Available online at www.sciencedirect.com
ScienceDirect
Toxicology and Applied Pharmacology 218 (2007) 135–142
Toxicology and Applied Pharmacology
www.elsevier.com/locate/ytaap

Arsenic methylation capability and hypertension risk in subjects living in arseniasis-hyperendemic areas in southwestern Taiwan

Yung-Kai Huang [a,1], Chin-Hsiao Tseng [b,c,d,1], Ya-Li Huang [e], Mo-Hsiung Yang [f], Chien-Jen Chen [g,h], Yu-Mei Hsueh [e,*]

[a] Graduate Institute of Medical Sciences, School of Medicine, Taipei Medical University, Taipei, Taiwan
[b] National Taiwan University College of Medicine, Taipei, Taiwan
[c] Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan
[d] Department of Medical Research and Development, National Taiwan University Hospital Yun-Lin Branch, Yun-Lin, Taiwan
[e] Department of Public Health, School of Medicine, Taipei Medical University, No. 250 Wu-Hsing Street, Taipei 110, Taiwan
[f] Department of Nuclear Science, National Tsing-Hua University, Hsinchu, Taiwan
[g] Genomic Research Center, Academia Sinica, Taipei, Taiwan
[h] Graduate Institute of Epidemiology, College of Public Health, National Taiwan University Taipei, Taiwan

Received 17 June 2006; revised 3 October 2006; accepted 9 October 2006
Available online 28 October 2006

Abstract

Background: Cumulative arsenic exposure (CAE) from drinking water has been shown to be associated with hypertension in a dose–response pattern. This study further explored the association between arsenic methylation capability and hypertension risk among residents of arseniasis-hyperendemic areas in Taiwan considering the effect of CAE and other potential confounders.
Method: There were 871 subjects (488 women and 383 men) and among them 372 were diagnosed as having hypertension based on a positive history or measured systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg. Urinary arsenic species were determined by high-performance liquid chromatography-hydride generator and atomic absorption spectrometry. Primary arsenic methylation index [PMI, defined as monomethylarsonic acid (MMA^V) divided by ($As^{III}+As^V$)] and secondary arsenic methylation index (SMI, defined as dimethylarsinic acid divided by MMA^V) were used as indicators for arsenic methylation capability.
Results: The level of urinary arsenic was still significantly correlated with cumulative arsenic exposure (CAE) calculated from a questionnaire interview ($p=0.02$) even after the residents stopped drinking the artesian well water for 2–3 decades. Hypertensive subjects had higher percentages of MMA^V and lower SMI than subjects without hypertension. However, subjects having CAE >0 mg/L-year had higher hypertension risk than those who had CAE=0 mg/L-year disregard a high or low methylation index.
Conclusion: Inefficient arsenic methylation ability may be related with hypertension risk.
© 2006 Elsevier Inc. All rights reserved.

Keywords: Urinary arsenic species; Hypertension; Arsenic methylation capability

Introduction

The main source of exposure to inorganic arsenic in humans is drinking water. There are more than 38 million people exposed to ground water with high concentrations of arsenic in Asian countries (Nordstrom, 2002). The range of total arsenic level in artesian well water in blackfoot disease (BFD) endemic areas in Taiwan is 470–897 μg/L with about 95% in inorganic forms, predominantly As^{III} (Chen et al., 1994). Subjects with prolonged exposure to inorganic arsenic from drinking the artesian well water carried a significantly higher risk of hypertension in a dose–response pattern in both Taiwan (Chen et al., 1995) and Bangladesh (Rahman et al., 1999). Exposure to high arsenic levels in artesian well water were associated with BFD (Ch'i and Blackwell, 1968) as well as

* Corresponding author. Fax: +886 2 27384831.
E-mail address: ymhsueh@tmu.edu.tw (Y.-M. Hsueh).
[1] YK Huang and CH Tseng contributed equally to this work and both should be considered as first authors.

0041-008X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2006.10.022

cardiovascular diseases such as peripheral vascular disease (Tseng et al., 1996, 1997), ischemic heart disease (Chen et al., 1996; Hsueh et al., 1998b; Tseng et al., 2003), cerebrovascular disease (Chiou et al., 1997) and carotid atherosclerosis (Wang et al., 2002).

The metabolism of inorganic arsenic involves reduction and oxidative methylation (Thomas et al., 2001; Kitchin, 2001; Vahter, 2002; Styblo et al., 2002; Thomas et al., 2004). After exposure to inorganic arsenic, As^V is readily reduced to As^{III} in red blood cells (Vahter, 1981) and subsequently methylated to monomethylarsonic acid (MMA^V) and dimethylarsinic acid (DMA^V) (Buchet et al., 1981a,1981b). Arsenic methylation process is catalyzed by a 42-kDa protein encoded by the cyt19 genes of mouse and human genomes and the methyl donor has been identified as *S*-adenosylmethionine (Thomas et al., 2004). Methylation was previously considered as a detoxification mechanism (Cullen and Reimer, 1989; Styblo et al., 1999). However, the key metabolic intermediates, MMA^{III} and DMA^{III} have been recently identified in human urine (Mandal et al., 2001) and these trivalent methylated arsenicals are demonstrated to be more toxic than inorganic compounds (Styblo et al., 2000; Petrick et al., 2001). Nesnow et al. (2002) showed that DNA damage is induced by MMA^{III} and DMA^{III}, mediated by reactive oxygen species formed.

Evaluation of arsenic methylation efficiency is mainly based on the relative amounts of the different metabolites in urine. Approximately 60–90% of an exposure dosage of inorganic arsenic is excreted in the urine in mammals, in the forms of inorganic arsenic (10–30%), MMA^V (10–20%), and DMA^V (60–80%). The arsenic metabolic capability varies in different animal species (Vahter, 1999) and MMA^V was found to be a major metabolite only in humans.

