Chin-Hsiao Tseng, Choon-Khim Chong, Ching-Ping Tseng and Jose A. Centeno

Blackfoot Disease in Taiwan: Its Link with Inorganic Arsenic Exposure from Drinking Water

INTRODUCTION

Blackfoot disease (BFD) was an endemic peripheral vascular disease confined to the southwestern coast of Taiwan. Typical symptoms and signs of progressive arterial occlusion were mainly found in the lower extremities, but the upper extremities might also be involved in rare cases. Ulceration, gangrene, and spontaneous or surgical amputation were typical results (1, 2). Sporadic cases of BFD occurred as early as in the early 20th century, and peak incidence was noted between 1956 and 1960, with prevalence rates ranging from 6.51 to 18.85 per 1000 population in different villages. The disease could afflict patients in a wide range of ages, from 2 to 87 y, but the mean age of onset was 52 y (1). The sex ratio for men to women was 1.5:1 (1). The etiology of this endemic disease has been extensively sought during the past 50 y. This paper reviews the link between the exposure to arsenic from drinking artesian well water and the development of the disease.

ARSENIC EXPOSURE AND PREVALENCE OF BLACKFOOT DISEASE

BFD is an endemic disease confined to the southwestern coast of Taiwan. The disease is characterized by progressive narrowing of the peripheral vessels involving mainly the lower extremities. In rare cases, the upper extremities can also be involved (1). Clinical manifestations are characteristic of ischemia involving the lower legs, such as numbness or coldness, intermittent claudication, or absence of peripheral pulsation in the initial stage (1). The afflicted subjects might develop ulceration and gangrene in the involved extremities in a later stage, and spontaneous or surgical amputation was the typical result. Sporadic cases were noted since the early 20th century. However, not much attention had been paid until after the mid-20th century when cumulative case numbers became noticeable. Peak incidence of BFD was found between 1956 and 1960 (2, 3). The concentration of arsenic in the drinking water from the artesian wells in the endemic areas has been found to be high, with the median concentration ranging from 0.70 to 0.93 mg L^{-1} (4, 5), in comparison with the shallow well water in other areas of Taiwan, which ranged between nondetectable and 0.30 mg L^{-1} with a median of 0.04 mg L^{-1} (5). Tseng collected a series of more than 1600 cases and followed the patients for more than 30 y (1). According to his early analyses, a dose-responsive relationship between arsenic concentration in the artesian wells and the prevalence of BFD was found in different age groups of residents in the endemic areas. In villages where the arsenic concentrations in well water were <0.30 mg L^{-1}, 0.30–0.59 mg L^{-1}, and more than 0.60 mg L^{-1}, the prevalence rates of BFD for residents aged 20–39 y were 0.5%, 1.3%, and 1.4%, respectively; for residents aged 40–59 y, 1.1%, 3.2%, and 4.7%, respectively; and for residents aged over 60 y, 2.0%, 3.2%, and 6.1%, respectively (1). This study suggested that the prevalence of BFD increased in proportion to increasing arsenic concentrations of the well water.

ARSENIC EXPOSURE AND PERIPHERAL VASCULAR DISEASE

Since the early 1990s Tseng et al. carried out a series of studies by using more objective tools such as Doppler ultrasound and laser Doppler flowmetry in combination with exercise test for diagnosis of subclinical defects in arterial flow and microcirculation and by considering the cumulative exposure dosage of arsenic and the potential effect of confounders. Subclinical arterial insufficiency (6) and microcirculatory defects (7) were clearly demonstrated before the development of clinically full-blown BFD in seemingly normal subjects living in the endemic areas after prolonged exposure to the well water containing high arsenic concentrations. A dose-response relationship between indices of long-term arsenic exposure dosage at individual levels and peripheral vascular disease was also clearly demonstrated, which was independent of the confounding effects of lipid profiles and other traditional cardiovascular risk factors (8, 9). The prevalence of peripheral vascular disease for those with a cumulative arsenic exposure of 0, 0.1–19.9, and $\geq$20 mg $L^{-1} \times$ y were 4.4, 11.6, and 19.8%, respectively; and the respective odds ratios (95% confidence interval) were 1.00, 2.77 (0.84–9.14), and 4.28 (1.26–14.54) after adjustment for potential confounders (2). The prevalence of peripheral vascular disease for those living in the endemic areas for $\geq$60 y could be as high as 28.4% and the multivariate-adjusted odds ratio was 10.54 (2.68–41.37), compared with those living in the endemic areas for less than 40 y (2). These later studies fortified the link between arsenic exposure and the development of peripheral vascular disease in the BFD-endemic areas in Taiwan.

