Biological Gradient Between Long-Term Arsenic Exposure and Carotid Atherosclerosis

Chih-Hao Wang, MD; Jiann-Shing Jeng, MD; Ping-Keung Yip, MD; Chi-Ling Chen, PhD; Lin-I Hsu, PhD; Yu-Mei Hsueh, PhD; Hung-Yi Chiou, PhD; Meei-Mann Wu, PhD; Chien-Jen Chen, ScD

Background—Long-term exposure to ingested arsenic has been documented to induce peripheral vascular disease, ischemic heart disease, and cerebral infarction in a dose-response relationship. This study further examined the biological gradient between ingested inorganic arsenic and carotid atherosclerosis.

Methods and Results—We studied 199 male and 264 female adult residents from the southwestern area of endemic arseniasis in Taiwan. The extent of carotid atherosclerosis was assessed by duplex ultrasonography. Diabetes mellitus was determined by oral glucose tolerance test, hypertension by mercury sphygmomanometers, and serum lipid profiles by autoanalyzers. Information regarding the consumption of high-arsenic artesian well water, cigarette smoking, and alcohol consumption was obtained through standardized questionnaire interviews. Logistic regression analysis was used to estimate the odds ratio and its 95% CI of carotid atherosclerosis for various risk factors. Three indices of long-term exposure to ingested arsenic, including the duration of consuming artesian well water, the average arsenic concentration in consumed artesian well water, and cumulative arsenic exposure, were all significantly associated with prevalence of carotid atherosclerosis in a dose-response relationship. The biological gradient remained significant after adjustment for age, sex, hypertension, diabetes mellitus, cigarette smoking, alcohol consumption, waist-to-hip ratio, and serum levels of total cholesterol and LDL cholesterol. The multivariate-adjusted odds ratio was 3.1 (95% CI 1.3 to 7.4) for those who had a cumulative arsenic exposure of ≥20 mg/L-years compared with those without exposure to arsenic from drinking artesian well water.

Conclusions—Carotid atherosclerosis is associated with ingested inorganic arsenic, showing a significant biological gradient. (Circulation. 2002;105:1804-1809.)

Key Words: arsenic ■ atherosclerosis ■ dose-response relationship

Arsenic is a ubiquitous element present in various compounds. It is transported in the environment mainly by water. The general population is exposed to inorganic and organic arsenic through environmental, occupational, and medicinal sources. In the arseniasis-endemic areas of the world, the main exposure to inorganic arsenic is through ingestion of high-arsenic drinking water.1 Arseniasis is becoming an emerging epidemic in Asia, and more than 100 million people are exposed to underground water with high concentrations of arsenic.2

Ingested arsenic has long been associated with the development of blackfoot disease (BFD), a unique peripheral vascular disease that was endemic in the southwestern coastal area of Taiwan.3-4 Clinically, the disease begins with coldness and/or numbness of the lower extremities, progresses over several years to intermittent claudication, and ends with dry gangrene and spontaneous amputation of the affected extremities.3 The pathological change of BFD is compatible with arteriosclerosis obliterans (70%) and thromboangiitis obliterans (30%), and the fundamental vascular change of BFD is an unduly developed severe atherosclerosis.5 Long-term exposure to arsenic has been found to be associated with an increased risk of diabetes mellitus, hypertension, ischemic heart disease, and cerebral infarction in a dose-response relationship.6-9

Ultrasonographic evaluation of the superficial carotid artery is a noninvasive and cost-effective tool for the assessment of carotid atherosclerosis. Subclinical lesions such as stenosis and plaque formation are relatively late manifestations of the atherosclerosis process.10,11 Progression of intimal-medial thickness (IMT) may reflect earlier changes.11 The specific aim of this study was to assess the dose-response
relationship of carotid atherosclerosis with long-term exposure to ingested inorganic arsenic through the consumption of artesian well water. High-resolution color duplex ultrasonography was used in this study to evaluate the extent and severity of the extracranial carotid artery (ECCA) as an indicator of atherosclerosis.

