ARSENIC SPECIES IN DRINKING WATER, HAIR, FINGERNAILS, AND URINE OF PATIENTS WITH BLACKFOOT DISEASE
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Published online: 30 Nov 2010.

To cite this article: Te-Hsien Lin Yeou-Lih Huang Ming-Yuh Wang (1998) ARSENIC SPECIES IN DRINKING WATER, HAIR, FINGERNAILS, AND URINE OF PATIENTS WITH BLACKFOOT DISEASE, Journal of Toxicology and Environmental Health, Part A: Current Issues, 53:2, 85-93, DOI: 10.1080/009841098159376

To link to this article: http://dx.doi.org/10.1080/009841098159376
An endemic peripheral vascular disease called blackfoot disease occurs in a large number of inhabitants on the southwest coast of Taiwan. The disease has an insidious onset, with numbness or coldness as the initial symptoms, followed by progressive development of localized ulceration and subsequent gangrenous changes, as evidenced by the characteristic black coloration of dry gangrene. The symptoms and signs of blackfoot disease are similar to those found in arteriosclerosis and Buerger’s disease (thromboangiitis obliterans). However, the severity...
of these symptoms is much greater in blackfoot disease (Yeh & How, 1963). In blackfoot patients there are vascular changes in the affected skin region characterized by a thickening and fibrinoid degeneration of the blood-vessel wall in the subcutaneous arterioles during the gangrenous phase (Yeh et al., 1969). Consequently, most blackfoot disease patients are permanently maimed as a result of spontaneous or artificial amputation of the affected extremity (Lo et al., 1977). The association of blackfoot disease with long-term arsenic exposure due to drinking water from high-arsenic artesian wells has been investigated. Although Tseng (1989) reported a concentration-response relationship between the prevalence of blackfoot disease and various arsenic concentrations in well water ingested by villagers afflicted with blackfoot disease, the etiology of this disease still needs clarification.

In the environment arsenic may occur in several forms including arsenite, arsenate, monomethylarsonic acid (MMAA), and dimethylarsinic acid (DMAA). The rank order of arsenical toxicity, as reflected by carcinogenesis and vascular disorders, is as follows: arsenite > arsenate > MMAA > DMAA (Lewis & Tatken, 1978). The methylation of inorganic arsenic in mammals is a detoxification process, which occurs primarily in the kidney. This results in the formation of metabolites that possess a diminished binding affinity for cellular macromolecules and are thus excreted (Chatterjee et al., 1995). When inorganic arsenicals are ingested, the main route of metal elimination is via the urine, with the metabolites DMAA and MMAA forming the predominant species in urine (Lagerkvist et al., 1986, 1988; Vahter & Lind, 1986). Arsenic is normally found in higher concentrations in human hair and nails than in other tissues, which may be attributed to the high content of keratin in these tissues, as keratin is known to bind to trivalent arsenic (Lin, 1986). Thus, measurement of arsenical species in urine, hair, and nails in blackfoot patients may provide evidence of a correlation between metal exposure and disease. This study was carried out to further clarify the association of blackfoot disease with long-term oral arsenic ingestion in endemic areas in Taiwan in a population that stopped consumption of high-arsenic artesian well water at least two decades earlier.

**MATERIALS AND METHODS**

**Apparatus**

A Perkin-Elmer (Norwalk, CT) model 5100pc atomic absorption spectrophotometer equipped with electrodeless discharge lamp and a mercury hydride system (MHS-10) was used. 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.
Reagents

High-purity water (18 MΩ-cm) was used throughout this work. All the acids and bases used were of suprapure grade (E. Merck, Germany). The sodium borohydride and antifoam 110 A emulsion (Darmstadt, E. Merck, Darmstadt, Germany) were of analytical reagent grade. A reducing solution containing 3% sodium borohydride in 2% sodium hydroxide solution was prepared daily by dissolving NaOH flakes in deionized water and subsequently adding this mixture to the solution with an appropriate amount of sodium borohydride powder. The solution was then passed through a fluted filter paper. A stock solution containing 100 ppm arsenic was prepared from the arsenic standard solution (1g Merck Titrisol standard solution) by diluting to 1 L with deionized water. The other standard solutions (1 ppm) were prepared according to Buratti et al. (1984). Cationic ion-exchange resin AG50W-X8, 100–200 mesh, was obtained from BioRad (Richmond, CA).