Previous epidemiological studies suggested that arsenic exposure could be associated with a higher risk of hypertension (Chen et al., 1995; Rahman et al., 1999). However, whether the metabolism of arsenic could have an effect on the risk of hypertension is an interesting issue that has not been investigated. The purpose of this study was to evaluate the relationship between the capability of arsenic methylation and the risk of hypertension considering the potential effects of arsenic exposure dosage and other confounders.

Methods

Study areas and subjects. Study areas and subjects were described in details in our previous studies (Chen et al., 1995; Tseng et al., 2005). In brief, the study area included Homei, Fuhsing, and Hsinming Villages in Putai Township of Chiayi County located along the southwestern coast of Taiwan. The population with an age of 30 years or older in the studied villages as registered in the household registration office was 2258. Among them, 1571 (70%) living in the study villages 5 or more days a week was considered as eligible subjects. From September to December 1988, a total of 1081 (69%) of the eligible subjects were interviewed with a structured questionnaire. All of the 1081 subjects were invited to participate in the first health examination during January and February 1989 and 941 (87%) actually participated. Biannual health examinations were then carried out. The urinary samples used for the assay of arsenic metabolites in the present study were collected during the first health examination. A total of 871 (81%) subjects recruited in 1989 received blood pressure measurement and serum and urine samples were taken from these subjects (Chen et al., 1995). The prevalence of hypertension defined as a positive history and/or a systolic blood pressure (SBP) $\geq$160 mm Hg and/or a diastolic blood pressure (DBP) $\geq$90 mm Hg among the 871 subjects in the age groups of 30–39, 40–49, 50–59, and $\geq$60 years old are 5.9%, 15.5%, 25.6%, and 25.6% in men, and 0.7%, 12.9%, 25.0%, and 29.9% in women is similar to Chen et al. (1995) study. An institutional review committee approved this study, and the procedures followed the institutional guidelines.

Since early 1900s residents in the BFD area had been using artesian well water as drinking water because of the high salinity of shallow well water. A tap water supply system was not available until early 1960s in the study villages, but its coverage remained low until early 1970s. Artesian well water was no longer used for drinking and cooking after the mid-1970s. The first health examination was carried out in villages where BFD was hyperendemic and the tap water supply system has been implemented for more than 20–30 years.

Questionnaire interview and arsenic exposure index. Two public health nurses well trained in interview techniques carried out the standardized personal interview based on a structured questionnaire. Information obtained from the interview included the consumption history of high arsenic-containing artesian well water, residential history, socioeconomic and demographic characteristics, and life style of alcohol consumption, cigarette smoking, and consumption frequency of various dietary items, as well as personal and family histories of hypertension, diabetes, and cardiovascular diseases.

The detailed residential history and duration of consuming the artesian well water were used to derive the cumulative arsenic exposure (CAE) for each study subject. Arsenic levels of artesian well water in the study area were found to be reasonably constant in two surveys carried out by the Taiwan Provincial Institute of Environmental Sanitation (Wu et al., 1961). The CAE index in mg/L-year for a given subject was defined by the following formula: $\sum(Ci \times Di)$, where Ci is the median arsenic concentration of artesian well water in mg/L in the village where the subject lived, and Di is the duration of consuming the artesian well water in years. For example, if a subject lived in 3 different villages throughout his or her lifetime for 10, 20, and 30 years, respectively, and the arsenic concentrations in artesian well water of the respective villages were 0.3, 0.5, and 0.7 mg/L, then the CAE was calculated as $(0.3\times10)+(0.5\times20)+(0.7\times30)=34$ mg/L-year. The CAE for a given subject was considered to be unknown if the median arsenic concentration of the artesian well water in any one or more villages where the subject had lived was unknown. CAE was not calculable in 215 out of the 871 subjects (24.7%) in this study.

Blood pressure measurement and diagnosis of hypertension. The standard protocol for measuring blood pressure recommended by the World Health Organization was used in this study (Rose et al., 1982). Blood pressure was measured three times with a mercury sphygmomanometer on a sitting position after resting for 20 min. SBP and DBP were defined at the first and fifth Korotkoff sounds, respectively. The average of the three measurements was used for analysis. Hypertension was defined in this study as an average SBP of 140 mm Hg or greater, or an average DBP of 90 mm Hg or greater and/or a history of hypertension under regular treatment with antihypertensive agents.

Biospecimen collection and laboratory examinations. Fasting blood samples were collected from study subjects for the measurement of serum concentrations of cholesterol and triglycerides using an autoanalyzer (Hitachi 737, USA) with reagents obtained from Boehringer Mannheim Diagnostics (Indianapolis, IN, USA).

Urine collection and determination of arsenic species in urine. The mid-stream of the first void urine after waking up in the morning was collected. Urinary samples were stored at −20 °C without any additive. The samples were retrieved for the determination of urinary arsenic species within 6 months after collection.

Urine was thawed at room temperature, mixed by ultrasonic waves, and filtered through a Sep-Pak C_{18} column. Analytical methods for As^{III}, As^V, MMA^V and DMA^V were described in detail in our previous study (Hsueh et al., 1998a). In brief, 200 μl of treated urine sample was used to separate As^{III}, As^V, MMA^V and DMA^V by HPLC (Waters 501; Waters Associates, Milford, MA, USA) equipped with an anion column (Phenomenex, Nucleosil 10sB, Torrance, CA, USA), which was on-line linked to HG-AAS to quantify the levels of various species of inorganic arsenic and its metabolites. Standard solutions of arsenite, arsenate,

MMA^V and DMA^V were prepared by appropriate dilution with deionized water from 1000 mg/L stock solutions. Standard solutions containing 1–50 μg of As/L were freshly prepared by serial dilution with deionized water to set up the calibration curve for the quantification of arsenic species. Recovery rates of As^{III}, DMA^V, MMA^V and As^V ranged from 93.8% to 102.2%, with detection limits of 0.02, 0.06, 0.07, and 0.10 μg/L, respectively. Freeze-dried urine SRM 2670, containing 480±100 μg/L arsenic was obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) and analyzed together with urine samples to assess the validity of the method. An experimental result of 507±17 μg/L (n=4) was recorded.