In a recent study, Tseng et al. further demonstrated the influence of the interaction between arsenic exposure and urinary arsenic species on the risk of peripheral vascular disease diagnosed by Doppler ultrasound in 479 (220 men and 259 women) adults residing in the BFD areas in Taiwan (10). Arsenic exposure was estimated by cumulative arsenic exposure; and urinary levels of total arsenic, inorganic arsenite and arsenate, monomethylarsonic acid (MMA^V), and dimethylarsinic acid (DMA^V) were determined; and primary methylation index ($PMI = MMA^V$/urinary inorganic arsenic) and secondary methylation index ($SMI = DMA^V/MMA^V$) were calculated. The association between peripheral vascular disease and urinary arsenic parameters was evaluated considering the interaction with cumulative arsenic exposure and the confounding effects of age, sex, body mass index, total cholesterol, triglycerides, cigarette smoking, and alcohol consumption. The results showed that the risk of peripheral vascular disease increased with a higher cumulative arsenic exposure and a lower capacity to methylate arsenic to DMA^V after taking into account the potential effect of confounders. The multivariate-adjusted odds ratios for cumulative arsenic exposure of 0, 0.1–15.4, and $>$15.4 mg $L^{-1} \times$ y were 1.00, 3.41 (0.74–15.78), and 4.62 (0.96–22.21), respectively ($p < 0.05$, trend test); and for PMI $\leq$ 1.77 and SMI $>$ 6.93, PMI $>$ 1.77 and SMI $>$ 6.93, PMI $>$ 1.77 and SMI $\leq$ 6.93, and PMI $\leq$ 1.77 and SMI $\leq$ 6.93 were 1.00, 2.93 (0.90–9.52), 2.85 (1.05–7.73), and 3.60 (1.12–11.56), respectively ($p <$

0.05, trend test). It was concluded that individuals with a higher arsenic exposure and a lower capacity to methylate inorganic arsenic to DMA^V have a higher risk of developing peripheral vascular disease in BFD areas in Taiwan. This is the first study showing an effect of arsenic methylation capacity on the development of arsenic-induced peripheral vascular disease. Therefore, the results of recent studies suggested that susceptibility to peripheral vascular disease is not only related to the exposure dosage of arsenic in the BFD areas. The capacity to metabolize and detoxify inorganic arsenic plays a significant role on the susceptibility and development of peripheral vascular disease in subjects chronically exposed to arsenic. Subjects with a higher capacity to methylate inorganic arsenic to DMA^V would have a lower risk of developing clinical disease.

REVERSIBILITY OF INCIDENCE OF BFD AND MORTALITY FROM PERIPHERAL VASCULAR DISEASE

The association between arsenic exposure and BFD is also supported by the observation that patients with BFD had a high co-occurrence of arsenic-related skin lesions such as hyperpigmentation, hyperkeratosis, and skin cancer (11). This co-occurrence of skin lesions and BFD could not be attributed to chance alone, and chronic arsenic exposure is the common cause or underlying factor (11). In a recent study, seemingly normal subjects living in the BFD-endemic areas were also found to have subclinical sensory nerve defects. While comparing the current perception thresholds measured by a Neurometer® at the trigeminal, median, and superficial peroneal nerves with frequencies of 5, 250, and 2000 Hz, 85 seemingly normal subjects living in the BFD-endemic areas were noted to have significantly (1.28- to 2.23-fold) higher current perception thresholds than 75 external normal controls without arsenic exposure (12). Since neuropathy is a common feature of arsenic intoxication, the findings of subclinical defects in sensory nerves also give supportive information for the possible link between arsenic exposure and the etiology of BFD.

Another way to demonstrate the link between arsenic exposure and BFD was to use the reversibility criterion in the evaluation of a cause-effect relationship. The different rates of BFD between residents consuming well water and those consuming tap water in the same areas and the dramatic decline of BFD after implementation of tap water supply systems to the endemic villages identifies arsenic from artesian well water as a causative agent of BFD. Tap water supply to the endemic areas was not available before the 1960s, and its coverage remained low until the 1970s. The incidence rates per 100 000 person-years for men and women who consumed artesian well water were 44.3 and 36.5, respectively; and were 2.9 and 3.1 for men and women who used tap water in the same areas (2). Most new cases of BFD after the 1970s occurred in people above 50 y of age in both genders, while BFD might occur in those below 30 y of age before the 1950s (2). By analyzing the mortality attributed to peripheral vascular disease in the BFD-endemic areas for the years 1971–2003, Yang also demonstrated a gradual decrease of mortality from peripheral vascular disease over a period of 25–27 y after the cessation of consumption of the artesian well water (13). The decline of the incidence of BFD and the reversibility of mortality from peripheral vascular disease in the endemic areas after cessation of the use of the artesian well water strongly suggested a link between the high arsenic-containing well water and the development of BFD.