Methods

Study Areas

The BFD study group has launched research on arseniasis since the early 1980s. The study area included Homei, Fuhsin, and Hsinning villages in Putai Township in southwestern Taiwan. The prevalence of BFD in the study area was the highest in Taiwan. Residents in the study areas had consumed high-arsenic artesian well water for more than 50 years. The median arsenic concentration of artesian well water measured in the early 1960s ranged from 0.7 to 0.93 mg/L. A tap water supply system was implemented in the early 1960s, but its coverage remained low until the early 1970s. Artesian well water was no longer used for drinking and cooking after the mid-1970s.

Study Subjects

The recruitment of study subjects was described in detail previously. In brief, all adult residents who lived >6 months in the study area were selected from records of the local household registration bureau, where demographic status and events including birth, marriage, education, migration, employment, and death of all family members in every household are mandatorily registered and updated annually. Household visits were carried out to interview residents who lived in the study area ≥5 days a week and to invite them to participate in health examination. Six follow-up health examinations have been carried out since the initial recruitment. The ultrasonographic assessment of ECCA atherosclerosis was conducted in the sixth examination in 1996. During this examination, a total of 463 cohort members were invited, and 436 (94%) of them completed the ultrasonographic assessment of ECCA.

Questionnaire Interview and Arsenic Exposure

A standardized personal interview of study subjects based on a structured questionnaire was carried out by well-trained public health nurses. Information obtained from the interview included socioeconomic and demographic characteristics, alcohol intake, cigarette smoking, physical activities, dietary consumption frequency, residential and water consumption history, and personal and family history of hypertension, diabetes, and cardiovascular diseases. Information regarding cigarette smoking habits was obtained, including age at starting cigarette smoking, average number of cigarettes smoked per day, and age at stopping smoking. Age at starting regular consumption of alcoholic beverages, average frequency of alcohol intake per week, average quantity of alcohol consumption, and age at stopping alcohol drinking were also obtained.

A detailed residential history, including villages of residence and duration of residence, and a history of water consumption, including water source and duration of consumption, were obtained on the basis of the questionnaire interview. The levels of arsenic in artesian well water of the villages where the study subjects had lived were obtained from reports of previous studies carried out in the 1960s. Some study subjects had moved from one village to another, and the arsenic concentrations in the artesian well water of these villages were different. An index of cumulative arsenic exposure was derived to reflect the overall exposure to arsenic for each study subject. The cumulative arsenic exposure (in mg/L-years) was defined as the sum of products derived by multiplying the arsenic concentration in well water (mg/L-years) by the duration of consuming the artesian well water (years) during consecutive periods of living at different villages. In other words, the cumulative arsenic exposure equaled \( \Sigma(C_i D_i) \), where \( C_i \) was the median arsenic concentration in artesian well water of a village in which a given subject lived during the period \( i \), and \( D_i \) was the duration of drinking artesian well water in the village during the period \( i \). An index of average arsenic concentration in artesian well water was also derived by the formula \( \Sigma(C_i D_i)/\Sigma(D_i) \).

Assessment of Carotid Atherosclerosis

An ultrasonographic assessment of ECCA atherosclerosis was performed with a Hewlett-Packard Sono 1000 equipped with a 7.5-MHz frequency in B-mode and 5.6-MHz frequency in pulsed Doppler mode. The duplex scanning was performed by neurologists and a cardiologist while study subjects were lying in the supine position with the head slightly extended and rotated 45° away from the side being examined. Transverse and longitudinal scans were performed to assess the common carotid artery, carotid bifurcation, and internal and external carotid arteries.

Plaque of the carotid artery was defined as irregular surface, lumen encroachment, significant wall thickening ≥50% of adjacent IMT, and/or structure heterogeneity such as acoustic shadow. The IMT was measured in the far wall of 3 segments of bilateral ECCAs. Three segments were identified on each side: the distal 1.0 cm of the common carotid artery proximal to the bifurcation, the bifurcation itself, and the proximal 1.0 cm of the internal carotid artery. The IMT

![Figure 1. Prevalence of carotid atherosclerosis by age and sex. CAIs as defined in text.](image-url)
was estimated as the mean of these 6 measurements. Three carotid atherosclerosis indices (CAIs) were defined as follows: CAI-1, presence of plaque or IMT ≥ 1.0 mm; CAI-2, IMT ≥ 1.0 mm; and CAI-3, presence of plaque.