Sampling

Most of the wells in the endemic areas where blackfoot disease was prevalent are now closed. Therefore, only three nearby wells in Putai that are still being used were selected, since these wells are typical of the regions in terms of the depth (100–200 m) and arsenic concentration (about 0.5 ppm). The physicochemical characteristics of these wells are reported elsewhere (Chen & Wu, 1962a, 1962b). In addition, two nearby wells in one of the two control areas, Chai-Li (south of the blackfoot disease area), where the arsenic concentration in the groundwater is not the same as the blackfoot disease endemic area, were also chosen. In the Chai-Li area people drank the well water almost as long as those in the blackfoot disease endemic area, yet no blackfoot disease was reported. Since the boundaries of the regions of the endemic area might be criticized due to the sporadic occurrence of blackfoot disease, two nearby wells in Kaohsiung (the south coast of Taiwan) were selected as the other control area where the arsenic concentration in the groundwater appears normal.

High-density polyethylene containers were used for groundwater collection. These were precleaned with 10% nitric acid and then rinsed with distilled water. Groundwater sampling was done by allowing the well water to flow through the pumping pipe for about 10 min prior to the collection of water. Well water (1 L) used for arsenic speciation was treated at the sampling site by filtration with a 0.45-μm Millipore membrane and then acidifying with sulfuric acid (Chen et al., 1995). All of the well-water samples were stored at 4°C prior to analysis.

The urine, hair, and fingernail specimens were collected from 25 volunteer individuals in Kaohsiung city and 25 patients with blackfoot disease (inpatients at the Prevention and Treatment Center for Blackfoot
Disease in Pei-Men, Tainan). The subjects in both groups included 13 men and 12 women with a mean age of 68.3 ± 6.6 yr (range of 54–80 yr). The spot urine specimens were collected, acidified with 0.5 ml of 6 mol/L sulfuric acid, and then either kept at 4°C for no longer than 2 d or stored for a few weeks at −20°C. Hair sampling and washing were carried out by the procedure recommended by the International Atomic Energy Agency (IAEA) (Ryabukhin, 1978). About 100 mg of each hair sample was placed in Teflon beakers, mixed with acetone, washed with double-distilled water, and the liquid was decanted off. The hair samples were then weighed prior to analysis. After brushing, the fingernail specimens were treated in the identical manner as hair.

**Pretreatments and Procedures**

The total arsenic in the urine was analyzed as described by Buratti et al. (1984). The ion-exchange chromatographic separation of metabolic forms of As in urine, hair, and fingernails was carried out according to Buratti et al. (1984) and Yamauchi and Yamamura (1984b). Urine samples were acidified with concentrated HCl and passed through chromatographic columns filled with AG50W-X8 resin. About 100 mg of hair and fingernail samples were used for the assay for arsenic by atomic absorption spectrophotometry (Buratti et al., 1984). Creatinine concentrations, necessary for the correction of arsenic concentration for fluctuations in urinary volume, were routinely determined, using an automated method based on the Jaffe reaction using a Hitachi model 736-40 automatic analyzer (Lin & Huang, 1995).

**Statistical Analysis**

All data are reported as mean values ± SD. Data were statistically analyzed using Student’s *t*-test, with *p* values <0.05 for statistically significant difference.

**RESULTS**

Total arsenic concentration and the distribution of arsenite, arsenate, MMAA, DMAA, and trimethylarsenic acid (TMA) in the well waters are given in Table 1. In the blackfoot disease regions (wells 1–3) the concentrations of total As predominantly in the inorganic form were markedly higher than in the control well areas. The present recommended limit for arsenic in drinking water as total arsenic is 0.01 mg/L and the maximum permissible limit for total arsenic is 0.05 mg/L. The average value of total arsenic (614.6 ppb) in waters of the Putai area found in this study was about 12 times greater than the maximum permissible limit for arsenic in drinking water. In comparing the total As and inorganic As concentrations from the Chali region to Kaohsiung city there was an approximate twofold higher level in Chali water, which exceeded the maximum permissible limit. However, the As concentrations present in Chali waters were one-tenth less than those found
Data in Table 2 show that individuals drinking well water from a blackfoot disease region excrete significantly higher total urinary As. The increase in total urinary As was comprised of significant elevation in the concentrations of inorganic arsenic (InAs), MMAA, and TMA. A similar significant rise in total As was seen in the hair and fingernails of blackfoot-afflicted patients (Table 2). In contrast to urine, where MMAA and TMA were elevated, only InAs was significantly increased in hair and fingernails of blackfoot patients. Regardless of the water source, the concentration of DMAA was not markedly different between control and blackfoot-afflicted individuals. It is of interest that for within- or between-group comparisons of male versus female there were no significant differences (date not shown).