The total arsenic level was the sum of As^{III}, As^V, MMA^V and DMA^V. In addition to the expression of the percentage of each arsenic species in reference to the total arsenic level, 2 indices were calculated as indicators for arsenic methylation efficiency. The primary methylation index (PMI) was defined as the ratio between the MMA^V level and InAs (inorganic arsenic, $As^{III}+As^V$) levels; and the secondary methylation index (SMI) as the ratio between the DMA^V and MMA^V levels.

Statistical analyses. Statistical analyses were performed using SAS 8.2 software. χ^2 test was used to test the difference of categorical variables and Student's t-test for continuous variables between cases with and without hypertension. We used linear regression to elucidate the relationship between internal arsenic exposure (urinary arsenic parameters) and CAE. Logistic regression models were further used to estimate the multivariate-adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) for hypertension risk.

Results

Relationship between CAE and urinary arsenic levels

Fig. 1 showed the relationship between internal arsenic levels (urinary total arsenic level, InAs percentage, MMA^V percentage and DMA^V percentage) and CAE. Although the residents had stopped drinking the well water for 2–3 decades, we still found a significant relationship between CAE and urinary total arsenic level and MMA^V percentage. The regression coefficients for CAE to estimate urinary total arsenic level and MMA^V percentage were 0.50 (p=0.02) and 0.09 (p<0.01), respectively.

Arsenic methylation indices and conventional risk factors for hypertension

There were 383 men and 488 women in this study. Table 1 compares the conventional risk factors, CAE and urinary arsenic indices between subjects with and without hypertension. For categorical variables, the frequency of alcohol consumption in subjects with hypertension was significantly higher than those without (χ^2=5.72, p=0.01), but gender and cigarette smoking status were not significantly different. For the continuous variables, age, body mass index, serum triglyceride level, CAE, and percentage of MMA^V were significantly higher and SMI was lower with borderline significant in the hypertensive subjects.

Arsenic methylation indices in different strata of conventional risk factors for hypertension

Age, sex, cigarette smoking, alcohol consumption and serum lipid profiles are considered as conventional risk factors for hypertension (Kornitzer et al., 1999). We compared the differences of arsenic methylation indices between different strata of age, sex, body mass index, lifestyle, and lipid profiles. Subjects younger than 50 years of age or of female sex had significantly lower MMA^V percentage, higher DMA^V percentage and higher SMI, indicating a more efficient capacity to methylate inorganic arsenic to DMA^V, than subjects with an older age and of the male sex, respectively. Cigarette smokers had a lower capability to methylate inorganic arsenic to DMA^V than nonsmokers and subjects with a higher triglyceride level

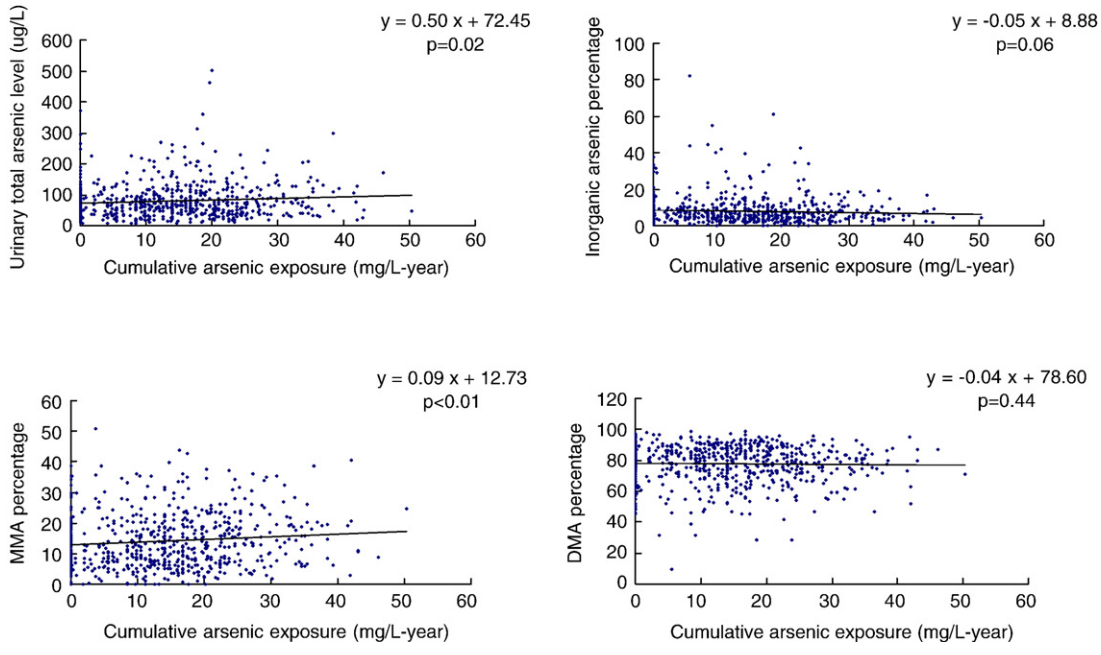

Fig. 1. Scatterplots showing the relationship between urinary arsenic parameters and cumulative arsenic exposure.