**Laboratory Examinations**

Fasting blood samples were collected from study subjects for testing of serum levels of total cholesterol, HDL cholesterol, triglycerides, and uric acid by standardized autoanalyzers. Glucose tolerance tests were also performed. The status of diabetes mellitus was defined as (1) a fasting glucose level of ≥ 7.8 mmol/L, (2) a 2-hour glucose level ≥ 11.1 mmol/L, or (3) a history of diabetes mellitus regularly treated with oral hypoglycemic agents or insulin. Anthropometric characteristics, including height, weight, waist circumference, hip circumference, systolic blood pressure, and diastolic blood pressure, were measured according to a standard protocol. Blood pressures were measured 3 times with a mercury sphygmomanometer with study subjects sitting. The average of 3 measurements was used for analysis. Hypertension was defined as (1) an average systolic blood pressure ≥ 160 mm Hg, (2) an average diastolic blood pressure ≥ 95 mm Hg, or (3) a history of hypertension regularly treated with antihypertensive agents.

**Statistical Analysis**

The age-and-sex-specific prevalences of carotid atherosclerosis were estimated for CAI-1, CAI-2, and CAI-3, respectively. A χ² test for trend was used to examine the statistical significance of the biological gradient of CAIs with age. The differences in CAIs between men and women were tested by Mantel-Haenszel χ² tests with adjustment for age. The dose-response relationships between CAIs and long-term arsenic exposure were assessed after adjustment for age and sex. The biological gradients were assessed by the duration of consuming artesian well water, average arsenic concentration in artesian well water, and cumulative arsenic exposure, respectively. Multiple logistic regression analysis was used to examine the associations between CAIs and arsenic exposure with further adjustment for various cardiovascular risk factors. All continuous variables of cardiovascular risk factors were categorized into 2 to 4 groups. The regression coefficients and their standard errors were used to derive odds ratios (ORs) and their 95% CIs for various variables.
Results

Age-and-Sex–Specific Prevalence of Carotid Atherosclerosis

Age-and-sex–specific prevalences of CAIs are shown in Figure 1. The prevalences of CAIs increased with age (all probability values for trend <0.0001) and were significantly higher in men than women (all probability values <0.03).

Cardiovascular Risk Factors and Carotid Atherosclerosis

Table 1 shows the prevalence of CAIs by various cardiovascular risk factors. A dose-response relationship was observed between CAIs and serum level of total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides. All the biological gradients were statistically significant after adjustment for age and sex (all probability values <0.05). Those who had higher body mass index or waist-to-hip ratio had a higher prevalence of CAIs. The associations were more striking for waist-to-hip ratio than for body mass index. Study subjects affected with diabetes mellitus or hypertension had increased prevalences of CAIs.

Long-Term Arsenic Exposure and Carotid Atherosclerosis

Figure 2 shows the prevalences of CAIs by long-term exposure to ingested arsenic. Three indices of long-term exposure to ingested arsenic, including the duration of consuming artesian well water, the average arsenic concentration in consumed artesian water, and cumulative arsenic exposure, were all significantly associated with carotid atherosclerosis showing a dose-response relationship (all probability values for trend <0.05) after adjustment for age and sex.

Discussion

In the arseniasis-endemic areas, the main exposure to inorganic arsenic is through ingestion of high-arsenic drinking water. Ingestion of inorganic arsenic through drinking water has been known to be associated with peripheral artery disease, diabetes mellitus, hypertension, ischemic heart disease, and cerebrovascular disease in Taiwan. Occupational exposures to inorganic arsenic have also been associated with an increased mortality from cardiovascular disease among copper smelter workers and chimney sweeps. Morbidity and mortality from ischemic heart disease and cerebral infarction may be considered late clinical manifestations of long-term arsenic exposure. These late health end points result from the interaction of both predisposing and precipitating factors for cardiovascular diseases. The risk assessment based on these late cardiovascular events might be underestimated because of the competing causes of death and the accuracy in the diagnosis of sudden death from ischemic heart disease or stroke. Carotid atherosclerosis indexed by IMT and/or plaque is a subclinical lesion and can be detected by ultrasonography before clinical events de-
The pathology of BFD had been extensively studied in 63 amputated parts of extremities from 51 BFD patients from southwest Taiwan. The pathology of BFD was compatible with 2 distinct types: arteriosclerosis obliterans and thromboangiitis obliterans.5 In 3 autopsy cases of BFD, the common pathological finding is generalized atherosclerosis involving large, medium-size, and small arteries. Interestingly, in an autopsy case of a 24-year-old woman with thromboangiitis obliterans—type BFD, severe atherosclerosis of coronary and other medium-size arteries was found.5 An autopsy finding on children in Antofagasta, Chile, with chronic arsenic intoxication had shown systemic arterial intimal thickening in small and medium-size arteries involving the heart, gastrointestinal tract, liver, skin, and pancreas.16 Therefore, accelerated atherosclerosis can occur under chronic arsenic exposure in the absence of traditional coronary risk factors. Moreover, researchers also found abnormal peripheral microcirculation in clinically normal subjects with a past history of chronic arsenic exposure.17