The sensitivity and reliability of the use of fingernails as a marker of exposure were examined. Clearly the use of hair as a biomarker of As contamination is well known (Ryabukhin, 1978). Data in our study show that a similar pattern of As exposure occurred in hair and fingernails (Table 2). Regression analysis of InAs in hair and fingernails is presented in Figure 1. A significant correlation was found between InAs concentrations in hair and fingernails, indicating that these tissues can be used as biomarkers of metal exposure.

**DISCUSSION**

The endemic area of blackfoot disease was on the southwest coast of Taiwan, where once agriculture, fisheries, and the salt industry flourished. Further development of agriculture and an increase of population in Putai. The methylarsenical concentrations were relatively low in all the well waters.

**TABLE 1.** Arsenic species concentrations in artesian well water obtained from blackfoot disease and control regions of Taiwan

<table>
<thead>
<tr>
<th>Well number</th>
<th>InAs (µg/L)</th>
<th>MMAA (µg/L)</th>
<th>DMAA (µg/L)</th>
<th>TMA (µg/L)</th>
<th>Total-As (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>720.8 ± 10.1</td>
<td>3.39 ± 1.1</td>
<td>2.07 ± 0.9</td>
<td>3.32 ± 1.0</td>
<td>729.6 ± 10.1</td>
</tr>
<tr>
<td>2</td>
<td>529.2 ± 20.9</td>
<td>3.49 ± 1.3</td>
<td>6.88 ± 1.6</td>
<td>3.67 ± 0.6</td>
<td>543.2 ± 19.3</td>
</tr>
<tr>
<td>3</td>
<td>559.9 ± 20.8</td>
<td>2.90 ± 0.5</td>
<td>3.00 ± 2.1</td>
<td>5.08 ± 1.5</td>
<td>570.9 ± 19.4</td>
</tr>
<tr>
<td>4</td>
<td>65.7 ± 2.8</td>
<td>2.40 ± 0.6</td>
<td>3.00 ± 0.9</td>
<td>3.70 ± 1.1</td>
<td>74.8 ± 2.1</td>
</tr>
<tr>
<td>5</td>
<td>64.8 ± 4.3</td>
<td>4.21 ± 1.2</td>
<td>2.34 ± 1.1</td>
<td>4.05 ± 0.6</td>
<td>75.4 ± 4.8</td>
</tr>
<tr>
<td>6</td>
<td>20.2 ± 1.2</td>
<td>0.54 ± 0.2</td>
<td>2.89 ± 1.1</td>
<td>4.10 ± 1.6</td>
<td>27.4 ± 1.4</td>
</tr>
<tr>
<td>7</td>
<td>24.1 ± 2.1</td>
<td>0.74 ± 0.3</td>
<td>3.00 ± 1.3</td>
<td>4.65 ± 1.2</td>
<td>32.5 ± 3.1</td>
</tr>
</tbody>
</table>

*Note.* Data presented are the mean ± SD of three separate sampling determinations. Abbreviations used were as follows: Total-As, total arsenic; InAs, inorganic arsenic; MMAA, monomethylarsonic acid; DMAA, dimethylarsinic acid; TMA, trimethylarsenic acid.