Table 1
Comparison of cumulative arsenic exposure, urinary arsenic indices and potential confounders between subjects with and without hypertension.

	Hypertension						p-value [a]
	Yes			No			
	N (%)			N (%)			
Categorical variables							
Gender							
Female	195 (52.42)			293 (58.72)			0.06
Male	177 (47.58)			206 (41.28)			
Cigarette smoke							
Never	282 (75.81)			391 (78.36)			0.37
Current or former	90 (24.19)			108 (21.64)			
Alcohol consumption							
Never	312 (83.87)			466 (89.38)			0.01
Current or former	60 (16.13)			53 (10.62)			
Continuous variables	N	Mean	S.E.	N	Mean	S.E.	p-value [b]
Age	372	53.33	0.46	499	45.54	0.45	<0.001
Body Mass Index (kg/m^2)	372	25.25	0.18	499	23.87	0.14	<0.001
Serum total cholesterol level (mg/dL)	372	229.62	3.68	499	221.34	2.97	0.07
Serum triglyceride level (mg/dL)	372	148.90	4.77	499	123.32	3.79	<0.001
CAE	285	16.68	0.60	371	11.44	0.54	<0.001
Urinary Arsenic level (μg/L)							
InAs	372	4.96	0.21	499	5.59	0.28	0.07
MMA^V	372	10.02	0.46	499	10.13	0.48	0.87
DMA^V	372	58.91	2.46	499	62.85	2.13	0.23
Total Arsenic	372	73.89	2.80	499	78.56	2.55	0.22
Urinary Arsenic Percentage		8.33					
InAs	372		0.38	499	8.20	0.35	0.81
MMA^V	372	14.32	0.46	499	13.07	0.38	0.03
DMA^V	372	77.35	0.66	499	78.73	0.53	0.10
PMI	369	3.37	0.48	494	2.87	0.31	0.37
SMI	368	9.57	0.58	485	13.23	1.81	0.05

SE: standard error.
CAE: Cumulative arsenic exposure.
PMI: Primary Methylation Index.
SMI: Secondary Methylation Index.
[a] χ^2 test.
[b] Student's t-test.

had a lower PMI than those with a lower triglyceride level (Table 2).

Arsenic methylation profiles and hypertension risk

Table 3 shows the results of the logistic regression analyses. Subjects with MMA^V percentage in the upper tertile carried a 1.5-fold higher risk of hypertension while compared with subjects with MMA^V percentage in the lowest tertiles (OR: 1.47; 95% CI=1.04–2.07) before adjustment for potential confounders (model I). However, the higher risk became non-significant after adjusting for the hypertension risk factors (Model II and Model III), suggesting that the risk of hypertension associated with a higher MMA^V percentage was explained by other risk factors.

Joint effects of cumulative arsenic exposure and urinary arsenic methylation capability on hypertension risk

In Table 4, for subjects with CAE=0 mg/L-year, the odds ratios differed without statistical significance between different subgroups of urinary arsenic indices. On the other hand, subjects with CAE >0 mg/L-year had higher risk of hypertension than those with CAE=0 mg/L-year at the same level of all the arsenic methylation indices. However, the p values for the test for trend for each combination of CAE and urinary arsenic parameters showed statistical significance. The results of these analyses suggested that although the risk of hypertension in the group with CAE >0 mg/L-year was consistently higher than the group with CAE=0 mg/L-year, a trend was noted with each of the joint effects of arsenic methylation index and CAE.

Discussion

This is the first study demonstrating the relationship between arsenic methylation capability and hypertension risk in a large community-based cohort characterized by chronic arsenic exposure from drinking water. The findings suggested that hypertensive subjects had higher urinary MMA^V percentage and lower SMI than subjects without hypertension (Table 1). An increasing trend of risk with increasing tertiles of MMA^V percentage was also observed in

Table 2
Distribution of arsenic species in different strata of life style and lipid profiles

	InAs %	MMA^V %	DMA^V %	PMI	SMI
	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.
Age (year)					
<50 (*N*=422)	8.68±0.44	11.52±0.38***	79.80±0.61***	2.99±0.46	15.31±2.15**
≥50 (*N*=449)	7.87±0.28	15.55±0.43***	76.58±0.56***	3.17±0.29	8.30±0.43**
Sex					
Female (*N*=590)	9.29±0.33	11.17±0.30***	79.54±0.45***	3.12±0.40	13.75±1.81*
Male (*N*=440)	9.45±0.33	14.72±0.44***	75.83±0.55***	3.04±0.35	9.03±0.75*
Cigarette smoke					
Never (*N*=799)	9.39±0.28	12.21±0.28**	78.40±0.40*	3.01±0.29	12.41±1.36*
Current or former (*N*=231)	9.27±0.40	14.34±0.59**	76.39±0.72*	3.33±0.65	9.15±0.82*
Alcohol consumption					
Never (*N*=894)	9.31±0.25	12.5±0.27†	78.18±0.37	3.14±0.31	11.96±1.21
Current or former (*N*=136)	9.65±0.60	13.9±0.82†	76.44±1.01	2.72±0.29	9.59±0.99
Body mass index (kg/m^2)					
<23 (*N*=305)	8.68±0.5	14.12±0.49	77.2±0.72	3.16±0.41	11.55±2.55
≥23 (*N*=566)	8.04±0.3	13.32±0.37	78.64±0.51	3.04±0.35	11.71±0.9
Total cholesterol level (mg/dL)					
<200 (*N*=352)	8.63±0.41	13.27±0.47	78.10±0.68	3.45±0.63	14.11±2.43†
≥200 (*N*=519)	8.01±0.33	13.82±0.38	78.16±0.52	2.84±0.15	9.99±0.67†
Triglyceride level (mg/dL)					
<150 (*N*=593)	8.24±0.32	13.52±0.35	78.24±0.49	3.40±0.39*	11.27±1.41
≥150 (*N*=278)	8.31±0.45	13.77±0.56	77.92±0.79	2.41±0.13*	12.46±1.43