In many species, inorganic arsenic is methylated to monomethylated, dimethylated, and trimethylated metabolites.18,19 S-adenosylmethionine is the major source of methyl group donors.20 Because of the involvement of S-adenosylmethionine, the homocysteine level may be increased through the methylation pathway of inorganic arsenic. Hyperhomocysteinemia has been known to be associated with increased risk of cardiovascular disease.21–23

The inhibitory effects of arsenic on individual cells are in the mitochondrial respiratory function and are highly preferential in the NAD-linked substrates, such as pyruvate dehydrogenase.24,25 The changes in mitochondrial protein synthesis and inner-membrane structural integrity might also play an important role for arsenic toxicity.24,25 Disruption of oxidative phosphorylation and a decrease in cellular production of ATP might result in formation of reactive oxygen species and induction of stress proteins.26,27 Disruption of mitochondrial respiratory function, oxidative stress, and alterations in the mitochondrial structure might result in cellular injury, necrosis, and/or apoptosis.24–27

Moreover, epidemiological studies have demonstrated that arsenic, ionizing radiation, and vinyl chloride monomer may induce various cancers and vascular diseases, including angiosarcoma and atherosclerotic plaques.28 These findings suggest that somatic mutation and cell proliferation may play important roles in the dual effects on carcinogenesis and atherosclerosis. In support of arsenic-induced cell proliferation, researchers have demonstrated that sodium arsenite may induce increased mRNA transcripts of growth factors, including granulocyte-macrophage colony-stimulating factor, transforming growth factor-α, and the inflammatory cytokine tumor necrosis factor-α.29 Therefore, inorganic arsenic may cause atherosclerosis through its roles in the induction of chromosomal abnormalities, oxidative stress, gene amplification, and alterations of growth factors and DNA repair.19 In addition, mercury accumulation in the human body was reported to be associated with accelerated progression of carotid atherosclerosis.30

From the strong dose-response relationship and biological plausibility for the association between arsenic exposure and carotid atherosclerosis, we conclude that long-term arsenic exposure is an independent risk factor for atherosclerosis and that carotid atherosclerosis is a novel biomarker for arseniasis. The impact of arsenic exposure on atherosclerosis and carcinogenesis needs to be considered simultaneously in the health risk assessment of arsenic. Regulatory levels such as the US Environmental Protection Agency’s maximum contaminant level (MCL) for arsenic in drinking water have been lowered from 50 to 10 μg/L. In addition to the cancer risk, atherosclerosis has also been considered in the setting of the MCL by the Environmental Protection Agency on a qualitative basis. To determine the adequacy of the new MCL for protecting public health, the atherosclerotic effect needs to be
quantified for the comprehensive health risk assessment of ingested inorganic arsenic at levels between 5 and 50 μg/L. Because of the small number of subjects at these levels in this study, we could not examine the dose-response relationship between ingested arsenic and carotid atherosclerosis by further categorizing the lowest exposure group of <50 μg/L into subgroups. Our present study in northeastern Taiwan, however, is collecting data on the prevalence of carotid atherosclerosis at lower arsenic concentrations, in particular 5 to 50 μg/L. In this study, we have established for the first time the biological gradient between ingested arsenic and carotid atherosclerosis before the development of clinical events such as acute myocardial infarction and stroke.

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