CONCLUSIONS

Exposure to inorganic arsenic can be associated with a variety of human diseases involving different organs and systems. The link between BFD and arsenic exposure from drinking water has been demonstrated for nearly 50 y in Taiwan. More recent studies also demonstrated the existence of subclinical defects in the arterial system and microcirculatory flow in seemingly normal subjects living in the BFD-endemic areas with prolonged arsenic exposure. The link between peripheral vascular disease before full-blown BFD and arsenic exposure has fortified the potential risk of atherogenicity associated with arsenic exposure. Actually the atherogenicity of arsenic is systemic and not limited to the lower extremities. Our recent study also demonstrated a link between arsenic exposure and ischemic heart disease (14). Environmental exposure to a variety of chemicals can be causative to cancers (15–20); arsenic is also a carcinogen found in environmental, occupational, and medicinal exposure (21–24). Exposure to arsenic is not only atherogenic, it can also be associated with a higher risk of developing diabetes (25–27) and hypertension (28), both of which can accelerate the atherogenicity of arsenic and clinical development of cardiovascular disease.

References and Notes

1. Tseng, W.P. 1989. Blackfoot disease in Taiwan: A 30-year follow-up study. *Angiology 40*, 547–558.
2. Tseng, C.H. 2002. An overview on peripheral vascular disease in blackfoot disease–hyperendemic villages in Taiwan. *Angiology 53*, 529–537.
3. Tseng, C.H. 2005. Blackfoot disease and arsenic: a never-ending story. *J. Environ. Sci. Health C 23*, 55–74.
4. Kuo, T.L. 1964. Arsenic content of artesian well water in endemic area of chronic arsenic poisoning. *Rep. Inst. Pathol. Taipei Taiwan Natl. Taiwan Univ. Coll. Med. 20*, 7–13.
5. Chen, K.P., Wu, H.Y. and Wu, T.C. 1962. Epidemiologic studies on blackfoot disease in Taiwan: III. Physicochemical characteristics of drinking water in endemic blackfoot disease areas. *Mem. Coll. Med. Natl. Taiwan Univ. 8*, 115–129.
6. Tseng, C.H., Chen, C.J., Lin, B.J. and Tai, T.Y. 1994. Abnormal response of ankle pressure after exercise in seemingly normal subjects living in blackfoot disease–hyperendemic villages in Taiwan. *Vasc. Surg. 28*, 607–617.
7. Tseng, C.H., Tai, T.Y., Lin, B.J. and Chen, C.J. 1995. Abnormal peripheral microcirculation in seemingly normal subjects living in blackfoot disease–hyperendemic villages in Taiwan. *Int. J. Microcirc. 5*, 21–27.
8. Tseng, C.H., Chong, C.K., Chen, C.J. and Tai, T.Y. 1996. Dose-response relationship between peripheral vascular disease and ingested inorganic arsenic among residents in blackfoot disease endemic villages in Taiwan. *Atherosclerosis 20*, 125–133.
9. Tseng, C.H., Chong, C.K., Chen, C.J. and Tai, T.Y. 1997. Lipid profile and peripheral vascular disease in arseniasis-hyperendemic villages in Taiwan. *Angiology 48*, 321–335.
10. Tseng, C.H., Huang, Y.K., Huang, Y.L., Chung, C.J., Yang, M.H., Chen, C.J. and Hsueh, Y.M. 2005. Arsenic exposure, urinary arsenic speciation and peripheral vascular disease in blackfoot disease–hyperendemic villages in Taiwan. *Toxicol. Appl. Pharmacol. 206*, 299–308.
11. Tseng, W.P., Chu, H.M., How, S.W., Fong, J.M., Lin, C.S. and Yeh, S. 1968. Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. *J. Natl. Cancer Inst. 40*, 453–463.
12. Tseng, C.H. 2003. Abnormal current perception thresholds measured by Neurometer among residents in blackfoot disease hyperendemic villages in Taiwan. *Toxicol. Lett. 146*, 27–36.
13. Yang, C.Y. 2006. Does arsenic exposure increase the risk of development of peripheral vascular diseases in humans? *J. Toxicol. Environ. Health A 69*, 1797–1804.
14. Tseng, C.H., Chong, C.K., Tseng, C.P., Hsueh, Y.M., Chiou, H.Y., Tseng, C.C. and Chen, C.J. 2003. Long-term arsenic exposure and ischemic heart disease in arseniasis-hyperendemic villages in Taiwan. *Toxicol. Lett. 137*, 15–21.
15. Sedman, R.M., Beaumont, J., McDonald, T.A., Reynolds, S., Krowech, G. and Howd, R. 2006. Review of the evidence regarding the carcinogenicity of hexavalent chromium in drinking water. *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 24*, 155–182.
16. Ahmed, F.E. 2006. Gene-gene, gene-environment and multiple interactions in colorectal cancer. *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 24*, 1–101.
17. Thorpe, N. and Shirmohammadi, A. 2005. Herbicides and nitrates in groundwater of Maryland and childhood cancers: a geographic information systems approach. *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 23*, 261–278.
18. Clark, H.A. and Snedeker, S.M. 2005. Critical evaluation of the cancer risk of dibromochloropropane (DBCP). *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 23*, 215–260.
19. McDonald, T.A. and Komulainen, H. 2005. Carcinogenicity of the chlorination disinfection by-product MX. *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 23*, 163–214.
20. Yang, M. and Pyo, M.Y. 2005. Molecular epidemiology of lung cancer in female passive smokers. *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 23*, 75–97.
21. Fukushima, S., Morimura, K., Wanibuchi, H., Kinoshita, A. and Salim, E.I. 2005. Current and emerging challenges in toxicopathology: carcinogenic threshold of phenobarbital and proof of arsenic carcinogenicity using rat medium-term bioassays for carcinogens. *Toxicol. Appl. Pharmacol. 207*, (2 Suppl), 225–229.
22. Wanibuchi, H., Salim, E.I., Kinoshita, A., Shen, J., Wei, M., Morimura, K., Yoshida, K., Kuroda, K. et al. 2004. Understanding arsenic carcinogenicity by the use of animal models. *Toxicol. Appl. Pharmacol. 198*, 366–376.
23. Mizoi, M., Takabayashi, F., Nakano, M., An, Y., Sagesaka, Y., Kato, K., Okada, S. and Yamanaka, K. 2005. The role of trivalent dimethylated arsenic in dimethylarsinic acid-promoted skin and lung tumorigenesis in mice: tumor-promoting action through the induction of oxidative stress. *Toxicol Lett. 158*, 87–94.
24. Basu, A., Som, A., Ghoshal, S., Mondal, L., Chaubey, R.C., Bhilwade, H.N., Rahman, M.M. and Giri, A.K. 2005. Assessment of DNA damage in peripheral blood