* $p<0.05$ by Student's *t*-test.
** $p<0.01$ by Student's *t*-test.
*** $p<0.0001$ by Student's *t*-test.
† $0.05<p<0.1$ by Student's *t*-test.

the logistic regression model before adjustment for confounders (Model I of Table 3). Although none of the other urinary arsenic indices was found to be significantly associated with hypertension when categorized into tertiles in models before or after adjustment for potential confounders (Table 3), the trends were all significant when both CAE and urinary arsenic indices were considered together (Table 4). Therefore, the results suggest that both arsenic exposure dosage and arsenic methylation capability may have a joint effect on the risk of hypertension.

One of the advantages of using CAE as an exposure index is that it may well reflect the cumulative dosage of long-term exposure to arsenic in individual subjects. However, one possible limitation is that the use of the medians of arsenic levels in the artesian well water in different villages in the calculation of CAE might not be accurate if the ranges were too wide. Although urinary arsenic level reflects arsenic exposure within a few days (ATSDR, 2000), a correlation between CAE and urinary total arsenic level could also be demonstrated in this study (Fig. 1), indicating that arsenic metabolites could also be detected in the urine after a prolonged exposure at a time even when the exposure is terminated for decades (about 20–30 years in this study). In addition, even after ceasing to drink artesian well water for about 20–30 years, the urinary total arsenic levels of subjects in the BFD areas were still higher than those who lived in non-arseniasis areas in Taiwan (Hsueh et al., 2002). From the results of these studies, it is rationale to say that the accumulated arsenic burden in the human body after a long duration of exposure would still be released in the urine after more than a few decades' termination of exposure.

The distribution of urinary arsenic species in the subjects in this study was similar to our previous study in Taiwan (Hsueh et al., 1998a). Percentage of urinary InAs, MMA^V, and DMA^V in northern Argentina people who were exposed to arsenic from drinking water at the time of study was 25–49%, 2–4%, and 54–74%, respectively (Concha et al., 1998). In contrast, they were 7–10%, 20–23%, and 67–73%, respectively, in the residents of the BFD endemic areas, who have ceased to drink artesian well water for 2 to 3 decades (Hsueh et al., 1998a). Native Andes women exposed to arsenic from drinking water excreted a lower level of MMA^V (2.3–3.5%) in urine (Vahter et al., 1995). Therefore, a substantial inter-individual variation in arsenic metabolism was found in different ethnicities, which might indicate a genetic role in the regulation of enzymes involved in arsenic metabolism. On the other hand, subjects ceased to drink high arsenic-containing water and shifted to consume lower levels of arsenic-containing water showed sequential decrease in the percentage of urinary MMA^V and increase in DMA^V percentage (Hopenhayn-Rich et al., 1996).

In this study, the subjects with hypertension had a higher CAE and a higher percentage of urinary MMA^V than subjects without hypertension (Table 1). This is quite compatible with our previous study which showed patients with skin cancer having higher As^V and MMA^V percentage, lower DMA^V percentage, and lower PMI than healthy controls (Hsueh et al., 1997); and also compatible with the study of Chen et al. showing lower SMI and higher CAE in patients with skin and bladder cancer (Chen et al., 2003a, 2003b). Arsenic methylated metabolites in urine have also been shown to be biomarkers for

Table 3
Logistic regression analyses of internal arsenic exposure markers associated with hypertension

Variable	Control	HT	Model I	Model II	Model III
	N	*N*	OR (95% CI)	OR (95% CI)	OR (95% CI)
Inorganic arsenic percentage					
<4.53	180	107	1.0	1.0	1.0
4.53–8.00	188	83	0.74 (0.52–1.05)$^{+}$	0.74 (0.50–1.08)	0.72 (0.46–1.11)
≥8.00	184	129	1.17 (0.84–1.63)	1.11 (0.78–1.60)	1.21 (0.79–1.85)
p for trend			*p*=0.29	*p*=0.50	*p*=0.35
MMA^{V} percentage					
<8.14	184	85	1.0	1.0	1.0
8.14–15.55	184	109	1.28 (0.90–1.81)	1.20 (0.82–1.76)	1.35 (0.86–2.12)
≥15.55	184	125	1.47 (1.04–2.07) *	1.00 (0.68–1.49)	1.04 (0.66–1.62)
p for trend			*p*=0.02	*p*=0.99	*p*=0.97
DMA^{V} percentage					
<75.80	184	125	1.0	1.0	1.0
75.80–85.25	184	96	0.76 (0.54–1.07)	0.91 (0.63–1.31)	0.90 (0.59–1.37)
≥85.25	184	98	0.78 (0.56–1.09)	1.09 (0.75–1.60)	1.05 (0.68–1.63)
p for trend			*p*=0.14	*p*=0.66	*p*=0.83
PMI					
<1.21	182	92	1.0	1.0	1.0
1.21–2.65	182	111	1.20 (0.85–1.70)	1.05 (0.71–1.54)	1.04 (0.67–1.62)
≥2.65	183	113	1.22 (0.86–1.72)	0.88 (0.59–1.29)	0.86 (0.55–1.33)
p for trend			*p*=0.26	*p*=0.49	*p*=0.47
SMI					
<4.87	180	123	1.0	1.0	1.0
4.87–9.82	179	95	0.77 (0.55–1.08)	1.06 (0.73–1.54)	1.31 (0.85–2.02)
≥9.82	179	97	0.79 (0.56–1.11)	1.13 (0.77–1.66)	1.06 (0.68–1.65)
p for trend			*p*=0.16	*p*=0.52	*p*=0.71

$^{+}0.1<p<0.05$; *$p<0.05$; compared with the reference group.
PMI: primary methylation index, MMA^{V} level/(InAs level).
SMI: secondary methylation index, DMA^{V} level/MMA^{V} level.
Model I: univariate logistic regression model.
Model II: age, gender, body mass index, cigarette smoke, alcohol consumption and triglyceride level were adjusted in logistic regression model.
Model III: age, gender, body mass index, cigarette smoke, alcohol consumption, triglyceride level, and cumulative arsenic exposure were adjusted in logistic regression model.

disease state and disease susceptibility in other ethnicities (Valenzuela et al., 2005).