*Numbers 1–3, Putai; 4–5, Chali; 6–7, Kaohsiung; wells 1–3 are in the blackfoot disease endemic area and 4–7 are in the control region.*
in this area caused pollution of surface water, and residents were recommended to dig artesian wells (100–300 m deep) around 1900. In the 1920s a number of patients with gangrene extremities were found. Early in the 1950s many cases of blackfoot disease were reported and drew public attention. The etiology is still unknown, but is generally attributed to the high concentration of arsenic found in the deep well waters (Tseng, 1989). Construction of a water-supply system began in 1956 in the identified endemic area. However, even after switching to this water system, a risk of blackfoot disease development remains among those who drank artesian well water in the pre-1956 era. About 50 new patients per year visit the Center for Prevention and Treatment of Blackfoot Disease, which was established in 1979 in Pei-Men. In 1982, a total of 1600 disease-affected patients were identified in this area. In the endemic area, many cases of malignant neoplasms of skin, lung, liver, or bladder, as well as circulatory diseases including blackfoot disease, were noted among those with chronic arsenicism (Yeh, 1973; Chen et al., 1985; Tseng et al., 1996).

Arsenic contamination in groundwater is primarily in the form of inorganic arsenic, and subsequent ingestion by humans was reported to produce carcinogenesis and cardiovascular disorders (Astorì et al., 1981; Chatterjee et al., 1995; Das et al., 1995). The chemical species of arsenic believed to produce the observed toxicity was inorganic arsenical compounds (Thompson, 1993). In agreement with these findings, the predominant species of arsenic found in a disease-identified drinking water was inorganic arsenic. High inorganic well-water arsenic concentrations were associated with elevated inorganic arsenic urinary excretion in blackfoot-affected patients. A similar marked rise in inorganic arsenic concentrations was detected in hair and fingernails of diseased patients. These data, taken together, clearly indicate that elevated inorganic arsenical compound levels are associated with blackfoot dis-

TABLE 2. Arsenic concentration in urine, hair, and fingernails from individuals living in blackfoot disease or control regions of Taiwan

<table>
<thead>
<tr>
<th>Specimen examined</th>
<th>Group</th>
<th>InAs (µg/g creatinine)</th>
<th>MMAA (µg/dry weight)</th>
<th>DMAA (µg/dry weight)</th>
<th>TMA (µg/dry weight)</th>
<th>Total AS (µg/dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>Control</td>
<td>3.3 ± 2.5</td>
<td>3.7 ± 1.9</td>
<td>6.0 ± 4.3</td>
<td>27.0 ± 6.9</td>
<td>39.9 ± 8.7</td>
</tr>
<tr>
<td></td>
<td>Blackfoot</td>
<td>6.5 ± 2.7*</td>
<td>6.1 ± 2.3*</td>
<td>7.9 ± 4.5</td>
<td>35.9 ± 10.2*</td>
<td>56.4 ± 13.6*</td>
</tr>
<tr>
<td>Hair</td>
<td>Control</td>
<td>0.16 ± 0.08</td>
<td>ND</td>
<td>0.07 ± 0.03</td>
<td>ND</td>
<td>0.22 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Blackfoot</td>
<td>0.32 ± 0.22*</td>
<td>ND</td>
<td>0.09 ± 0.04</td>
<td>ND</td>
<td>0.41 ± 0.24*</td>
</tr>
<tr>
<td>Fingernails</td>
<td>Control</td>
<td>0.38 ± 0.28</td>
<td>ND</td>
<td>0.13 ± 0.08</td>
<td>ND</td>
<td>0.51 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>Blackfoot</td>
<td>0.5 ± 0.32*</td>
<td>ND</td>
<td>0.16 ± 0.07</td>
<td>ND</td>
<td>0.71 ± 0.34*</td>
</tr>
</tbody>
</table>

Note. Data are the mean ± SD of 25 per group. For abbreviations, see Table 1. ND, not detectable. *Significantly different from control (p < .05).
ease. Although blackfoot patients also excreted higher amounts of urinary MMAA and TMA, this may be attributed to greater ingestion of seafood, and both these species do not produce toxicity (Cannon et al., 1981; Yamato, 1988). It is of interest that chronic arsenic poisoning is persistent, in that ingestion of contaminated well water ended 20 years ago yet arsenic, predominantly in the form of inorganic arsenical compounds, is still present in well water. In addition, there are still cases of blackfoot disease reported in subjects residing in the Putai region of Taiwan whose content of hair, fingernail, and urine arsenic remains significantly higher.

REFERENCES