lymphocytes of individuals susceptible to arsenic induced toxicity in West Bengal, India. *Toxicol. Lett. 159*, 100–112.
25. Tseng, C.H. 2004. The potential biological mechanisms of arsenic-induced diabetes mellitus. *Toxicol. Appl. Pharmacol. 197*, 67–83.
26. Tseng, C.H., Tseng, C.P., Chiou, H.Y., Hsueh, Y.M., Chong, C.K. and Chen, C.J. 2002. Epidemiologic evidence of diabetogenic effect of arsenic. *Toxicol. Lett. 133*, 69–76.
27. Tseng, C.H., Tai, T.Y., Chong, C.K., Tseng, C.P., Lai, M.S., Lin, B.J., Chiou, H.Y., Hsueh, Y.M. et al. 2000. Long-term arsenic exposure and incidence of non-insulin-dependent diabetes mellitus: a cohort study in arseniasis-hyperendemic villages in Taiwan. *Environ. Health Perspect. 108*, 847–851.
28. Chen, C.J., Hsueh, Y.M., Lai, M.S., Shyu, M.P., Chen, S.Y., Wu, M.M., Kuo, T.L. and Tai, T.Y. 1995. Increased prevalence of hypertension and long-term arsenic exposure. *Hypertension 25*, 53–60.
29. The authors thank the Department of Health (DOH89-TD-1035) and the National Science Council (NSC-90–2320-B-002–197, NSC-92–2320-B-002–156, NSC-93–2320-B-002–071, NSC-94–2314-B-002–142, and NSC-95–2314-B-002–311) of Taiwan for their continuous financial support for epidemiological studies on arsenic-related health hazards and diabetes mellitus carried out in Taiwan.

Chin-Hsiao Tseng Department of Internal Medicine National Taiwan University Hospital, No. 7 Chung-Shan South Road Taipei, Taiwan; Division of Endocrinology and Metabolism, Department of Internal Medicine National Taiwan University Hospital, Taipei, Taiwan; Department of Medical Research and Development National Taiwan University Hospital Yun-Lin Branch Yun-Lin, Taiwan; Division of Environmental Health and Occupational Medicine National Health Research Institutes Taipei, Taiwan
ccktsh@ms6.hinet.net

Choon-Khim Chong Department of Rehabilitation, and Ching-Ping Tseng School of Medical Technology Chang Gung University Taoyuan, Taiwan

Jose A. Centeno Armed Forces Institute of Pathology, Division of Biophysical Toxicology Department of Environmental and Infectious Disease Sciences Washington, DC, USA
centeno@afip.ods.mil