Because the assay of arsenic species was performed within 6 months after collection of the urinary samples and all samples were stored at a temperature of −20 °C, we believed that the arsenic species as measured in the present study should be reliable. The detection of the transient metabolites of MMA^{III} and DMA^{III} depends on the conditions and temperature of sample storage and the concentration in the urine, which was beyond the analytical setting of this study in 1989. We did not observe trivalent methylated metabolites in this study due to the fact that the chemical forms of trivalent methylated arsenic were unknown and the analysis method for these trivalent metabolites was not developed at the time when our urinary samples were collected and analyzed. MMA^{V} and DMA^{V} are generally considered as non-toxic previously. However, we cannot exclude the possibility that the higher MMA^{V} in the urine is a marker of higher MMA^{III} in the blood or inside the cells, where the injuries incurred by arsenic occur. Studies also showed that people with a lower MMA^{V} excretion in the urine tend to have a lower retention of arsenic (Vahter, 2002). This could also possibly explain why people with a lower MMA^{V} percentage tend to have a lower risk of developing arsenic-related diseases. Several studies have shown that a higher MMA^{V} percentage would indicate a higher risk of cancer in higher CAE group (Hsueh et al., 1997; Chen et al., 2003a, 2003b). This study may suggest that inefficient arsenic methylation capability was related to serious disease in high CAE group even after a long period of termination of exposure (Table 4). Therefore, the incidence of arsenic-related disease is determined by the arsenic methylation capability and the exposure dosage after a long-term exposure to arsenic from drinking water.

Cigarette smoking is a risk factor for hypertension, but we did not find that the smoking prevalence differed significantly between the subjects with and without hypertension (Table 1). One of the possible explanations is that smoking rate is increasing in the younger generation who are at a lower risk of developing hypertension than the older subjects. Another explanation is that arsenic effect might have exceeded the effect of tobacco.

The measurement of arsenic methylation profiles at or after the diagnosis of hypertension in this study raises the concern on the temporal correctness between arsenic methylation capability and disease development. Therefore the effects seen in this study might not be due to the impact of methylation patterns on disease, but rather, due to the impact of disease or disease treatment on methylation patterns.

Table 4
Joint effects of cumulative arsenic exposure and urinary arsenic methylation capability index on hypertension risk

	CAE=0 mg/L-year		CAE>0 mg/L-year	
	HT subjects/healthy subjects	OR (95% CI)	HT subjects/healthy subjects	OR (95% CI)
InAs %				
<6.10	13/48	1.0	103/164	2.31 (1.19–4.48)*
≥6.10	13/46	1.04 (0.43–2.48)	117/152	2.84 (1.47–5.46)**
Test for trend $p=0.0001$				
MMA^V %				
<11.30	9/48	1.0	96/148	3.45 (1.62–7.37)**
≥11.30	17/46	1.97 (0.79–4.86)	124/168	3.93 (1.86–8.32)**
Test for trend $p<0.0001$				
DMA^V %				
≥81.24	10/48	1.0	99/154	3.08 (1.49–6.38)**
<81.24	16/46	1.67 (0.68–4.05)	121/164	3.59 (1.74–7.31)**
Test for trend $p<0.0001$				
PMI				
<1.85	12/50	1.0	105/151	2.89 (1.47–5.71)**
≥1.85	14/44	1.33 (0.55–3.16)	114/162	2.93 (1.49–5.75)**
Test for trend $p=0.0005$				
SMI				
≥6.91	9/46	1.0	91/146	3.18 (1.48–6.81)**
<6.91	16/44	1.86 (0.74–4.64)	126/166	3.89 (1.83–8.20)**
Test for trend $p<0.0001$				

CAE: cumulative arsenic exposure.
PMI; primary methylation index.
SMI: secondary methylation index.
* $p<0.05$.
** $p<0.01$.

Dietary components might have an effect on the methylation capability of arsenic. DMA^V percentage was positively associated with plasma folate and MMA^V percentage negatively with plasma folate (Gamble et al., 2005). Low intake of calcium, animal protein, folate, and fiber was found to increase the susceptibility to arsenic-induced skin lesions (Mitra et al., 2004). However, our previous study did not find that the frequencies of dietary intake of fish, shellfish and seaweed were significantly correlated with urinary arsenic species in subjects who drank tap water, and arsenic methylation pattern were similar before and after refraining from eating seafood for 3 days (Hsueh et al., 2002). The lack of data collection on some hypertensive risk factors such as sodium intake and physical activity was a limitation of this study. However, because the subjects in this study were recruited from three neighboring villages and they showed similar physical activity and dietary patterns (Chen et al., 1995). Therefore, the impact of the difference in these factors might not exert significant influence on the arsenic methylation profiles between subjects with and without hypertension. Another limitation is that CAE could not be calculated in about 25% of the subjects. However, the odds ratios for hypertension for those without CAE were between the odds ratios for the lowest and the highest CAE (data not shown), suggesting that the association between hypertension risk and urinary arsenic species cannot be explained by the lack of CAE in some subjects.

