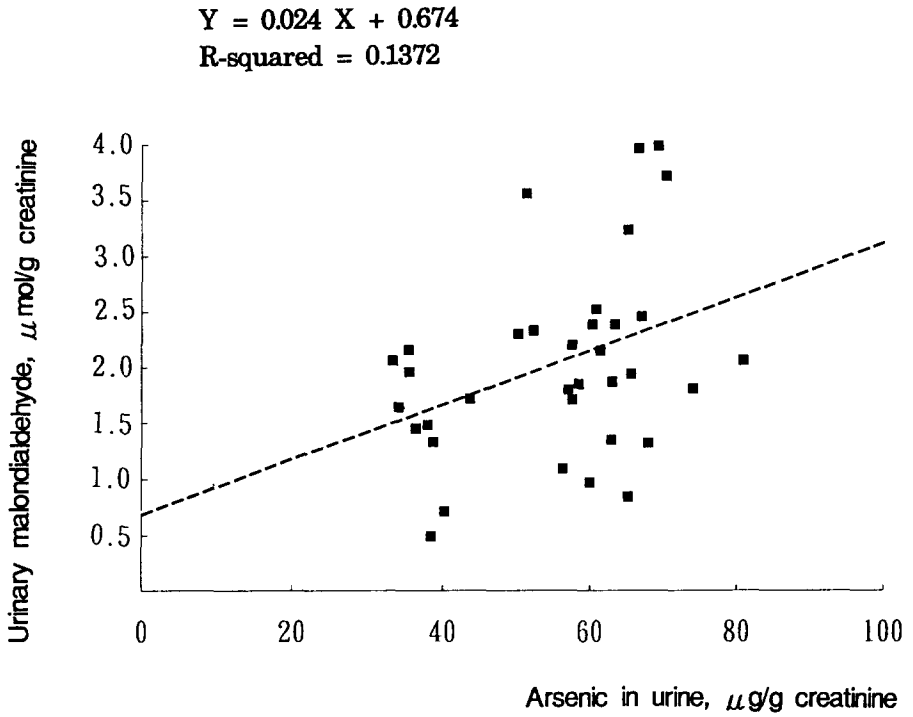

Figure 1. Regression curve of urinary arsenic versus malondialdehyde concentration

supplemented vitamin E, Se or Zn ameliorated their concentrations (Dougherty and Hoekstra 1982; Fukino et al. 1984; Levander et al. 1977; Sugawara and Sugawara 1984). In our previous study, we found that Zn and Se were deficient in patients with Blackfoot disease (Lin and Yang 1988). It therefore may enhance the damage of arsenic and lipid peroxidation to the cells of the organisms. Further studies on the effects of reactive oxygen systems, antioxidants, arsenic species and lipid peroxidation on the Blackfoot disease are required.

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