In summary, after adjustment for hypertension risk factors, a relationship between the joint effect of arsenic exposure dosage and arsenic methylation capability and the risk of hypertension was noted among subjects exposed to arsenic from consuming the artesian well water for a long period of time. This risk association is observed at a time when the exposure has been terminated for 20–30 years. This study provided another direction for assessing the risks of arsenic-related diseases.

Acknowledgments

The study was supported by grants NSC-86-2314-B-038-038, NSC-87-2314-B-038-029, NSC-88-2314-B-038-112, NSC-88-2318-B-038-002-M51, NSC-89-2320-B-038-013, NSC-89-2318-B-038-M51, NSC-89-2314-B-038-049, NSC-90-2320-B-038-021, NSC-91-3112-B-038-001, NSC-92-3112-B-038-001, NSC-92-2321-B-038-004 and NSC-94-2314-B-002-142 and NSC-95-2314-B-002-311 from the National Science Council of the ROC.

References

ATSDR, 2000. Toxicological profile for arsenic. US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

Buchet, J.P., Lauwerys, R., Roels, H., 1981a. Comparison of the urinary excretion of arsenic metabolites after a single oral dose of sodium arsenite, monomethylarsonate, or dimethylarsinate in man. Int. Arch. Occup. Environ. Health 48, 71–79.

Buchet, J.P., Lauwerys, R., Roels, H., 1981b. Urinary excretion of inorganic arsenic and its metabolites after repeated ingestion of sodium metaarsenite by volunteers. Int. Arch. Occup. Environ. Health 48, 111–118.

Chen, S.L., Dzeng, S.R., Yang, M.H., Chiu, K.H., Shieh, G.M., Wai, C.M.,

1994. Arsenic species in groundwaters of the blackfoot disease area, Taiwan. Environ. Sci. Technol. 28, 881–887.

Chen, C.J., Hsueh, Y.M., Lai, M.S., Shyu, M.P., Chen, S.Y., Wu, M.M., Kuo, T.L., Tai, T.Y., 1995. Increased prevalence of hypertension and long-term arsenic exposure. Hypertension 25, 53–60.

Chen, C.J., Chiou, H.Y., Chiang, M.H., Lin, L.J., Tai, T.Y., 1996. Dose–response relationship between ischemic heart disease mortality and long-term arsenic exposure. Arterioscler., Thromb., Vasc. Biol. 16, 504–510.

Chen, Y.C., Guo, Y.L., Su, H.J., Hsueh, Y.M., Smith, T.J., Ryan, L.M., Lee, M.S., Chao, S.C., Lee, J.Y., Christiani, D.C., 2003a. Arsenic methylation and skin cancer risk in southwestern Taiwan. J. Occup. Environ. Med. 45, 241–248.

Chen, Y.C., Su, H.J., Guo, Y.L., Hsueh, Y.M., Smith, T.J., Ryan, L.M., Lee, M.S., Christiani, D.C., 2003b. Arsenic methylation and bladder cancer risk in Taiwan. Cancer Causes Control 14, 303–310.

Ch'i, I.C., Blackwell, R.Q., 1968. A controlled retrospective study of blackfoot disease, an endemic peripheral gangrene disease in Taiwan. Am. J. Epidemiol. 88, 7–24.

Chiou, H.Y., Huang, W.I., Su, C.L., Chang, S.F., Hsu, Y.H., Chen, C.J., 1997. Dose–response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. Stroke 28, 1717–1723.

Concha, G., Nermell, B., Vahter, M.V., 1998. Metabolism of inorganic arsenic in children with chronic high arsenic exposure in northern Argentina. Environ. Health Perspect. 106, 355–359.

Cullen, W.R., Reimer, K.J., 1989. Arsenic speciation in the environment. Chem. Rev. 89, 713–764.

Gamble, M.V., Liu, X., Ahsan, H., Pilsner, R., Ilievski, V., Slavkovich, V., Parvez, F., Levy, D., Factor-Litvak, P., Graziano, J.H., 2005. Folate, homocysteine, and arsenic metabolism in arsenic-exposed individuals in Bangladesh. Environ. Health Perspect. 113, 1683–1688.

Hopenhayn-Rich, C., Biggs, M.L., Kalman, D.A., Moore, L.E., Smith, A.H., 1996. Arsenic methylation patterns before and after changing from high to lower concentrations of arsenic in drinking water. Environ. Health Perspect. 104, 1200–1207.

Hsueh, Y.M., Chiou, H.Y., Huang, Y.L., Wu, W.L., Huang, C.C., Yang, M.H., Lue, L.C., Chen, G.S., Chen, C.J., 1997. Serum beta-carotene level, arsenic methylation capability, and incidence of skin cancer. Cancer Epidemiol., Biomarkers Prev. 6, 589–596.

Hsueh, Y.M., Huang, Y.L., Huang, C.C., Wu, W.L., Chen, H.M., Yang, M.H., Lue, L.C., Chen, C.J., 1998a. Urinary levels of inorganic and organic arsenic metabolites among residents in an arseniasis-hyperendemic area in Taiwan. J. Toxicol. Environ. Health, Part A 54, 431–444.

Hsueh, Y.M., Wu, W.L., Huang, Y.L., Chiou, H.Y., Tseng, C.H., Chen, C.J., 1998b. Low serum carotene level and increased risk of ischemic heart disease related to long-term arsenic exposure. Atherosclerosis 141, 249–257.

Hsueh, Y.M., Hsu, M.K., Chiou, H.Y., Yang, M.H., Huang, C.C., Chen, C.J., 2002. Urinary arsenic speciation in subjects with or without restriction from seafood dietary intake. Toxicol. Lett. 133, 83–91.

Kitchin, K.T., 2001. Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites. Toxicol. Appl. Pharmacol. 172, 249–261.

Kornitzer, M., Dramaix, M., De Backer, G., 1999. Epidemiology of risk factors for hypertension: implications for prevention and therapy. Drugs 57, 695–712.

Mandal, B.K., Ogra, Y., Suzuki, K.T., 2001. Identification of dimethylarsinous and monomethylarsonous acids in human urine of the arsenic-affected areas in West Bengal, India. Chem. Res. Toxicol. 14, 371–378.

Mitra, S.R., Mazumder, D.N., Basu, A., Block, G., Haque, R., Samanta, S., Ghosh, N., Smith, M.M., von Ehrenstein, O.S., Smith, A.H., 2004. Nutritional factors and susceptibility to arsenic-caused skin lesions in West Bengal, India. Environ. Health Perspect. 112, 1104–1109.

Nesnow, S., Roop, B.C., Lambert, G., Kadiiska, M., Mason, R.P., Cullen, W.R., Mass, M.J., 2002. DNA damage induced by methylated trivalent arsenicals is mediated by reactive oxygen species. Chem. Res. Toxicol. 15, 1627–1634.

Nordstrom, D.K., 2002. Public health. Worldwide occurrences of arsenic in ground water. Science 296, 2143–2145.

Petrick, J.S., Jagadish, B., Mash, E.A., Aposhian, H.V., 2001. Monomethylarsonous acid (MMA(III)) and arsenite: LD(50) in hamsters and in vitro inhibition of pyruvate dehydrogenase. Chem. Res. Toxicol. 14, 651–656.

Rahman, M., Tondel, M., Ahmad, S.A., Chowdhury, I.A., Faruquee, M.H., Axelson, O., 1999. Hypertension and arsenic exposure in Bangladesh. Hypertension 33, 74–78.

Rose, G.A., Blackburn, H., Gillum, R.F., Prineas, R.J., 1982. Cardiovascular Survey Methods, 2nd ed. World Health Organization, Geneva, Switzerland, pp. 82–85.

Styblo, M., Vega, L., Germolec, D.R., Luster, M.I., Del Razo, L.M., Wang, C., Cullen, W.R., Thomas, D.J., 1999. Matabolism and toxicity of arsenicals in cultured cells. In: Chappell, W.R., Abernathy, C.O., Calderon, R.L. (Eds.), Arsenic Exposure and Health Effect. Elsevier, pp. 311–323.

Styblo, M., Del Razo, L.M., Vega, L., Germolec, D.R., LeCluyse, E.L., Hamilton, G.A., Reed, W., Wang, C., Cullen, W.R., Thomas, D.J., 2000. Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. Arch. Toxicol. 74, 289–299.

Styblo, M., Drobna, Z., Jaspers, I., Lin, S., Thomas, D.J., 2002. The role of biomethylation in toxicity and carcinogenicity of arsenic: a research update. Environ. Health Perspect. 110 (Suppl. 5), 767–771.

Thomas, D.J., Styblo, M., Lin, S., 2001. The cellular metabolism and systemic toxicity of arsenic. Toxicol. Appl. Pharmacol. 176, 127–144.

Thomas, D.J., Waters, S.B., Styblo, M., 2004. Elucidating the pathway for arsenic methylation. Toxicol. Appl. Pharmacol. 198, 319–326.

Tseng, C.H., Chong, C.K., Chen, C.J., 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 120, 125–133.

Tseng, C.H., Chong, C.K., Chen, C.J., Tai, T.Y., 1997. Lipid profile and peripheral vascular disease in arseniasis-hyperendemic villages in Taiwan. Angiology 48, 321–335.

Tseng, C.H., Chong, C.K., Tseng, C.P., Hsueh, Y.M., Chiou, H.Y., Tseng, C.C., Chen, C.J., 2003. Long-term arsenic exposure and ischemic heart disease in arseniasis-hyperendemic villages in Taiwan. Toxicol. Lett. 137, 15–21.

Tseng, C.H., Huang, Y.K., Huang, Y.L., Chung, C.J., Yang, M.H., Chen, C.J., 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.

Vahter, M., 1981. Biotransformation of trivalent and pentavalent inorganic arsenic in mice and rats. Environ. Res. 25, 286–293.

Vahter, M., 1999. Methylation of inorganic arsenic in different mammalian species and population groups. Sci. Prog. 82, 69–88.

Vahter, M., 2002. Mechanisms of arsenic biotransformation. Toxicology 181–182, 211–217.

Vahter, M., Concha, G., Nermell, B., Nilsson, R., Dulout, F., Natarajan, A.T., 1995. A unique metabolism of inorganic arsenic in native Andean women. Eur. J. Pharmacol. 293, 455–462.

Valenzuela, O.L., Borja-Aburto, V.H., Garcia-Vargas, G.G., Cruz-Gonzalez, M.B., Garcia-Montalvo, E.A., Calderon-Aranda, E.S., Del Razo, L.M., 2005. Urinary trivalent methylated arsenic species in a population chronically exposed to inorganic arsenic. Environ. Health Perspect. 113, 250–254.

Wang, C.H., Jeng, J.S., Yip, P.K., Chen, C.L., Hsu, L.I., Hsueh, Y.M., Chiou, H.Y., Wu, M.M., Chen, C.J., 2002. Biological gradient between long-term arsenic exposure and carotid atherosclerosis. Circulation 105, 1804–1809.

Wu, H.Y., Chen, K.P., Tseng, W.P., Hsu, C.L., 1961. Epidemiologic studies on Blackfoot disease: I. Prevalence and incidence of the disease by age, sex, occupation and geographical distribution. Mem. Coll. Med. Natl. Taiwan Univ. 7, 33–50.